Class 1 Integrons and Their Impact on the Mobility of Antibiotic Resistance in Clinical Environments

Miranda Christopher (BBiotech)

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Department of Biological Sciences,

Macquarie University, Australia.

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Master of Philosophy

Statement of Candidature

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of a requirement for a degree to any other university or institute other than Macquarie University. I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research, work and preparation of the thesis itself have been appropriately acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signed

Miranda Christopher

Date

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Abstract

Antibiotic resistance in pathogenic bacteria is becoming an increasingly important issue in the management of infectious diseases. Mortalities resulting from the inability to treat infections caused by resistant organisms have been recorded in significant numbers around the world. Mobile genetic elements such as integrons, transposons and gene cassettes, along with the lateral gene transfer which occurs between them, are largely responsible for the spread of resistance genes amongst pathogenic Gram negative bacteria.

Integrons are gene expression systems capable of capturing and disseminating mobile gene elements such as gene cassettes. These gene cassettes, when associated with clinical integrons, predominantly code for antibiotic resistance genes. The fact that integrons allow both the integration and excision of multiple gene cassettes leads to the efficient spread of multi-drug resistance amongst pathogenic bacteria.

This project investigates antibiotic resistance in *Escherichia coli* by examining class 1 integrons within several contexts. A collection of 718 *E. coli* isolates were obtained from clinical sources, primarily originating from urinary tract infections. These isolates were typed for antibiotic resistance capabilities and tested for class 1 and 2 integron presence. Integron cassette arrays were characterized, sequenced and mapped. In the collection, 23% of isolates carried class 1 integrons, while 3% carried a class 2 integron. Eight distinct cassette arrays were detected, consisting of gene cassettes solely from the *aadA* and *dfrA* families. Since identical integrons and cassette arrays appeared in a range of *E. coli* phylotypes and clonal lines, there appears to be significant and ongoing lateral gene transfer between *E. coli* lineages.

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Chapter 1

Introduction

1.1 - Antibiotic Resistance

Antibiotic resistance in clinically relevant strains of bacteria is a major issue in the control of infectious diseases. Pathogens possessing genes which code for antibiotic resistance may be immune to standard antibiotic treatments, resulting in reduced ability to treat or contain outbreaks and infections. This inability to treat infections leads to a mortality and morbidity rate which is increasing world-wide. The spread of such antibiotic resistance has been increasing since the first clinical use of antibiotics.

The discovery of antibiotics made it possible to treat many infectious diseases previously thought to be incurable. As a result, antibiotics were prescribed for almost any illness, regardless of whether it was caused by bacteria that were sensitive to such treatment. Unfortunately, the use of antibiotics selected for organisms which were resistant to their effects (Oakberg & Luria 1947). In more recent times, the use of combination antibiotic therapy has helped to lengthen the effective lifetime of antibiotics. However, combination therapy has also

resulted in a considerable increase in the number of organisms that exhibit co-resistance, or have multi-resistance traits (Rowe-Magnus & Mazel 2002).

Until recently, it was not known that genes could easily be transferred between distantly related groups of microorganisms (Salyers & Amabile-Cuevas 1997). We now know that one of the major mechanisms that drives the spread of antibiotic resistance is lateral gene transfer (LGT), where mobile genetic elements disseminate antibiotic resistance genes by moving between cells (Beiko *et al.* 2005).

Sharing of antibiotic resistance traits via LGT can occur between resistant and non-resistant strains. This results in the generation of increasingly resistant pathogens and the introduction of resistance traits into organisms that would previously respond to treatment. LGT can also transfer genes involved in heavy metal resistance and genes conferring the ability to survive high concentrations of disinfectants such as quaternary ammonium compounds (Hall & Stokes 1993). The over-prescription and misuse of antibiotics by healthcare professionals and patients provides a strong selective pressure, favouring the emergence of resistant strains. This ensures the fixation of genes mobilised by lateral transfers, often involving resistance genes (Wright & Poinar 2012).

Decreasing the use of antibiotics does not necessarily result in a return to a pre-selection population of bacteria. Resistant strains do not lose their resistance genes when selective pressures have been relaxed (Salyers & Amabile-Cuevas 1997). This is believed to be because the presence of such genes is not significantly detrimental to the organisms' survival. That is, when there is no selective pressure, having the ability to resist antibiotics is not harmful to the organism. As such, there is no evolutionary advantage to losing the resistance genes.

Unfortunately, even if the level of resistant strains could be diminished, it would be a short period of time before reuse of antibiotics raised the frequency of resistance again.

The presence of antibiotic resistance genes in environmental isolates which do not naturally encounter antibiotic selection indicates that such resistance genes can be stably maintained without having to be under continuous selective pressure (Salyers & Amabile-Cuevas 1997). Long term exposure of clinical bacteria to low concentrations of antibiotics is a condition which is most likely to allow the maintenance of resistance genes, suggesting that the overuse of antibiotics by medical professionals and the agricultural industry could be as important as the use of antibiotics in hospitals (Salyers & Amabile-Cuevas 1997).

Genetic elements such as integrons are capable of carrying many genes for antibiotic resistance in the form of mobile gene cassettes. Such resistance genes can be easily moved between organisms and quickly integrated into their genomes. This results in a continuously changing diversity in resistance capabilities amongst infectious organisms and poses a major threat to our ability to treat infectious diseases.

1.2 - Lateral Gene Transfer

LGT is a process which involves the movement of genetic material from one organism to another, resulting in one or more genes being relocated, while maintaining functionality (Davis *et al.* 1980). LGT can occur via one of three methods; transformation, transduction or conjugation (Figure 1.1) (Furuya & Lowy 2006). Natural transformation is where a bacterium is inherently able to take up DNA from its environment and integrate it into its own genome.

Transduction involves a phage which infects the host cell, takes up host DNA, is released by lysogeny, then infects a new cell and integrates the DNA taken from the previous host. Finally, conjugation occurs when two bacterial cells, one carrying a plasmid, link via a sex pilus (de la Cruz & Davies 2000). A copy of the plasmid in the donor cell then passes through the sex pilus into the recipient cell, resulting in the lateral transfer of all genes present on that plasmid.

One genetic element that plays a major role in LGT and the dissemination of resistance genes is the integron. Integrons are gene capture and expression systems now found in 20-60% of Gram negative bacteria isolated from clinical contexts (Labbate *et al.* 2009). Integrons are able to function in diverse locations and have been found located on bacterial chromosomes, within transposons and on plasmids (Ochman *et al.* 2000). When carried within transposons or plasmids, integrons can be highly mobile, as both transposons and plasmids are themselves mobile. This facilitates both intra- and interspecies gene transmission, and has major implications for the spread of antibiotic resistance genes, and for other phenotypic traits carried on mobile gene cassettes (Glenn *et al.* 2012). Complex DNA elements composed of transposons, integrons and gene cassettes are responsible for the spread of antibiotic resistance genes via conjugation (Ochman *et al.* 2000).

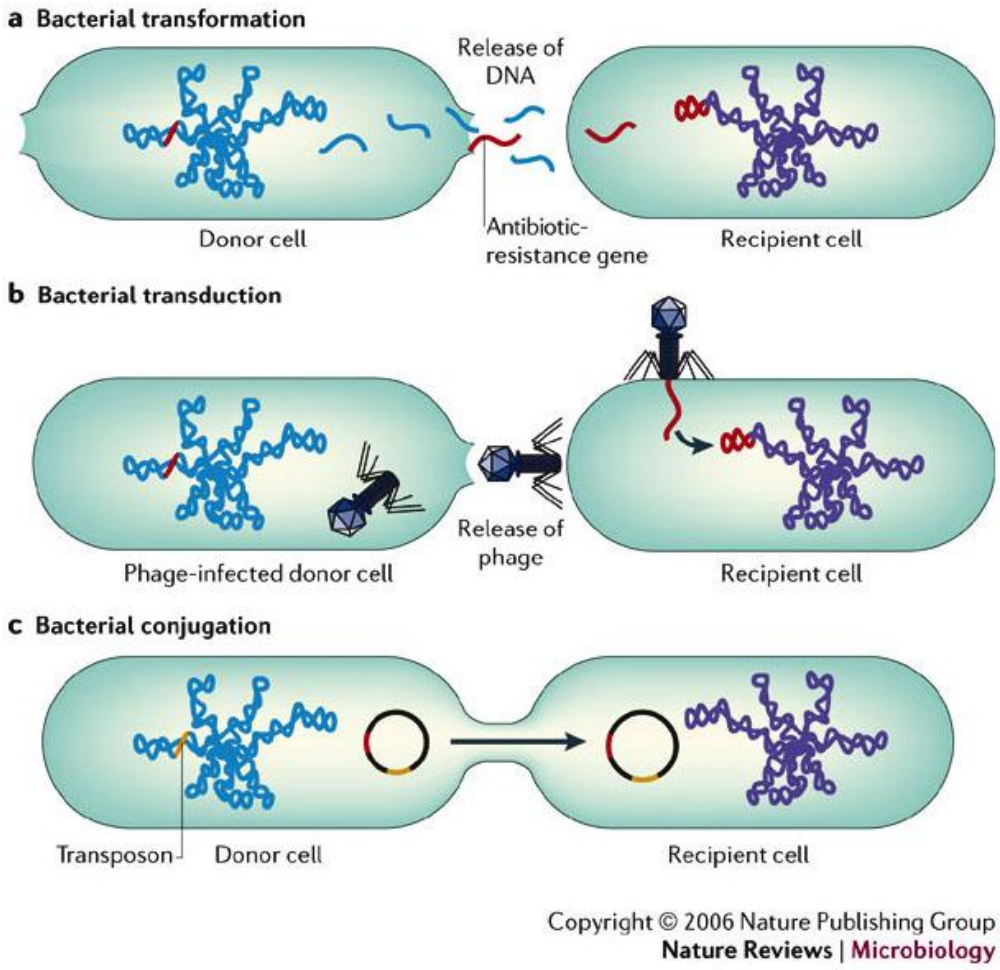

Figure 1.1 The three different mechanisms responsible for lateral gene transfer in bacteria

Taken from (Furuya & Lowy 2006).

1.3 - Mobile Genetic Elements

1.3.1 - Plasmids & Transposons

Plasmids are circular extra-chromosomal DNA molecules (Bauman 2009). They are capable of replicating independently of chromosomal DNA, and can be found in almost all microorganisms (Garret & Grisham 1999). Plasmids can encode various traits, and can also carry further mobile elements such as transposons and integrons. Their size can vary considerably, from 1 to 1000 kb. As shown in figure 1.1, entire plasmids can pass from one organism to another, potentially carrying resistance genes and other mobile genetic elements.

Transposons are also independently mobilisable genetic elements (Bauman 2009). However, unlike plasmids, families of transposons can be either replicative or non-replicative. As such, transposons can be located on a chromosome, and by excising themselves, can relocate to another chromosome or onto a plasmid within the same cell, or vice-versa (Garret & Grisham 1999). This non-replicative form of transposition results in the introduction of genes carried within the transposon into either the chromosome or plasmid DNA. Particular classes of integrons are known to be associated with, or inserted into, specific families of transposons. For example, clinical class 1 integrons have been associated with the Tn*402*/Tn*5090* transposons (Radström *et al.* 1994).

1.3.2 - Integrons

Integrons are genetic platforms that are capable of integrating and excising gene cassettes. The most basic form of integron contains an integron-integrase gene (*intI*) and a contiguous recombination site (*attI*) proximal to *intI* (Figure 1.2) (Stokes *et al.* 1997). The integron integrase is responsible for mediating the orientation and specific integration of gene cassettes by

catalysing the recombination between the *attI* site of an integron and the *attC* recombination site of a gene cassette (Stokes *et al.* 1997). A promoter within the integrase gene often drives the expression of genes within inserted cassettes. However, it has also been found that some gene cassettes carry their own promoters (Michael & Labbate 2010). Integration of gene cassettes leads to the insertion of the cassette-encoded gene downstream of the promoter. The strength of the promoter can significantly affect the expression of that gene (Boucher *et al.* 2007). In all integrons that have been investigated so far, gene cassettes are able to be successfully expressed from the integrase-associated promoter (Lévesque *et al.* 1994; Collis & Hall 1995), and it is presumed that this applies to all integrons. Consequently, integrons can sample gene cassettes, integrate them into the bacterial genome without interrupting existing genes, and express the gene cassette immediately (Stokes & Hall 1989).

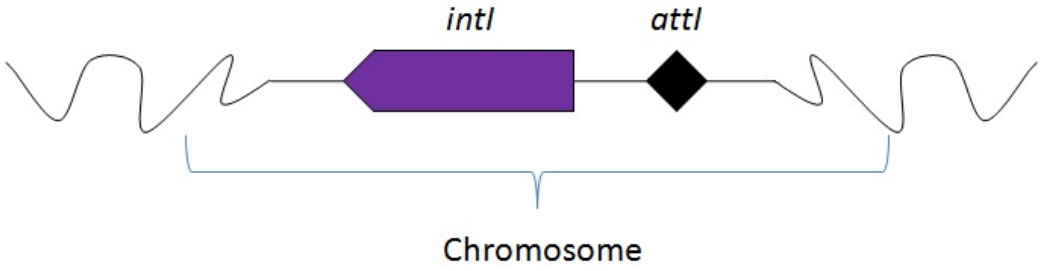

Figure 1.2 Basic structure of an integron. The purple solid arrow indicates the integrase gene, pointing in the direction of transcription. The black diamond represents the *attI* recombination site. The chromosomal DNA is indicated at each end of the integron. The insertion of an integron into chromosomal DNA may or may not interrupt the transcription of the genes around it.

Integrons were initially grouped into numbered classes based on the sequence homology of their respective integrase genes (Recchia & Hall 1995). Using this criterion, there would now be over 100 classes of integrons. Consequently, the formal numbering of new integron classes has been abandoned. However, the clinically important integrons, classes 1, 2 and 3 are still referred to by this nomenclature.

Class 1 integrons are the best studied class to date, and are considered to be the primary group responsible for the capture of antibiotic resistance gene cassettes in clinical contexts (Recchia & Hall 1995). However, all three numbered classes (1, 2 & 3) are considered to be important in mediating and disseminating antibiotic resistance.

1.3.3 - Gene Cassettes

Gene cassettes are some of the smallest known mobile genetic elements next to MITES. They are mobilized by site-specific recombination, and are capable of both integrating into and excising from integron cassette arrays (Hall *et al.* 1991; Collis & Hall 1992b; Rowe-Magnus *et al.* 1999). Gene cassettes found in pathogens and human commensals primarily encode different forms of antibiotic resistance (Partridge *et al.* 2009). In contrast, the gene cassettes carried by environmental integrons often encode functions which are yet to be determined (Mazel 1998; Stokes *et al.* 2001; Michael *et al.* 2004; Koenig *et al.* 2009).

Gene cassettes consist of a promotorless open reading frame (orf) which usually encodes a single polypeptide, and a recombination site known as *attC* (Figure 1.3)(Boucher *et al.* 2006; Partridge *et al.* 2009). Sometimes, there is a promoter within the orf, although, due to the presence of promoters within the integron integrase gene, this is not necessary for cassette

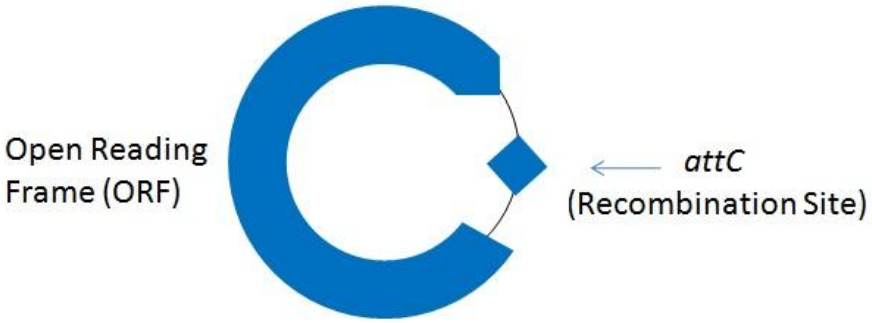

Figure 1.3 Genetic organization of a circularised gene cassette, showing a single orf and recombination site (Collis & Hall 1992a). The solid blue arrow represents the gene carried by the gene cassette. The cassette bound recombination site, *attC*, a blue diamond, is represented in the same colour as the gene because each ORF is associated with a particular *attC* site.

expression. Gene cassettes can exist in two forms; as free circular molecules, or in linear form within an integron or conjugative transposon (Recchia & Hall 1997). These genes cannot be successfully expressed unless inserted into a functional integron (Recchia & Hall 1995).

Gene cassettes can be inserted into or excised from integrons via recombination between the *attI* site of an integron and its *attC* site, or between the *attC* sites of multiple gene cassettes (Figure 1.4) (Holmes *et al.* 2003; Boucher *et al.* 2006). The recombination of multiple cassettes with *attI* results in the production of cassette arrays which can contain from 2 to over 200 cassettes. The ability of gene cassettes to sequentially integrate into an integron generates cassette arrays which are capable of conferring multiple resistance phenotypes. It has also been found that the recombination sites of gene cassettes can be highly variable in both length and sequence, in some cases, each *attC* being unique (Nield *et al.* 2001). The *attC* determines the orientation of the cassette upon integration; this ensures the cassette encoded gene is integrated in the correct orientation for transcription (Rowe-Magnus *et al.* 1999).

Although many thousands of gene cassettes have been described, only 100 or so are known to encode antibiotic resistance. The majority of cassettes described from natural environments encode hypothetical proteins or proteins of unknown function (Stokes *et al.* 2001; Michael *et al.* 2004; Partridge *et al.* 2009). It is understood that despite the fact that most gene cassettes have unknown function, they do produce functional proteins (Nield *et al.* 2001; Robinson *et al.* 2005). The collective gene cassette pool is potentially shared between all organisms containing integrons, thus allowing the transfer of diverse genetic material between many different species (Holmes *et al.* 2003).

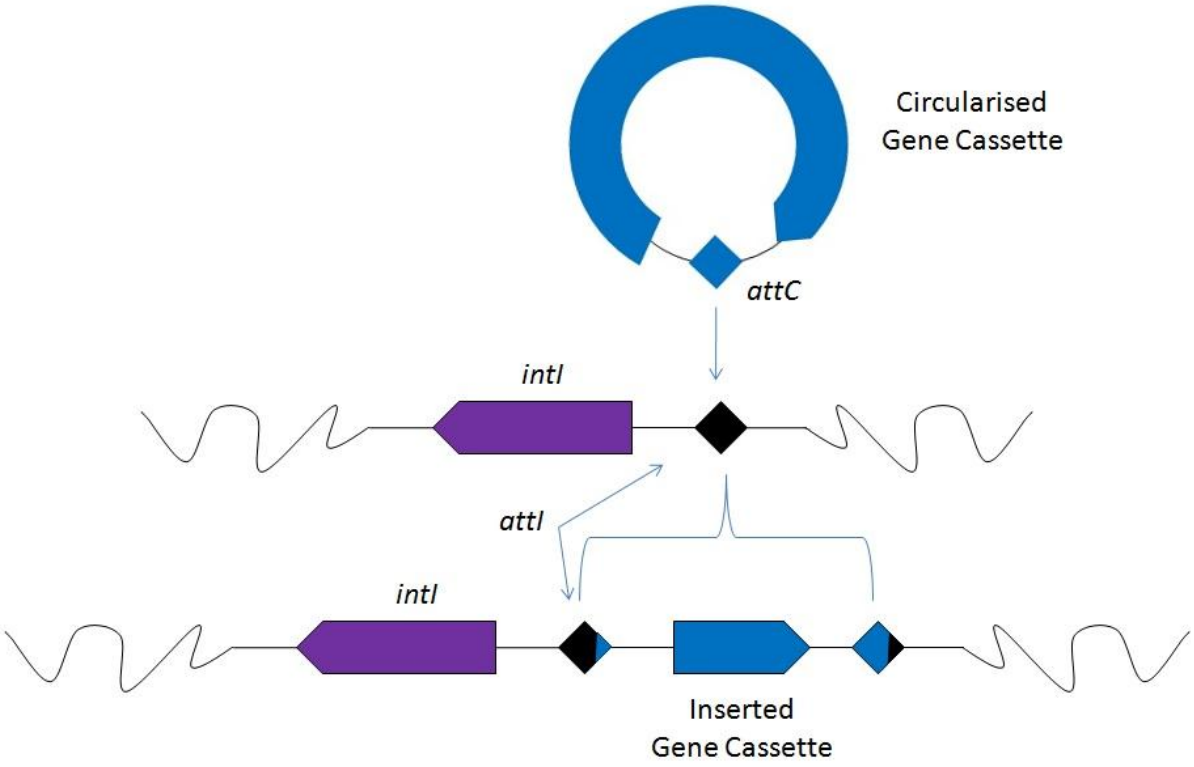

Figure 1.4 The integration of a gene cassette into an integron via recombination of the *attC* and *attI* sites. The purple solid arrow indicates the integrase gene, pointing in the direction of transcription. The black diamond represents the *attI* recombination site. The chromosomal DNA is indicated at each end of the integron. The solid blue arrow represents the gene carried by the gene cassette and *attC* is a blue diamond. Post integration, the *attI* and *attC* recombination sites have been joined, represented by a black diamond with small piece of blue (*attI*) and the blue diamond with a small piece of black (*attC*). Further gene cassettes may continue to be inserted at *attI.*

Novel resistance patterns can be created by the capture and insertion of new gene cassettes, or shuffling and deletion of one or more pre-existing cassettes within an integron bound cassette array. Integrons are capable of sampling gene cassettes from environmental, commensal and clinical bacterial communities (Fluit & Schmitz 1999). This ability to freely sample genes from almost any environment increases both the diversity and likelihood of genetic dissemination.

One piece of evidence for the diverse origins of gene cassettes is that the codon usage of the genes found in gene cassettes is highly variable (Rowe-Magnus *et al.* 2002). As different groups of organisms have a tendency to favour certain codon types, this indicates that gene cassettes originated in many different organisms and were generated by a currently unknown mechanism (Recchia & Hall 1997; Holmes *et al.* 2003). As yet, there are only hypotheses for gene cassette formation (Léon & Roy, 2009). It has been proposed that they are formed by reverse transcription of mRNA molecules, which is believed to explain the presence of a complete gene with little in the way of flanking sequence (Collis & Hall 1995; Recchia & Hall 1997). However, the reverse transcriptase responsible for this process is yet to be identified and there is still little known about the origins of the *attC* site of gene cassettes (Recchia & Hall 1997). In all, it is clear that there is still a great deal to be learnt about these mobile genetic elements and their role in bacterial genome evolution. The gene pool created by gene cassettes needs to be more extensively characterized to help predict the potential consequences of lateral gene transfer mediated by integrons.

1.4 - Clinically Relevant Integrons

Class 1, 2 and 3 integrons from clinical contexts are all believed to have been recently derived from environmental sources (Gillings *et al.* 2008b). Each are associated with transposons and/or plasmids, which facilitate their rapid movement between cells and species. These three classes of integron have received the most attention, and our understanding of integron function is primarily based on these classes (Boucher *et al.* 2007). Integron classes 1, 2 and 3 are differentiated from each other by the amino acid homology of their respective integrases (Boucher *et al.* 2007). Other classes of integrons are found on chromosomes and are not known to be mobile over short evolutionary time frames. Such chromosomal integrons are usually named after the organism in which they were originally found.

1.5 - Class 1 Integrons

Class 1 integrons were the first group of integrons to be discovered. They are responsible for a substantial proportion of multi-drug resistance in hospital acquired infections (Boucher *et al.* 2007). Class 1 integrons are now present in 20-60% of gram negative pathogens isolated from clinical contexts, or from livestock (Oppegaard *et al.* 2001; Wu *et al.* 2011). They are important in the spread of antibiotic resistance genes amongst pathogenic bacteria as they can serve as vehicles for the intra- and inter-species transmission of resistance genes (Martinez & de la Cruz 1988). This may be due to their presence in both environmental and clinical bacteria, thus providing the mechanism for the transfer of genes from one environment to another.

Many different structural compositions of the class 1 integron have been recorded. It is believed that these record the evolutionary path that may have been taken between environmental class

1 integrons and the clinically important class 1 integrons found commonly today. Although the general classification of a class 1 integron is strict in regards to the genetic backbone, there is still a large amount of potential variability with respect to the number, type and order of gene cassettes they may carry, and the structure of the region beyond the cassette array (Gillings *et al.* 2008b). More than one gene cassette may be integrated.

A basic class 1 integron consists of an integrase gene (*intI1*) and a recombination site (*attI1*), much like the simple integron discussed earlier. A class 1 integron may carry an array of gene cassettes, or none at all. When no gene cassettes are integrated, the 5' conserved segment (5'CS) is adjoined directly to the 3' conserved segment (3'CS) with the recombination site (*attI*) being complete rather than recombined with an *attC* (Figure 1.5) (Collis *et al.* 1993).

As previously mentioned, gene cassettes integrated into an integron rely on a pre-existing promoter (Pc) for their expression (Lévesque *et al.* 1994; Collis & Hall 1995). The level of gene cassette expression is affected by the distance of the cassette from the promoter located within the integrase gene (Lévesque *et al.* 1994; Collis & Hall 1995). The expression of cassettes is also affected by the strength of the promoters located upstream in the integron (Collis & Hall 1995).The strength of promoters varies amongst clinical class 1 integrons, coinciding with sequence changes within the class 1 integrase gene. In some cases it has been found that more than one promoter can be present in a class 1 integron (Collis & Hall 1995; Rowe-Magnus & Mazel 2002). Ultimately, the closer a gene cassette is to the promoter, the more likely it is to be expressed. Genes further down the cassette array may not be expressed at all, especially if the array contains many cassettes, as is often the case in integrons found in *Vibrio spp,* however, these are not class 1 integrons (Rowe-Magnus *et al.* 1999).

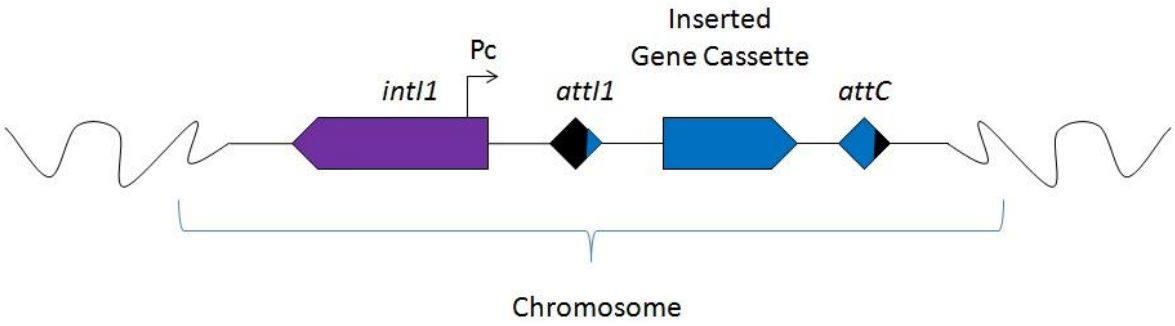

Figure 1.5 Genetic organization of a basic class 1 integron with inserted gene cassette, showing the internal promoter (Pc). The solid purple arrow indicates the class 1 integron integrase gene and direction of transcription. The partial black diamond represents the *attI* recombination site after the integration of a gene cassette (blue solid arrow). Since the promoter is located within the integrase gene, expression of gene cassettes inserted downstream is affected by its strength. The promoters' ability to drive expression is stronger for proximal cassettes. The further away cassettes are inserted, the more they have to rely on internal promoters for expression.

1.6 - The Evolution of the Clinical Class 1 Integron

Class 1 integrons are believed to have been in existence for an extremely long time. Their involvement in the dissemination of resistance genes began when antibiotics were first used to treat infections. Since that time, over 50 years ago, this clinically important class of integrons has evolved, becoming more efficient in conferring resistance. They can also confer resistance to other treatments including quaternary ammonium compounds and sulphonamides (Gillings *et al.* 2009b).

The exact origin of the clinical class 1 integron platform is unknown. However, the discovery of different conformations of class 1 integrons allows inferences about their evolutionary origins to be made (Gillings *et al.* 2008a). If the transition from environmental class 1 integrons to the clinically relevant forms can be characterised, a better understanding of the development of such genetic elements can be obtained. The mechanisms involved in their production and the relevant selective pressures that direct their evolution would also be valuable information if identified.

Class 1 integrons have been characterised from many different environments. It has been found that class 1 integrons can have many different genetic conformations. The one thing that all class 1 integrons have in common is simply a class 1 integron-integrase gene. While there is a common structure to the different conformations of a class 1 integron, the genes which surround it can vary. These variations have occurred over time due to random deletions, insertions and gene rearrangements, many made possible by the very nature of the integron itself, or by the transposons they are often associated with.

The basic class 1 integron (Figure 1.5) is most commonly found in environmentally isolated organisms. It is this integron that is referred to as an environmental class 1 integron; often carrying gene cassettes of no clinical significance. At some point in the past, the basic class 1 integron was

captured by a Tn*402* transposon (Figures 1.6, 1.7). The discovery of class 1 integrons which predate the association with a Tn*402* like transposon indicates that the ancestor to this class was most likely chromosomally located, like most other forms of environmental integrons found to date (Stokes *et al.* 2006). It has been suggested that the evolution of today's clinical class 1 integrons began with a chromosomal integron being incorporated into a plasmid-borne Tn*402* transposon during a mobilization event (Gillings *et al.* 2008a). As a result of this insertion event, clinical class 1 integrons became associated with the transposition functions of the Tn*402* transposon, which itself transposed into the larger Tn*21* element (Mazel 2006; Gillings *et al.* 2008b). This association with transposons and transposition elements increases the potential for mobility. The mechanism for chromosomal class 1 integron movement is still unknown, although it was originally assumed to be due to class 1 integrons' common association with transposons. However, the discovery of environmental isolates containing class 1 integrons which lacked any Tn*402* features suggests this is not the case (Gillings *et al.* 2008b).

As a part of the insertion into the Tn*402* transposon, clinical class 1 integrons possess 2 conserved non-coding 25bp sequences, one located at the left hand end of the *intI1* gene (IRi) and a reverse repeat of this sequence at the right hand end of the entire integron (IRt) (Gillings *et al.* 2008a). These two sequences therefore define the limits of a clinical class 1 integron. Integrons which have not become associated with the Tn*402* transposon do not possess these sequences (Gillings *et al.* 2009a). This division between pre Tn*402* and post Tn*402* association helps to separate the broader groups of environmental and clinical integrons, as all clinical integrons are thought to be descendants of the initial event that inserted a chromosomal class 1 integron into a Tn*402* like element.

Recently, some environmental isolates from freshwater sediments were found to contain class 1 integrons which were unlike any seen before (Stokes *et al.* 2006). These unusual integrons shared 99% or more nucleotide sequence identity in their integrase genes and recombination sites with those found in clinical isolates, but at the same time lacked association with the Tn*402* transposition system (Stokes *et al.* 2006). These environmental class 1 integrons were found to contain gene cassettes, however, these cassettes did not code for any form of antibiotic resistance. The high level of similarity between the two forms of class 1 integrons (environmental versus clinical) indicate that the clinical integrons seen today may have evolved from the environmental form.

It is believed that after the insertion event with a Tn*402* transposon, the integration of a gene cassette encoding for resistance to quaternary ammonium compounds (*qacE*) occurred. Shortly after this, a gene for resistance to sulfonamides was also integrated, truncating the *qacE* gene, now termed *qacEΔ.* The insertion of the *sul* gene also resulted in a truncation of the *tni* module, reducing the mobility of the transposition module.

An immediate common ancestor to the typical clinical class 1 integron has been suggested as being similar to a Tn*402*-like transposon with an arrangement like that shown in figure 1.8 (Stokes *et al.* 2006; Gillings *et al.* 2008a). Isolates containing integrons that exhibit deletions in the *qacE* gene, incorporation of the *sul* gene and partial deletions of the *tni* module are most commonly found in clinical contexts (Gillings *et al.* 2008a). These class 1 integron modifications have been illustrated in figures 1.9 and 1.10. It is important to note that while class 1 integrons vary in their 3'CS, the 5'CS remains the same in all clinical isolates (Brown *et al.* 1996).

Figure 1.6 The Tn*402* transposon, showing the complete *tni* module (Radström *et al.* 1994). Green triangles represent the terminal inverted repeats at the boundaries of the transposon; the yellow, orange, red and pink solid arrows represent the four genes of the *tni* module, pointing in the direction of transcription. The *tniABQ* genes mediate transposition via cointegration of their products. This process is catalysed by the product of the *tniC* gene, which is a member of the invertase/resolvase family (Kholodii *et al.* 1995).

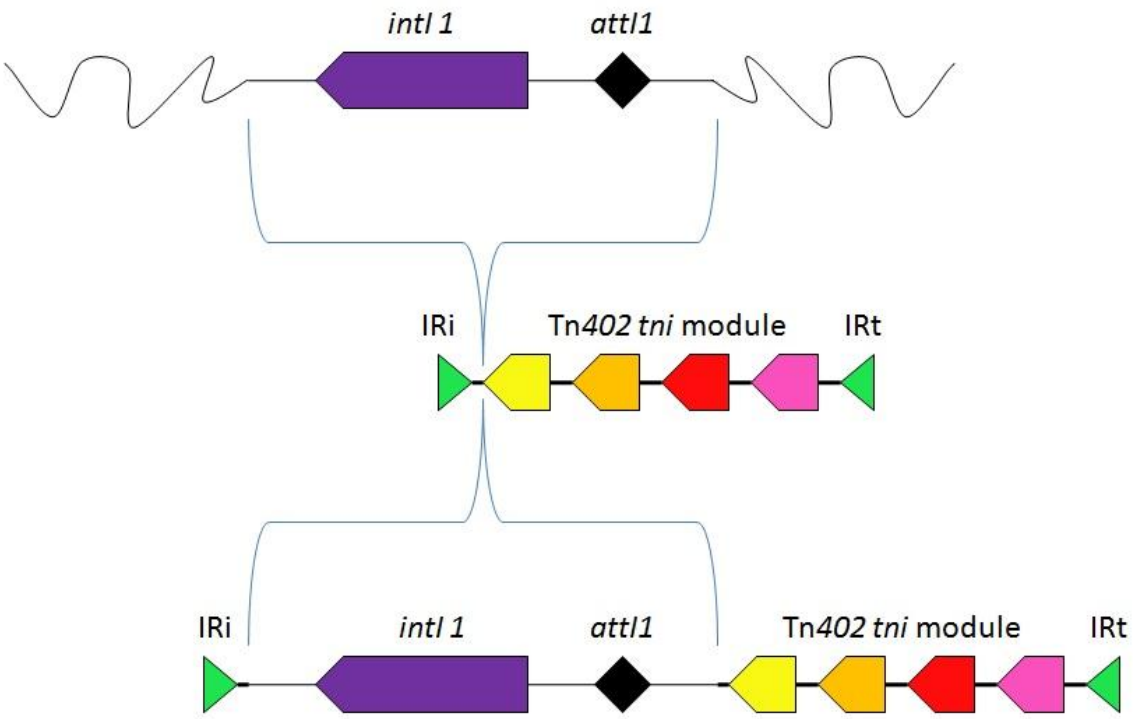

Figure 1.7 Insertion of a class 1 integron into the Tn*402* transposon, forming the Tn*402*-like class 1 integron (Sajjad *et al.* 2011). Green triangles represent the terminal inverted repeats at the boundaries of the transposon; the yellow, orange, red and pink solid arrows represent the four genes of the tni module, pointing in the direction of transcription. The purple solid arrow indicates the integron-integrase gene, pointing in the direction of transcription. The black diamond represents the *attI* recombination site. The chromosomal DNA is indicated at each end of the integron pre-insertion. The insertion of a class 1 integron into the Tn*402* transposon significantly increased the likelihood of recombination events, as the entire integron is now capable of being moved, while also being capable of sampling the vast array of gene cassettes in the environment.

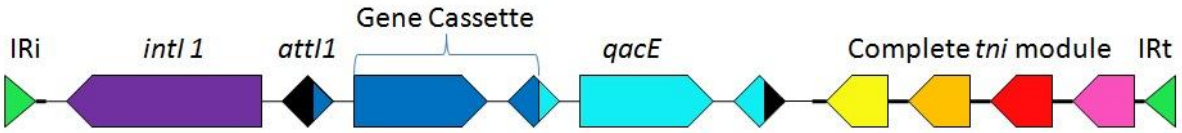

Figure 1.8 Insertion of the quaternary ammonium compound resistance gene cassette (*qacE*) into the Tn*402*-like class 1 integron (Gillings *et al.* 2009b). The aqua solid arrow represents the *qacE* gene and its direction of transcription. *qacE* is believed to have been a gene cassette, as such; its insertion did not interrupt the arrangement of the integron itself.

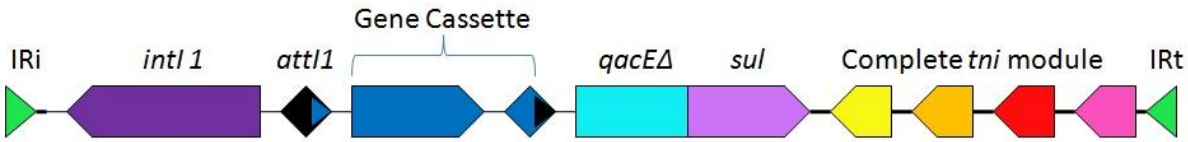

Figure 1.9 Insertion of the sulfonamide resistance gene (*sul*) into the Tn*402*-like class 1 integron. The light purple solid arrow indicates the *sul* gene and its direction of transcription. The insertion of the sul gene resulted in the truncation of the *qacE* gene, removing the *attC* site and thus preventing *qacEΔ* mobilisation.

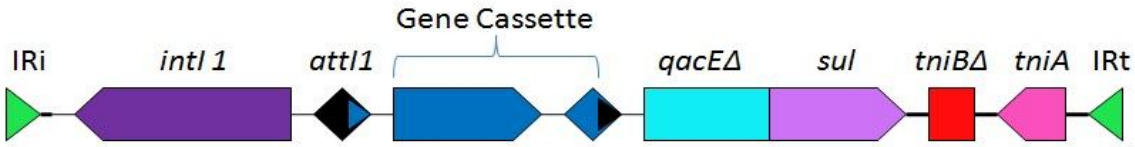

Figure 1.10 Clinical class 1 integron, showing truncated *tni* module and *qacEΔ* gene (Stokes & Hall 1989). Later deletion events are believed to have occurred, resulting in a truncated *tni* module. This resulted in a decreased capacity for transposition via the Tn*402* transposon. This is the most common form of class 1 integron found in clinical isolates, often carrying one or more resistance cassettes.

1.7 - Class 2 & 3 Integrons

Class 2 and 3 integrons, like class 1 integrons, are considered to be clinically important. The scope of this project covered only class 1 and 2 integrons, as class 3 integrons are rare and have only been found in *Serratia marcescens* and *Klebsiella pneumoniae.* This study investigates integrons in *Escherichia coli* and therefore class 3 integrons are unlikely to be present.

Class 2 integrons are associated with the Tn*7* transposon, possess a defective integrase gene and lack a 3' conserved region. Class 2 integrons are also capable of carrying antibiotic resistance genes, but are only known to be associated with 6 different gene cassettes; as opposed to the pool of over 100 cassettes that class 1 integrons are known to access (Mazel 2006). They are less commonly found in clinical isolates in comparison to class 1 integrons, but can be found in the same organism as one or more class 1 integrons (Bennett 1999; White *et al.* 2001; Sunde 2005a).

The fact that class 2 integrons are only known to have sampled a pool of 6 gene cassettes explains the lack of more extensive research on their involvement in antibiotic resistance since they are less common and pose less of a threat than class 1 integrons. The apparent reason for the lack of cassette diversity is that clinical class 2 integrons have a non-functional integrase gene (Bennett 1999). The class 2 integrase is rendered inactive by a point mutation that generates a stop codon within the coding sequence. This results in a non-functional integrase gene and the lack of ability to excise or integrate gene cassettes (White *et al.* 2001). As such, the array of gene cassettes within most class 2 integrons is fixed and cannot be changed, unless by integron-integrase activity supplied in *trans*. Class 2 integrons have been found in both clinical and agricultural environments, however, they are far less common than class 1 integrons (Barlow & Gobius 2006; Marquez *et al.* 2008a).

1.8 - Impact of Integrons on Antibiotic Resistance

Understanding the evolutionary route taken by class 1 integrons is important in illuminating the rapid spread of novel phenotypes between bacterial species. The potential for further evolution could greatly impact the treatment of infectious diseases in the future. The misuse of antibiotics to treat bacterial infections can effectively select for resistant strains, killing off susceptible organisms, leaving only the resistant organisms able to reproduce. This results in a bacterial population that, as a whole, contains a greater portion of resistant organisms and a greater diversity of resistance genes. In particular, the use of antibiotics at concentrations which are high enough to have a selective effect, but low enough to allow bacterial replication, can result in adaptive mutations and the spread of resistance genes (Salyers & Amabile-Cuevas 1997). Antibiotic resistance is now a permanent and escalating problem for the health systems of the world. The activities of integrons have assisted the penetration of antibiotic resistance into pathogens and commensals of humans, and are a major problem for hospital acquired infections (Martinez-Freijo *et al.* 1998).

For these reasons, it is important to understand how integrons became what they are, how they are so efficient in the lateral transfer of resistance genes, what genes they are capable of transferring, and what plasmids and other mobile elements vector them between cells and species. Such information will help us to better manage the problem at hand. Basic knowledge of integron biology and dynamics may pre-empt further resistance transmission or help in the discovery of new forms of treatment for such infections. As such, the purpose of this study was to describe the versatility of integrons and their ability to affect the resistance capabilities of *E. coli* in the clinical setting. Particular attention was given to the following questions:

- What is the diversity of gene cassettes in clinical strains of *E. coli* isolated from urinary tract infections?

- Can the frequency and mode of transmission of gene cassettes be examined by comparing arrays and genomic backgrounds in isolates collected over time, from a single area?
- Does the presence of an integron affect an organisms overall ability to resist antibiotic treatments?
- Are there common phylotypes or clonal lines associated with the presence of an integron?
- Will examination of integrons in a comprehensive collection of strains aid in the understanding of ongoing rearrangements occurring in integron containing strains?

In order to address these questions, a large cohort of *E. coli* strains isolated from urinary tract infections was studied. These isolates were chosen as *E. coli* is an organism which exists not only as an infective agent, but also as normal flora in the human gastrointestinal system. Being such a prolific organism increases the likelihood of being affected by mobile genes, especially considering that *E.coli* are amongst the most promiscuous organisms in terms of ability to accept foreign genetic material, but they are not naturally competent, unlike many other bacteria. This species of microorganism is also known to frequently carry integrons and high levels of antibiotic resistance. All of these factors make *E. coli* an ideal organism for the study of integrons and antibiotic resistance in a clinical setting. Recovered integrons and associated cassette arrays were characterised using various molecular techniques followed by phylogenetic and clonal typing of the host organisms. Antibiotic resistance profiles were recorded and compared to the genetic data collected.

Chapter 2

Materials & Methods

2.1 Class 1 Integrons in clinical *Escherichia coli* isolates

2.1.1 - Collection of clinical isolates

Escherichia coli samples were collected from the Sydney Adventist Hospital (SAN) on a weekly basis during the period of late January (24/01/10) to early September (05/09/10) of 2010. Samples were received on ISO-Sensitest agar plates (Oxoid)**,** having been tested for antibiotic susceptibility by the hospital pathology laboratory. Samples were primarily collected from urinary tract infections, however, some isolates were from wound/abscess swabs, sputum or blood cultures. Each sample was collected from a single sampling event. Upon collection, samples were catalogued and allocated a sample number to aid in the tracking of sample data. The patient number, sample site, sample date and antibiotic resistance, including extended spectrum beta-lactamases (ESBLs) and inducible beta-lactamases (IBLs) were recorded in an Excel spreadsheet (Appendix A). No further patient data or history was available in accordance with maintaining patient confidentiality.

2.1.2 - Sample screening for integrons

For the purpose of screening large numbers of samples, crude DNA extractions were carried out using a boiling method. Bacterial samples were processed in batch sizes according to how many were received from the hospital. Suspensions of the bacterial cultures were prepared by mixing one loop (approximately 5-10mg) of bacterial culture scraped from an ISO-Sensitest agar plate (Oxoid) using a calibrated microbiological loop, this was then mixed with 100µL of sterile PCR water. Suspensions were then heated to 99°C for 5 min to lyse the cells and release the bacterial DNA, followed by snap chilling on ice and centrifugation of cell debris. Using 1µL of undiluted DNA supernatant, each sample was then screened by PCR, using primers specific for class 1 or class 2 integrase genes (Appendix B). Those samples which were positive for either class 1 or class 2 integrons were re-cultured for storage as glycerol stocks and subsequently processed using Fastprep^{TM} DNA extraction (see below) to generate a DNA stock for further testing and storage. Glycerol stocks were prepared by growing a pure culture of the isolate in 5mL of LB broth (10g tryptone, 5g yeast extract and 5g NaCl per litre in H_2O) in a 10mL falcon tube for 12 hours at 37°C with shaking. These tubes were then spun down at 3,500rpm for 7 min, the supernatant carefully discarded and the pellet re-suspended in 600µL of M9 salts (7g $Na_2HPO_4 \bullet 7H_2O$, 3g KH_2PO_4, 0.5g NaCl and 1g NH_4Cl per litre in H_2O) and 600µL of 50% glycerol. This mixture was then pipetted into labelled cryo-tubes and stored at -80°C.

2.1.3 - DNA Extractions

DNA was extracted from all integron positive samples using a modified FastPrep™ DNA extraction method, outlined by Yeates and Gillings (1998) (Yeates *et al.* 1998). Cells (5-10mg of pure *E.coli* culture) were lysed in 1 ml of CLS-VF buffer (Fastprep) using a Bio101/Savant FP120 FastPrep machine and lysing matrix E. The quality of extracted DNA was assessed using electrophoresis on 1% agarose gels. Gels were stained with GelRed™ (Biotium) and DNA visualized using transmitted UV light. (Appendix C, figure 2.1.3)

2.1.4 - Class 1 Integrase gene (*intI1*) PCR

To test for the presence of class 1 integrons, primers HS915 and HS916 were used (Table 2.1). These primers target conserved regions of the *intI1* gene, resulting in a 289 bp product (Marquez *et al.* 2008b) (Figure 2.1). Primers HS464 and HS463a (Table 2.1) were then used to confirm the presence of a class 1 integron, as primers HS915 and HS916 can occasionally produce weak false positives from boiled lysates. The HS464 HS463a primer pair amplify a conserved region of the class 1 integrase gene to generate a 473 bp product. Class 1 amplifications used KC2 as a positive control. This is an isolate of *E. coli* that contains a previously characterized class 1 integrase gene (Gillings et al. 2008). These PCR's are represented in Appendix C, figure 2.1.4a and 2.1.4b.

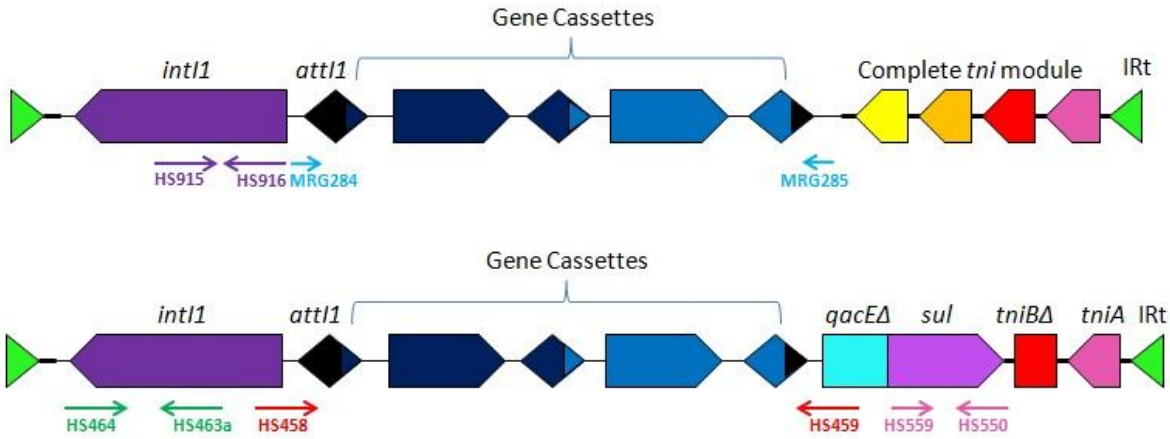

Figure 2.1 Map showing the regions targeted by each primer set used for class 1 integron screening in this research

Symbols are as follows: Green triangles, the *Tn*402 inverted repeats IRi and IRt; purple solid arrow, class 1 integrase gene (*intI1*); black diamond, integron recombination site (*attI1*); blue solid arrows, gene cassettes with associated (blue diamonds) recombination sites (*attC*); yellow, orange, red and pink solid arrows, the complete *tni* module; aqua rectangle, truncated form of the *qacE* gene (*qacEΔ*); magenta solid arrow, gene for sulfonamide resistance (*sul1*); red rectangle, truncated *tni* module. The direction of the solid arrows indicates the direction of transcription. The direction of the arrows alongside the primer names indicate the direction of primer binding. These two maps represent an environmetal class 1 integron (with a complete *tni* module), and a clinical class 1 integron (bottom, with inserted genes and trucated *tni* module). Primers used in this research targeted both types of integron with the aim of singling out any integrons that may have been in an evolutionary stage between these two forms of class 1 integron.

Table 2.1 Primers used in this research

Primer Name	Sequence (3'-5')	Target region	Source
HS915	CGTGCCGTGATCGAAATCCAG	*intI1* gene	Marquez et al. 2008
HS916	TTCGTGCCTTCATCCGTTTCC		
HS464	ACATGCGTGTAAATCATCGTCG	*intI1* gene	Holmes et al. 2003
HS463a	CTGGATTTCGATCACGGCACG		
MRG284	GTTACGCCGTGGGTCGATG	*intI1* cassette array	Gillings et al. 2009
MRG285	CCAGAGCAGCCGTAGAGC		
HS458	GTTTGATGTTATGGAGCAGCAACG	5'-CS *attI1* end	Holmes et al. 2003
HS459	GCAAAAAGGCAGCAATTATGAGCC	3'-CS	
HS549	ACTAAGCTTGCCCCTTCCGC	*sul1* gene	Marquez et al. 2008
HS550	CTAGGCATGATCTAACCCTCGG		
ERIC IR	ATGTAAGCTCCTGGGGATTCAC	Enterobacterial Repetitive Intergenic Consensus sequences	Versalovic et al. 1991
ERIC2	AAGTAAGTGACTGGGGTGAGCG		
INTI2F	CACGGATATGCGACAAAAAGGT	*intI2* gene	Mazel et al. 2000
INTI2R/HS502	GTAGCAAACGAGTGACGAAATG		
HS914	GCGCCTAATCCCAGTAATAAAAC	Specific for functional version of *intI2*	Marquez et al. 2008
HS913	CGCCTAATCCCAGCAATAAAAT		
ChuA1	GACGAACCAACGGTCAGGAT	*chuA* gene	Clermont et al. 2000
ChuA2	TGCCGCCAGTACCAAAGACA		
YjaA1	TGAAGTGTCAGGAGACGCTG	*yjaA* gene	
YjaA2	TGGAGAATGCGTTCCTCAAC		
TspE4C21	GAGTAATGTCGGGGCATTCA	DNA fragment TSPE4.C2	
TspE4C22	CGCGCCAACAAAGTATTACG		
M13F	ATTCCCCAAGAGACACGGAGAGG	plasmid polylinker	Promega, pGem-T Easy Vector System
M13R	CCTCTCCGTGTCTCTTGGGGAAT		

2.1.5 – Class 2 Integrase Gene (*intI2*) PCR

To test for the presence of class 2 integrons, the primers INTI2F and INTI2R were used (Table 2.1). These target a conserved region of the *intI2* gene, resulting in a product which is 788bp long (Mazel *et al.* 2000) (Figure 2.2). As Class 2 integrons are known to exist in two forms, functional and non-functional, secondary PCRs were carried out on all *intI2* positive isolates, to determine which variant of the *intI2* gene they carried (Marquez *et al.* 2008a). This required two further PCRs using the primers HS502 and HS913 for detection of the non-functional variant of the class 2 integrase gene, using *Tn*7 as a positive control. This primer set generated a product 308bp in length. For the detection of the functional class 2 integrase gene, primers HS502 and HS914 were used with EC8157 as a positive control (Marquez *et al.* 2008a). These primers produced a product 309bp in length.

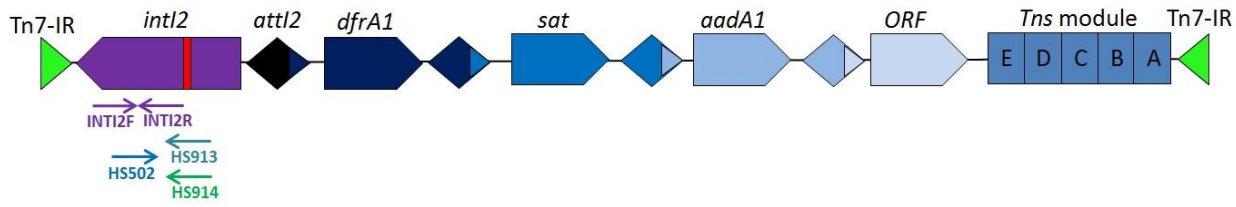

Figure 2.2 Map showing regions targeted using the class 2 integrase gene primer sets

Symbols are as follows: Green triangles, the *Tn*7 inverted repeats Tn7-IR; purple solid arrow, class 2 integrase gene (*intI2*); red stripe, representation of stop codon which makes the integrase gene non-functional (may or may not be present); black diamond, integron recombination site (*attI2*); blue solid arrows, gene cassettes with associated (blue diamonds) recombination sites (*attC*'s); blue rectangles, *tns* module. The direction of the solid arrows indicate the direction of transcription. The direction of the arrows alongside the primer names indicate the direction of primer binding. This map represents the most common form of the class 2 integron.

2.1.6 - *sul1* Gene PCR

Sul gene PCRs using primer HS549/HS550 (Table 2.1) was performed in 20µL volumes using GoTaq White (Promega), in the buffer supplied with the enzyme, with 25pmol of each primer and RNAse A to a final concentration of 10µg/mL. The temperature cycles for the PCR were: 94°C for 3min for 1 cycle, 94°C for 30sec, 65°C for 30 sec, 72°C for 1min and 30sec for 35 cycles, and 72°C for 5min for 1 cycle. Amplifications used KC2 as a positive control (Gillings *et al.* 2008a).

The presence of PCR products was assessed using electrophoresis on 2% agarose gels run at 110V for 1 hour. Gels were stained with GelRed™ (Biotium) and DNA visualized using transmitted UV light. (Appendix C, figure 2.1.6)

2.2 - Phylotyping and Cassette Analysis

2.2.1 - Gene Cassette PCR

To amplify gene cassettes, two primer combinations were used. The primers HS458 and HS459 (Table 2.1) were used to amplify cassette arrays in samples containing clinical class 1 integrons with a typical 3'-CS (Stokes & Hall 1989) (Figure 2.1). These primers amplify the gene cassettes between *attI* and the 3'-CS, including *qacEΔ*. Primers MRG284/285 (Table 2.1) amplify cassette regions in integrons that contain a complete *tni* module, without a typical 3'-CS (pre-clinical integrons). HS458/459 amplifications used KC2 as a positive control (Gillings *et al.* 2008a), while MRG284/285 amplifications used B4CC2 (Gillings *et al.* 2008a). These PCR's are represented in Appendix C, figure 2.2.1a and 2.2.1b.

2.2.2 - Enzyme digests of Gene cassette PCR

PCR products resulting from the amplification of cassette regions of class 1 integrons were digested using the enzyme *Rsa*I at 37°C for 18 hours, according to the manufacturers' instructions. Resulting digests were electrophoresed in a 2% agarose gel at 80V for 8hours in order to maximize visibility of banding patterns. (Appendix C, figure 2.2.2)

2.2.3 - Gene Cassette Sequencing

Gene cassette array digestions were used to select several examples of each RFLP type for further characterization. HS458/HS459 PCR products were purified using the Wizard SV Minipreps DNA purification System (Promega) as per the manufacturers' instructions (centrifuge protocol). Once purified, 7µl of extracted material was added to 3.2 pmol of primer HS458 or HS459, sterile PCR water was added to a final volume of 12µl. Purified PCR products were sequenced using BigDye v3.1 chemistry (Applied Biosystems) with either the forward or reverse primer (HS458/HS459), in order to sequence the entire cassette array. Sequences were determined using ABI 3130x/Genetic Analyzer. Sequencing reactions, unincorporated dye-terminator removal and capillary electrophoresis were carried out at the Macquarie University DNA Analysis Facility. The resultant DNA sequences were edited and assembled using Geneious version 4.8.5. Sequences were analysed using the NCBI blastx translated nucleotide protein query (http://blast.ncbi.nlm.nih.gov/blast). Identities of cassettes were recorded according to percentage homology with sequences matched via blast, with a cut off of 95%.

2.2.4 - Phylogenetic Grouping PCR

Phylotype grouping of *E. coli* allows the samples to be categorized into broad phylogenetic groups, useful for tracking and epidemiology. There are four named phylotypes in *E. coli*, phylotypes A, B1, B2 and D. In order to determine which group each sample was a member of, a multiplex PCR targeting three different regions (*chuA, yjaA,* DNA fragment TSPE4.C2) (Table 2.1) was performed (Clermont *et al.* 2000). These amplifications used isolate 9A as a positive control for phylotype A , isolate MQ224 as a positive control for phylotype B1 , isolate TA207 as a positive control for phylotype B2 and isolate MQ116 as a positive control for phylotype D. Control isolates were kindly provided by Tiffany Delport from the Power Lab, Macquarie University. These were received as purified DNA, and were stored at -20°C until use. These PCR's are represented in Appendix C, figure 2.2.4a and 2.2.4b.

2.2.5 - ERIC PCR

Enterobacterial Repetitive Intergenic Consensus (ERIC) sequence PCRs were utilized to determine if there were any prominent *E. coli* clonal lines present in the sample collection. Amplifications used KC2 as a positive control (Gillings et al. 2008).

ERIC PCRs were performed in 20µL volumes using GoTaq White (Promega), in the buffer supplied with the enzyme, with 25pmol of each primer and RNAse A to a final concentration of 10µg/mL. The temperature cycles for the PCR's were: 94°C for 3min for 1 cycle, 94°C for 30sec, 52°C for 30 sec, 68°C for 8min for 35 cycles, and 68°C for 15min for 1 cycle.

Resulting PCR products were electrophoresed in a 2% agarose gel at 80V for 8 hours in order to maximize visibility of distinct banding patterns. The individual banding patterns produced by each isolate were inspected and compared visually. An attempt was made to group the isolates in to clonal lines using these unique profiles. (Appendix C, figure 2.2.5)

2.2.6 - Cloning of MRG284/MRG285 PCR Products for Sequencing

PCR products were purified using the Wizard SV Minipreps PCR purification system as per the manufacturer's protocols. The purified samples were then ligated into the pGEM-T EASY Vector (Promega) as per the manufacturers' instructions. The ligated product was transformed into *E. coli* JM109 competent cells (Promega) by heat shock as per the manufacturer's protocol. After successful ligation and transformation, the clones were grown on LB plates with added 64µg/mL X-Gal, 20µg/mL ampicillin and 100µL of 100µg/mL IPTG spread on the surface of the plates once set.

After being grown at 37°C overnight on LB plates containing X-Gal and IPTG, individual white colonies were selected to create a clone library for each reaction. Clones were grown overnight in LB broth containing ampicillin. To determine if cloning was successful, 20µl of

overnight broth was heated for 5 min at 99°C before immediately being placed on ice. The lysate was then spun for 3 min at 14000 rpm. PCR was performed using 2 µL of the boiled clone DNA with the mastermix (20ul): 25pmol of each primer, a final concentration of 10ug/mL of RNase A, 10µlGoTaq White (Promega). Temperature cycles for the MRG284/285 reactions were: 94°C for 3min for 1 cycle, 94°C for 30sec, 65°C for 30 sec, 72°C for 2min for 35 cycles, and 72°C for 5min for 1 cycle.

RsaI restriction digests were carried out in order to identify different amplicons. Restriction fragments were analysed on a 2% agarose gel to determine which clones carried different cassette regions.

Unique clones were grown by inoculating a 5mL broth containing 10µL ampicillin (1mg/mL) with 5µL of broth containing cloned cells. The culture was incubated overnight at 37°C in a shaker at 150 rpm. Plasmids were extracted using the Wizard SV Minipreps DNA purification System (Promega) as per the manufacturers' instructions (centrifuge protocol). Once extracted, 7µl of extracted material was added to 3.2pM of primer M13F and/or M13R, depending if both forward and reverse sequences were required. Sterile PCR water was added to make a final volume of 12µl. These preparations were sent to the Macquarie University Sequencing facility for sequencing using an ABI Prism 377 Sequencer. Once received, successful sequences were edited and assembled using the program Geneious version 4.8.5. Sequences were analysed using the NCBI blastx translated nucleotide protein query (http://blast.ncbi.nlm.nih.gov/blast). The identities of gene cassettes were recorded according to percentage homology with sequences matched via blast, with a cut off criterion of 95% homology.

2.3 - Novel Class 1 Integrons

Of all the isolates which tested positive for the class 1 integrase gene, two were unique in that they did not carry the normal features of a class one integron. These two isolates, namely 24 and 213, tested positive for a class one integrase gene, but not for a cassette region, or any of the features in the 3' conserved segment. There was no *sul* gene and no *qacEΔ* detected. The class 1 integron integrase gene was present, but it could not be determined in what context, as all other standard tests were negative.

2.3.2 - Fosmid Libraries

In order to determine the genetic context of potential novel class 1 integrons 24 and 213, Fosmid library construction was carried out using the CopyControl™ Fosmid Library Production Kit with pCC1FOS™ Vector (Epicentre® Biotechnologies), according to the manufacturers' instructions. Briefly, the target DNA is sheared into approximately 40kb sized fragments, then, using a vector, these fragments are inserted into competent *E. coli* cells which are then screened for the presence of the DNA fragment of interest.

2.3.3 - Phenol/Chloroform DNA Extractions

Overnight cultures of each *E. coli* sample to be further tested were grown in 10mL of LB broth overnight at 37°C with shaking at 100 rpm. These cultures were aseptically sub-cultured from glycerol stocks prepared previously (section 2.1.2). Cell suspensions were then spun down in a microcentrifuge at 14000 rpm for 10 min. Excess media was discarded, leaving only the pellet of cells, which was then re-suspended in 200µL of 50mM Tris pH 7.5. Once sufficiently re-suspended, 400µL of 10% SDS was added, and the tube gently inverted 5 times. 500µL of phenol was then added, lid closed, then inverted 2 times to mix, and the tubes were left at room temperature overnight to ensure complete lysis of all cells. Tubes

were spun down at 14000 rpm for 10 min. approximately 500µL of the aqueous layer was harvested into a fresh tube, and extracted with 300µL of chloroform: isoamyl alcohol (24:1). Tubes were then spun at 14000 rpm for 10 min. The aqueous phase (400µL) was recovered and transferred to a fresh tube. DNA was precipitated with 800µL of ice cold 100% ethanol. The tube was gently inverted 5 times, and then placed in a -20°C freezer for 15 min. After this, the tubes were spun down at 14000 rpm for 5 min, and the supernatant poured off and discarded. The DNA pellet was then washed 3 times with cold 70% ethanol/100mM sodium acetate solution, spun down at 14000 rpm for 5 min, then left to air dry for 10 min. DNA pellets were re-suspended in 75µL of TE buffer pH 7.6 (10mM Tris HCL, 1mM EDTA) with 1µL of 1mg/mL RNase added, and left at 4°C overnight to ensure complete re-suspension. DNA extractions were then run out on a 1% agarose gel for confirmation of successful extraction.

2.3.4 - High Molecular Weight Plasmid DNA Extractions (<50kb)

High molecular weight plasmid DNA was extracted using the rapid alkaline extraction method outlined by (Birnboim & Doly, 1979). Extracted DNA was electrophoresed on a 1% agarose gel for confirmation of successful extraction.

2.3.5 - Low Molecular Weight Plasmid DNA Extractions (<20kb)

Low molecular weight plasmid DNA was extracted using the Wizard® *Plus* SV Minipreps DNA Purification Kit (Promega). These extractions were carried out in accordance with the manufacturers' instructions. Extracted DNA was electrophoresed on a 1% agarose gel for confirmation of successful extraction.

2.3.6 - Very High Molecular Weight Plasmid DNA Extractions (<105kb)

Very High molecular weight plasmid DNA was extracted using the QIAGEN® Large-Construct Kit (QIAGEN). These extractions were carried out in accordance with the kit manufacturers' instructions. Extracted DNA was electrophoresed on a 1% agarose gel for confirmation of successful extraction.

Chapter 3

Class 1 Integrons & Antibiotic Resistance in Clinically Isolated *Escherichia coli*

3.1 - Introduction

Antibiotic resistance in microorganisms that are capable of causing infectious diseases is a significant and ongoing global health challenge. The incidence of multi-resistance traits amongst such organisms is increasing. In Gram negative organisms, gene transfer systems such as integrons and their gene cassettes are responsible for a large proportion of acquired resistance mechanisms (Ito *et al.* 2009; Phongpaichit *et al.* 2011; Stewart & Rozen 2011; Zhang *et al.* 2011).

Microorganisms such as *E. coli* are a particularly important in the dissemination of resistance determinants because they are human commensals which are also capable of causing disease. Being commensal organisms, they are frequently exposed to antibiotics, even when not the target of treatment. In such situations, this leads to the inadvertent acquisition of resistance genes amongst the commensal community within a host, potentially turning the normal microbiota into pathogens (Bailey *et al.* 2010; Meyer *et al.* 2010).

E. coli is the primary causative organism of urinary tract infections in humans. Infection is often caused by contamination from the patients' own normal flora (Barber *et al.* 2013). For this reason, such isolates can be used as an indicative measure of the level of antibiotic resistance

present in commensal *E. coli*, and the diversity of resistance genes carried within them. Recent studies have noted increasing levels of resistance and multi-resistance traits in enterobacterial organisms. Such resistance traits can often be attributed to mobile gene systems involving integron associated gene cassettes that encode resistance determinants (Blahna *et al.* 2006; Rijavec *et al.* 2006; Solberg *et al.* 2006; Marquez *et al.* 2008b). Many antibiotic resistance cassettes have been identified and isolated from clinical, commensal and environmental organisms (Partridge *et al.* 2009). Dissemination of these resistance genes can occur rapidly across diverse environments.

Many studies into the antibiotic resistance of infections caused by *E. coli* have focused on the presence of a CTX-M gene, coupling this with characterization of plasmid type and occasionally phylotype analysis (Machado *et al.* 2005; Guiral *et al.* 2011; Pitout 2012; Sun *et al.* 2012). Other mechanisms of mobile genetic transfer, such as integrons, have not been as extensively investigated. In particular, little is known about how resistance is acquired in *E. coli*, and what mechanisms are involved in gaining these new capabilities. Multiple transfer mechanisms are possible, and resistance can also arise by mutation (Toprak *et al.* 2012). It is important to determine the relative contribution of each mechanism to the problem of antibiotic resistance in pathogens.

This study reports on the relationships between integron presence and antibiotic resistance phenotypes in *E. coli* isolated from urinary tract infections over a period of 9 months spanning January to September, 2010. All integrons, cassette arrays and resistance profiles from each of 718 isolates were characterized and recorded. This study included a large number of samples as it was presumed that a large cohort would make resistance patterns more obvious. Understanding the dynamics of the integron/gene cassette system is important in designing strategies to avoid dissemination of resistance genes in the future.

3.2 - Methods

3.2.1 - Bacterial isolates

Escherichia coli samples were collected from the Sydney Adventist Hospital (SAN) on a weekly basis during the period of late January (24/01/10) to early September (05/09/10) of 2010. Samples were received on ISO-Sensitest agar plates (Oxoid). Samples were primarily collected from urinary tract infections, however, some isolates were from wound/abscess swabs, sputum or blood cultures. Upon collection, samples were catalogued and allocated a sample number to aid in the tracking of sample data (Appendix A).

3.2.2 - Antibiotic Resistance

Isolates were received on ISO-Sensitest agar plates (Oxoid) and tested against a standard array of relevant antibiotics using the disk diffusion method. The Kirby-Bauer method of measuring resistance was used to determine the resistance profile of each isolate collected. These tests included extended spectrum beta-lactamases (ESBLs) and inducible beta-lactamases (IBLs). Resistance profiles, patient identity number, sampling site and sample date were recorded in an Excel spreadsheet (Appendix A).

3.2.3 - Detection and Characterization of Class 1 and 2 Integrons

PCRs were carried out to determine the characteristics of any class 1 or 2 integrons present in the isolates (Table 3.1). Briefly, this involved testing isolates for: presence of class 1 or 2 integron-integrase genes (*intI1, intI2*); gene cassettes associated with these integrons; the presence of a 3' conserved segment characteristic of clinical class 1 integrons; and the resistance gene *sul1*, encoding resistance to sulphonamides (Table 3.1). The presence of PCR products was assessed using electrophoresis on 2% agarose gels run at 110V for 1 hour. Gels

were stained with GelRed™ (Biotium) and DNA visualized using transmitted UV light. Results of all tests carried out on these isolates were tabulated to aid in analysis (Appendix B). Further details on the methods used can be found in Chapter 2.

PCR products resulting from the amplification of cassette regions of class 1 integrons were digested using the restriction enzyme *Rsa*I at 37°C for 18 hours, according to the manufacturers' instructions. Resulting digests were electrophoresed on a 2% agarose gel at 80V for 8 hours in order to maximize visibility of banding patterns. These patterns were then used to group the isolates by cassette array type in order to minimise the amount of sequencing required.

Representative isolates of each RFLP type were chosen for sequence analysis. HS458/HS459 PCR products were purified using the Wizard SV Minipreps DNA purification System (Promega) as per the manufacturers' instructions (centrifuge protocol). An aliquot of 7µl of extracted material was added to 3.2 pmol of primer HS458 or HS459, sterile PCR water was added to a final volume of 12µl. Purified PCR products were sequenced using BigDye v3.1 chemistry (Applied Biosystems) with either the forward or reverse primer (HS458/HS459), in order to sequence the entire cassette array. Sequences were determined using an ABI 3130x/Genetic Analyzer.

3.2.4 - Statistical Analysis

Each isolate was regarded as an independent sample of the *E. coli* strains causing UTIs and other infections in humans. Antibiotic resistance abilities between groups of isolates were compared using the chi-square test or, when samples were small, Fisher's exact test. A *p*-value of <0.05 was considered significant.

Table 3.1 List of primers used in PCR screening of *E. coli* isolates

Primer Name	Sequence (3'-5')	Amplicon Size (bp)	Annealing Temperature	Target region	Source
HS915	CGTGCCGTGATCGAAATCCAG	289	60	*intI1* gene	Marquez et al, 2008
HS916	TTCGTGCCTTCATCCGTTTCC				
HS464	ACATGCGTGTAAATCATCGTCG	473	60	*intI1* gene	Stokes et al, 2006
HS463a	CTGGATTTCGATCACGGCACG				
MRG284	GTTACGCCGTGGGTCGATG	Variable	65	*intI1* cassette array	Gillings et al, 2009
MRG285	CCAGAGCAGCCGTAGAGC				
HS458	GTTTGATGTTATGGAGCAGCAACG	Variable	65	5'-CS *attI1* end 3'-CS	Holmes et al, 2003
HS459	GCAAAAAGGCAGCAATTATGAGCC				
HS549	ACTAAGCTTGCCCCTTCCGC	1100	65	*sul1* gene	Stokes et al, 2006
HS550	CTAGGCATGATCTAACCCTCGG				
INTI2F	CACGGATATGCGACAAAAAGGT	788	60	*intI2* gene	Mazel et al, 2000
INTI2R	GTAGCAAACGAGTGACGAAATG				

3.3 - Results

3.3.1 - Antibiotic resistance

E. coli isolates were forwarded to the laboratory on a weekly basis over a period of 9 months. A total of 713 isolates were received on Sensitest agar plates, having been subjected to standardized disk–diffusion antibiotic resistance testing using the Kirby-Bauer method for cut-off values. Resistance was recorded for the 13 primary antibiotics of interest for Gram negative organisms (Table 3.2). Some isolates were not tested against the whole array of antibiotics, while others were tested against additional antibiotics. The antibiotic testing was carried out at the discrepancy of that hospitals pathology staff, no information regarding the reasoning involved in testing particular selections could be obtained. To standardize the results, the focus was placed on tests for the 13 primary antibiotics only (Appendix A).

There were several resistance patterns which were common amongst the isolates, regardless of integron presence. Resistance to augmentin, chloramphenicol and cefazolin often occurred together, 308 isolates exhibited this pattern. There was also common resistance to nitrofurantoin, amikacin and gentamicin together, 97 isolates exhibited this resistance pattern. In 52 isolates, these patterns were combined to result in resistance to all 6 antibiotics (Figure 3.1). While none of the isolates exhibited a detectable presence of extended spectrum β-lactamases, there was notable resistance to β-lactam antibiotics amongst the class 1 integron positive isolates (Appendix A).

It was found that 82% of all isolates collected were resistant to at least one antibiotic, while 75% were multi-resistant. Overall, isolates that carried a class 1 integron were more likely to be resistant to at least one of the antibiotics tested than those which did not carry an

integron (*p*-value <0.05). This is clearly illustrated in Figure 3.2, where a much higher proportion of integron positive isolates exhibit antibiotic resistance than do integron negative isolates. However, there were some cases where resistances to particular antibiotics were more common in isolates without an integron. Such was the case with amikacin and imipenem (Table 3.3). Isolates with a class 1 integron were on the whole more likely to be multi-resistant than those without an integron (*p*-value <0.05) (Table 3.3).

Table 3.2 List of antibiotics used to test for resistance

Antibiotic/Concentration	Diffusion Disk
Cefazolin 30µg	KZ30
Cefotaxim 5µg	CTX5
Cefepime 10µg	FEP10
Ampicillin 25µg	AMP25
Augmentin (Amoxycillin/Clavulanic Acid) 60µg	AMC60
Imipenem 10µg	IPM10
Amikacin 30µg	AK30
Gentamicin 10µg	CN10
Norfloxacin 10µg	NOR10
Ciprofloxacin 2.5µg	CIP2.5
Chloramphenicol 100µg	CL100
Nitrofurantoin 200µg	F200
Trimethoprim 5µg	W5

Table 3.3 Percentage of antibiotic resistance phenotypes in clinically isolated *E. coli*. *p* = Results of statistical test on whether the presence of an integron increases the likelihood of resistance to that group of antibiotics. NS = not significant. * Too few isolates tested for reliable statistical comparison between integron presence and absence. Refer to table 3.2 for antibiotic explanations.

Antibiotic	Total Resistant Isolates (n = 718)	*intI1* Positive Isolates (n = 167)	*intI1* Negative Isolates (n = 548)	*p*
Cephalosporins				
KZ30	24.23	34.34	21.20	
CTX5	9.47	18.67	6.70	<0.05
FEP10	4.46	12.65	1.99	
Penicillins				
AMP25	39.69	62.05	32.97	NS
AMC60	22.01	34.94	18.12	
Carbapenems				
IPM10	2.92	1.81	3.26	*
Aminoglycosides				
AK30	57.38	57.23	57.43	NS
CN10	53.06	54.22	52.72	
Quinolones				
NOR10	8.36	17.47	5.62	NS
CIP2.5	7.49	17.47	5.07	
Others				
CL100	17.55	28.89	15.94	*
F200	64.21	57.23	66.30	*
W5	21.59	52.41	12.32	*

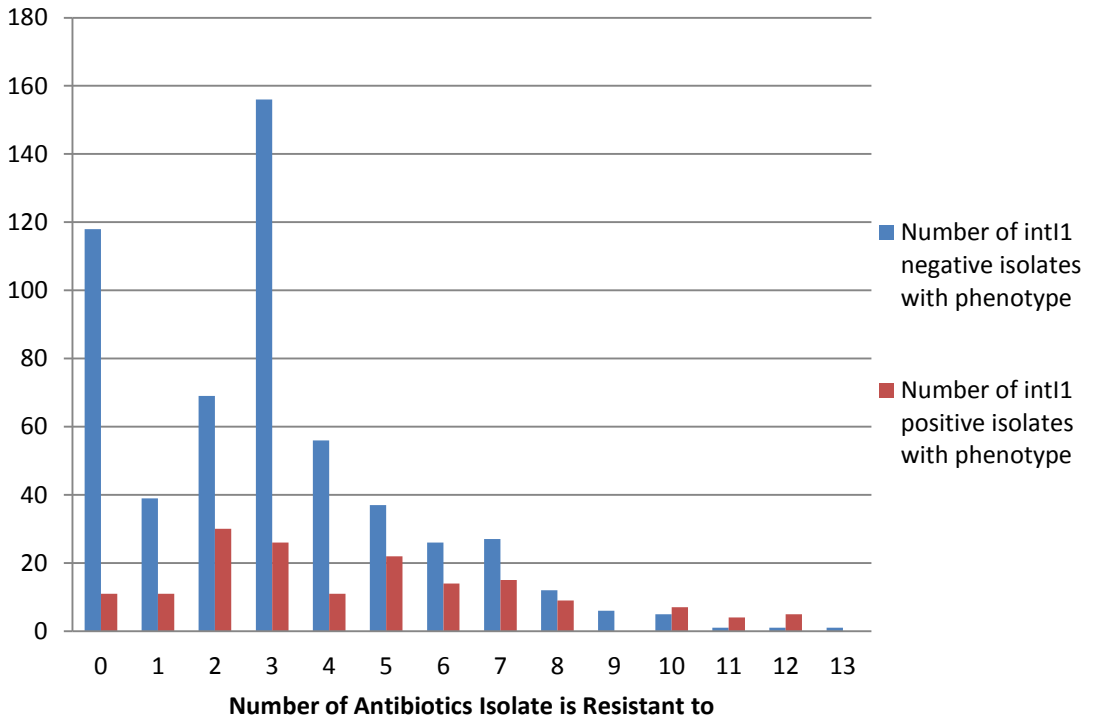

Figure 3.1 The number of isolates which exhibit multi-resistance phenotypes.

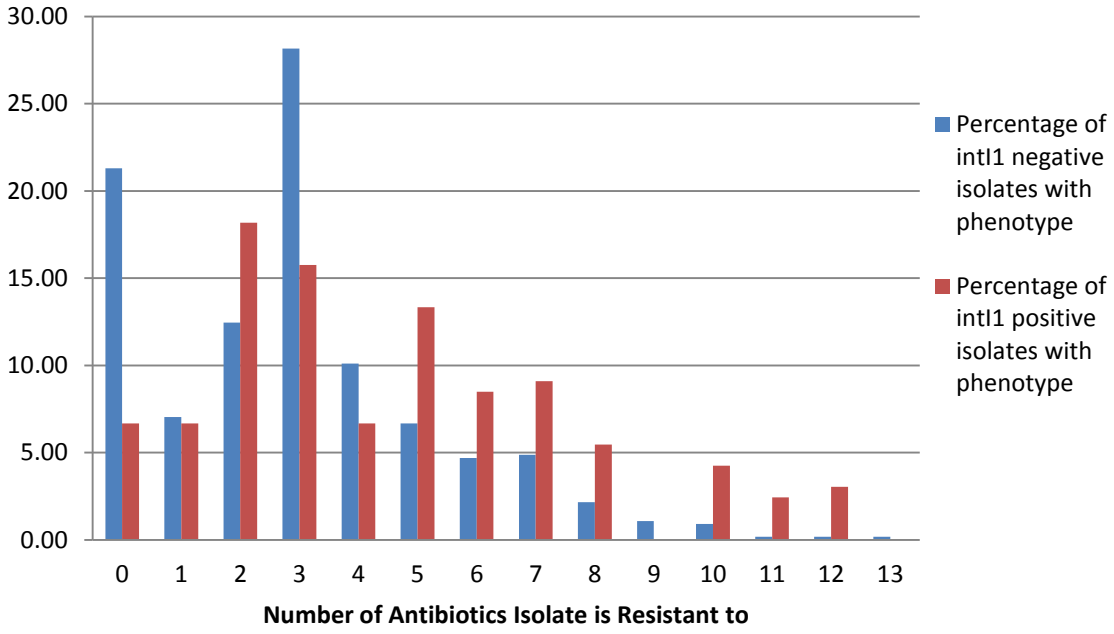

Figure 3.2 The percentage of isolates which exhibit multi-resistance phenotypes.

3.3.2 - Detection of class 1 integrons

Each week, isolates received at the laboratory were characterized for integron content. As an initial test, suspensions of bacteria were boiled and the resulting lysate screened with primers specific for the class 1 integron-integrase gene (*intI1*). Of 718 isolates, a total of 167 isolates generated the 289bp product expected for PCR with primers HS915/HS916 (Appendix C, Figure 2.1.4). These 167 isolates were then re-plated from single colonies and genomic DNA generated for further testing (Appendix C, figure 2.1.3).

To confirm the detection of class 1 integrons, a second PCR was performed using primer set HS463a/HS464, also targeting the class 1 integron-integrase gene. All isolates generated the 473bp fragment expected of integron positive isolates (Appendix C, figure 2.1.4). A selection of these PCR products was sequenced. All *intI1* genes detected exhibited 100% homology with previously described class 1 integrons isolated from clinical contexts (Koh *et al.* 2013; Touati *et al.* 2013). A representative sequence of an *intI1* gene from this study was submitted to Genbank (Genbank accession: KF534915).

Analysis of the relative frequency of class 1 integrons over the 9 month period was performed. There was no evidence of seasonal variation in the frequency of integron-carrying strains. The average proportion of integron positive strains in the collection was 23.22% (SD =5.25%) (Fig. 3.3).

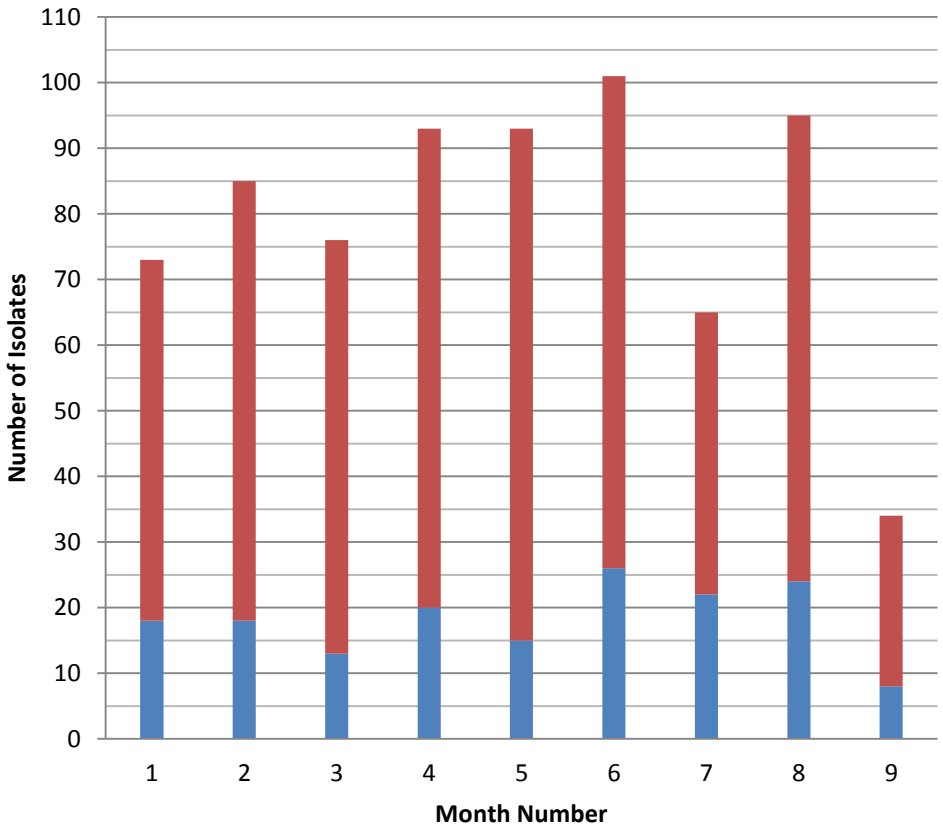

Figure 3.3 Frequency of Class 1 integrons found per month of sampling. The relative proportions of isolates containing class one integrons are plotted for each time period. Blue bars show the proportion of isolates carrying a class one integron, the Red bars show the total number of isolates tested. During month 9, samples were collected for only two weeks.

3.3.3 - Characterization of class 1 integrons

To understand the epidemiology of *E. coli* infections and the transmission of integrons and their gene cassettes, further characterization of the integron positive strains was conducted. These analyses included determination of the integron cassette content and flanking sequences. To understand transmission between lineages, phylotyping and ERIC-PCR were conducted (see Chapter 4).

Many class 1 integrons from clinical sources carry a 3' conserved segment (3'-CS). This consists of a partially deleted *qacE* gene attached to a gene for sulfonamide resistance, *sul1* (Stokes & Hall 1989). To test if isolates in this study contained a classical 3'-CS, PCR was targeted at *sul1*, using primers HS549/HS550. A proportion of the *intI1* positive isolates (77/167) generated the 1100bp band expected for isolates containing the 3'-CS (Appendix C figure 2.1.6). The remaining isolates were set aside for further testing to determine whether their integrons dated from before acquisition of the 3'-CS, or whether this segment had subsequently been deleted.

The cassette arrays carried by the class 1 integrons were investigated using PCRs that target the boundaries of this variable region. Since there are a number of different conformations of the 3'-CS, two primer pairs were used; one which targets *qacEΔ* and the end of *intI1* (HS458/HS459) and the other which targets the *attI1* region of *intI1* and a region distal to the final *attC* site in an array (MRG284/MRG285) (Table 3.1). Due to the variability of the cassette region, the resultant PCR products varied in length (1100-2100bp) depending on the number and type of gene cassettes carried in the array. A total of 69 isolates had characterizable cassette arrays (Appendix B). The remaining 98 isolates gave no product with either of the cassette PCR's. In all cases, positive and negative controls were run and showed successful PCR with no contamination. Isolates that simply had an empty cassette array

would present a product of approximately 100bp for both MRG284/MRG285 and HS458/HS459 as in both cases the primers target the boundaries of the cassette array and are not dependent on the presence of cassettes. The lack of cassette array product for these isolates could be explained by deletions, rearrangements or substitutions in the 3' CS of such integrons.

The cassette array PCR products were subjected to restriction digestion in order to group them according to the resulting RFLP profile. This minimized the number of isolates which subsequently required sequencing. Two representative isolates from each RFLP profile group were sequenced and analyzed. Five individual cassette arrays were identified (Table 3.7). A representative sequence from each of the five arrays characterized was submitted to Genbank (Genbank accessions: KF534910, KF534911, KF534912, KF534913, and KF534914). Only two families of antibiotic resistance gene cassettes were found, encoding dihydrofolate reductases (*dfrA*) or aminoglycoside adenyltransferases (*aadA*). These cassettes are responsible for conferring resistance to trimethoprim and streptomycin/spectinomycin respectively (Table 3.6). At the terminus of cassette arrays, *qacEΔ* cassettes were also identified (Table 3.6), these cassettes are responsible for conferring resistance to quaternary ammonium compounds when in the complete form. The *qacEΔ* cassettes found in this study were in the degenerate form and therefore likely non-functional, as denoted by the delta (*Δ*) symbol. The majority of the cassette arrays characterized consisted of either *dfrA1*|*aadA1*|*qacEΔ* or *dfrA17*|*aadA5* |*qacEΔ* cassettes (Table 3.7). With this information, gene maps of these integrons were constructed (Figure 3.5).

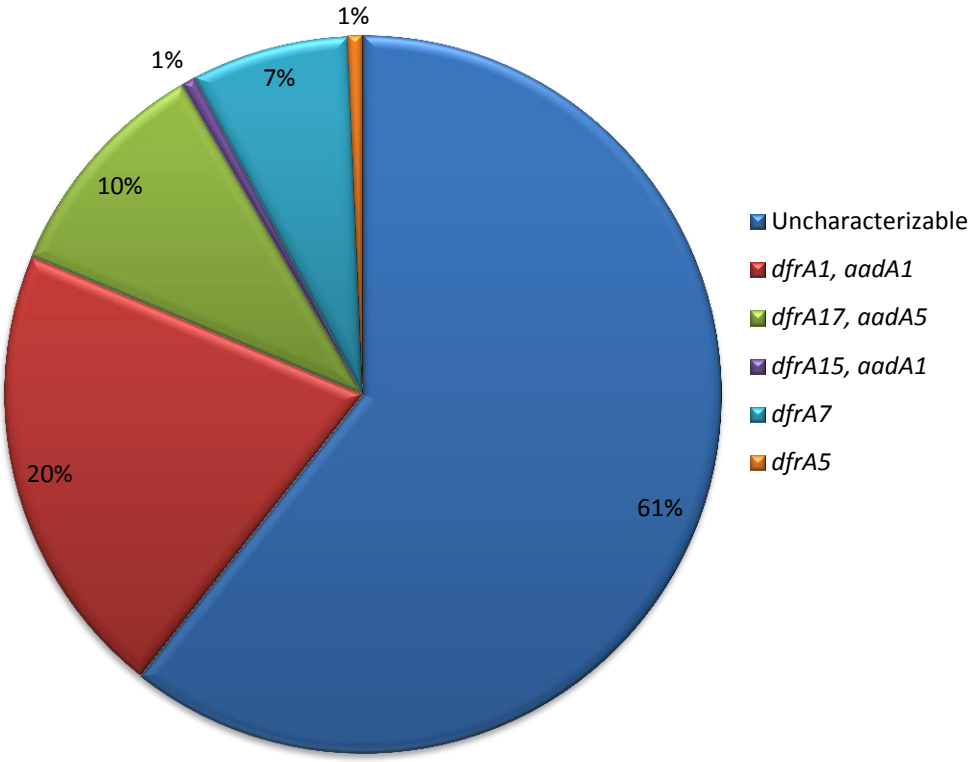

Figure 3.4 Identities of the various cassette arrays identified in the class 1 integrons detected by screening clinical *E. coli* isolates. The majority of integron positive isolates (61%) did not generate cassette array products with the primers used in this study.

3.3.4 - Detection of class 2 integrons

The genomic DNA used for screening class 1 integrons was also used to screen for class 2 integrons. Using primers INTI2F/INTI2R, which target the class 2 integrase gene (*intI2*), 21 isolates generated the 788bp band expected from class 2 integron carrying strains (Mazel *et al.* 2000). There was a single isolate found to carry both a class 1 and class 2 integron.

3.3.5 - Characterization of class 2 integrons

Further PCR analysis was carried out in order to determine if class 2 integrons were of the functional or non-functional form (Marquez *et al.* 2008a). The primer pair HS502/HS914 targets the functional *intI2*, while the primer pair HS502/HS915 was used to target the *intI2* variant which carries an internal stop codon that results in loss of integrase function. It was found that all 21 isolates were the non-functional variant of the class 2 integron-integrase gene. Sequences of IntI2 genes recovered in this project were identical to each other and were 100% homologous to those previously lodged in Genbank. A representative sequence from this study was submitted (Genbank accession: KF534916).

Table 3.5 Characterized Integron Genetic Content. The fragment profiles represent the fragments in base pairs achieved through RsaI digests for all those with HS 458/459 PCR products, and PCR product size for all MRG 284/285 PCR products.

intI1	HS 458/459	MRG 284/285	Fragment profile	Cassette array	*sul1*	*intI2*	n	Comments
+	+	-	1100	*dfrA7*	+	-	3	Typical 3'CS
+	+	-	100, 600, 1300	*dfrA17, aadA5*	+	-	16	Typical 3'CS
+	+	-	150, 450, 650	*dfrA1, aadA1*	+	-	26	Typical 3'CS
+	+	-	500, 700, 800	*dfrA15, aadA1*	+	-	1	Typical 3'CS
+	+	-	150, 200, 700	*dfrA5*	+	-	1	Typical 3'CS
+	-	-	N/A	N/A	+	-	19	No amplification of cassette array
+	-	-	N/A	N/A	-	-	75	No amplification of cassette array
+	+	-	1100	*dfrA7*	-	-	1	*qacE* + (Possibly predating *sul1*)
+	+	-	150, 450, 650	*dfrA1, aadA1*	-	-	3	*qacE* + (Possibly predating *sul1*)
+	+	-	100, 600, 1300	*dfrA17, aadA5*	-	-	9	*qacE* + (Possibly predating *sul1*)
+	-	+	1500	Unable to sequence	-	-	5	Pre-3'CS or pre *Tn402*
+	-	+	800	Unable to sequence	-	-	7	Pre-3'CS or pre *Tn402*
+	+	-	150, 450, 650	*dfrA1, aadA1*	+	+	1	Class 1 and class 2 integrons present

Table 3.6 Gene cassettes in class 1 integrons identified by PCR and sequencing

Gene Cassette	Gene Product	Presumptive Phenotype
dfrA1 *dfrA5* *dfrA7* *dfrA15* *dfrA17*	Dihydrofolate reductase	trimethoprim resistance
aadA1 *aadA5*	Aminoglycoside adenyltransferase	streptomycin/spectinomycin resistance
qacEΔ	Quaternary ammonium compound efflux	Quaternary ammonium compound resistance

Table 3.7 Proportions of isolates with particular cassette arrays and their resistance to antibiotics. The presence of a *dfrA* gene cassette and/or an *aadA* cassette was compared with the isolate's resistance to trimethoprim and/or streptomycin.

Cassette array	Number with cassette array	Number with phenotypic resistance	Percentage that show phenotypic resistance
dfrA1, aadA1	32	32	100
dfrA17, aadA5	16	16	100
dfrA15, aadA1	1	1	100
dfrA7	11	2	18
dfrA5	1	1	100

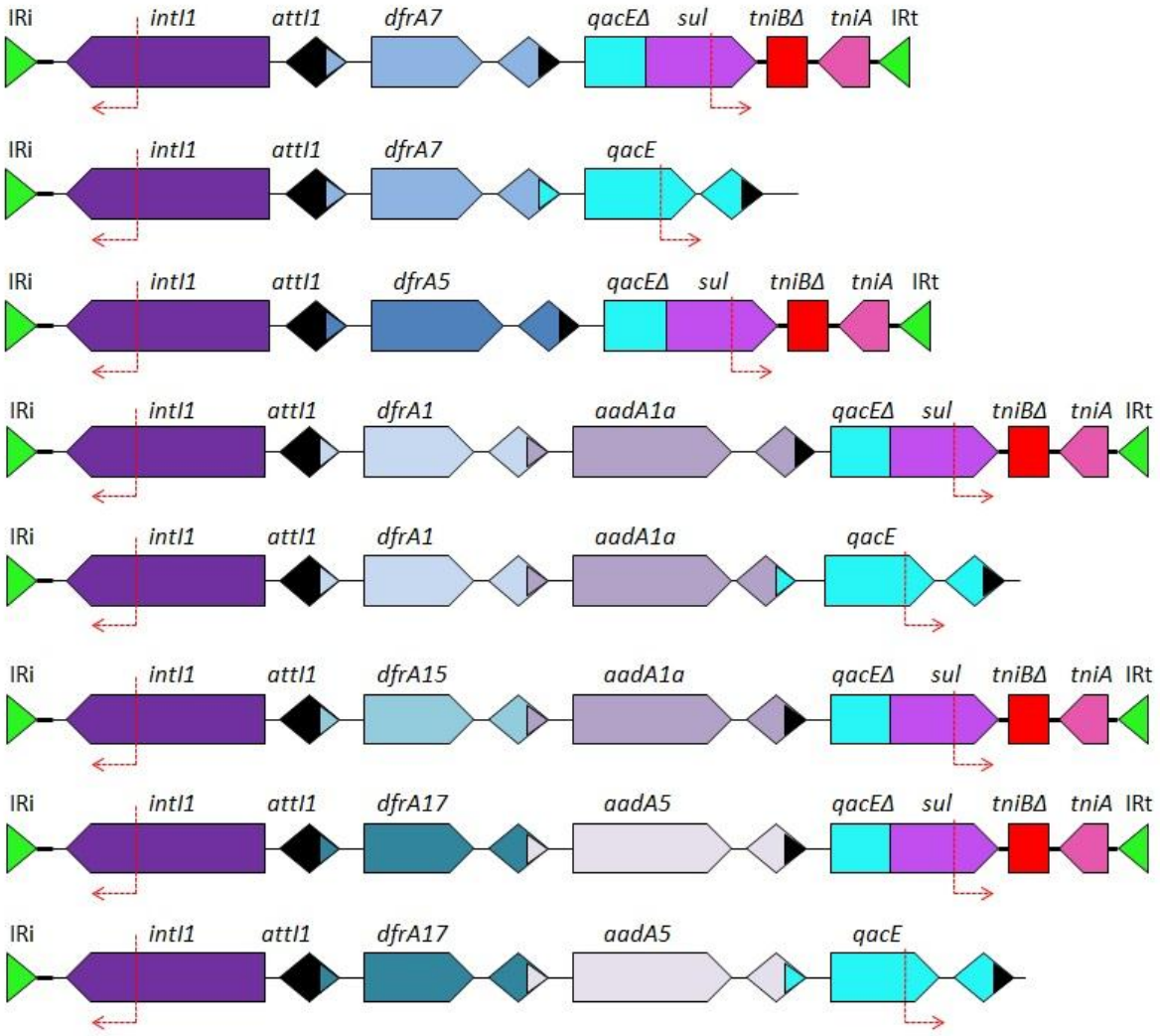

Figure 3.5 Gene maps of class 1 integrons identified in clinical *E. coli* isolates. From left to right, the symbols are as follows: green triangles represent the terminal inverted repeats; IRi and IRt, at the boundaries of the integron. Solid black lines represent the junction of the integron with the Tn*402* transposon sequence. The purple solid arrow indicates the integrase gene; *intI1*, pointing in the direction of transcription. The black diamond represents the *attI* recombination site. The gene cassettes found are in different colours and face the direction of transcription. The dotted red arrow indicates the boundary of assumed knowledge, that is; the genes beyond this arrow are assumed to be present, but were not tested. The red and pink solid arrows represent the remaining genes of the *tni* module, pointing in the direction of transcription.

3.4 - Discussion

The study of integron carriage and antibiotic resistance exhibited by clinically isolated *E. coli* shows that there is a significant relationship between these two factors. A number of common antibiotic resistance patterns were observed, and isolates containing integrons were more likely to exhibit antibiotic resistance. However, there was not any relationship between the specific gene cassettes carried on integrons and the range of resistance phenotypes found in an isolate. This may not be surprising, since the antibiotics tested are used to treat completely different infections, they have been in clinical use for different time periods and they have different modes of action. Further, they are not used in combination therapy. The only common element amongst the antibiotics in these groups is that they are all likely to have been overused/overprescribed or used in livestock (Davis *et al.* 1980). These resistance patterns are not likely to be directly related to integrons as the integrons characterized here did not carry gene cassettes that conferred resistance to the antibiotics in question. However, isolates with integrons are statistically more likely to be resistant to the antibiotics tested than those without an integron, regardless of what gene cassettes they carry. That is, integrons detected in this study with cassette arrays only carried cassettes that confer resistance to streptomycin and trimethoprim, but those isolates were also more likely to be resistant to the other antibiotics as well. In particular, there was a significant correlation between the presence of an integron and the isolates' ability to resist the action of cephalosporin antibiotics. This indicates that the evolutionary history of isolates containing integrons involved a complex series of selection events, making these particular isolates more susceptible to gene transfer and mutation events, allowing them to adapt to environments containing high doses of antibiotics.

Aminoglycoside resistance levels amongst these isolates were quite concerning, especially when paired with percentage of multi-resistant organisms. The two antibiotics with the greatest number of resistant strains were ampicillin and gentamicin. These antibiotics are used as a combination therapy in complicated urinary tract infections (long lasting, extensive infections that may have spread to the bladder or kidneys), which explains the high level of co-resistance recorded in this study (Ronald & Harding 1997). These isolates are likely to have been exposed to ampicillin and gentamicin treatments. The prescription of such combination therapies has clearly affected the resistance profiles directly, since these isolates were primarily isolated from urinary tract infections from patients who were hospital bound. Therefore, the spread of nosocomial infection may be an issue. In other studies, up to 75% of aminoglycoside resistance has been found to be encoded by cassettes embedded in integrons (Levesque *et al.* 1995).

Resistance mechanisms other than those attributable to the presence of integrons and gene cassettes are the likely cause of the high levels of resistance detected in this isolate collection. There was more diverse antibiotic resistance present than the gene cassettes in integrons were capable of conferring. *E. coli* has the ability to develop resistance via random mutation over extremely short periods of time, and is capable of accessing a diversity of plasmids which can confer resistance to many antibiotics concurrently (Amábile-Cuevas & Chicurel 1992; Bergstrom *et al.* 2000; Toprak *et al.* 2012). *E. coli* are commonly known to carry up to 4 plasmids, and this can increase the likelihood of harboring resistance genes, especially when coupled with the presence of integrons, as gene transfer can occur between plasmids, chromosomes and integrons. Resistance determinants to each of the antibiotic groups tested have all been identified on plasmids (Heffron *et al.* 1975; Horinouchi & Weisblum 1982; Breeze & Obaseiki-Ebor 1983; Huovinen *et al.* 1995; Davies & Wright 1997;

Martínez-Martínez *et al.* 1998; Goldstein 2002; Sandegren *et al.* 2008; Gupta *et al.* 2011). As such, it is likely that the resistance properties displayed by the organisms here were encoded on resistance plasmids. The common co-occurrence of resistance phenotypes to subsets of antibiotics in this collection of isolates suggests that multiple resistance determinants might be linked on plasmids not specifically examined during this project.

Of the antibiotics tested in this study, isolates showed least resistance to imipenem. It is a relatively new antibiotic in comparison with those to which isolates showed high levels of resistance (chloramphenicol, ampicillin etc). The most logical explanation for the lack of resistance to imipenem is that it is a controlled antibiotic with restricted use (Rahal *et al.* 1998). These restrictions are likely to increase the longevity of its effectiveness, since by not over-exposing bacterial communities to its presence, less resistance is likely to build up.

There was no detectable pattern in the number of integrons that appeared each month/week of sampling. The proportion of class 1 integrons remained relatively constant throughout the sample period. That is, class 1 integrons showed no significant clustering over the sampling period, nor did they show seasonal variation. However, a longer sampling period would be needed to make any firm conclusions about relative abundance over time.

Not all of the integrons detected had the standard clinical structure. Many of the integrons did not carry a *sul* gene, or did not test positive for a cassette region with the primers sets used in this study. This indicates the presence of a number of class 1 integrons with different distal regions. Such arrangements might be caused by deletion of the 3'-CS, or by movement of *intI1* and its associated cassette array into a different plasmid or chromosomal location. The cassette arrays of over 60% of the class 1 integrons in this study could not be successfully characterized with commonly used primer sets, including sets designed to detect arrays in pre-Tn*402* integrons, and Tn*402* integrons prior to the formation of the 3'-

CS. Clearly there is significant diversity in the arrangement and array conformations of clinical integrons that is not being detected using standard molecular testing protocols. Further investigation is required to better understand the arrangements and genetic contexts of these unusual integrons.

The cassette arrays within the integrons described here were not diverse. However, since the majority of isolates had cassette arrays that were unable to be characterized, there may be hidden cassette diversity in the isolate collection that remains to be characterized. We can be confident that these were not isolates with empty cassette arrays, since the PCR tests utilized here would have detected such arrangements. As these cassette arrays did not amplify with the PCR protocols described, deletions or rearrangements in the boundaries of the cassette array have probably changed or deleted the primer binding sites. Once again, further investigation is required to better identify the characteristics of these integrons.

Other studies have found similar cassette arrays to those successfully detected here, and these were present in both commensal and clinical isolates (Skurnik *et al.* 2005; Cocchi *et al.* 2007; Ajiboye *et al.* 2009; Partridge *et al.* 2009; El-Najjar *et al.* 2010; Glenn *et al.* 2012). In particular, cassette arrays containing |*dfrA7*|, |*drfA1*|*aadA1a*| and |*dfrA17*|*aadA5*| are amongst the commonest arrays flanked by the 5'-CS and 3'-CS (Partridge *et al.* 2009). Because these cassette arrays are common in both clinical and commensal organisms, it is likely that the antibiotics they confer resistance to (trimethoprim and streptomycin) have been overused for a substantial amount of time.

Class 2 integrons were found, but were uncommon in comparison to class 1 integrons. All the class 2 integron-integrase genes contained an internal stop codon and were non-functional. At this time and in this community, they appear to not pose a major threat, as they are present at low frequency and are incapable of rearranging their cassette arrays

without integrase activity supplied in *trans* (Hansson *et al.* 2002). While functional class 2 integrons have been detected elsewhere, it would appear that their introduction to the clinical environment is limited. It may be quite some time before the presence of class 2 integrons is as much of a threat as class 1 integrons.

In conclusion, this study showed that *E.coli* isolated from clinical contexts carries a significant level of antibiotic resistance, and that this resistance covered many of the common families of antibiotics. Class 1 integrons were common in the collection, but did not confer resistance to the antibiotic classes routinely tested in hospital microbiology programs. Nevertheless, the presence of integrons was strongly associated with an increased probability of multiple antibiotic resistance. This probably reflects the evolutionary history of selection upon the clonal lineages examined in this study.

Chapter 4

Integron Associated Antibiotic Resistance and Phylogenetic grouping of *Escherichia coli* from Clinical Environments

4.1 - Introduction

Integrons and the gene cassettes they capture play an integral role in the spread of antibiotic resistance amongst clinical *Escherichia coli* strains. This is due to their ability to capture, integrate and express gene cassettes which carry antibiotic resistance determinants (Hall & Collis 1994). The prevalence of integrons in clinical *E. coli* isolates ranges from 20 to 60%, and isolates exhibiting high levels of antibiotic resistance are more likely to carry an integron (Maguire *et al.* 2001)(Chapter 3).

Studies of *E. coli* populations have found a mainly clonal structure (Selander & Levin 1980). There are several ways that clonal groupings can be defined and detected. Clonal lineages can be defined using enterobacterial repetitive intergenic consensus sequences (via ERIC PCR) or phylotyping (Versalovic *et al.* 1991; Clermont *et al.* 2000). By investigating the clonal groups present in clinically isolated *E. coli*, including integron presence and cassette distribution, a better understanding of the population dynamics involved in the spread and expression of antibiotic resistance might be gained.

Phylogenetic types of *E. coli* are structured into four major groups, A, B1, B2 and D. These groups are defined by the presence of different virulence factors characterized by the genes *chuA*, *yjaA* and the DNA fragment TspE4C2. The four phylogenetic groups are defined by the presence or absence of each of these genes/fragments. Groups A and B1 are generally found in commensal *E. coli* isolated from the gastro-intestinal tract, while groups B2 and D are more frequently isolated from sites of infection (Zhang *et al.* 2002; Kotlowski *et al.* 2007; Skurnik *et al.* 2009). Isolates from the B2 group have been found to be the least resistant to antibiotics, despite carrying more virulence factors and being the most common phylotype of *E.coli* isolated from clinical settings (Skurnik *et al.* 2005).

This study reports on the relationships between integron presence, cassette array types, phylotypes and clonal lines in *E. coli* isolated from urinary tract infections over a period of 9 months spanning January to September, 2010. All integrons, cassette arrays, phylotypes and clonal lines for the 167 isolates were characterized. Understanding the dynamics of the integron/gene cassette system and its interactions with *E. coli* phylotypes is important in understanding the dissemination of resistance genes and how the environment in which they are captured affects their impact.

4.2 - Methods

4.2.1 - Bacterial Isolates

Escherichia coli samples were collected from the Sydney Adventist Hospital (SAN) on a weekly basis during the period of late January (24/01/10) to early September (05/09/10) of 2010. Samples were received on ISO-Sensitest agar plates (Oxoid), having been tested for antibiotic susceptibility by the hospital pathology laboratory. Samples were primarily collected from urinary tract infections, however, some isolates were from wound/abscess swabs, sputum or blood cultures. Upon collection, samples were catalogued and allocated a sample number to aid in the tracking of sample data. The patient number, sample site, sample date and antibiotic resistance, including extended spectrum beta-lactamases (ESBLs) and inducible beta-lactamases (IBLs) were recorded in an Excel spreadsheet (Appendix A).

4.2.2 - Detection of class 1 integrons

Class 1 integrons were detected via the presence of the IntI1 gene. Two primer sets were utilized; HS915 (5'-CGTGCCGTGATCGAAATCCAG-3') / HS916 (5'-TTCGTGCCTTCATCCGTTTCC-3'), and HS464 (5'-ACATGCGTGTAAATCATCGTCG-3') / HS463a (5'-CTGGATTTCGATCACGGCACG-3'), as previously described (Stokes *et al.* 2006; Marquez *et al.* 2008b). The presence of PCR products was assessed using electrophoresis on 2% agarose gels run at 110V for 1 hour. Gels were stained with GelRed^{TM} (Biotium) and DNA visualized using transmitted UV light. Class 1 integron presence or absence was recorded depending on PCR products recovered. The 167 isolates used in this study all carried a class 1 integron; all other isolates were discarded with no further testing.

4.2.3 - Phylogenetic typing of *E. coli*

Phylogenetic groups were assigned using multiplex PCR based on the presence of three DNA products associated with virulence factors in *E. coli*, namely, *chuA*, *yjaA* and TspE4C2, as previously described (Clermont *et al.* 2000). The presence of multiplex PCR products was assessed using electrophoresis on 2% agarose gels run at 110V for 1 hour. Gels were stained with GelRed^{TM} (Biotium) and DNA visualized using transmitted UV light. Individual phylotypes were then determined by visual inspection and comparison to control isolates of known phylotype (Appendix C, figure 2.2.4).

4.2.4 - Clonal Typing of *E.coli*

To investigate the potential presence of dominant clonal lines, all isolates were typed using enterobacterial repetitive intergenic consensus (ERIC)-PCR with the primers ERIC-IR (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC-2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') as described (Versalovic *et al.* 1991). Resulting PCR products were electrophoresed in a 2% agarose gel at 80V for 8 hours in order to maximize visibility of distinct banding patterns. Clonal lines were subsequently grouped by measurement and visual inspection of the banding patterns presented by the ERIC PCR products for each isolate.

4.2.5 - Statistical Analysis

Each isolate was regarded as an independent sample. Many isolates had a distinctive ERIC-PCR profile. While isolates grouped by ERIC-PCR could not be definitively proven to be a clonal group (Meacham *et al.* 2003), for the purposes of this project they were treated as clonal. Abundance of cassette array types and antibiotic resistance phenotypes were compared within phylogenetic groupings using the chi-square test or, when samples were small, Fisher's exact test. A p-value of <0.05 was considered significant.

4.3 - Results

4.3.1 - Phylogenetic grouping of *E. coli* isolates

Clinical *E. coli* isolates previously characterized as containing class 1 integrons (Chapter 3) were subjected to a multiplex PCR designed to target genes involved in *E. coli* virulence. The resulting PCR products allowed the isolates to be grouped according to a previously described phylotyping system (Clermont *et al.* 2000). Of 164 isolates successfully typed, 2 belonged to group A, 2 to group B1, 54 to group B2 and 109 to group D (Figure 4.1).

The small numbers of phylotype A and B1 isolates recovered precluded any detailed analysis of these phylotypes. The commonly recovered groups belonged to phylotypes B2 and D. There was no clear pattern in their tendency to be isolated from particular infection sites, each being found in blood cultures, swabs and urinary tract infections (Appendix A).

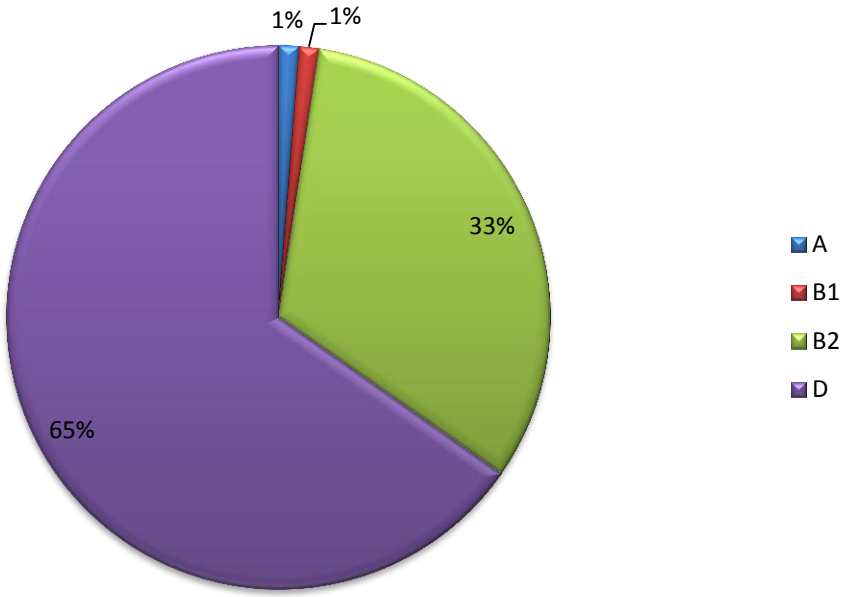

Figure 4.1 Proportions of phylotypes amongst *E. coli* isolates carrying class 1 integrons. Phylotyping was based on the scheme of Clermont et al. (2000).

4.3.2 - Investigation of *E. coli* clonal lines

A PCR designed to target the Enterobacterial Repetitive Intergenic Consensus (ERIC) sequences (Hulton *et al.* 1991) was carried out on the same cohort of *E. coli* samples as above. The resulting PCR products varied in size and number, generating complex DNA banding profiles from each line. In all, 117 distinct clonal groups were identified based on the ERIC banding pattern generated (Appendix B). Many isolates exhibited unique profiles, and the maximum number of isolates sharing an ERIC profile was 8. There were similarities between some groups of samples and several band sizes appeared to be dominant and frequent (Appendix C, Figure 2.2.5).

4.3.3 - Data Comparisons

Cassette array data for these isolates were collected and recorded previously (chapter 3). There was no correlation between clonal line and the presence of a class 1 integron, the cassette array it carried or the phylotype of the isolate. This was reinforced by the fact that clonal lines did not form large groups, with many individual isolates being unique. It is clear, however, that the prominent phylotype was group D, and the majority of those isolates either carried a cassette array that did not amplify with the primers used in this study, or carried the |*dfrA1*|*aadA1*| cassette array (Table 4.1).

Those clonal groups that were identified on the basis of ERIC patterns were not isolated in clusters, rather their collection dates varied, indicating no particular pattern in the spread of these lines. Some clonal lines appeared together (e.g.: samples belonging to the B2-5 clonal group), while others appeared scattered over several months (e.g.: samples belonging to the D-4 clonal group). As expected, all members of an individual clonal group carried the same cassette array (Appendix D). Major similarities between some of the clonal lines found in

phylotypes D and B2 were found, where the ERIC PCR profiles appeared extremely similar between phylotypes, often only varying by a single band.

It was found that phylotype D and B2 isolates exhibited similar level of resistance to the antibiotics tested (*p*-Value = 0.15). Other studies have found B2 isolates to be more sensitive to antibiotics than group D isolates (Picard *et al.* 1999; Johnson *et al.* 2003). There were too few members of the B1 and A groups found in this cohort to include these isolates in the statistical analysis.

Table 4.1 Cassette arrays exhibited by *E. coli* phylogenetic groups. Bracketed numbers represent the number of individual clonal lines identified for each group of isolates.

		Phylotype				
		A	B1	B2	D	Total
Cassette array	Uncharacterizable	1(1)	2 (2)	21 (17)	69 (37)	93 (57)
	dfrA1, aadA1	0	0	23 (19)	16 (15)	39 (34)
	dfrA17, aadA5	0	0	8 (7)	14 (8)	22 (15)
	dfrA15, aadA1	0	0	1 (1)	0	1 (1)
	dfrA7	1 (1)	0	0	10(9)	11 (10)
	dfrA5	0	0	0	1 (1)	1 (1)
	Total	2 (2)	2 (2)	53 (44)	110 (70)	167 (118)

Table 4.2 Antibiotic resistance phenotypes exhibited by phylogenetic groups. The numbers of isolates from each phylotype that exhibit resistance to each tested antibiotic are given.

	Phylotype			
Antibiotic	A (2)	B1 (2)	B2 (54)	D (109)
Cephalosporins				
KZ30	1	1	22	34
CTX5	1	0	8	20
FEP10	0	0	7	13
Penicillins				
AMP25	1	2	35	60
AMC60	1	2	20	36
Carbapenems				
IPM10	1	0	1	0
Aminoglycosides				
AK30	1	1	24	76
CN10	0	1	27	68
Quinolones				
NOR10	1	0	6	19
CIP2.5	1	0	10	20
Others				
CL100	1	1	18	22
F200	1	2	21	75
W5	1	1	28	56

4.3.4 - Cassette associated antibiotic resistance

Upon collection of the original isolates (described in chapter 3), the phenotypic antibiotic resistance profiles, as recorded by disk-diffusion assays, were recorded. The phenotypic resistance profiles were compared with the known genotypic resistance determinants found in any integrons present, where appropriate. Of the characterizable cassette arrays, only two types of gene that potentially encoded antibiotic resistance were found, these being several genes from the dihydrofolate reductase and aminoglycoside adenyltransferase families. These genes potentially encode resistance to trimethoprim and streptomycin respectively. It is not standard protocol to test Gram negative isolates against streptomycin. However, trimethoprim resistance was recorded in the isolate collection and could be compared to the presence of a dihydrofolate reductase gene in the cassette array (Appendix A and B).

Of the 61 isolates known to carry a gene cassette for trimethoprim resistance, 9 were sensitive to trimethoprim according to the Kirby-Bauer method of antibiotic sensitivity testing (Table 4.3). The sequences of these dihydrofolate reductase gene cassettes were analyzed. All 9 isolates carried a cassette array that consisted solely of a single *dfrA7* gene cassette. The sequence data for these cassettes were identical between the isolates in question and exhibited 100% similarity to functional *dfrA7* cassettes lodged in Genbank. The promoter regions of the integrons in these isolates were of the 'weak' + second configuration (Lévesque *et al.* 1994).

Table 4.3 Phenotypic resistance to trimethoprim

Cassette array	Number that have cassette array	Number that show phenotypic resistance	Percentage that show phenotypic resistance
Uncharacterizable	94	N/A	N/A
dfrA1, aadA1	31	31	100.00
dfrA17, aadA5	16	16	100.00
dfrA15, aadA1	1	1	100.00
dfr7	11	2	18.00
dfr5	1	1	100.00

4.4 - Discussion

In this project, a collection of *E. coli* isolates from a clinical setting was examined. Isolates that tested positive for the presence of a class 1 integron-integrase gene were further characterized to determine what gene cassettes they carried, and what genetic background these integrons occurred in, as tested using phylotyping and ERIC-PCR. The rationale behind this analysis was to understand the potential for transmission of isolates between hospital patients and to understand the potential for lateral gene transfer of integrons and gene cassettes between *E. coli* lineages.

Five different cassette arrays could be identified, consisting of *dfr* and *aadA* genes, either singly or in pairs (Table 4.1). However, the majority of class 1 integron positive isolates could not be characterized as to their array contents. These integrons did not have the standard 3'-CS, and thus represent genetic arrangements where the 3'-CS has been lost, or these integrons represent an earlier stage in the evolution of the typical clinical class 1 integron, prior to the

acquisition of the 3'-CS. In either case, reliance on standard PCR tests to characterize cassette array contents would fail to detect such integrons.

All integron positive isolates were characterized using the phylotyping system of Clermont (Clermont *et al.* 2000). The majority of isolates were of phylotype group D (65%). The second largest group, B2, consisted of 33% of the isolates, with the remaining groups, B1 and A consisting of 1% each. Most studies of *E. coli* isolates from urinary tract infections find phylotype B2 to be the largest group (Skurnik *et al.* 2009; Gündoğdu *et al.* 2011; Agarwal *et al.* 2013; Kõljalg *et al.* 2013). This discrepancy with our results is probably due to the exclusion of isolates which did not contain a class 1 integron from the current study, since in commensal *E. coli*; phylotype B2 isolates are less likely to carry an integron (Skurnik *et al.* 2005).

It is common for clinical cohorts of *E. coli* to consist of fewer phylotype A and B1 isolates than phylotypes B2 and D, as also found in this study (Duriez *et al.* 2001; Nowrouzian *et al.* 2006; Kõljalg *et al.* 2013). Several other studies have investigated phylotype structure in *E. coli,* using commensal isolates or isolates taken from different infections and patient populations. Results from those studies differ somewhat to those reported here, but this is probably a consequence of our concentration on UTIs and integron positive isolates (Duriez *et al.* 2001; Zhang *et al.* 2002; Nowrouzian *et al.* 2006; Kotlowski *et al.* 2007; Skurnik *et al.* 2009; Gündoğdu *et al.* 2011; Agarwal *et al.* 2013; Kõljalg *et al.* 2013; Kudinha *et al.* 2013).

Where the class 1 integron could be successfully characterized, the integron-integrase and cassette array were completely DNA sequenced for a subset of isolates, and their likely identity to the remaining un-sequenced isolates confirmed by PCR and restriction digestion. This

analysis showed that the individual integrons, as assessed by the identity of the most commonly recovered cassette arrays, could be found in both B2 and D phylotypes, and within a large diversity of ERIC-PCR types within phylotypes. The most parsimonious conclusion for these observations is that individual integrons have penetrated into a large and diverse set of *E*. coli lineages, and that consequently, the transmission of integrons between such lineages is common, and in some senses, now a matter of history.

The investigation of clonal groups and their integrons did not show any significant correlation between clonal group and integron presence, cassette types, phylogenetic grouping, antibiotic resistance profile or time of isolation. This is primarily due to the fact that there were a large number of distinct clonal groups identified using ERIC-PCR amongst the 167 isolates tested. The majority of isolates had unique ERIC-PCR patterns, thus fell into a clonal group of their own. The lack of common ERIC-PCR patterns clustered by time of isolation, infection site or integron features indicates that little or no transmission of specific clonal lines of *E. coli* occurred between these patients over the 9 months of sampling. Since the majority of isolates primarily originated from urinary tract infections, this observation is not surprising, since the primary mode of infection for urinary tract infections relies on contamination with the host's own flora (Barber *et al.* 2013). Our observations further suggest that each patient is carrying their own identifiable line of *E. coli*. The presence of the same integron (by 100% sequence identity) in multiple phylotypes and clonal lines of *E. coli* from different patients suggests that there has been frequent lateral gene transfer involving the integrons characterized here. The mechanism by which such integrons penetrate diverse clonal lineages in different hosts is worth investigating further.

Comparison of the antibiotic resistance profiles of each phylogenetic group (primarily B2 and D) showed little difference in resistance capabilities. However, the phylotype B2 group was consistently more frequently resistant to β-lactam antibiotics than the group D isolates. Previous studies have found that the B2 group members are often more sensitive to antibiotics than the other phylotypes (Johnson *et al.* 1991; Picard *et al.* 1999; Johnson *et al.* 2003; Kuntaman *et al.* 2005; Moreno *et al.* 2006). While the groups A and B1 did not consist of enough members for a reliable statistical comparison, previous studies have found these groups to exhibit high levels of resistance, which was also seen in the few isolates recovered here (Picard *et al.* 1999; Johnson *et al.* 2002). Phylogenetic group B2 is also reported to be more sensitive to quinolone antibiotics (Johnson *et al.* 2003). In this study, they were found to be only slightly more sensitive than the group D isolates. Once again, these discrepancies may be due to our concentration on isolates containing a class 1 integron.

A number of isolates found to be carrying a *dfrA7* gene cassette, usually responsible for conferring resistance to trimethoprim, were found to be phenotypically sensitive to trimethoprim. The sequence for these gene cassettes did not indicate a problem with the *dfrA7* gene. The integrons that carried these cassettes were found to have the promoter combination of “weak + second”; however, this promoter combination should have resulted in a decreased transcription rate and lowered resistance rather than complete sensitivity. There were no other cassettes present in these arrays, as such; distance from the promoters was not an issue. It is likely that other factors were involved in the prevention of expression of these *dfrA7* gene cassettes. The cause for the lack of expression experienced by these gene cassettes requires further investigation.

In conclusion, this study confirmed that fewer *E. coli* of phylotype B2 carry a class 1 integron, while class 1 integrons are more prevalent in the phylogenetic group D. Antibiotic resistance is comparable between the groups D and B2, and as with previous studies, phylotypes A and B1 are far less common in a clinical cohort. The integrons characterized in this study have made their way into diverse *E. coli* clonal lines, indicating a rapid transmission of integrons between phylotypes and lineages within this group. Conversely, there was no evidence of significant transmission of *E. coli* between individuals or within the hospital environment, at least for the kinds of infections examined here. The conclusion must be reached that the majority of UTIs originate from contamination with the patient's own flora, and that collectively, these flora consist of diverse phylotypes and clonal lines, each of which may carry integrons and other resistance determinants.

Chapter 5

General Conclusion - The Involvement of Class 1 Integrons in the Clinical Environment and Their Impact on the Spread of Resistance Genes

5.1 Discussion

Antibiotic resistance in pathogenic bacteria is increasingly important in the management of infectious disease. Mortalities resulting from the inability to treat infections caused by resistant organisms have been recorded in significant numbers around the world. The spread of resistance genes amongst pathogenic Gram negative bacteria is largely driven by the lateral gene transfer of mobile genetic elements, and their associated integrons, transposons and gene cassettes.

In this study, class 1 integrons were commonly found in isolates of *E. coli* from a major metropolitan hospital. The carriage of integrons was 23%, a frequency similar to that seen in previous studies (El-Najjar *et al.* 2010; Gündoğdu *et al.* 2011). In some studies, much higher

rates of carriage of class 1 integrons have been reported, indicating that their presence in clinical isolates is highly dependent on the environment and on the isolate collections examined (Vromen *et al.* 1999; Blahna *et al.* 2006; Solberg *et al.* 2006; Ajiboye *et al.* 2009; Skurnik *et al.* 2009; Gündoğdu *et al.* 2011). Class 2 integrons were also detected in the current study, but these were present at a much lower frequency, also as previously reported in various surveys of *E. coli* (Barlow & Gobius 2006; Solberg *et al.* 2006; Marquez *et al.* 2008b). All class 2 integrons detected in this study carried the non-functional class 2 integron-integrase gene (Hansson *et al.* 2002). This suggests that direct lateral gene transfer of the original class 2 integron was responsible for its presence in the various phylotypes of *E. coli* detected in this project.

The low frequency of class 2 integrons, and their carriage of a limited number of resistance determinants suggests that they represent a much lower threat for infection control than do the more numerous and diverse class 1 integrons (Sunde 2005b; Vinue *et al.* 2008). Functional class 2 integrons have recently been detected in both environmental and clinical contexts, but were not detected in this study (Barlow & Gobius 2006; Marquez *et al.* 2008a). It appears that their penetration into the clinical environment is currently limited. It is important to monitor the presence of class 2 integrons all the same, as once a functional form makes its way into clinical isolates, their opportunity to gain new gene cassettes will quickly increase their importance in resistance gene transfer.

No detectable pattern was apparent in the rate of integron carriage month by month across the duration of sampling undertaken in this study. Since the proportion of integron positive isolates remained relatively constant, there appears to be no seasonal relationship between the

presence of integrons and the occurrence of infection caused by *E. coli*. Currently, only one previous study has investigated the seasonality of class 1 integron occurrence, this being in environmental samples, from oysters and fresh water (Barkovskii *et al.* 2010). Continual monitoring of integron occurrence in clinical isolates over time could allow the first infiltration of new mobile genetic elements into the *E. coli* population to be detected. Such an observation would then allow subsequent lateral transfers to be documented, along with times, locations and contexts. This would provide powerful information about the modes and rates of gene transfer amongst organisms in a clinical environment.

A significant number of class 1 integrons detected in this study contained the conserved 5' and 3' segments, typical of most class 1 integrons described from clinical contexts (Hall *et al.* 1994). Detection methods for integrons often rely on the presence of such conserved regions. For instance, the presence of class 1 integrons can be determined based on PCR targeting *intI1* (Recchia & Hall 1995), while the identity of the gene cassettes carried in an array can be determined by PCR anchored in *attI* and the *sul1* gene (Gillings *et al.* 2009b). However, a significant proportion of class 1 integrons, including those detected in this study, do not carry a *sul1* gene (Gillings *et al.* 2008b), or may have deletions of other potential PCR targets such as the *tni* transposition module (Stokes *et al.* 2006). Integrons with this structure may represent deletion events derived from the typical clinical class 1 integron, or may be class 1 integrons still circulating in hospitals that represent an earlier stage of integron evolution, before the formation of the 3' conserved segment (Stokes *et al.* 2006; Gillings *et al.* 2008a). Further investigation is required to better understand the structure of these integrons, to determine their frequency and to develop methods for their detection and characterization. The presence

of such variant integrons presents a problem for molecular diagnostics, since they may not be detected using popular screening protocols.

This study found antibiotic resistance is common in clinically isolated *E. coli* samples, and resistance to multiple antibiotics is also common. This finding is not unusual, but the rate of resistance is alarming none the less. In this case, the gene cassettes carried by integrons were only one component of the resistance profiles exhibited by clinical isolates. Antibiotic resistance in our collection of isolates was also encoded by genes other than those carried on integrons, but these were not characterized in this study. Loci other than integrons can encode resistance mechanisms, for example, by mutation to the genes encoding antibiotic target molecules (Toprak *et al.* 2012; Johnson *et al.* 2013), or the presence of resistance plasmids which carry genes conferring resistance to many antibiotics (Amábile-Cuevas & Chicurel 1992; Bergstrom *et al.* 2000). *E. coli* can carry up to 4 plasmids; this can increase the likelihood of harboring resistance genes, especially when coupled with the presence of integrons, as transfer can occur between plasmids and chromosomally or plasmid borne integrons (Stokes & Hall 1989; Davies 2007).

A number of common resistance profiles were identified during this study. Evidence for the sequential development of these patterns in particular could not be found, as each antibiotic is normally used to treat completely different infections (Bauman 2009). The antibiotics in question have all been in clinical use for different time periods, all have different modes of action, none are used in combination therapies with each other and none of the integrons detected carried gene cassettes for resistance to these specific combinations of antibiotics

(Bauman 2009). The only common factor is that they are all likely to have been overused, overprescribed and/or used in livestock (Davis *et al.* 1980). The co-occurrence of resistance to particular antibiotics might be explained by the carriage of diverse resistance genes on a single plasmid; however this was not examined in the current study.

The resistance patterns of our isolate collection were not directly related to the integrons and gene cassettes in the collection. However, isolates with integrons were statistically more likely to be resistant to any suite of antibiotics tested than those without an integron, regardless of what gene cassettes they carried. That is, the integrons and cassette arrays detected in this study only carried cassettes that conferred resistance to streptomycin and trimethoprim, but those isolates were also more likely to be resistant to a range of other antibiotics as well. This presumably reflects the evolutionary history of the isolates in question. Isolates that carried integrons were more likely to be the descendants of bacterial lineages that had undergone multiple rounds of selection for resistance to a diverse range of antibiotics.

The levels of aminoglycoside resistance in our collection were quite concerning, especially when the proportion of multi-resistant organisms was considered. The two antibiotics to which isolates exhibited most frequent resistance were ampicillin and gentamicin. Both are used in combination therapy to treat complicated urinary tract infections (long lasting, extensive infections that may have spread to the bladder or kidneys) (Ronald & Harding 1997). The prescription of such combination therapies has clearly affected resistance profiles, particularly since our isolates were primarily recovered from urinary tract infections in patients who were hospital bound. Statistically, there was no evidence of clustering of ampicillin or gentamicin

resistance amongst phylotypes (*p*-Value = 0.15) and since so few clonal groups could be identified there was also no clustering of resistance in comparison to clonality. Other studies have found that up to 75% of aminoglycoside resistance was due to the presence of integrons (Levesque *et al.* 1995), but no gene cassettes conferring aminoglycoside resistance were detected in this study.

Imipenem was the antibiotic to which the *E. coli* isolates exhibited the least amount of resistance. It is a relatively new antibiotic, having only been released for medical use in the 1980's (Birnbaum *et al.* 1985). One potential explanation for the lack of resistance to this particular compound is that imipenem is a controlled antibiotic with restricted use (Rahal *et al.* 1998). These restrictions may be prolonging its effectiveness by not over-exposing bacterial communities to its presence. This imposes less selection pressure, and therefore less resistance has developed in the target organisms.

The cassette arrays found in this study are known to be common amongst class 1 integrons, and in clinical settings (Ajiboye *et al.* 2009; Partridge *et al.* 2009; El-Najjar *et al.* 2010). The two families of gene cassettes found were *aadA* and *dfrA* variants, which confer resistance to two of the most commonly used antibiotics; streptomycin and trimethoprim respectively. Since *E. coli* is a normal component of human microbial flora, isolates are likely to have fixed resistance genes through unintentional selection for lateral transfer events during treatment for an unrelated infection. Via simple contamination, these *E. coli* could subsequently cause urinary tract infections, while carrying previously acquired resistance genes.

A number of isolates were found to be carrying a *dfrA7* gene cassette, usually responsible for conferring resistance to trimethoprim, but these were phenotypically sensitive to trimethoprim. The sequence of these genes cassettes did not indicate any mutations that would affect the activity of the *dfrA7* gene. Integrons that carried these *dfrA7* cassettes were found to have the promoter combination of "weak + second" (Lévesque *et al.* 1994), however, this promoter combination should have resulted in a decreased transcription rate and lowered resistance rather than complete sensitivity. There were no other cassettes present in these arrays, and as such, distance from the promoters should also not be an issue (Collis & Hall 1995; Jacquier *et al.* 2009). It is likely that other factors were involved in the prevention of expression of these *dfrA7* gene cassettes. The cause for the lack of expression experienced by these gene cassettes requires further investigation.

The cassette arrays found in the class 1 integrons of this study were not diverse. However, the majority of isolates had cassette arrays that were unable to be characterized. They were not isolates with empty cassette arrays, as those would have been detected with our protocols. These isolates had cassette arrays that did not amplify with the primer pairs conventionally used to target the cassette regions of class 1 integrons. Such a lack of amplification indicates that there have likely been deletions or rearrangements in the cassette array boundaries which resulted in the cassette array primers not binding. Once again, further investigation is required to better identify the characteristics of these integrons.

The frequency and identity of the gene cassette arrays found in this study suggests considerable and frequent lateral movement of integrons and gene cassettes between *E. coli*

phylotypes and strains. Integrons and cassette arrays with identical DNA sequences could be detected in different phylotypes and in different clonal lines as defined by ERIC-PCR. For gene cassettes and integrons with the same DNA sequence to appear in different clonal lines and phylotypes of *E. coli,* there must have been lateral gene transfer between these organisms. Further, because each of the isolates investigated in this study came from individual patients, and these isolates in turn exhibited considerable genetic diversity, integrons must be transferring between the distinctive commensal *E. coli* strains in these patients, at least in the long-term.

The proportions of each *E. coli* phylotype amongst the isolates showed that the majority of isolates belonged to phylotype group D (65%), with B2 being the second most frequent phylotype. Phylotypes B1 and A accounted for only 1% of integron containing isolates. Most studies of *E. coli* isolates from urinary tract infections find phylotype B2 to be the most common group (Zhang *et al.* 2002; Skurnik *et al.* 2009; Gündoğdu *et al.* 2011; Agarwal *et al.* 2013; Kõljalg *et al.* 2013; Kudinha *et al.* 2013).The discrepancy with our results is probably due to the exclusion of isolates which did not contain a class 1 integron from our study. It has been previously reported that in commensal *E. coli*, phylotype B2 isolates are less likely to carry an integron (Skurnik *et al.* 2005). It is common for clinical cohorts of *E. coli* to consist of fewer phylotype A and B1 isolates than B2 and D (Skurnik *et al.* 2009; Gündoğdu *et al.* 2011; Agarwal *et al.* 2013; Kõljalg *et al.* 2013), and this study showed no exception. Although our study examined clinical isolates, urinary tract infections are often caused by contamination with the commensal enterobacterial flora (Nicolle 2002; Meacham *et al.* 2003). The inherent diversity of *E. coli* strains between individual patients in our study strongly supports this conclusion. If, in

contrast, patients were infected in the community or the hospital with *E. coli* that caused UTIS, one might expect considerably more homogeneity in the various isolates than we observed here.

The investigation of clonal groups present did not show any significant correlation between clonal group and integron presence, cassette types, phylogenetic group or antibiotic resistance profile. This is primarily due to the fact that there were so many clonal groups identified on the basis of ERIC PCR amongst the 167 isolates tested, with most individual isolates falling into a clonal group of their own. The lack of definitive groups indicates that little transmission of specific clonal lines of *E. coli* occurred between these patients over the 9 months of sampling, which considering the isolates primarily originated from urinary tract infections is perhaps not surprising. Since the primary mode of contamination for urinary tract infections is contamination by the hosts own flora, it is not surprising that each individual carries their own line of *E. coli*. However, the presence of the same integron in various phylotypes and clonal lines suggests frequent lateral gene transfer of the integron between these clonal lines and phylotypes. Integrons with 100% sequence identity could be found in multiple clonal lines and phylotypes.

Comparison of the antibiotic resistance profiles between phylogenetic groups (primarily B2 and D) showed little difference in resistance capabilities. However, phylotype B2 was consistently more resistant to β-lactam antibiotics than group D. Previous studies found that the B2 group is usually more sensitive to antibiotics than the other phylotypes (Picard *et al.* 1999; Johnson *et al.* 2003). While the groups A and B1 did not have enough members for a reliable statistical

comparison, previous studies have found these groups to exhibit high levels of resistance (Johnson *et al.* 2003), which was also seen in isolates from this study. Phylogenetic group B2 are also known to be more sensitive to quinolone antibiotics (Johnson *et al.* 2003). In our isolates, they were found to be only slightly more sensitive than group D. Once again, these discrepancies may be due to specific selection of isolates containing a class 1 integron during this study.

In conclusion, class 1 integrons are an important and current threat in the realm of antibiotic resistance in clinically relevant bacteria. Their ability to acquire resistance determinants and their inherent mobility allow them to rapidly spread resistance in commensal bacteria. This can lead to the conversion of organisms from normal components of flora into pathogens. The continued over use of antibiotics and disregard for the fact that treatment of infectious agents targets more than just the agent of infection will continue to select for increasing levels of resistance in all organisms. This study focused on *E. coli* in a clinical setting however; there are many more organisms which reside naturally in the human host. All are potentially capable of becoming pathogenic in the future. Alternative methods of treatment need to be investigated, including improved hygiene control (Rosenthal *et al.* 2012; Derde *et al.* 2013), phage therapy (Brüssow 2012; Kutter *et al.* 2013; Trigo *et al.* 2013) and the potential for personalized antibiotic treatments based on the screening of resistance determinants already extant in the patient. There may even be the potential for co-treatment with probiotics; however, limited success has been experienced thus far and no studies have covered the link between commensal gastrointestinal micro-flora and urinary tract infections (Fukuda *et al.* 2002; Manley *et al.* 2007; Oudhuis *et al.* 2011). Methods to ensure minimal effect on the native micro-flora

during treatment with antibiotics need to be considered further. This is important as in situations like the occurrence of urinary tract infections, where the primary mode of infection is via contamination by the hosts' normal flora, and the resistance capabilities of the normal flora become extremely relevant. This is a topic which is often completely overlooked, as interest in organisms is usually elicited after they have caused an infection rather than before they do so. At this point, we need to pursue strategies that minimize the transfer of resistance genes and slow down the spread of resistance.

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Appendix A: Raw Sample data

All dates sampled were within the year 2010. Antibiotic abbreviations as follows; W5 trimethoprim 5µg, CTX5 cefotaxim 5µg, F200 nitrofurantoin 200µg, CIP2.5 ciprofloxacin 2.5µg, IPM10 imipenem 10 µg, AK30 amikacin 30µg, NOR10 norfloxacin 10µg, AMP25 ampicillin 25µg, CN10 gentamicin 10µg, FEP10 cefepime 10µg, AMC60 augmentin (amoxycillin/clavulanic acid) 60µg, CL100 chloramphenicol 100µg, KZ30 cefazolin 30µg. ESBL; Extended spectrum β-lactamase, IBL; Inducible β-lactamase. *intI1*; Class 1 integron presence, *intI2*; Class 2 integron presence.

Sample Data					Antibiotic Resistance Profiles															Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
1	16/2/10	2/2	csu-m,c&s	1	+	+	-	-	-	-	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
2	16/2/10	5/2	swab -low vaginal	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
3	16/2/10	10/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	16/2/10	10/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	16/2/10	1/2	urine-midstream	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
6	16/2/10	3/2	urine-midstream	2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	16/2/10	5/2	urine-midstream	2	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
8	16/2/10	5/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
9	16/2/10	8/2	urine-midstream	3	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-
10	16/2/10	10/2	urine-unknown meth	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	16/2/10	10/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	16/2/10	1/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	16/2/10	5/2	urine-unknown meth	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	16/2/10	5/2	swab-vaginal	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	16/2/10	8/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	16/2/10	9/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	16/2/10	9/2	urine-midstream	3	+	-	-	+	-	-	+	+	-	-	+	+	+	-	-	+	-
18	16/2/10	13/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	16/2/10	2/2	urine-midstream	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
20	16/2/10	5/2	urine-midstream	2	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	16/2/10	5/2	urine-midstream	2	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
22	16/2/10	9/2	urine-midstream	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
23	16/2/10	1/2	Sputum-mucosalivar	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	16/2/10	1/2	urine-midstream	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
25	16/2/10	1/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	16/2/10	1/2	urine-midstream	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
27	16/2/10	28/1	urine-midstream	2	-	-	-	-	-	N/T	-	-	-	-	-	-	-	-	-	-	-
28	16/2/10	2/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	16/2/10	5/2	sputum-mucopurulen	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	16/2/10	5/2	csu-m,c&s	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	16/2/10	5/2	csu-m,c&s	2	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-
32	16/2/10	2/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	16/2/10	5/2	csu-m,c&s	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
34	16/2/10	3/2	swab-right leg	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
35	16/2/10	6/2	swab-perineal absc	2	+	-	±	-	-	-	-	+	-	-	+	-	-	-	-	+	-
36	16/2/10	5/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	16/2/10	5/2	urine-midstream	2	-	+	+	-	-	-	-	+	-	-	+	+	+	-	-	-	-
38	16/2/10	8/2	Sputum-mucosalivar	1	N/T	N/T	N/T	-	+	N/T	N/T	N/T	+	N/T	N/T	N/T	N/T	-	-	+	-
39	16/2/10	8/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	16/2/10	8/2	urine-midstream	3	+	-	-	+	-	-	+	+	+	-	-	-	-	-	-	+	-
41	16/2/10	8/2	csu-m,c&s	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-

Sample Data					Antibiotic Resistance Profiles															Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
42	16/2/10	10/2	swab-wound	2	-	-	+	-	-	+	-	-	-	-	+	+	+	-	-	-	+
43	16/2/10	10/2	urine-midstream	2	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	16/2/10	9/2	swab-left foot ulc	1	-	-	-	+	-	-	+	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
45	16/2/10	9/2	blood culture x1	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
46	16/2/10	9/2	csu-m,c&s	2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
47	16/2/10	9/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
48	16/2/10	10/2	swab-thoracotomy w	2	+	-	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-
49	16/2/10	10/2	swab-left foot toe	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	-	-	+	+	+	-	-	-	-
50	16/2/10	8/2	swab-toe wound	3	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-
51	16/2/10	8/2	urine-midstream	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	17/2/10	28/1	swab-low vaginal	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
53	17/2/10	25/1	urine-unknown meth	2	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-	+	-
54	17/2/10	28/1	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55	17/2/10	29/1	urine-unknown meth	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
56	17/2/10	18/1	urine-unknown meth	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
57	17/2/10	30/1	urine-unknown meth	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58	17/2/10	24/1	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59	17/2/10	24/1	urine-midstream	2	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	17/2/10	24/1	urine-midstream	2	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
61	17/2/10	24/1	C	1	-	-	+	-	-	-	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
62	17/2/10	1/2	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	+	-	-	-	-	-	-	-	-
63	17/2/10	1/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
64	17/2/10	31/1	urine-midstream	2	+	+	-	-	-		-	N/T	N/T	-	-	N/T	N/T	-	-	-	-
65	17/2/10	30/1	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66	17/2/10	29/1	urine-midstream	3	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-
67	17/2/10	30/1	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
68	17/2/10	28/1	urine-midstream	4	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-
69	17/2/10	28/1	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70	17/2/10	28/1	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
71	17/2/10	28/1	blood culture x1	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
72	17/2/10	25/1	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
73	22/2/10	15/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
74	22/2/10	15/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75	22/2/10	16/2	urine-midstream	2	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-
76	22/2/10	13/2	blood culture x1	2	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
77	22/2/10	14/2	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	-	-	-	-	-	-	-	-	-
78	22/2/10	15/2	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	±
79	22/2/10	16/2	csu-m,c&s	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
80	1/3/10	17/2	urine-midstream	1	-	-	-	-	-	-	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
81	1/3/10	17/2	urine-midstream	2	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	+	-
82	1/3/10	18/2	urine-midstream	3	-	-	-	+	-		+	-	+	-	-	-	-	-	-	-	-
83	1/3/10	17/2	urine-midstream	2	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Sample Data					Antibiotic Resistance Profiles														Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
84	1/3/10	12/2	tissue-right leg u	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
85	1/3/10	14/2	csu-m,c&s	2	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-
86	1/3/10	17/2	urine-unknown meth	2	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	1/3/10	17/2	urine-midstream	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	1/3/10	17/2	urine-midstream	2	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
89	1/3/10	17/2	urine-midstream	2	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	15/3/10	22/2	urine-midstream	2	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
91	15/3/10	3/3	urine-midstream	2	+	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-
92	15/3/10	9/3	urine-unknown meth	2	+	-	+	-	-	-	+	+	+	-	+	+	+	-	-	-	+
93	15/3/10	23/2	urine-unknown meth	2	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	-
94	15/3/10	23/2	swab-vaginal	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
95	15/3/10	8/3	urine-midstream	2	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
96	15/3/10	8/3	urine-midstream	2	-	-	-	-	-	+	-	+	+	-	+	+	+	-	-	+	-
97	15/3/10	9/3	swab-dog bite	2	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
98	15/3/10	2/3	urine-midstream	2	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
99	15/3/10	10/3	swab-vaginal	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
100	15/3/10	1/3	blood culture x1	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
101	15/3/10	2/3	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
102	15/3/10	2/3	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
103	15/3/10	2/3	urine-midstream	2	+	-	+	-	-	-	-	+	-	-	+	+	+	-	-	+	-
104	15/3/10	2/3	swab-left cheek	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
105	15/3/10	8/3	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
106	15/3/10	8/3	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
107	15/3/10	6/3	urine-unknown meth	3	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	+	-
108	15/3/10	10/3	swab-right lateral	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
109	15/3/10	23/2	swab-right groin a	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
110	15/3/10	23/2	urine-midstream	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
111	15/3/10	22/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
112	15/3/10	21/2	urine-midstream	2	-	-	-	+	-	-	+	+	+	-	-	-	+	-	-	-	-
113	15/3/10	23/2	swab-deep right gr	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
114	15/3/10	28/2	urine-midstream	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
115	15/3/10	28/2	urine-midstream	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
116	15/3/10	28/2	urine-midstream	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
117	15/3/10	24/2	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
118	15/3/10	24/2	urine-midstream	2	-	+	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-
119	15/3/10	24/2	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	+	-	-	-	-	-
120	15/3/10	3/3	swab-left anterior	2	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-
121	15/3/10	24/2	urine-midstream	2	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
122	15/3/10	24/2	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	+	-	-	-	+	-	-	-	-
123	15/3/10	23/2	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-

Sample Data					Antibiotic Resistance Profiles															Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
124	15/3/10	2/3	swab-spc site pus	3	N/T	+	N/T	N/T	+	+	N/T	N/T	N/T	+	+	N/T	N/T	-	-	+	-
125	15/3/10	2/3	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
126	15/3/10	2/3	urine-unknown meth	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
127	15/3/10	2/3	bilateral lobe bronc	2	+	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-
128	15/3/10	1/3	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
129	15/3/10	3/3	urine-midstream	2	-	-	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-
130	15/3/10	3/3	swab-right ankle	2	+	+	+	+	-	-	+	+	+	-	+	+	+	-	-	+	-
131	15/3/10	3/3	urine-unknown meth	2	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
132	15/3/10	3/3	c	2	+	+	-	+	-	+	+	+	+	+	+	+	+	-	-	+	-
133	15/3/10	3/3	blood culture x1	1	+	+	-	+	-	+	+	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
134	15/3/10	6/3	csu-m,c&s	4	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
135	15/3/10	7/3	c	2	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-
136	15/3/10	7/3	csu-m,c&s	1	+	-	-	-	-	-	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
137	15/3/10	7/3	urine-midstream	2	-	-	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-
138	15/3/10	7/3	urine-midstream	3	+	+	+	+	+	-	+	+	-	-	+	+	+	-	-	-	-
139	15/3/10	7/3	urine-midstream	2	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
140	15/3/10	7/3	urine-midstream	1	-	-	-	-	-	-	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
141	15/3/10	7/3	sputum-mucopurulen	2	-	-	+	-	-	+	-	-	-	-	+	+	+	-	-	-	-
142	15/3/10	8/3	blood culture x1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
143	15/3/10	8/3	swab-suture line	2	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
144	15/3/10	8/3	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
145	15/3/10	8/3	blood culture x1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
146	15/3/10	9/3	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
147	15/3/10	9/3	urine-midstream	1	-	-	-	-	-	-	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
148	15/3/10	9/3	tissue-left hip	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
149	15/3/10	9/3	swab-perineal absc	2	-	+	-	-	-	-	-	+	-	-	+	+	+	-	-	-	-
150	15/3/10	8/3	urine-midstream	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
151	15/3/10	8/3	blood culture x1	2	-	-	+	-	-	-	-	+	-	-	+	+	+	-	-	-	-
152	15/3/10	8/3	swab-abdominal abs	2	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
153	15/3/10	8/3	fluid-gall bladder	1	-	-	+	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
154	15/3/10	8/3	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-	-	-	-	-	-	-
155	15/3/10	7/3	urine-midstream	1	-	-	-	-	-	N/T	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
156	15/3/10	9/3	tissue-left hip	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
157	15/3/10	10/3	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
158	15/3/10	10/3	urine-midstream	2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
159	22/3/10	16/3	urine-midstream	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
160	22/3/10	17/3	urine-midstream	2	+	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-
161	22/3/10	16/3	urine-midstream	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
162	22/3/10	16/3	urine-midstream	2	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
163	22/3/10	17/3	urine-midstream	2	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-

Sample Data					Antibiotic Resistance Profiles															Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
164	22/3/10	16/3	urine-midstream	2	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
165	22/3/10	17/3	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
166	22/3/10	17/3	swab-scrotal absce	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
167	22/3/10	17/3	urine-midstream	2	+	-	+	-	-	+	-	-	+	-	-	-	+	-	-	-	+
168	22/3/10	17/3	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-
169	22/3/10	14/3	blood culture x1	2	+	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-
170	22/3/10	14/3	csu-m,c&s	2	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
171	22/3/10	14/3	urine-midstream	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
172	22/3/10	15/3	urine-midstream	2	-	+	+	-	-	-	-	+	+	-	+	+	+	-	-	-	-
173	22/3/10	16/3	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	+	-	+	-	-	-	-
174	22/3/10	15/3	urine-midstream	2	-	-	-	-	-	+	-	+	+	-	-	+	+	-	-	-	-
175	22/3/10	15/3	swab-toe	2	-	-	+	-	-	+	-	-	+	-	+	+	+	-	-	-	-
176	22/3/10	17/3	urine-midstream	2	+	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-
177	22/3/10	17/3	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-
178	22/3/10	16/3	urine-midstream	2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
179	30/3/10	22/3	swab-high vaginal	4	+	+	+	-	-	+	-	+	+	+	+	+	+	-	-	+	-
180	30/3/10	24/3	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
181	30/3/10	24/3	urine-midstream	2	-	-	+	+	-	+	-	-	+	-	-	+	-	-	-	-	-
182	30/3/10	22/3	urine-midstream	5	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-
183	30/3/10	22/3	csu-m,c&s	3	+	-	+	+	-	+	+	+	-	-	-	-	+	-	-	+	-
184	30/3/10	18/3	blood culture x1	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
185	30/3/10	18/3	blood culture x1	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
186	30/3/10	19/3	urine-midstream	4	-	+	+	-	-	+	-	+	+	-	+	+	+	-	-	+	-
187	30/3/10	22/3	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
188	30/3/10	22/3	tissue-content of	6	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
189	30/3/10	22/3	swab-high vaginal	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
190	30/3/10	21/3	urine-midstream	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
191	30/3/10	21/3	blood culture x1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
192	30/3/10	21/3	m	2	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	+	-
193	30/3/10	21/3	blood culture x1	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
194	30/3/10	23/3	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
195	30/3/10	23/3	swab-low vaginal	2	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-
196	30/3/10	23/3	swab-surgical site	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
197	30/3/10	24/3	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	+	+	+	-	-	+	-
198	30/3/10	24/3	urine-midstream	1	N/T	N/T	N/T	-	-	N/T	N/T	N/T	-	N/T	N/T	N/T	N/T	-	-	-	-
199	14/4/10	26/3	urine-midstream	2	+	+	+	+	-	+	+	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
200	14/4/10	29/3	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
201	14/4/10	6/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-
202	14/4/10	7/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
203	14/4/10	7/4	urine-midstream	2	-	+	+	-	-	-	-	+	-	-	+	+	+	-	-	-	+

Sample Data					Antibiotic Resistance Profiles															Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
204	14/4/10	29/3	urine-midstream	2	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
205	14/4/10	30/3	urine-midstream	2	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
206	14/4/10	30/3	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
207	14/4/10	7/4	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
208	14/4/10	7/4	urine-midstream	2	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
209	14/4/10	27/3	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	+	+	+	+	+	-	-	-	-
210	14/4/10	29/3	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
211	14/4/10	29/3	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
212	14/4/10	30/3	c	2	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-
213	14/4/10	29/3	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	+	+	+	-	-	+	-
214	14/4/10	29/3	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
215	14/4/10	4/4	swab-right leg	2	-	-	+	-	-	-	-	+	+	-	+	+	+	-	-	-	-
216	14/4/10	4/4	urine-midstream	3	+	+	+	+	-	+	+	+	+	-	+	+	+	-	-	+	-
217	14/4/10	4/4	blood culture x1	3	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-	+	-
218	14/4/10	4/4	swab-abdominal wou	2	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
219	14/4/10	30/3	swab-wound	1	-	-	+	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
220	14/4/10	30/3	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
221	14/4/10	30/3	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
222	14/4/10	30/3	urine-midstream	2	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
223	14/4/10	30/3	swab-right eye	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	+	-
224	14/4/10	30/3	urine-midstream	2	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
225	14/4/10	30/3	fluid-bile	4	-	+	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
226	14/4/10	5/4	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-	-	-	-	-	-	-
227	14/4/10	5/4	csu-m,c&s	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	+	+	+	+	+	-	-	+	-
228	14/4/10	7/4	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	+	+	-	+	+	-	-	-	-
229	14/4/10	7/4	urine-midstream	2	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-
230	14/4/10	6/4	swab-leg wound	2	+	+	+	-	-	+	+	+	+	-	+	+	+	-	-	-	-
231	14/4/10	6/4	urine-midstream	2	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-
232	14/4/10	6/4	blood culture x1	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
233	14/4/10	6/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
234	14/4/10	5/4	urine-midstream	1	-	-	-	-	-	-	-							-	-	-	-
235	30/4/10	20/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
236	30/4/10	28/4	urine-midstream	2	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-
237	30/4/10	21/4	urine-midstream	2	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
238	30/4/10	28/4	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
239	30/4/10	28/4	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	-	-	-	-	-	-	-	+	-
240	30/4/10	28/4	urine-midstream	2	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
241	30/4/10	20/4	urine-midstream	2	-	-	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-
242	30/4/10	19/4	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-

Sample Data					Antibiotic Resistance Profiles															Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
243	30/4/10	28/4	urine-unknown meth	2	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-
244	30/4/10	28/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
245	30/4/10	19/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
246	30/4/10	21/4	urine-midstream	2	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
247	30/4/10	20/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
248	30/4/10	19/4	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
249	30/4/10	20/4	swab-right leg ulc	2	-	-	+	-	-	-	-	+	+	-	+	+	+	-	+	-	-
250	30/4/10	18/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
251	30/4/10	13/4	urine-midstream	2	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
252	30/4/10	19/4	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	+	-
253	30/4/10	19/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
254	30/4/10	11/4	blood culture x1	2	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	+
255	30/4/10	11/4	csu-m,c&s	2	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
256	30/4/10	21/4	urine-unknown meth	2	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
257	30/4/10	19/4	IUD	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	+	-
258	30/4/10	19/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+
259	30/4/10	13/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-
260	30/4/10	24/4	culture-cord blood	2	-	-	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-
261	30/4/10	25/4	urine-catheter	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
262	30/4/10	26/4	csu-m,c&s	2	-	-	+	-	+	+	-	+	+	-	+	+	+	-	+	-	-
263	30/4/10	26/4	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	-	+	-	-	-	-
264	30/4/10	17/4	blood culture x1	2	-	-	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-
265	30/4/10	13/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
266	30/4/10	21/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-
267	30/4/10	21/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	+	-	-
268	30/4/10	19/4	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	-	+	-	-	-	-
269	30/4/10	13/4	csu-m,c&s	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
270	30/4/10	20/4	urine-midstream	2	+	-	+	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
271	30/4/10	28/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
272	30/4/10	12/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-
273	30/4/10	12/4	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	+	-	+	+	+	-	-	-	-
274	30/4/10	12/4	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
275	30/4/10	21/4	urine-unknown meth	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
276	30/4/10	13/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	+	+	+	-	-	-	-
277	30/4/10	13/4	urine-midstream	2	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
278	30/4/10	16/4	urine-midstream	2	N/T	-	N/T	N/T	-	N/T	N/T	N/T	N/T	-	+	N/T	N/T	-	-	+	-
279	30/4/10	16/4	swab-left fourth t	2	N/T	+	N/T	N/T	-	N/T	N/T	N/T	N/T	N/T	+	-	N/T	-	-	-	-
280	30/4/10	20/4	urine-midstream	2	-	-	+	+	-	+	+	+	+	-	+	+	+	-	-	-	-

Sample Data					Antibiotic Resistance Profiles														Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
281	30/4/10	17/4	blood culture x1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
282	30/4/10	13/4	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
283	30/4/10	12/4	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	+	-	-	-	-	-
284	30/4/10	13/4	blood culture x1	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
285	30/4/10	13/4	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
286	30/4/10	17/4	urine-midstream	3	N/T	+	N/T	N/T	-	N/T	N/T	N/T	N/T	+	+	N/T	N/T	+	-	+	-
287	30/4/10	18/4	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	+	+	+	-	-	+	-
288	30/4/10	18/4	urine-midstream	2	-	+	+	-	-	+	-	+	+	-	+	+	+	-	+	-	-
289	30/4/10	27/4	mouth drain	2	-	-	+	-	-	+	-	+	+	-	+	+	+	+	+	-	-
290	30/4/10	28/4	csu-m,c&s	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
291	30/4/10	28/4	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	+	-	+	-	-	+	-
292	30/4/10	28/4	swab-nasal skin pu	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	+	-	-
293	30/4/10	19/4	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
294	30/4/10	13/4	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
295	30/4/10	13/4	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	+	-
296	30/4/10	19/4	swab-right ear	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-
297	30/4/10	20/4	urine-midstream	2	+	+	+	-	+	+	-	+	+	-	+	+	+	-	-	-	-
298	30/4/10	19/4	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
299	30/4/10	12/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
300	30/4/10	14/4	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
301	30/4/10	14/4	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
302	10/5/10	4/5	sputum-mucopurulen	1	N/T	N/T	N/T	+	N/T	N/T	N/T	+	N/T	N/T	N/T	N/T	N/T	-	-	-	-
303	10/5/10	5/5	urine-midstream	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
304	10/5/10	5/5	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
305	10/5/10	5/5	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
306	10/5/10	5/5	urine-midstream	2	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
307	10/5/10	29/4	blood culture x1	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
308	10/5/10	1/5	csu-m,c&s	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
309	10/5/10	3/5	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	-	-	-	-	-	-	-	-	-
310	10/5/10	2/5	csu-m,c&s	2	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
311	10/5/10	4/5	urine-midstream	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
312	10/5/10	4/5	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
313	10/5/10	4/5	urine-midstream	2	-	-	+	-	-	-	-	+	+	-	+	+	+	-	-	+	-
314	10/5/10	4/5	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	+	+
315	10/5/10	4/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
316	10/5/10	4/5	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
317	10/5/10	3/5	csu-m,c&s	1	+	+	+	+	-	+	+							-	-	+	-
318	10/5/10	4/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-

Sample Data					Antibiotic Resistance Profiles															Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
319	10/5/10	3/5	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	+	-	+	-	-	-	+
320	10/5/10	3/5	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	-	+	-	-	+	-
321	10/5/10	4/5	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
322	10/5/10	1/5	urine-catheter	4	+	-	+	-	-	+	-	+	+	-	+	-	-	-	-	+	-
323	10/5/10	2/5	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
324	10/5/10	2/5	csu-m,c&s	3	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-
325	10/5/10	3/5	urine	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
326	10/5/10	3/5	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
327	10/5/10	3/5	swab-vaginal	2	+	-	+	-	-	+	-	+	+	-	+	-	+	-	-	-	+
328	31/5/10	24/5	urine-unknown meth	1	N/T	N/T	N/T	N/T	N/T	-	N/T	+	-	-	-	-	-	-	-	-	-
329	31/5/10	24/5	urine-midstream	1	N/T	N/T	N/T	-	-	-	N/T	N/T	-	N/T	N/T	N/T	N/T	-	-	-	-
330	31/5/10	8/5	right femoral-poplit	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	+	-	-
331	31/5/10	26/5	swab-left ankle	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	-	-	+	+	+	-	-	-	-
332	31/5/10	9/5	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	+	-	+	+	+	-	-	-	-
333	31/5/10	8/5	right femoral-poplit	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
334	31/5/10	17/5	swab-high vaginal	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	-	-	-	-	-	-	-	+	-
335	31/5/10	25/5	urine-unknown meth	3	-	-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-
336	31/5/10	25/5	urine-unknown meth	3	+	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-
337	31/5/10	25/5	trapped sputum-muc	3	+	+	+	-	+	+	-	-	+	-	-	+	+	-	+	-	-
338	31/5/10	17/5	urine-midstream	2	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-
339	31/5/10	15/5	blood culture x1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
340	31/5/10	17/5	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
341	31/5/10	26/5	urine-midstream	2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
342	31/5/10	26/5	csu-m,c&s	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
343	31/5/10	11/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
344	31/5/10	23/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
345	31/5/10	19/5	urine-midstream	2	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
346	31/5/10	19/5	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
347	31/5/10	15/5	blood culture x1	2	-	-	+	+	-	+	+	-	+	-	-	+	-	-	-	-	-
348	31/5/10	25/5	urine-unknown meth	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
349	31/5/10	24/5	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
350	31/5/10	24/5	csu-m,c&s	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
351	31/5/10	24/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
352	31/5/10	25/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
353	31/5/10	25/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-
354	31/5/10	10/5	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
355	31/5/10	10/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-

Sample Data					Antibiotic Resistance Profile															Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
356	31/5/10	17/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
357	31/5/10	23/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
358	31/5/10	17/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
359	31/5/10	23/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
360	31/5/10	23/5	blood culture x1	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
361	31/5/10	24/5	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
362	31/5/10	24/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
363	31/5/10	25/5	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
364	31/5/10	25/5	swab-vaginal	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
365	31/5/10	25/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
366	31/5/10	17/5	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	-	-	-	-	-	-
367	31/5/10	24/5	sputum-mucopurulen	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
368	31/5/10	18/5	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	+	-
369	31/5/10	18/5	sputum-mucopurulen	2	+	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-
370	31/5/10	23/5	urine-midstream	2	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	+	-
371	31/5/10	18/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
372	31/5/10	12/5	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
373	31/5/10	12/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
374	31/5/10	12/5	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
375	31/5/10	10/5	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
376	31/5/10	18-5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
377	31/5/10	12/5	fluid-perinephric	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	+	-
378	31/5/10	19/5	urine-midstream	2	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-
379	31/5/10	19/5	urine-midstream	2	+	-	-	-	-	-	-	+	-	-	+	-	+	-	-	+	-
380	31/5/10	19/5	sputum-mucopurulen	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
381	31/5/10	19/5	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
382	31/5/10	19/5	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
383	31/5/10	17/5	urine-midstream	2	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-
384	31/5/10	11/5	urine-midstream	2	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
385	31/5/10	11/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
416	15/6/10	31/5	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	+	-	+	-	-	-	-
417	15/6/10	31/5	swab-left scrotum	2	+	-	+	-	+	+	-	+	+	-	+	+	+	-	+	-	-
418	15/6/10	31/5	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
419	15/6/10	1/6	urine-unknown meth	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
420	21/6/10	7/6	swab-left ankle la	3	N/T	+	N/T	N/T	+	-	N/T	N/T	N/T	-	+	N/T	N/T	-	-	-	-
421	21/6/10	9/6	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	+	-	-	-	-	-	-	-	-
422	21/6/10	9/6	ulcer right lower le	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	+	-	-	-	+	-	-	-	-
423	21/6/10	9/6	csu-m,c&s	1	-	-	+	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
424	21/6/10	7/6	urine-midstream	1	-	-	+	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
425	21/6/10	7/6	urine-midstream	1	-	-	+	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
426	21/6/10	10/6	c	2	+	+	+	+	-	N/T	+	+	N/T	+	+	N/T	N/T	+	-	+	-
427	21/6/10	5/6	swab-peritoneal fl	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-

Sample Data					Antibiotic Resistance Profile															Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
428	21/6/10	4/6	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
429	21/6/10	7/6	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
430	21/6/10	6/6	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
431	21/6/10	9/6	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
432	21/6/10	9/6	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
433	21/6/10	9/6	csu-m,c&s	2	+	-	+	-	+	+	-	+	+	-	+	+	+	-	+	-	-
434	21/6/10	9/6	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
435	21/6/10	7/6	swab-left ankle me	2	N/T	N/T	N/T	N/T	N/T	-	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
436	21/6/10	7/6	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	+	+	-	-	-	-
437	21/6/10	8/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	-	-	-	-	-	-
438	21/6/10	7/6	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
439	21/6/10	5/6	blood culture x1	2	-	-	+	-	-	+	-	+	+	-	+	-	-	-	-	-	-
440	21/6/10	9/6	urine-midstream	2	+	+	+	-	-	-	-	+	-	-	+	+	+	+	+	-	-
441	21/6/10	9/6	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	+	-
442	21/6/10	9/6	csu-m,c&s	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	+	-
443	21/6/10	8/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	-	+	-	-	+	-
444	21/6/10	8/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	-	+	-	-	+	-
445	21/6/10	9/6	urine-midstream	2	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-
446	28/6/10	18/6	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
447	28/6/10	18/6	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
448	28/6/10	18/6	urine-unknown meth	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
449	28/6/10	12/6	urine-midstream	1	N/T	N/T	N/T	N/T	-	N/T	N/T	N/T	N/T	-	+	N/T	N/T	-	-	+	-
450	28/6/10	16/6	urine-midstream	3	+	+	+	-	+	+	-	+	-	-	+	+	+	-	+	-	-
451	28/6/10	15/6	urine-midstream	3	+	-	+	+	-	+	+	+	-	-	+	-	-	-	-	+	-
452	28/6/10	13/6	culture-cord blood	2	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
453	28/6/10	18/6	urine-midstream	2	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
454	28/6/10	15/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
455	28/6/10	12/6	blood culture x1	2	-	-	+	-	-	+	-	N/T	N/T	-	+	N/T	N/T	-	-	+	-
456	28/6/10	16/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
457	28/6/10	15/6	fluid-knee	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
458	28/6/10	15/6	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
459	28/6/10	15/6	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
460	28/6/10	15/6	fluid-synovial flu	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
461	28/6/10	15/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
462	28/6/10	N/S	urine-unknown meth	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
463	28/6/10	16/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-
464	28/6/10	16/6	sputum-mucopurulen	2	+	+	+	+	+	-	+	+	-	+	+	+	+	-	-	-	-
465	28/6/10	15/6	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
466	28/6/10	14/6	sputum-mucopurulen	2	+	+	+	-	-	-	-	+	-	-	+	+	+	-	-	-	-
467	28/6/10	15/6	tissue-left knee b	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-

Sample Data					Antibiotic Resistance Profile														Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
468	28/6/10	N/S	urine-midstream	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
469	28/6/10	16/6	urine-unknown meth	2	-	+	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
470	28/6/10	16/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
471	28/6/10	16/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	-	+	-	-	+	-
472	28/6/10	12/6	blood culture x1	2	+	+	+	+	-	+	+	+	-	-	+	-	-	-	-	-	-
473	28/6/10	15/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
474	12/07/10	23/6	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-	-	-	-	-	+	-
475	12/07/10	22/6	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	-	-	-	-	+	-	-	-	-
476	12/07/10	28/6	m	3	-	-	+	+	-	N/T	+	+	+	-	-	-	-	-	-	-	-
477	12/07/10	28/6	swab-laparotomy wo	4	+	+	-	+	-	-	+	+	+	+	+	+	+	+	-	+	-
478	12/07/10	26/6	urine-midstream	4	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-
479	12/07/10	15/6	urine-midstream	2	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
480	12/07/10	15/6	urine-midstream	2	-	-	-	-	-	+	-	+	+	-	-	-	+	-	-	-	-
481	12/07/10	22/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
482	12/07/10	22/6	blood culture x1	2	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
483	12/07/10	21/6	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
484	12/07/10	21/6	urine-midstream	2	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
485	12/07/10	23/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	--	-
486	12/07/10	23/6	csu-m,c&s	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
487	12/07/10	21/6	csu-m,c&s	2	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
488	12/07/10	23/6	urine-midstream	2	-	-	+	-	-	-	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
489	12/07/10	21/6	urine-unknown meth	2	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-
490	12/07/10	21/6	urine-midstream	2	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-
491	12/07/10	23/6	urine-midstream	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
492	12/07/10	28/6	swab-ulcer	2	+	+	+	-	-	-	-	+	-	-	+	+	+	-	-	-	-
493	12/07/10	23/6	urine-midstream	2	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	+	-	-	-	-	-	-	-	-
494	12/07/10	29/6	urine-midstream	2	+	-	+	-	-	+	+	-	+	-	-	-	-	-	-	+	-
495	12/07/10	29/6	urine-midstream	2	+	-	-	-	-	+	-	+	+	-	-	-	-	-	-	+	-
496	12/07/10	20/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	-	+	-	-	-	-
497	12/07/10	14/6	blood culture x1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
498	12/07/10	22/6	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
499	12/07/10	22/6	urine-unknown meth	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-
500	12/07/10	29/6	urine-midstream	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
501	12/07/10	14/6	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
502	12/07/10	21/6	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
503	12/07/10	27/6	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
504	12/07/10	21/6	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
505	12/07/10	21/6	urine-unknown meth	2	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
506	12/07/10	22/6	fluid-ascitic	2	+	+	+	-	-	-	-	+	-	+	+	+	+	-	-	-	-
507	12/07/10	27/6	csu-m,c&s	2	+	-	-	+	-	+	+	+	+	-	+	-	+	-	-	+	-

Sample Data					Antibiotic Resistance Profiles														Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
508	12/07/10	23/6	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
509	12/07/10	27/6	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
510	19/07/10	4/7	urine-midstream	1	N/T	-	N/T	N/T	-	N/T	N/T	N/T	N/T	-	+	N/T	N/T	-	+	-	-
511	19/07/10	22/6	urine-midstream	1	+	-	+	+	-	-	+	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
512	19/07/10	11/7	blood culture x1	1	-	-	-	-	-	-	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
513	19/07/10	26/6	urine-midstream	1	N/T	+	N/T	N/T	-	N/T	N/T	N/T	N/T	+	+	N/T	N/T	-	-	+	-
514	19/07/10	21/6	trapped sputum-muc	1	+	-	+	-	-	-	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
515	19/07/10	23/6	urine-midstream	1	-	-	+	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
516	19/07/10	30/6	urine-midstream	1	+	-	+	+	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
517	19/07/10	6/7	urine-unknown meth	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
518	19/07/10	30/6	urine-midstream	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-
519	19/07/10	6/7	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
520	19/07/10	6/7	urine-unknown meth	2	-	+	+	-	-	+	-	+	+	+	+	+	+	-	-	-	-
521	19/07/10	4/7	urine	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
522	19/07/10	5/7	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	+	+	+	-	-	+	-
523	19/07/10	4/7	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	+	-	+	-	-	+	-
524	19/07/10	6/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-
525	19/07/10	2/7	swab-low vaginal	2	-	-	+	-	-	+	-	+	+	-	-	+	+	-	-	-	-
526	19/07/10	30/6	urine-midstream	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-
527	19/07/10	27/6	urine-catheter	2	N/T	N/T	N/T	N/T	-	N/T	N/T	N/T	N/T	-	+	N/T	N/T	-	-	+	-
528	19/07/10	30/6	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
529	19/07/10	5/7	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	-	+	-	-	-	-
530	19/07/10	5/7	csu-m,c&s	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	+	+	-
531	19/07/10	9/7	urine-unknown meth	2	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
532	19/07/10	6/7	urine-midstream	2	+	-	+	-	-	+	-	-	+	-	-	+	-	-	-	+	-
533	19/07/10	5/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
534	19/07/10	7/7	swab-right leg	2	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-
535	19/07/10	7/7	urine-midstream	2	-	-	+	-	-	-	-	+	+	-	+	+	+	-	-	-	-
536	19/07/10	7/7	urine-midstream	2	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
537	19/07/10	5/7	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	-	-	-	-	-	-
538	19/07/10	30/6	blood culture x1	2	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-
539	19/07/10	29/6	blood culture x1	2	+	-	-	-	-	+	-	+	+	-	-	-	+	-	-	+	-
540	19/07/10	29/6	iud	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
541	26/7/10	13/7	urine-midstream	1	+	-	+	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
542	26/7/10	9/7	csu-m,c&s	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	+	-	-	-	-	-	-	-	-
543	26/7/10	7/7	swab-left ankle me	1	N/T	+	N/T	N/T	+	N/T	N/T	N/T	N/T	-	+	N/T	N/T	-	-	+	-
544	26/7/10	9/7	ulcer-right lower le	1	-	-	-	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
545	26/7/10	9/7	urine-unknown meth	1	N/T	+	N/T	N/T	-	N/T	N/T	N/T	N/T	+	+	N/T	N/T	-	-	+	-
546	26/7/10	11/7	blood culture x1	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-	-	-	-	-	+	-
547	26/7/10	13/7	urine-unknown meth	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	+	+	-

Sample Data					Antibiotic Resistance Profiles															Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
548	26/7/10	13/7	swab-right leg	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
549	26/7/10	13/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-
550	26/7/10	13/7	swab-right leg	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-
551	26/7/10	13/7	csu-m,c&s	2	+	-	+	-	-	+	-	+	+	-	+	-	-	-	-	-	-
552	26/7/10	13/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
553	26/7/10	5/7	blood culture x1	2	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-
554	26/7/10	13/7	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-
555	26/7/10	12/7	swab-buttock rash	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
556	26/7/10	11/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
557	26/7/10	11/7	urine-midstream	2	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-
558	26/7/10	6/7	bronchial wash-lll	2	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
559	26/7/10	13/7	swab-right foot	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
560	26/7/10	13/7	urine-midstream	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
561	26/7/10	11/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-
562	26/7/10	7/7	urine-midstream	2	-	-	+	-	-	N/T	-	-	+	-	-	-	-	-	-	-	-
563	26/7/10	6/7	bronchial wash-lul	2	-	-	+	-	-	N/T	-	-	+	-	-	-	-	-	-	-	-
564	26/7/10	12/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-
565	26/7/10	5/7	blood culture x1	2	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
566	9/8/10	28/7	csu-m,c&s	4	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
567	9/8/10	26/7	urine-unknown meth	3	-	+	+	+	-	+	+	+	+	-	+	+	+	-	-	-	-
568	9/8/10	27/7	urine-midstream	3	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
569	9/8/10	19/7	urine-midstream	1	-	-	+	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	+	-
570	9/8/10	13/7	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	+	-	-	-	-	-	-	-	-
571	9/8/10	20/7	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
572	9/8/10	19/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
573	9/8/10	20/7	swab-right great t	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
574	9/8/10	20/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
575	9/8/10	20/7	urine	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
576	9/8/10	27/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-
577	9/8/10	19/7	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	-	+	-	-	-	-
578	9/8/10	19/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
579	9/8/10	18/7	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-
580	9/8/10	20/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
581	9/8/10	27/7	urine-midstream	2	-	-	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-
582	9/8/10	27/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
583	9/8/10	27/7	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
584	9/8/10	28/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
585	9/8/10	27/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
586	9/8/10	27/7	csu-m,c&s	2	+	-	+	+	-	+	+	-	+	-	-	+	-	-	-	+	-
587	9/8/10	27/7	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-

Sample Data					Antibiotic Resistance Profiles														Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
588	23/8/10	09/08	urine-midstream	1	-	-	+	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
589	23/8/10	09/08	urine-unknown meth	1	-	-	+	-	+	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
590	23/8/10	03/08	urine-midstream	3	+	-	+	-	-	+	-	+	+	-	+	+	+	-	-	+	-
591	23/8/10	11/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
592	23/8/10	10/08	urine-midstream	2	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+
593	23/8/10	11/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
594	23/8/10	11/08	urine-midstream	2	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
595	23/8/10	11/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
596	23/8/10	11/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
597	23/8/10	08/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
598	23/8/10	09/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
599	23/8/10	10/08	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	+	-
600	23/8/10	10/08	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	+	-
601	23/8/10	10/08	swab-left lower le	2	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-
602	23/8/10	10/08	sputum-mucoid	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
603	23/8/10	09/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-
604	23/8/10	10/08	bronchial washing	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
605	23/8/10	08/08	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	+	-	+	-	-	+	-
606	23/8/10	10/08	urine-midstream	2	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
607	23/8/10	11/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
608	23/8/10	10/08	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	+	-
609	23/8/10	10/08	urine-midstream	2	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-
610	23/8/10	10/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
611	23/8/10	10/08	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	+	-	-
612	23/8/10	09/08	m	2	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
613	23/8/10	10/08	urine-midstream	2	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-
614	23/8/10	09/08	csu-m,c&s	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
615	23/8/10	09/08	swab-nose cancer	2	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-
616	23/8/10	04/08	sputum-mucopurulen	2	+	+	+	+	-	+	+	-	+	+	-	+	+	-	-	-	-
617	30/08/10	16/08	swab-vaginal	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-	-	-	-	-	-	-
618	30/08/10	16/08	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	+	-	-	-	+	-	-	-	-
619	30/08/10	16/08	urine-midstream	1	-	-	+	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
620	30/08/10	09/09	urine-unknown meth	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	+	-	-	-	-	-	-	-	-
621	30/08/10	09/08	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	+	-	-	-	-	-	-	-	-
622	30/08/10	16/08	urine-midstream	5	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	+	-
623	30/08/10	09/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
624	30/08/10	16/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
625	30/08/10	18/08	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	+	-
626	30/08/10	18/08	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
627	30/08/10	18/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-

Sample Data					Antibiotic Resistance Profiles														Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
628	30/08/10	16/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
629	30/08/10	16/08	urine-midstream	2	+	-	+	-	-	+	-	+	+	-	+	-	-	-	-	+	-
630	30/08/10	16/08	urine-unknown meth	2	+	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-
631	30/08/10	17/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
632	30/08/10	18/08	blood culture x1	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
633	30/08/10	14/08	swab-perineal absc	2	+	+	-	-	-	-	-	+	+	-	+	+	+	-	-	+	-
634	30/08/10	17/08	swab-right groin	2	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	+	-
635	30/08/10	16/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
636	30/08/10	18/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
637	30/08/10	18/08	urine-midstream	2	+	-	+	-	-	+	+	+	+	-	-	-	+	-	-	+	-
638	30/08/10	17/08	csu-m,c&s	2	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-
639	30/08/10	17/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
640	30/08/10	14/08	csu-m,c&s	2	+	+	+	+	-	+	+	N/T	+	N/T	N/T	N/T	N/T	-	+	+	-
641	30/08/10	18/08	blood culture x1	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
642	30/08/10	17/08	urine-midstream	2	-	+	+	-	-	-	-	+	-	-	+	+	+	-	-	-	-
643	30/08/10	16/08	blood culture x1	2	+	+	+	+	+	+	+	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
644	30/08/10	14/08	csu-m,c&s	2	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	+	+	+	+	+	+	-	-	-
645	30/08/10	09/08	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
646	30/08/10	18/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
647	30/08/10	18/08	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-
648	30/08/10	18/08	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
649	30/08/10	16/08	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-
650	30/08/10	16/08	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-
651	30/08/10	17/08	csu-m,c&s	2	+	-	+	+	-	+	+	+	+	-	+	+	+	-	-	+	-
652	13/9/10	23/8	swab-right shin	1	N/T	N/T	N/T	-	+	N/T	N/T	N/T	-	N/T	N/T	N/T	N/T	-	-	-	-
653	13/9/10	5/9	urine-midstream	1	+	-	+	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
654	13/9/10	31/8	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	+	+	-	+	+	+	-	+	-
655	13/9/10	16/8	urine-midstream	1	-	-	+	-	-	+	-	N/T	N/T	N/T	N/T	N/T	N/T	-	-	-	-
656	13/9/10	16/8	urine-midstream	1	N/T	N/T	N/T	N/T	N/T	N/T	N/T	+	+	-	-	-	-	-	-	-	-
657	13/9/10	1/9	csu-m,c&s	4	+	+	-	-	-	+	-	+	-	-	-	+	+	+	-	-	+
658	13/9/10	4/9	blood culture x1	4	+	+	+	-	-	+	-	+	+	+	+	+	+	+	-	+	-
659	13/9/10	4/9	csu-m,c&s	4	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-
660	13/9/10	23/8	culture-cord blood	4	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-
661	13/9/10	24/8	urine-midstream	3	+	+	+	+	-	-	+	+	+	-	+	+	+	-	+	+	-
662	13/9/10	30/8	urine-midstream	3	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-
663	13/9/10	23/8	urine-midstream	3	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
664	13/9/10	21/8	csu-m,c&s	3	-	-	+	-	-	-	-	+	+	-	-	+	+	-	-	-	-
665	13/9/10	28/8	blood culture x2	3	-	-	-	+	-	-	+	+	+	-	+	-	+	-	-	-	-
666	13/9/10	25/8	sputum-mucoid	3	+	+	+	+	-	-	+	+	-	-	+	+	+	-	-	-	-
667	13/9/10	22/8	blood culture 1x	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-

Sample Data					Antibiotic Resistance Profiles														Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
668	13/9/10	25/8	urine-midstream	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
669	13/9/10	25/8	blood culture x1	2	-	-	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-
670	13/9/10	21/8	blood culture x1	2	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-
671	13/9/10	22/8	blood culture x1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
672	13/9/10	25/8	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-
673	13/9/10	22/8	urine-midstream	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
674	13/9/10	22/8	urine-midstream	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
675	13/9/10	22/8	csu-m,c&s	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
676	13/9/10	16/8	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
677	13/9/10	22/8	blood culture x1	2	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-
678	13/9/10	29/8	urine-catheter	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
679	13/9/10	30/8	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
680	13/9/10	23/8	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
681	13/9/10	23/8	csu-m,c&s	2	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+	-
682	13/9/10	25/8	fluid-abscess drai	2	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	-
683	13/9/10	25/8	swab-abscess	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
684	13/9/10	22/8	urine-midstream	2	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-
685	13/9/10	29/8	csu-m,c&s	2	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	+	-
686	13/9/10	23/8	csu-m,c&s	2	N/T	+	N/T	N/T	N/T	N/T	N/T	N/T	N/T	-	+	N/T	N/T	+	-	+	-
687	13/9/10	22/8	csu-m,c&s	2	-	-	+	-	-	-	-	+	-	-	-	+	+	+	-	-	-
688	13/9/10	21/8	sputum-mucopurulen	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-
689	13/9/10	30/8	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
690	13/9/10	23/8	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
691	13/9/10	30/8	csu-m,c&s	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
692	13/9/10	30/8	urine-midstream	2	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
693	13/9/10	25/8	urine-midstream	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
694	13/9/10	29/8	urine-midstream	2	+	-	-	+	-	-	+	+	-	-	-	-	-	-	-	+	-
695	13/9/10	23/8	urine-midstream	2	-	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-
696	13/9/10	29/8	csu-m,c&s	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-
697	13/9/10	16/8	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
698	13/9/10	30/8	urine-midstream	2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
699	13/9/10	30/8	urine-midstream	2	-	-	+	-	-	+	-	+	-	-	-	-	+	-	-	-	-
700	13/9/10	30/8	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
701	13/9/10	30/8	urine-midstream	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
702	13/9/10	30/8	csu-m,c&s	2	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-
703	13/9/10	05/9	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
704	13/9/10	05/9	urine-midstream	2	-	-	+	-	-	-	-	+	-	-	+	+	+	-	+	-	-
705	13/9/10	05/9	urine-midstream	2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
706	13/9/10	30/8	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
707	13/9/10	30/8	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Sample Data					Antibiotic Resistance Profiles															Integron	
Sample #	Date received	Date Sampled	Source	# of plates	W5	CTX5	F200	CIP2.5	IPM10	AK30	NOR10	AMP25	CN10	FEP10	AMC60	CL100	KZ30	ESBL	IBL	*IntI1*	*IntI2*
709	13/9/10	31/8	urine-unknown meth	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
710	13/9/10	4/9	sputum-mucopurulen	2	+	+	+	-	-	+	+	+	-	-	-	+	+	-	-	-	-
711	13/9/10	4/9	blood culture x1	2	+	-	+	-	-	+	-	+	+	-	-	-	+	-	-	+	-
712	13/9/10	5/9	urine-midstream	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
713	13/9/10	31/8	swab-abdominal wou	2	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	+	-
714	13/9/10	31/8	urine-midstream	2	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-
715	13/9/10	31/8	csu-m,c&s	2	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-
716	13/9/10	31/8	urine-midstream	2	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-
717	13/9/10	31/8	csu-m,c&s	2	-	-	+	-	-	+	-	-	-	-	+	-	-	-	+	-	-
718	13/9/10	31/8	blood culture x1	2	-	-	+	-	-	+	-	-	+	-	-	-	-	-	+	-	-

Appendix B: Experimental Sample Data. Including PCR screening results, gene cassettes found and Phylotype grouping. * Multiple cassette arrays were present, no sequence obtained.

Sample Number	Date Sampled	Source	HS915/ HS916	HS464/ HS463a	HS458/ HS459	MRG284/ MRG285	HS549/ HS550	Cassettes	phenotypic Resistance	Phylotype
1	2/2	csu-m,c&s	+	+	-	-	-	N/A	N/A	D
2	5/2	swab -low vaginal	+	+	-	-	-	N/A	N/A	B2
5	1/2	urine-midstream	+	+	+	-	+	*aadA1*, *dfrA1*	+	B2
7	5/2	urine-midstream	+	+	+	-	-	*dfrA7*	+	D
17	9/2	urine-midstream	+	+	+	-	+	*aadA5*, *dfrA17*	-	B2
19	2/2	urine-midstream	+	+	-	-	-	N/A	N/A	B2
22	9/2	urine-midstream	+	+	+	-	+	*aadA1*, *dfrA1*	-	B2
24	1/2	urine-midstream	+	+	-	-	-	N/A	N/A	B2
26	1/2	urine-midstream	+	+	+	-	+	*aadA5*, *dfrA17*	+	B2
35	6/2	swab-perineal absc	+	+	-	-	-	N/A	N/A	B1
38	8/2	Sputum-mucosalivar	+	+	-	-	+	N/A	N/A	B2
40	8/2	urine-midstream	+	-	+	-	+	*aadA5*, *dfrA17*	+	B2
41	8/2	csu-m,c&s	+	+	+	-	+	*dfrA1*, *aadA2*	+	B2
44	9/2	swab-left foot ulc	+	-	-	-	-	N/A	N/A	B2
45	9/2	blood culture x1	+	+	-	-	-	N/A	N/A	B2
46	9/2	csu-m,c&s	+	+	+	-	+	*aadA5*, *dfrA17*	+	B2
53	25/1	urine-unknown meth	+	+	+	-	+	*dfrA15*, *aadA1*	+	B2
55	29/1	urine-unknown meth	+	+	+	-	+	*aadA1*, *dfrA1*	-	B2
68	28/1	urine-midstream	+	-	+	-	+	*aadA5*, *dfrA17*	+	D
79	16/2	csu-m,c&s	+	+	-	-	-	N/A	N/A	B2
81	17/2	urine-midstream	+	+	+	-	+	*aadA1*, *dfrA1*	-	B2
93	23/2	urine-unknown meth	+	+	+	-	+	*aadA1*, *dfrA1*	-	B2
96	8/3	urine-midstream	+	+	+	-	+	*aadA1*, *dfrA1*	-	B2
103	2/3	urine-midstream	+	+	-	-	-	N/A	N/A	D
107	6/3	urine-unknown meth	+	+	+	-	+	*aadA5*, *dfrA17*	+	D
110	23/2	urine-midstream	+	+	-	-	-	N/A	N/A	B2
114	28/2	urine-midstream	+	+	-	-	-	N/A	N/A	B2
115	28/2	urine-midstream	+	+	-	-	-	N/A	N/A	B2
116	28/2	urine-midstream	+	+	-	-	-	N/A	N/A	B2
120	3/3	swab-left anterior	+	+	-	-	+	N/A	N/A	D
124	2/3	swab-spc site pus	+	+	+	-	+	*aadA1*, *dfrA1*	+	D
130	3/3	swab-right ankle	+	+	-	-	+	N/A	N/A	D

Sample Number	Date Sampled	Source	HS915/ HS916	HS464/ HS463a	HS458/ HS459	MRG284/ MRG285	HS549/ HS550	Cassettes	phenotypic Resistance	Phylotype
133	3/3	blood culture x1	+	+	+	-	+	*aadA5*, *dfrA17*	+	B2
136	7/3	csu-m,c&s	+	+	-	-	-	N/A	N/A	B2
146	9/3	urine-midstream	+	+	-	-	-	N/A	N/A	B2
147	9/3	urine-midstream	+	-	-	-	-	N/A	N/A	B2
179	22/3	swab-high vaginal	+	+	-	-	-	N/A	N/A	B2
182	22/3	urine-midstream	+	-	-	+	-	*aadA5*, *dfrA17*	+	B2
183	22/3	csu-m,c&s	+	+	+	-	+	*dfrA7*	+	A
186	19/3	urine-midstream	+	+	-	-	-	N/A	N/A	D
192	21/3	m	+	+	-	+	-	*aadA5*, *dfrA17*	+	D
197	24/3	urine-midstream	+	+	+	-	+	*aadA1*, *dfrA1*	+	B2
199	26/3	urine-midstream	+	-	-	+	-	*aadA5*, *dfrA17*	+	B2
213	29/3	urine-midstream	+	+	-	-	-	N/A	N/A	B2
216	4/4	urine-midstream	+	+	-	-	+	N/A	N/A	D
217	4/4	blood culture x1	+	+	-	-	+	N/A	N/A	D
219	30/3	swab-wound	+	+	-	-	+	N/A	N/A	B2
223	30/3	swab-right eye	+	+	+	-	+	*aadA1*, *dfrA1*	-	B2
227	5/4	csu-m,c&s	+	+	+	-	+	*aadA1*, *dfrA1*	*	D
236	28/4	urine-midstream	+	+	+	-	-	*	*	D
239	28/4	urine-midstream	+	+	-	-	+	N/A	N/A	D
243	28/4	urine-unknown meth	+	+	+	-	+	*dfrA5*	+	D
252	19/4	urine-midstream	+	+	+	-	+	*aadA1*, *dfrA1*	-	B2
257	19/4	IUD	+	+	+	-	+	*aadA1*, *dfrA1*	-	B2
259	13/4	urine-midstream	+	-	-	-	-	N/A	N/A	B2
266	21/4	urine-midstream	+	-	-	-	-	N/A	N/A	B2
270	20/4	urine-midstream	+	-	-	+	-	N/A	N/A	D
278	16/4	urine-midstream	+	-	-	+	-	*aadA5*, *dfrA17*	*	B2
286	17/4	urine-midstream	+	+	-	+	-	*aadA5*, *dfrA17*	*	D
287	18/4	urine-midstream	+	+	-	-	+	N/A	N/A	B2
291	28/4	urine-midstream	+	+	+	-	+	*	*	D
295	13/4	urine-midstream	+	+	-	-	+	N/A	N/A	D
310	2/5	csu-m,c&s	+	-	-	+	-	N/A	N/A	B2
313	4/5	urine-midstream	+	+	+	-	+	*aadA1*, *dfrA1*	-	B2
314	4/5	urine-midstream	+	+	+	-	+	*aadA1*, *dfrA1*	-	B2

Sample Number	Date Sampled	Source	HS915/ HS916	HS464/ HS463a	HS458/ HS459	MRG284/ MRG285	HS549/ HS550	Cassettes	phenotypic Resistance	Phylotype
317	3/5	csu-m,c&s	+	+	-	-	+	N/A	N/A	D
320	3/5	urine-midstream	+	+	+	-	+	*aadA1*, *dfrA1*	-	B2
322	1/5	urine-catheter	+	+	+	-	+	*aadA1*, *dfrA1*	+	D
324	2/5	csu-m,c&s	+	-	-	+	-	*aadA5*, *dfrA17*	+	B2
334	17/5	swab-high vaginal	+	+	-	-	-	N/A	N/A	D
341	26/5	urine-midstream	+	+	+	-	-	*aadA5*, *dfrA17*	+	D
368	18/5	urine-midstream	+	+	+	-	-	*aadA1*, *dfrA1*	-	D
370	23/5	urine-midstream	+	+	-	-	-	N/A	N/A	D
377	12/5	fluid-perinephric	+	+	+	-	-	*aadA5*, *dfrA17*	-	D
379	19/5	urine-midstream	+	+	-	-	-	N/A	N/A	D
383	17/5	urine-midstream	+	+	-	-	-	N/A	N/A	D
391	12/5	urine-midstream	+	+	-	-	-	N/A	N/A	D
392	1/6	swab-peritoneal fl	+	-	+	-	-	*aadA5*, *dfrA17*	+	D
393	31/5	urine-midstream	+	+	+	-	-	*aadA5*, *dfrA17*	N/T	D
395	1/6	csu-m,c&s	+	-	-	-	-	N/A	N/A	D
396	1/6	blood culture x1	+	-	-	-	-	N/A	N/A	D
398	1/6	urine-midstream	+	+	-	-	-	N/A	N/A	D
407	30/5	urine-midstream	+	+	-	-	-	N/A	N/A	D
408	30/5	csu-m,c&s	+	+	-	-	-	N/A	N/A	D
426	10/6	c	+	+	-	-	-	N/A	N/A	D
441	9/6	urine-midstream	+	+	-	-	-	N/A	N/A	D
442	9/6	csu-m,c&s	+	+	+	-	+	*aadA1*, *dfrA1*	-	D
443	8/6	urine-midstream	+	+	-	-	-	N/A	N/A	D
444	8/6	urine-midstream	+	+	-	-	-	N/A	N/A	D
446	18/6	urine-midstream	+	-	-	-	-	N/A	N/A	D
447	18/6	urine-midstream	+	-	+	-	-	*aadA5*, *dfrA17*	N/T	D
449	12/6	urine-midstream	+	+	-	-	-	N/A	N/T	D
451	15/6	urine-midstream	+	+	+	-	-	*aadA5*, *dfrA17*	+	D
455	12/6	blood culture x1	+	+	+	-	-	*aadA5*, *dfrA17*	-	D
471	16/6	urine-midstream	+	+	+	-	-	*aadA5*, *dfrA17*	-	D
474	23/6	urine-midstream	+	+	-	-	-	N/A	N/A	D
477	28/6	swab-laparotomy wo	+	+	-	-	+	N/A	N/A	D
478	26/6	urine-midstream	+	+	-	-	-	N/A	N/A	D
489	21/6	urine-unknown meth	+	+	+	-	-	*aadA5*, *dfrA17*	-	D

Sample Number	Date Sampled	Source	HS915/ HS916	HS464/ HS463a	HS458/ HS459	MRG284/ MRG285	HS549/ HS550	Cassettes	phenotypic Resistance	Phylotype
495	29/6	urine-midstream	+	-	-	-	-	N/A	N/A	D
507	27/6	csu-m,c&s	+	-	-	-	-	N/A	N/A	D
513	26/6	urine-midstream	+	+	-	-	-	N/A	N/A	D
515	23/6	urine-midstream	+	+	-	-	-	N/A	N/A	D
516	30/6	urine-midstream	+	-	-	-	-	N/A	N/A	D
518	30/6	urine-midstream	+	-	-	-	-	N/A	N/A	D
522	5/7	urine-midstream	+	+	+	-	+	*aadA1*, *dfrA1*	+	D
523	4/7	urine-midstream	+	+	-	-	-	N/A	N/A	D
524	6/7	urine-midstream	+	-	-	-	-	N/A	N/A	D
526	30/6	urine-midstream	+	-	+	-	+	*aadA5*, *dfrA17*	-	D
527	27/6	urine-catheter	+	-	-	-	-	N/A	N/A	D
528	30/6	urine-midstream	+	-	-	-	-	N/A	N/A	D
530	5/7	csu-m,c&s	+	-	-	-	-	N/A	N/A	D
531	9/7	urine-unknown meth	+	+	-	-	-	N/A	N/A	D
532	6/7	urine-midstream	+	-	-	-	-	N/A	N/A	D
534	7/7	swab-right leg	+	-	-	-	-	N/A	N/A	D
539	29/6	blood culture x1	+	+	-	-	-	N/A	N/A	D
543	7/7	swab-left ankle me	+	-	-	-	-	N/A	N/A	A
544	9/7	ulcer-right lower le	+	+	-	-	-	N/A	N/A	D
545	9/7	urine-unknown meth	+	+	-	-	-	N/A	N/A	D
546	11/7	blood culture x1	+	-	-	-	-	N/A	N/A	D
547	13/7	urine-unknown meth	+	-	-	-	-	N/A	N/A	D
549	13/7	urine-midstream	+	-	-	-	-	N/A	N/A	D
550	13/7	swab-right leg	+	+	-	-	-	N/A	N/A	D
553	5/7	blood culture x1	+	-	-	-	-	N/A	N/A	D
554	13/7	urine-unknown meth	+	-	-	-	-	N/A	N/A	D
557	11/7	urine-midstream	+	-	-	-	-	N/A	N/A	D
561	11/7	urine-midstream	+	-	-	-	-	N/A	N/A	D
564	12/7	urine-midstream	+	-	-	-	-	N/A	N/A	D
569	19/7	urine-midstream	+	+	-	-	-	N/A	-	D
576	27/7	urine-midstream	+	-	-	-	-	N/A	N/A	D
586	27/7	csu-m,c&s	+	+	+	-	+	*aadA1*, *dfrA1*	+	D
590	03/08	urine-midstream	+	+	-	+	-	N/A	N/A	D
599	10/08	urine-midstream	+	+	+	-	+	*dfrA7*	-	D

Sample Number	Date Sampled	Source	HS915/ HS916	HS464/ HS463a	HS458/ HS459	MRG284/ MRG285	HS549/ HS550	Cassettes	phenotypic Resistance	Phylotype
603	09/08	urine-midstream	+	+	+	-	+	*aadA1*, *dfrA1*	-	D
605	08/08	urine-midstream	+	+	-	+	-	N/A	N/A	B1
608	10/08	urine-midstream	+	+	+	-	+	*aadA1*, *dfrA1*	-	D
622	16/08	urine-midstream	+	-	+	-	+	*aadA1*, *dfrA1*	-	D
625	18/08	urine-midstream	+	+	+	-	+	*aadA1*, *dfrA1*	-	B2
629	16/08	urine-midstream	+	+	-	-	+	N/A	N/A	B2
633	14/08	swab-perineal absc	+	+	+	-	+	*aadA1*, *dfrA1*	+	D
634	17/08	swab-right groin	+	-	+	-	+	*aadA1*, *dfrA1*	+	B2
637	18/08	urine-midstream	+	-	+	-	+	*aadA1*, *dfrA1*	+	D
640	14/08	csu-m,c&s	+	+	+	-	-	*aadA1*, *dfrA1*	+	B2
651	17/08	csu-m,c&s	+	-	+	-	+	*aadA1*, *dfrA1*	+	B2
654	31/8	urine-midstream	+	-	+	-	+	*aadA1*, *dfrA1*	N/T	B2
658	4/9	blood culture x1	+	-	-	-	+	N/A	N/A	B2
661	24/8	urine-midstream	+	+	-	-	+	N/A	N/A	D
667	22/8	blood culture 1x	+	+	-	-	-	N/A	N/A	D
670	21/8	blood culture x1	+	+	-	-	-	N/A	N/A	D
672	25/8	urine-midstream	+	+	+	-	+	*aadA5*, *dfrA17*	-	D
677	22/8	blood culture x1	+	+	-	-	+	N/A	N/A	D
681	23/8	csu-m,c&s	+	+	-	-	+	N/A	N/A	D
682	25/8	fluid-abscess drai	+	+	+	-	+	*aadA1*, *dfrA1*	-	D
684	22/8	urine-midstream	+	+	-	-	-	N/A	N/A	D
685	29/8	csu-m,c&s	+	+	+	-	+	*aadA5*, *dfrA17*	-	D
686	23/8	csu-m,c&s	+	+	-	-	+	N/A	N/T	D
688	21/8	sputum-mucopurulen	+	+	+	-	+	*aadA1*, *dfrA1*	-	D
694	29/8	urine-midstream	+	-	+	-	+	*aadA1*, *dfrA1*	+	D
698	30/8	urine-midstream	+	+	-	-	+	N/A	N/A	D
705	05/9	urine-midstream	+	+	-	-	-	N/A	N/A	D
708	4/9	swab-lumbar drain	+	-	+	-	+	*aadA1*, *dfrA1*	N/T	D
711	4/9	blood culture x1	+	+	-	-	+	N/A	N/A	D
713	31/8	swab-abdominal wou	+	+	+	-	+	*aadA5*, *dfrA17*	-	D

Appendix C: Gel Images Corresponding to Molecular Methods Utilized in this Study

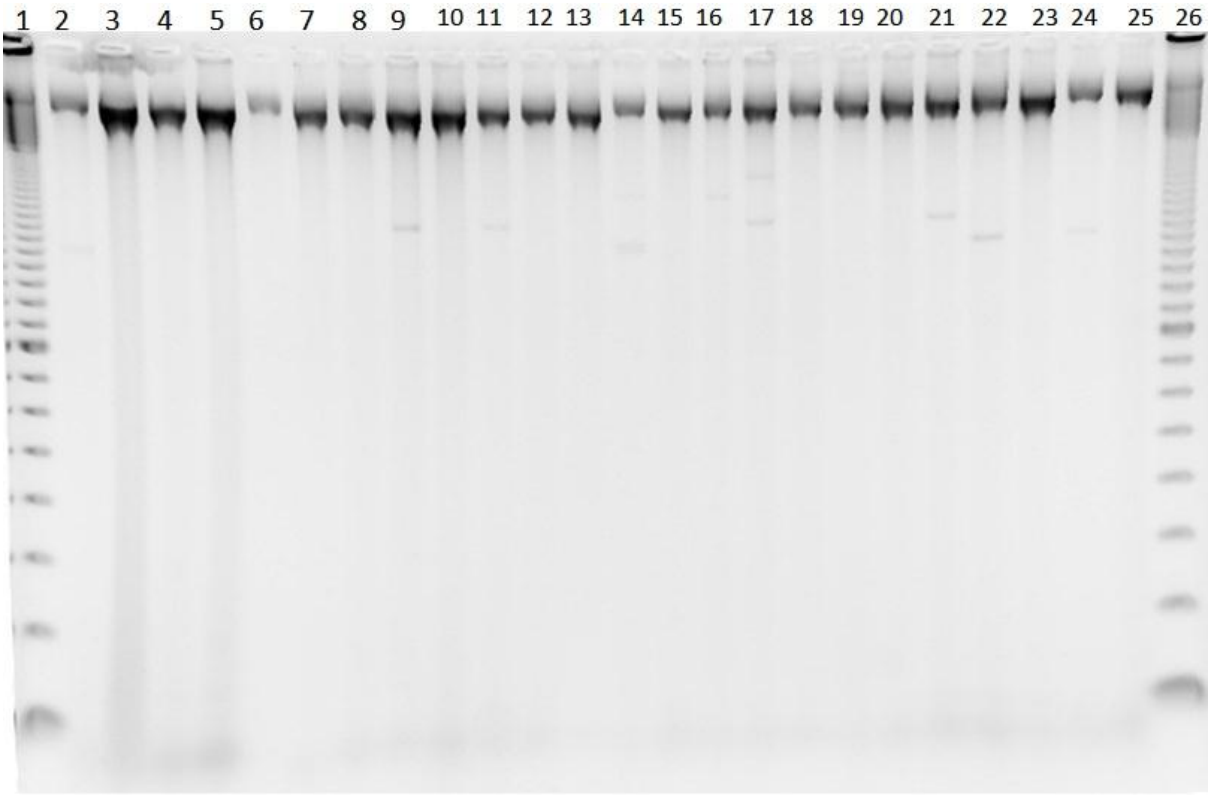

Figure 2.1.3 Representative Gel of Fast Prep DNA extracted from samples. 10µL of sample loaded into 0.8% agarose gel (Promega), post stained with Gel Red™ (Biotium). Lanes: 1: 100 base pair ladder (GE Healthcare); 2: Sample 1; 3: Sample 2a; 4: Sample 2b; 5: Sample 3a; 6: Sample 3b; 7: Sample 4a; 8: Sample 4b; 9: Sample5a; 10: Sample 5b; 11: Sample 6a; 12: Sample 6b; 13: Sample 7a; 14: Sample 7b; 15: Sample 8a; 16: Sample 8b; 17: Sample 9a; 18: Sample 9b; 19: Sample 9c; 20: Sample 10a; 21: Sample 10b; 22: Sample 11a; 23: Sample 11b; 24: Sample 12a; 25: Sample 12b; 26: 100 base pair ladder (GE Healthcare).

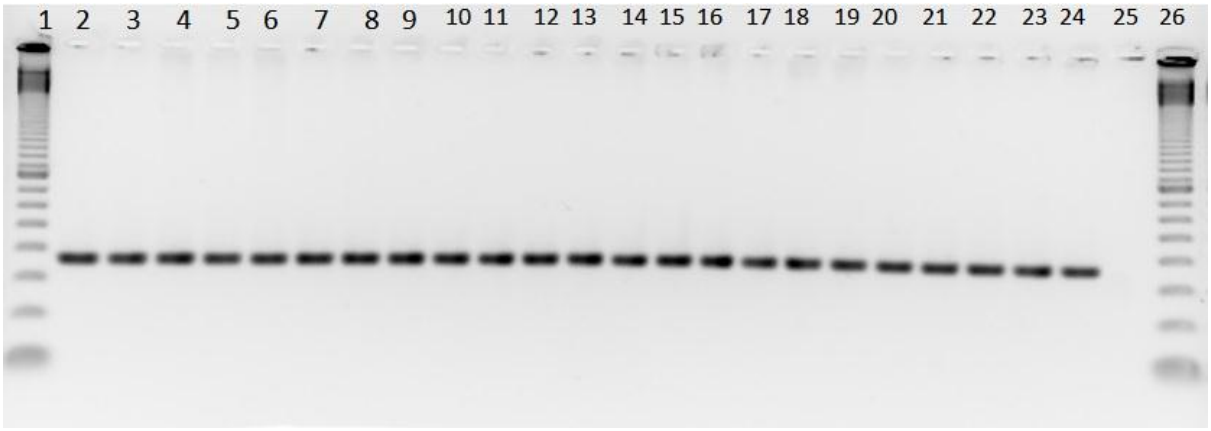

Figure 2.1.4a Representative Gel of Class 1 Integrase (*intI1*) PCR (HS915/HS916) on FastPrep DNA extracts from samples found to be class 1 integrase positive. 7µL of sample loaded into 2% agarose gel (Promega), post stained with Gel Red™ (Biotium). Lanes: 1: 100 base pair ladder (GE Healthcare); 2: Sample 1; 3: Sample 2a; 4: Sample 2b; 5: Sample 5a; 6: Sample 5b; 7: Sample 7a; 8: Sample 7b; 9: Sample19a; 10: Sample 19b; 11: Sample 22a; 12: Sample 22b; 13: Sample 24a; 14: Sample 24b; 15: Sample 26a; 16: Sample 26b; 17: Sample 35a; 18: Sample 35b; 19: Sample 38; 20: Sample 40a; 21: Sample 40b; 22: Sample 40c; 23: Sample 41a; 24: Positive control KC2; 25: Negative control; 26: 100 base pair ladder (GE Healthcare).

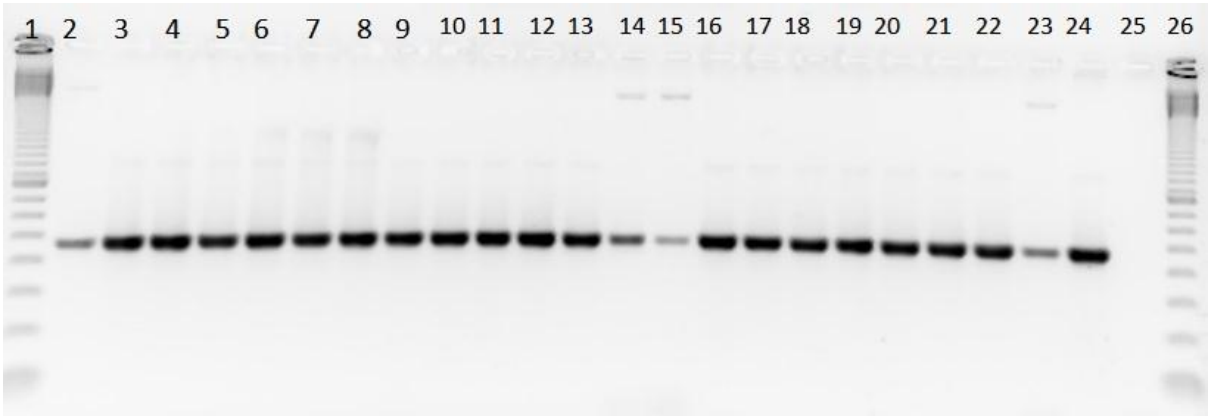

Figure 2.1.4b Representative Gel of Class 1 Integrase (*intI1*) PCR (HS464/HS463a) on FastPrep DNA extracts from samples found to be class 1 integrase positive. 7µL of sample loaded into 2% agarose gel (Promega), post stained with Gel Red™ (Biotium). Lanes: 1: 100 base pair ladder (GE Healthcare); 2: Sample 1; 3: Sample 2a; 4: Sample 2b; 5: Sample 5a; 6: Sample 5b; 7: Sample 7a; 8: Sample 7b; 9: Sample19a; 10: Sample 19b; 11: Sample 22a; 12: Sample 22b; 13: Sample 24a; 14: Sample 24b; 15: Sample 26a; 16: Sample 26b; 17: Sample 35a; 18: Sample 35b; 19: Sample 38; 20: Sample 40a; 21: Sample 40b; 22: Sample 40c; 23: Sample 41a; 24: Positive control KC2; 25: Negative control; 26: 100 base pair ladder (GE Healthcare).

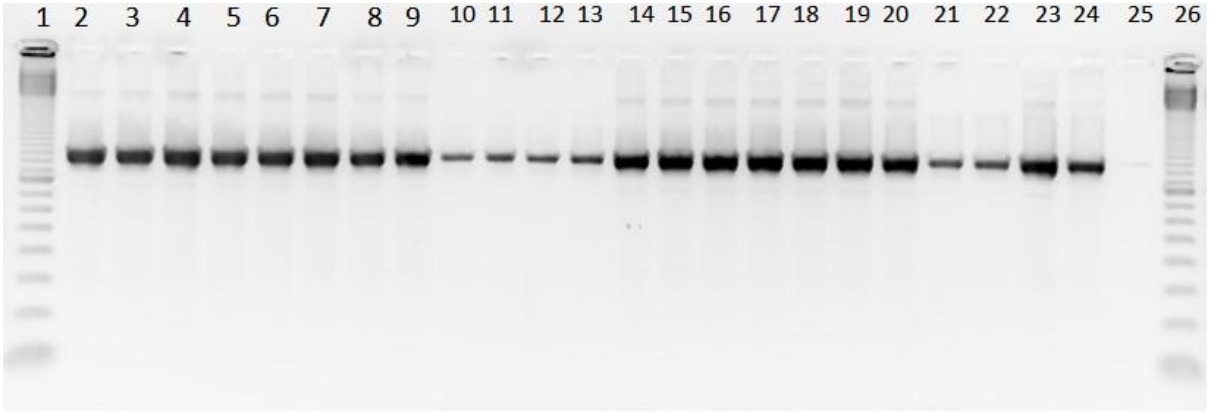

Figure 2.1.6 Representative Gel of *sul1* PCR HS549/HS550 PCR on FastPrep DNA extracts from samples found to be class 1 integrase gene positive. 7µL of sample loaded into 2% agarose gel (Promega), post stained with Gel Red™ (Biotium). Lanes: 1: 100 base pair ladder (GE Healthcare); 2: Sample 5a; 3: Sample 5b; 4: Sample 22a; 5: Sample 22b; 6: Sample 26a; 7: Sample 26b; 8: Sample 41a; 9: Sample 41b; 10: Sample 46a; 11: Sample 46b; 12: Sample 53a; 13: Sample 53b; 14: Sample 55a; 15: Sample 55b; 16: Sample 81a; 17: Sample 81b; 18: Sample 93a; 19: Sample 93b; 20: Sample 96a; 21: Sample 96b; 22: Sample 120a; 23: Sample 120b; 24: Positive control KC2; 25: Negative control; 26: 100 base pair ladder (GE Healthcare).

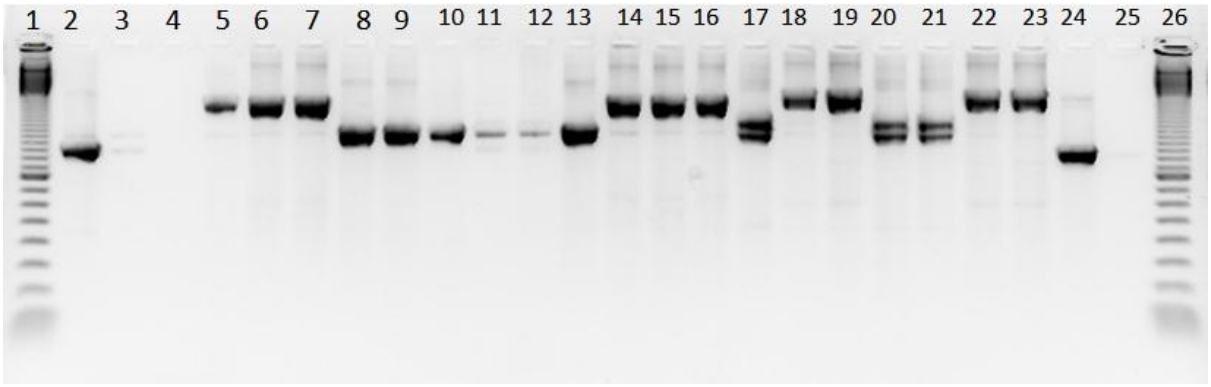

Figure 2.2.1a Representative Gel of Gene Cassette PCR (HS458/HS459) on FastPrep DNA extracts from samples found to be class 1 integrase gene positive. 7µL of sample loaded into 2% agarose gel (Promega), post stained with Gel Red™ (Biotium). Lanes: 1: 100 base pair ladder (GE Healthcare); 2: Sample 133; 3: Sample 2a; 4: Sample 2b; 5: Sample 17a; 6: Sample 17b; 7: Sample 17c; 8: Sample 40a; 9: Sample 40b; 10: Sample 40c; 11: Sample 2a; 12: Sample 2b; 13: Sample 227; 14: Sample 107a; 15: Sample 107b; 16: Sample 107c; 17: Sample 447; 18: Sample 96a; 19: Sample 96b; 20: Sample 132a; 21: Sample 132b; 22: Sample 192a; 23: Sample 192b; 24: Positive control KC2; 25: Negative control; 26: 100 base pair ladder (GE Healthcare).

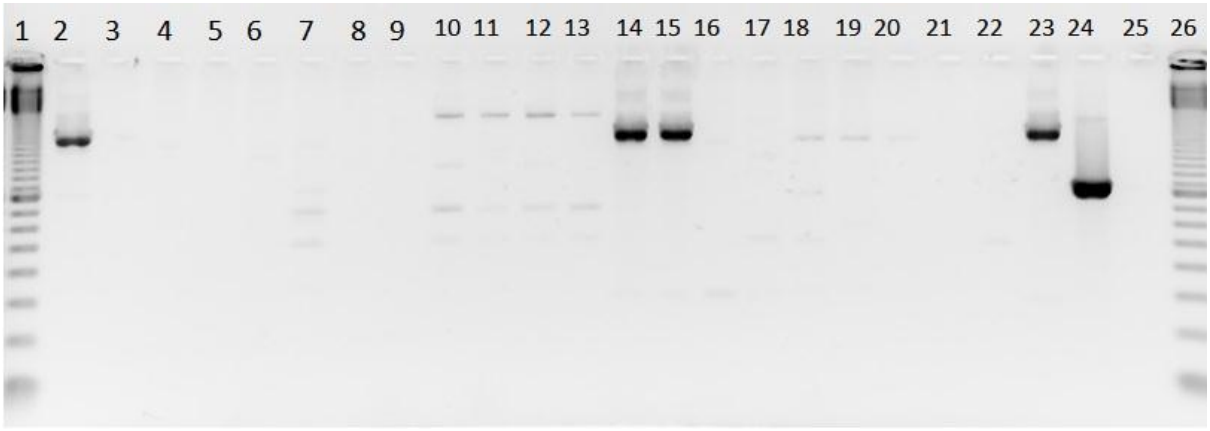

Figure 2.2.1b Representative Gel of Gene Cassette PCR (MRG284/MRG285) on FastPrep DNA extracts from samples found to be class 1 integrase gene positive. 7µL of sample loaded into 2% agarose gel (Promega), post stained with Gel Red™ (Biotium). Lanes: 1: 100 base pair ladder (GE Healthcare); 2: Sample 192a; 3: Sample 2a; 4: Sample 2b; 5: Sample 5a; 6: Sample 5b; 7: Sample 1; 8: Sample 7a; 9: Sample 7b; 10: Sample 68a; 11: Sample 68b; 12: Sample 68c; 13: Sample 68d; 14: Sample 199a; 15: Sample 199b; 16: Sample 19a; 17: Sample 19b; 18: Sample 22a; 19: Sample 22b; 20: Sample 40a; 21: Sample 40b; 22: Sample 40c; 23: Sample 192b; 24: Positive control B4CC2; 25: Negative control; 26: 100 base pair ladder (GE Healthcare).

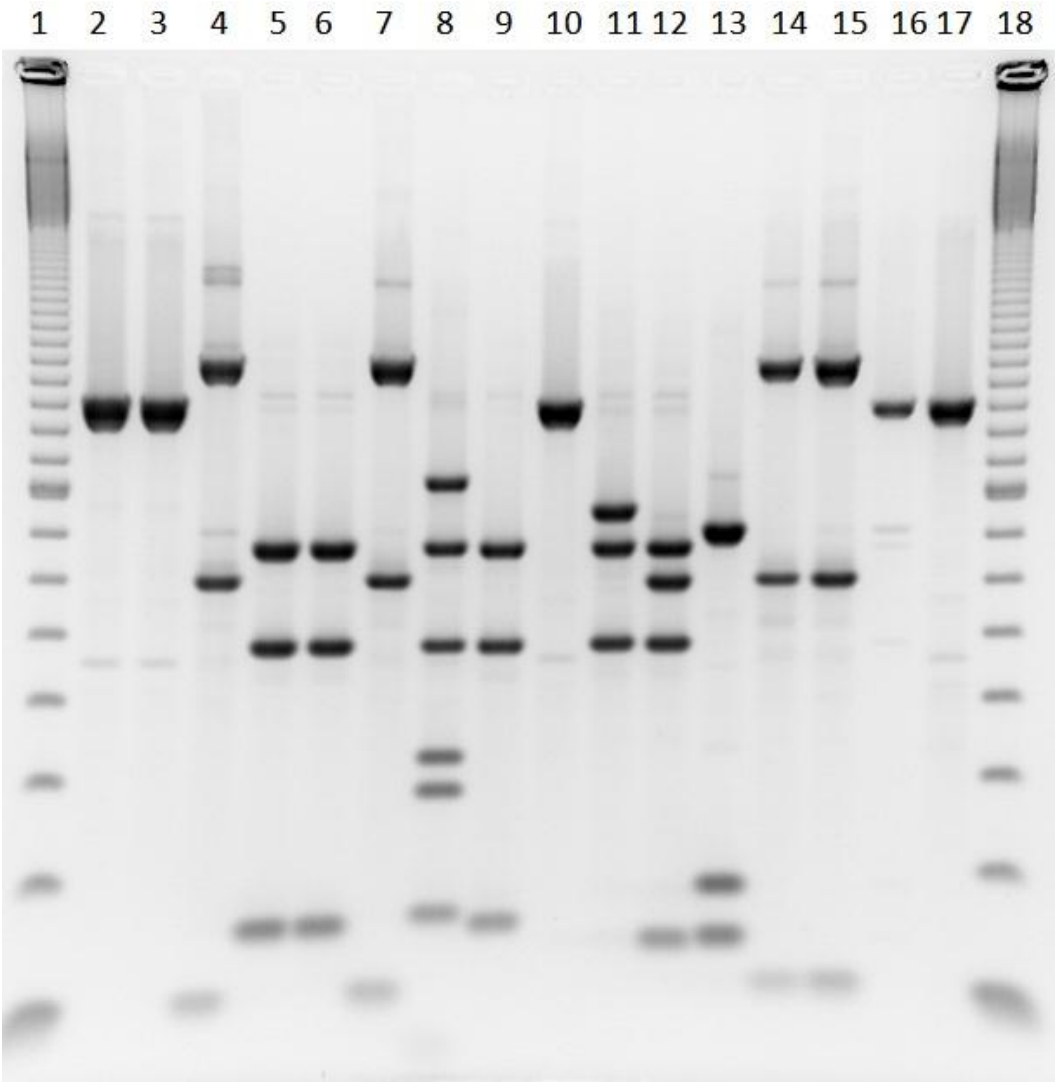

Figure 2.2.2 RsaI Enzyme digests of HS458/HS459 Gene cassette PCR. 25µL of sample loaded into 2% agarose gel (Promega), post stained with Gel Red™ (Biotium). Lanes: 1: 100 base pair ladder (GE Healthcare); 2: Sample 183; 3: Sample 599; 4: Sample 17; 5: Sample 5; 6: Sample 322; 7: Sample 658; 8: Sample 291; 9: Sample 672; 10: Sample 713; 11: Sample 53; 12: Sample 41; 13: Sample 243; 14: Sample 708; 15: Sample 310; 16: Sample 236; 17: Sample 586; 18: 100 base pair ladder (GE Healthcare).

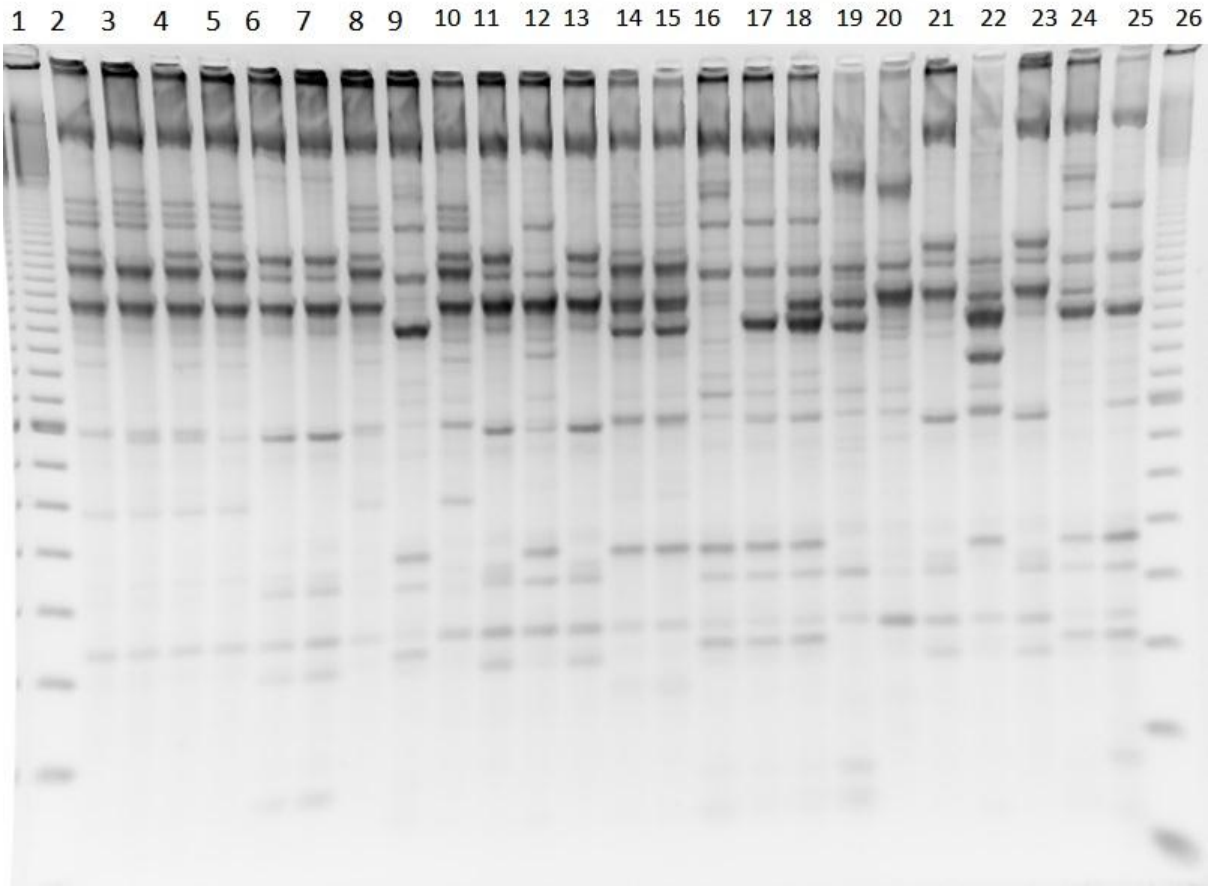

Figure 2.2.5 Representative Gel of ERIC PCR on FastPrep DNA extracts from samples found to be class 1 integrase gene positive. 10µL of sample loaded into 2% agarose gel (Promega), post stained with Gel Red™ (Biotium). Lanes: 1: 100 base pair ladder (GE Healthcare); 2: Sample 1a; 3: Sample 2a; 4: Sample 5a; 5: Sample 7a; 6: Sample 17a; 7: Sample 19a; 8: Sample 22a; 9: Sample 24a; 10: Sample 26a; 11: Sample 35a; 12: Sample 38a; 13: Sample 40a; 14: Sample 41a; 15: Sample 44a; 16: Sample 45a; 17: Sample 46a; 18: Sample 53a; 19: Sample 55a; 20: Sample 68a; 21: Sample 79a; 22: Sample 81a; 23: Sample 93a; 24: Sample 96a; 25: Sample 103a; 26: 100 base pair ladder (GE Healthcare)

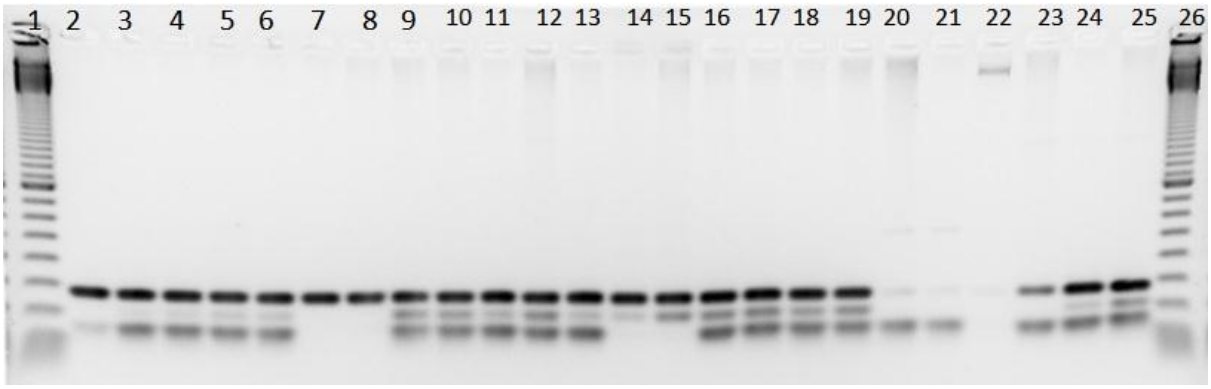

Figure 2.2.4 Representative Gel of Phylogenetic Grouping PCR on FastPrep DNA extracts from samples found to be class 1 integrase gene positive. 7µL of sample loaded into 2% agarose gel (Promega), post stained with Gel Red™ (Biotium). Lanes: 1: 100 base pair ladder (GE Healthcare); 2: Sample 1; 3: Sample 2a; 4: Sample 2b; 5: Sample 5a; 6: Sample 5b; 7: Sample 7a; 8: Sample 7b; 9: Sample19a; 10: Sample 19b; 11: Sample 22a; 12: Sample 22b; 13: Sample 24a; 14: Sample 24b; 15: Sample 26a; 16: Sample 26b; 17: Sample 35a; 18: Sample 35b; 19: Sample 38; 20: Sample 40a; 21: Sample 40b; 22: Sample 40c; 23: Sample 41a; 24:; 25:; 26: 100 base pair ladder (GE Healthcare).

Appendix D: Phylotype, Clonal Line & Cassette Array Data

Date	Sample #	Phylotype	Array	Eric Profile
22/3	183	A	*dfrA7*	A-1
7/7	543	A	Uncharacterizable	A-2
6/2	35	B1	Uncharacterizable	B1-1
08/08	605	B1	Uncharacterizable	B1-2
5/2	2	B2	Uncharacterizable	B2-1
1/2	5	B2	*aadA1*, *dfrA1*	B2-2
22/3	182	B2	*aadA5*, *dfrA17*	B2-3
16/4	278	B2	*aadA5*, *dfrA17*	B2-3
9/2	22	B2	*aadA1*, *dfrA1*	B2-4
3/3	133	B2	*aadA1*, *dfrA1*	B2-4
9/3	146	B2	*aadA1*, *dfrA1*	B2-4
1/2	24	B2	Uncharacterizable	B2-5
16/2	79	B2	Uncharacterizable	B2-5
28/2	114	B2	Uncharacterizable	B2-5
28/2	115	B2	Uncharacterizable	B2-5
3/3	132	B2	Uncharacterizable	B2-5
7/3	136	B2	*aadA1*, *dfrA1*	B2-6
8/2	38	B2	Uncharacterizable	B2-7
8/2	40	B2	*aadA5*, *dfrA17*	B2-8
8/2	41	B2	*dfrA1*, *aadA2*	B2-9
9/2	44	B2	Uncharacterizable	B2-10
17/2	81	B2	*aadA1*, *dfrA1*	B2-11
8/3	96	B2	*aadA1*, *dfrA1*	B2-11
23/2	110	B2	Uncharacterizable	B2-12
9/3	147	B2	Uncharacterizable	B2-13
22/3	179	B2	Uncharacterizable	B2-13
24/3	197	B2	*aadA1*, *dfrA1*	B2-14
30/3	223	B2	*aadA1*, *dfrA1*	B2-15
19/4	252	B2	*aadA1*, *dfrA1*	B2-16
19/4	257	B2	*aadA1*, *dfrA1*	B2-17
18/4	287	B2	Uncharacterizable	B2-18
2/5	310	B2	Uncharacterizable	B2-19
4/5	313	B2	*aadA1*, *dfrA1*	B2-20
4/5	314	B2	*aadA1*, *dfrA1*	B2-21
3/5	320	B2	*aadA1*, *dfrA1*	B2-22
18/08	625	B2	*aadA1*, *dfrA1*	B2-23
16/08	629	B2	Uncharacterizable	B2-24
17/08	634	B2	*aadA1*, *dfrA1*	B2-25
14/08	640	B2	*aadA1*, *dfrA1*	B2-26
17/08	651	B2	*aadA1*, *dfrA1*	B2-27
31/8	654	B2	*aadA1*, *dfrA1*	B2-28
30/3	219	B2	Uncharacterizable	B2-29
13/4	259	B2	Uncharacterizable	B2-30
21/4	266	B2	Uncharacterizable	B2-31
29/3	213	B2	Uncharacterizable	B2-32
2/5	324	B2	*aadA5*, *dfrA17*	B2-33
4/9	658	B2	Uncharacterizable	B2-34

Date	Sample #	Phylotype	Array	Eric Profile
23/2	93	B2	*aadA1*, *dfrA1*	B2-35
9/2	45	B2	Uncharacterizable	B2-36
25/1	53	B2	*dfrA1*5, *aadA1*	B2-37
28/2	116	B2	Uncharacterizable	B2-38
26/3	199	B2	*aadA5*, *dfrA17*	B2-39
9/2	46	B2	*aadA5*, *dfrA17*	B2-40
1/2	26	B2	*aadA5*, *dfrA17*	B2-41
29/1	55	B2	*aadA1*, *dfrA1*	B2-42
9/2	17	B2	*aadA5*, *dfrA17*	B2-43
2/2	1	D	Uncharacterizable	D-1
28/1	68	D	*aadA5*, *dfrA17*	D-2
6/3	107	D	*aadA5*, *dfrA17*	D-2
2/3	103	D	Uncharacterizable	D-3
03/08	590	D	Uncharacterizable	D-3
21/8	670	D	Uncharacterizable	D-3
3/3	120	D	Uncharacterizable	D-4
4/4	216	D	Uncharacterizable	D-4
6/7	524	D	Uncharacterizable	D-4
05/9	705	D	Uncharacterizable	D-4
3/3	130	D	Uncharacterizable	D-5
4/4	217	D	Uncharacterizable	D-5
19/3	186	D	Uncharacterizable	D-6
21/3	192	D	*aadA5*, *dfrA17*	D-7
26/5	341	D	*aadA5*, *dfrA17*	D-7
5/4	227	D	*aadA1*, *dfrA1*	D-8
28/4	236	D	Multiple arrays-no sequence	D-9
28/4	239	D	Uncharacterizable	D-10
5/7	530	D	Uncharacterizable	D-10
20/4	270	D	Uncharacterizable	D-11
17/4	286	D	*aadA5*, *dfrA17*	D-12
28/4	291	D	Multiple arrays-no sequence	D-13
13/4	295	D	Uncharacterizable	D-14
3/5	317	D	Uncharacterizable	D-15
23/5	370	D	Uncharacterizable	D-15
1/6	396	D	Uncharacterizable	D-15
8/6	444	D	Uncharacterizable	D-15
18/6	446	D	Uncharacterizable	D-15
1/5	322	D	*aadA1*, *dfrA1*	D-16
17/5	334	D	Uncharacterizable	D-17
18/5	368	D	*aadA1*, *dfrA1*	D-18
19/5	379	D	Uncharacterizable	D-19
17/5	383	D	Uncharacterizable	D-20
12/5	391	D	Uncharacterizable	D-21
1/6	392	D	*aadA5*, *dfrA17*	D-22
1/6	395	D	Uncharacterizable	D-23

Date	Sample #	Phylotype	Array	Eric Profile
6/7	532	D	Uncharacterizable	D-24
30/5	408	D	Uncharacterizable	D-25
26/6	478	D	Uncharacterizable	D-25
9/6	441	D	Uncharacterizable	D-26
8/6	443	D	Uncharacterizable	D-27
12/6	449	D	*aadA1*, *dfrA1*	D-28
16/6	471	D	*dfrA7*	D-29
23/6	474	D	Uncharacterizable	D-30
23/6	515	D	Uncharacterizable	D-30
27/7	576	D	Uncharacterizable	D-30
28/6	477	D	Uncharacterizable	D-31
29/6	495	D	Uncharacterizable	D-31
26/6	513	D	Uncharacterizable	D-31
30/6	516	D	Uncharacterizable	D-31
7/7	534	D	Uncharacterizable	D-31
11/7	561	D	Uncharacterizable	D-31
22/8	684	D	Uncharacterizable	D-31
30/8	698	D	Uncharacterizable	D-31
29/6	494	D	Uncharacterizable	D-32
27/6	507	D	Uncharacterizable	D-33
30/6	518	D	Uncharacterizable	D-34
4/7	523	D	Uncharacterizable	D-34
30/6	528	D	Uncharacterizable	D-34
27/6	527	D	Uncharacterizable	D-35
9/7	544	D	Uncharacterizable	D-35
11/7	546	D	Uncharacterizable	D-36
13/7	547	D	Uncharacterizable	D-37
11/7	557	D	Uncharacterizable	D-37
13/7	549	D	Uncharacterizable	D-38
27/7	586	D	*aadA1*, *dfrA1*	D-39
10/08	599	D	*dfrA7*	D-40
10/08	608	D	*aadA1*, *dfrA1*	D-41
18/08	637	D	*aadA1*, *dfrA1*	D-42
22/8	677	D	Uncharacterizable	D-43
23/8	681	D	Uncharacterizable	D-44
29/8	685	D	*dfrA7*	D-45
23/8	686	D	*aadA1*, *dfrA1*	D-46
12/5	377	D	*dfrA7*	D-47
9/6	442	D	*aadA1*, *dfrA1*	D-48
2/3	124	D	*aadA1*, *dfrA1*	D-49
13/7	550	D	Uncharacterizable	D-50
21/6	489	D	*dfrA7*	D-51
30/6	526	D	*dfrA7*	D-51
14/08	633	D	*aadA1*, *dfrA1*	D-52
9/7	531	D	Uncharacterizable	D-53
25/8	672	D	*dfrA7*	D-54
25/8	682	D	*aadA1*, *dfrA1*	D-55

Date	Sample #	Phylotype	Array	Eric Profile
5/7	522	D	*aadA1*, *dfrA1*	D-56
09/08	603	D	*aadA1*, *dfrA1*	D-56
21/8	688	D	*aadA1*, *dfrA1*	D-57
4/9	711	D	Uncharacterizable	D-58
1/6	398	D	Uncharacterizable	D-59
9/7	545	D	Uncharacterizable	D-59
16/08	622	D	*aadA1*, *dfrA1*	D-60
28/4	243	D	*dfrA5*	D-61
31/5	393	D	*aadA5*, *dfrA17*	D-62
15/6	451	D	*aadA5*, *dfrA17*	D-63
29/6	539	D	Uncharacterizable	D-64
5/2	7	D	*dfrA7*	D-65
19/7	569	D	*dfrA7*	D-65
10/6	426	D	Uncharacterizable	D-66
5/7	553	D	Uncharacterizable	D-66
13/7	554	D	Uncharacterizable	D-66
12/7	564	D	Uncharacterizable	D-66
10/08	600	D	Uncharacterizable	D-66
18/6	447	D	*aadA5*, *dfrA17*	D-67
12/6	455	D	*dfrA7*	D-67
24/8	661	D	Uncharacterizable	D-67
22/8	667	D	Uncharacterizable	D-68
29/8	694	D	*aadA1*, *dfrA1*	D-69
31/8	713	D	*aadA5*, *dfrA17*	D-70