The Comparative Risk of Antimicrobial Resistance Transfer from Pig, Poultry and Bovine Manure to Grassland

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Declaration of Authorship

This thesis has not been submitted in whole or in part to this or any other university for any degree and is original work of the author except for the whole genome sequence assembly and processing of these sequences through ABRicate, PointFinder, MLST and Platon, conducted by Dr. Robert Leigh.

Signed: _____

Date: _____

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Abbreviations

AMR	Antimicrobial Resistance
ARB	Antimicrobial Resistant Bacteria
ARG	Antibiotic Resistance Gene
AST	Antibiotic Susceptibility Testing
BM	Before Manure
Ca	Calcium
CDT	Combination Disk Test
CM	Cow manure
Cu	Copper
DM	Dry Matter
EDTA	Ethylenediaminetetraacetic acid
EMB	Eosin Methylene Blue
EPEC	Enteropathogenic *E. coli*
ESBL	Extended Spectrum β-lactamase
ETEC	Enterotoxigenic *E. coli*
EU	European Union
FSA	Food Safety Authority
GLASS	Global Antimicrobial Resistance Surveillance Systems
GLASS–AMC	Global Antimicrobial Resistance Surveillance Systems - Antibiotic Consumption
HGT	Horizontal Gene Transfer
HT-qPCR	High Throughput Quantitative Polymerase Chain Reaction
LAM	Leeds Acinetobacter Agar
LB	Luria- Bertani
LPS	Lipopolysaccharide
L-Ara4N	4-amino-4-deoxy-L-arabinose
MBL	Metallo - β-lactamase
MDR	Multidrug resistance
MGE	Mobile Genetic Element
MH	Mueller Hinton
MH^{2+}	Muller Hinton Cation Adjusted
MIC	Minimum Inhibitory Concentration

MLSB	Macrolide-lincosamide-streptogramin B
Mn	Manganese
MS	Mass spectrometry
Na	Sodium
NDM	New Delhi Metallo-β-lactamase
NMDS	Mon-metric multidimensional scaling
NTC	No Template Control
OM	Organic Matter
OMP	Outer Membrane Protein
OTU	Operational Taxonomic Unit
PBS	Phosphate Buffered Saline
PBP	Penicillin Binding Protein
PCR	Polymerase Chain Reaction
PEtN	Phosphoethanolamine
PIA	*Pseudomonas* Isolation Agar
PM	Chicken Manure
QRDR	Quinolone Resistance Determining Regions
RND	Resistance-Nodulation – Division
SCA	Simmons Citrate Agar
SM	Pig Manure
SNP	Single Nucleotide Polymorphism
SOP	Standard Operating Procedure
ST	Sequence Type
WGS	Whole Genome Sequencing
WHO	World Health Organisation
Zn	Zinc
16S-RMTases	16S rRNA methyltransferases

Abstract

Antimicrobial resistance (AMR) is a multifactorial issue involving an intertwining relationship between animals, humans and the environment. Therefore, it is critical to fully understand all potential routes of AMR transmission. Manure landspreading introduces bacteria, antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) into the environment as well as altering the indigenous resistome and microbiome. Grassland consists of ~70% of global agricultural land and is a vital source of food for livestock. The phyllosphere of plants is an under-researched area regarding the impact of agricultural management practices. Therefore, the grass phyllosphere is a possible source of AMR transmission to livestock, which may enter the food chain. Despite the important role grassland plays in food security, the temporal impact of manure application on its resistome and microbiome is unknown. Additionally, the comparative risk associated with different manure sources is unclear.

This thesis aimed to compare the temporal and contrasting impact pig, cow and chicken manure had on grassland microbiomes and resistomes using 16S rRNA amplicon sequencing and high-throughput qPCR (HT-qPCR). Additionally, through culture-dependent approaches the antimicrobial resistance profiles of the WHO priority pathogens *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolated from the manures and grassland were assessed and the molecular mechanisms of their resistance were investigated using PCR and whole genome sequencing (WGS).

The manures, soil and the grass phyllosphere, both pre and post manuring, contained a diverse range of ARGs, MGEs and opportunistic pathogens, including the priority pathogens *E. coli, K. pneumoniae* and *A. baumanii*. Additionally, manure application resulted in an increased diversity of ARGs and MGEs being detected in grass and soil. In

Ireland, current agricultural practices involve returning animals to land six weeks after splash-plate manure spreading. In this study, manure spreading altered the microbiome and resistome of grassland, however temporal analysis aligned with this current Irish agricultural practice, indicating that current guidelines are sufficient to reduce the spread of AMR to livestock. Pig manure was associated with the greatest change in the microbiome and resistome however overall, temporal patterns of manure types were similar, indicating that time had a greater impact than manure type. Overall, these results demonstrate the role of the grass phyllosphere as a novel reservoir of AMR and that current agricultural practices are sufficient to mitigate AMR dissemination.

CHAPTER 1

INTRODUCTION

Chapter 1. Introduction

1.1 The Origins and Dissemination of Antibiotic resistance (AMR)

The discovery of antibiotics was one of the greatest and transformative advancements in medicine due to their ability to treat infectious diseases (Adedeji, 2016). Since the revolutionary discovery of penicillin in 1929 (Fleming, 1929) and the sulphonamides in 1935 (Kirchhelle, 2018), many other classes of antibiotics have been discovered (Aminov, 2010). During the "Golden Age" of antibiotic discovery between 1950 and 1960, approximately 50% of the antibiotics used today were discovered (Rosenblatt-Farrell, 2009). However, since 1986 there has been no novel class of antibiotics discovered (Durand, Raoult and Dubourg, 2019). Despite the pre-emptive warning Alexander Fleming gave in 1945 that the misuse of antibiotics could lead to the selection of resistant bacteria, resistance has developed and antibiotic resistant infections have now become a global occurrence (Rosenblatt-Farrell, 2009; Chokshi *et al.*, 2019)**.** In 2017, the World Health Organisation (WHO) published a list of priority pathogens for which research and development of new antibiotics is greatly needed due to the emergence of antibiotic resistance in these pathogens (WHO, 2017). Of critical importance is the emergence of carbapenemase producing *Acinetobacter baumanii* and *Pseudomonas aeruginosa* and carbapenemase producing and extended spectrum β-lactamase (ESBL) producing *Enterobacterales*, demonstrating the threatening emergence of a post-antibiotic era.

Antibiotics are secondary metabolites, naturally produced by environmental bacteria and fungi as a method to compete in their dynamic and competitive environments (José L Martínez, 2012). For example, the soil commensal bacterial family, the *Actinomycetes*, produces many different clinically important antibiotics such as tetracycline and streptomycin (Clardy, Fischbach and Currie, 2009). Bacteria possess many different mechanisms to defend themselves against antibiotics, which can be categorised into two

main classes: Intrinsic resistance and acquired resistance. Intrinsic mechanisms of resistance are chromosomally encoded and confer a natural resistance to an antibiotic. Intrinsic resistance mechanisms include nonspecific efflux pumps or reduced permeability of the outer membrane by alteration in outer membrane proteins (OMPs) (Reygaert, 2018). An example of a soil bacterial species with intrinsic resistance to multiple antibiotics is *Stenotrophomonas maltophilia*. *Stenotrophomonas maltophilia* are environmental bacteria which are also opportunistic pathogens of immunocompromised patients. Concerningly, they are intrinsically resistant to aminoglycosides, β-lactams, carbapenems and quinolones by harbouring chromosomal efflux pumps (transport proteins involved in the movement of toxic substances out of cells e.g.: Resistance-Nodulation-Division (RND) family multidrug resistance (MDR) efflux pumps and antibiotic modifying enzymes (e.g.: β-lactamases L1 and L2) (Reygaert, 2018; Gil-Gil, Martínez and Blanco, 2020). This greatly limits the treatment options for *S. maltophilia* infections (Gil-Gil, Martínez and Blanco, 2020). Acquired resistance on the other hand, is when a bacterium acquires genetic material, such as a plasmid that contains a resistance gene, by horizontal gene transfer (HGT). Such plasmid mediated genes may result in specific efflux pumps to an antibiotic being expressed, or may encode genes involved in the production of antibiotic inactivating enzymes or target modifying enzymes (Peterson and Kaur, 2018; Reygaert, 2018). These genes responsible for AMR in environmental hosts are mainly chromosomal in origin, however their mobilisation onto plasmids by integrons has been attributed to the rapid dissemination of antibiotic resistance genes (ARGs) into the clinic (José L Martínez, 2012; Millan, 2018). Examples of this are the genes $bla_{\text{OXA-48}}$ and *qnrA*. Both $bla_{\text{OXA-48}}$ and *qnrA* have been found to originate in the environmental bacterium *Shewanella*, however they have also been detected on plasmids in *Enterobacterales* in clinical samples (Nazic, Poirel and Nordmann, 2005; Poirel *et al.*, 2005; Tamang *et al.*, 2008; Hidalgo *et al.*, 2019).

1.2 Resistance Mechanisms to WHO Priority Antibiotics

Of particular interest in this thesis were resistance mechanisms related to the WHO listed critically important antimicrobials: the aminoglycosides, polymyxins, quinolones and the β-lactams: the cephalosporins and the carbapenems (WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR), 2019). Resistance to all of these antimicrobials includes modification of the target site, decreased permeability of the cell membrane through modification of OMPs, enzyme mediated modification of the antimicrobial and efflux of the antimicrobial (Munita and Arias, 2016).

Aminoglycosides are broad spectrum antibiotics that act as protein synthesis inhibitors that target the 30S subunit of the ribosome. The ribosome, as the antibiotics target site, can undergo modifications therefore preventing action of the aminoglycoside. Additionally, the aminoglycoside binding site can undergo modification through the production of the aminoglycoside methyltransferases (16S-RMTases) (Doi, Wachino and Arakawa, 2016). The 16S-RMTases confer both intrinsic and plasmid mediated aminoglycoside resistance and cause aminoglycoside resistance through methylation of the 16S rRNA subunit of the 30s ribosomal subunit (Doi and Arakawa, 2007; Garneau-Tsodikova and Labby, 2016). There have been ten 16S RMTases identified (ArmA, RmtA – RmTH, and NpmA), with the most commonly identified being ArmA, NpmA (Lioy *et al.*, 2014) and RmTB (Cassu-Corsi *et al.*, 2018). However, the more common method of aminoglycoside resistance is through the production of the aminoglycoside degrading enzymes: the *N*-acetyltransferases, the *O*-nucleotidyltransferases and the *O*-phosphotransferases. Approximately, 100 aminoglycoside degrading enzymes have been identified and they are often associated with plasmid-mediated aminoglycoside resistance (Garneau-Tsodikova and Labby, 2016).

The quinolones and fluoroquinolones are synthetic, broad-spectrum antibiotics that are DNA synthesis inhibitors. The target site of these antibiotics are the topoisomerase

enzymes (DNA gyrase and topoisomerase IV), that are involved in supercoiling of DNA during DNA replication (Redgrave *et al.*, 2014; Hooper and Jacoby, 2015). Point mutations in the quinolone resistance determining region (QRDR) of the GyrA or GyrB subunit of DNA gyrase and the ParC or ParE subunit of topoisomerase IV are associated with resistance or reduced susceptibility to both the quinolones and fluroquinolones (Johnning *et al.*, 2015). Plasmid mediated quinolone resistance is associated mainly with the *qnr* genes (*qnrA, qnrB, qnrS, qnrC and qnrD*) and a variant of an aminoglycoside acetyl transferase (*aac-(6')-Ib-cr*). Additionally, the efflux pumps QepAB and OqxAB are associated with quinolone resistance. Plasmid mediated resistance is mostly associated with low level resistance, however can facilitate the selection of mutations that confer high level resistance (Hooper and Jacoby, 2015).

The β-lactams: the third generation cephalosporins and the carbapenems are broad spectrum antibiotics. As both are β-lactam antibiotics, they consist of a β-lactam ring and target the cell wall synthesis pathway by disrupting peptidoglycan crosslinking. These antibiotics do this by targeting the penicillin-binding proteins (PBPs) and by doing so, prevent cell wall synthesis that leads to lysis of the bacterial cells due to osmotic stress (Arumugham, Gujarathi and Cascella, 2022). Resistance or reduced susceptibility to these antibiotics can be due to overexpression of efflux pumps, mutations in OMPs or modifications in their PBPs (Codjoe and Donkor, 2017). Additionally, third generation cephalosporin and carbapenem resistance in gram negative bacteria is often associated with β-lactam modifying enzymes (β-lactamases) that hydrolyse the β-lactam ring. These enzymes can be classified as Ambler Class A, B, C or D (Ambler, Baddiley and Abraham, 1980; Tooke *et al.*, 2019). Class A β-lactamases have a serine active site and consist of cephalosporin degrading enzymes (TEM, SHV and CTX-M), as well as enzymes with carbapenem activity such as KPC (Codjoe and Donkor, 2017; Palzkill, 2018; Tooke *et al.*, 2019). Class B β-lactamases are known as metallo-β-lactamases (MBLs) due to their

zinc active site and have activity against both cephalosporins and carbapenems. Examples of MBLs are the enzymes: IMP, VIM and NDM (Codjoe and Donkor, 2017; Tooke *et al.*, 2019). Class C β-lactamases are mainly chromosomal and consist of the AmpC β-lactamases that confer cephalosporin resistance. These enzymes can confer reduced susceptibility or resistance to carbapenems when overexpressed or when paired with another resistance mechanisms such as increased efflux or porin loss (Jacoby, 2009; Tooke *et al.*, 2019). The Class D β-lactamases are a diverse family of β-lactamases and consist of enzymes active against cephalosporins (eg: OXA-28) (Poirel *et al.*, 2001) and carbapenems (eg: OXA-48) (Poirel *et al.*, 2004; Evans and Amyes, 2014; Tooke *et al.*, 2019).

Polymyxins, along with carbapenems, are considered one of the last lines of defence against gram-negative bacterial infections. Polymyxin antibiotics include polymyxin B and polymyxin E (colistin) and have a narrow range of activity; only having an effect on gram negative bacteria. Colistin targets the bacterial cell membrane by binding to lipid A of the lipopolysaccharide (LPS) layer and displacing calcium and magnesium ions from the membrane. This disruption of the bacterial cell membrane therefore leads to lysis of the bacterial cell. Resistance to colistin can be chromosomal or plasmid mediated. Chromosomal colistin resistance occurs through the addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) and phosphoethanolamine (PEtn) to lipid A, therefore modifying the colistin target site. Plasmid associated colistin resistance is mediated by, to date, ten variants of the *mcr* gene that alter lipid A (Liu *et al.*, 2016; AbuOun *et al.*, 2017; Borowiak *et al.*, 2017; Carattoli *et al.*, 2017; Yin *et al.*, 2017; Wang *et al.*, 2018; Yang *et al.*, 2018; Carroll *et al.*, 2019; C. Wang *et al.*, 2020).

1.3 The One Health Concept

The One Health Concept is a critical concept to understand when addressing AMR. One Health means that humans, animals, and the environment are all interlinked (Robinson *et al.*, 2016) as illustrated in Figure 1.1. It is widely accepted that human and animal health are closely linked. However, the mechanisms underpinning the transfer of resistance genes between bacteria in humans, animals and the environment are unclear.

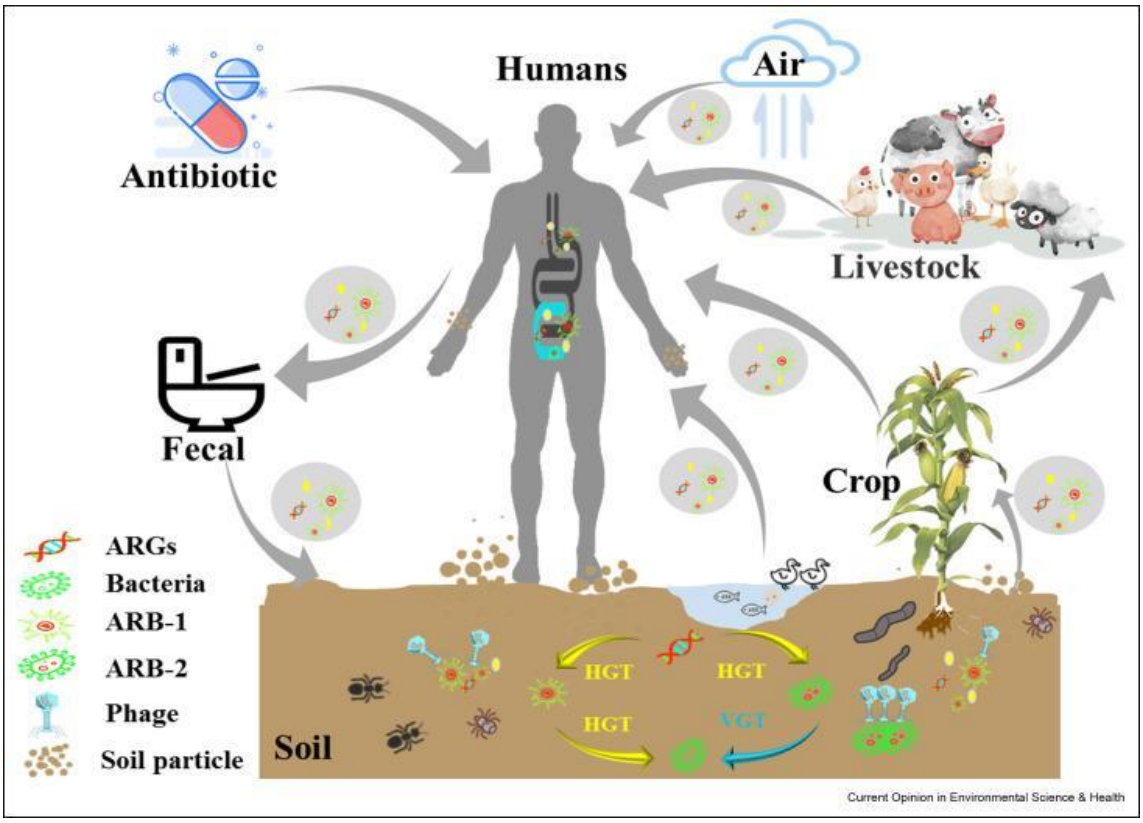

Figure 1.1: Schematic illustrating the various routes of AMR dissemination involved in the One Health concept. From: (Wang et al., 2021).

The spread of AMR has been facilitated through increased globalisation of industry and increased travel (Robinson *et al.*, 2016). Antibiotic resistance has also been documented to be spread by wildlife, particularly by migratory birds who travel long distances across the globe (Agnew *et al.*, 2016; Foti *et al.*, 2017). Notably, global dissemination and proliferation of ARGs has been observed through the dissemination of the plasmid mediated *mcr-1* gene.. Since its discovery in China in pigs in 2016 it has now been

identified globally (Liu *et al.*, 2016; Ling *et al.*, 2020). Additionally, new variants of the *mcr* gene (*mcr-2 – mcr-10*) (Abiodun *et al.*, 2017; Borowiak *et al.*, 2017; Carattoli *et al.*, 2017; Yin *et al.*, 2017; Wang *et al.*, 2018, 2020; Yang *et al.*, 2018; Carroll *et al.*, 2019) have been identified, illustrating the rapid evolution of this resistance gene. Another example is the rapid spread of the carbapenemase New Delhi metallo-β-lactamase (NDM-1) that was originally identified in a tourist in New Delhi in 2008 (Yong *et al.*, 2009) and is now found worldwide (Nordmann, Naas and Poirel, 2011). Of grave concern is the discovery of bacterial isolates containing multiple plasmids harbouring carbapenemases and *mcr* genes, resulting in very few therapeutic options being left available (Liu *et al.*, 2017; Long *et al.*, 2019).

Resistance genes from bacteria of zoonotic origin are thought to be transferred to human commensal bacteria or pathogens in two ways: by direct or indirect contact (Landers *et al.*, 2012; Muloi *et al.*, 2018). Direct contact can occur through exposure of humans to animals containing bacteria harbouring ARGs or their biofluids (such as faeces, blood and urine). Indirect contact involves the infection of humans with ARGs from contaminated food products or from the environment (Landers *et al.*, 2012). An example of this is the identification of ARG-containing bacteria in contaminated meat products and therefore pose a potential risk by entering the food chain (Leverstein-van Hall *et al.*, 2011; Thung *et al.*, 2016; Moawad *et al.*, 2017; Davis *et al.*, 2018). Additionally, there have been many studies showing that agricultural animals are reservoirs of antimicrobial resistant bacteria (ARB) (Dierikx *et al.*, 2013; Webb *et al.*, 2016; Birkegård *et al.*, 2017; Higuera-Llantén *et al.*, 2018). It has been found that individuals who are in frequent contact with agricultural animals, such as farm workers, are at a higher risk of acquiring ARB (Castillo Neyra *et al.*, 2012). The dissemination of ARGs through HGT and through clonal transmission between farm workers, animals and the farm environment has been documented for resistance to clinically important antimicrobials (Deng *et al.*, 2011;

Hammerum *et al.*, 2014; Dohmen *et al.*, 2015; Sun *et al.*, 2020). This identification of ARG and ARB in the food chain is of importance due to the potential of these ARGs to integrate into pathogens in the human gut microbiome and therefore reduce the therapeutic effect of antibiotics (Wang *et al.*, 2021).

1.4 The Occurrence of Critical Pathogens in the Environment

Many bacteria of clinical importance are known to survive, or naturally inhabit the environment. Of particular interest in this thesis are the WHO priority pathogens: *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumanii* and *Pseudomonas aeruginosa.* Both *P. aeruginosa* and *K. pneumoniae* are naturally found in the environment, in soil, water and plants (Deredjian *et al.*, 2014; Martin and Bachman, 2018), with *P. aeruginosa* being particularly associated with environments with high human activity (Crone *et al.*, 2020). While *K. pneumoniae* contains strains that can be either beneficial (Liu *et al.*, 2018) or pathogenic to plants (Huang *et al.*, 2016), *P. aeruginosa*, unlike other *Pseudomonas* spp., is thought to only be pathogenic (Walker *et al.*, 2004). *Escherichia coli* is a commensal of the gastrointestinal tract and is normally harmless. However, certain pathogenic strains such as enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) have been found in humans and animals (Gerba, 2015; Lupindu, 2017). Since *E. coli* is associated with the gastrointestinal tract of humans and animals it is used in environmental monitoring as an indicator of faecal contamination (Jang *et al.*, 2017). However, this practice has come under scrutiny in recent years due to the identification of *E. coli* in environmental reservoirs, such as soil (Ishii *et al.*, 2006; Brennan *et al.*, 2010), water (Power *et al.*, 2005) and crops (Jongman And Korsten, 2016). Additionally, *E. coli* have been found to become naturalised and persist in soils over multiple seasons (Ishii *et al.*, 2006) and years (Brennan *et al.*, 2010). Investigation of the presence of *E. coli* in plants has mainly focused on the occurrence of pathogenic strains in vegetables (Gagliardi and Karns, 2002; Ingham *et al.*, 2004), however little is known

about the occurrence of *E. coli* in other important crops, such as grass. Therefore, the role *E. coli* plays in natural ecosystems as a reservoir of AMR has yet to be fully understood. Similar to *E. coli*, there are conflicting views on the occurrence of *A. baumannii* in the natural environment. While other members of the *Acinetobacter* spp. are readily isolated from environmental samples such as soil and water, the natural reservoir of *A. baumannii* is relatively unknown (Towner, 2009). It has been stated that *A. baumannii* is an environmental commensal and can be readily isolated from soil and water (Ng *et al.*, 2018). However, this opinion is now considered to be outdated due to a lack of evidence regarding their natural environmental niches (Towner, 2009). It is now thought that *A. baumannii* is mainly associated with hospital outbreaks and can be found in terrestrial and aquatic environments due to waste discharge from these hospitals (Ferreira *et al.*, 2011; Hrenovic *et al.*, 2014). Outside of hospital related environments, *A. baumannii* has also been isolated from agricultural soil (Byrne-Bailey *et al.*, 2009) manure (Fernando *et al.*, 2016; Hrenovic *et al.*, 2019) and crops (Berlau *et al.*, 1999; Soliman *et al.*, 2021). However, its recovery from these environments is uncommon. Since the natural reservoir of *A. baumannii* is unknown, the ecological distribution of *A. baumannii* and its transmission between humans and the environment needs to be fully investigated to evaluate its risk as a transmission route of AMR.

1.5 Antibiotic Use and the Spread of Antibiotic Resistance in Agriculture

The world population is increasing at an unprecedented rate leading to increased pressure on food production and agriculture (Van Boeckel *et al.*, 2015). Antimicrobial use has become a central component of modern food animal production. In Ireland in 2018, 99.4 tonnes of antibiotics were sold for veterinary use with the main antibiotics purchased being the tetracyclines (33.95%), penicillins (24.2%), sulphonamides and trimethoprim (17%) (HPRA, 2018). The sales of macrolides and 3rd/4th generation cephalosporins have increased during the last 5 years despite being on the WHO list of priority antibiotics

(Martin *et al.*, 2020; HPRA, 2018). Antibiotics can be used several ways in animal husbandry: therapeutically, prophylactically, metaphylactically and for growth promotion (Phillips *et al.*, 2003; Van Epps and Blaney, 2016). The therapeutic use of antibiotics is important for the treatment of sick animals. However, of concern, is the use of antibiotics that are identical, or within the same class as those that are vital in human medicine (Catry, 2017). One example of this is the use of the third-generation cephalosporin, ceftiofur, to treat mastitis. However, since this class of antibiotics are on the WHO priority list the use of these antibiotics should be limited to extreme cases (Collignon and McEwen, 2019).

Due to the intensive nature of modern animal husbandry, antibiotics are often administered to livestock, most commonly through their feed or water, to prevent disease in animals during stressful periods, for example when piglets are weaned, in a practice known as prophylaxis. Metaphylaxis is also another commonly used practice and involves administering antibiotics as a disease preventive measure in healthy animals when one or a few members of the herd of flock is ill, commonly when a new animal in introduced to a herd (Phillips *et al.*, 2003). Antibiotics can also have growth promotion effects in livestock by modifying the animal gut microbiome and allowing for improved digestion and metabolism or through the prevention of disease (Phillips *et al.*, 2003). However, this practice of using antibiotics as growth promoters was banned in the European Union (EU) in 2006 and globally has been banned or heavily restricted in most countries (‘35 years of resistance’, 2012). However, antibiotics in livestock are still used heavily in some countries such as the United States, China and Russia (Zalewska *et al.*, 2021). Therefore, global harmonisation of antibiotic usage policies is needed. The non-therapeutic uses of antibiotics are often used in subtherapeutic concentrations, which is problematic due to these sub-inhibitory doses promoting virulence and HGT and providing a selection pressure for antibiotic resistant bacterial strains (Viswanathan, 2014). The dissemination and exchange of ARGs between animals is aided by modern agricultural practices such

as high-density populations of livestock living in confined spaces, which facilitates the spread of commensal and pathogenic bacteria that may contain ARGs (Landers *et al.*, 2012; Dierikx *et al.*, 2013; Kyselková *et al.*, 2015; Brower *et al.*, 2017). Additionally, the reuse of bedding contaminated with ARB can result in their spread from sick to healthy animals (J. Liu *et al.*, 2016; Singer *et al.*, 2016). In comparison, the impact of humans and animals on the environmental microbiome and resistome is not as well understood (Davies and Davies, 2010). However, it is thought that the use of manure and slurry in agriculture, for example in crop production, is a key contributor to the flow of ARGs between animals and the terrestrial and aquatic environments (Udikovic-Kolic *et al.*, 2014; Kivits *et al.*, 2018). It is estimated that 58% of veterinary antibiotics are transferred to the environment, the majority of which enter the soil (Xie, Shen and Zhao, 2018). However, the degree to which livestock and agricultural land act as reservoirs of ARB, and how these two factors interact is not well understood.

1.6 Manure as a Reservoir of ARGs

The landspreading of manure onto agricultural land is an integral agricultural practice. Due to the vast quantity of organic waste produced by livestock, landspreading of manure is not only a waste management strategy but is also vital for the recycling nutrients back into the land. Annually, in Ireland it is estimated that 40.3 million tonnes of manure are spread. However, due to the use of clinically important antibiotics in animal husbandry, manure from animals has been found to be a reservoir of ARGs and mobile genetic elements (MGEs) (Binh *et al.*, 2008a; Looft *et al.*, 2012; Jechalke *et al.*, 2013; Thanner, Drissner and Walsh, 2016; Pérez-Valera *et al.*, 2019), with the occurrence of ARGs in livestock waste being found to be up 28,000 times higher than in soil (Zhu *et al.*, 2013; He *et al.*, 2020).

The livestock gut harbours a diverse range of microorganisms, including those which are known to be potential human pathogens, such as *Salmonella t*yphimurium, *E. coli*

(Venglovsky *et al.*, 2018) and *Campylobacter coli* (Kempf *et al.*, 2017). The use of antibiotics in livestock has been shown to perturb the commensal gut microbiome which may provide pathogenic or ARB with a selective advantage to survive and proliferate. Additionally, these ARGs may transfer by HGT to commensal members of the microbiota (Zeineldin, Aldridge and Lowe, 2019). The livestock gut has been found to naturally contain ARGs and antibiotic use has been found to increase the diversity and relative abundance of ARGs in manure by creating a selective pressure for these resistance determinants in the livestock gut (Looft *et al.*, 2012). As a result, the faecal shedding of bacteria from livestock and the application of manure to land can directly introduce these resistant bacteria and ARGs into the environment (Moynihan *et al.*, 2015). Additionally, after excretion, antibiotics or their metabolites may retain some of their antibiotic activity. This can result in manure introducing residual amounts of antibiotics that may enhance the proliferation of resistance genes already present in the environmental resistome due to this selection pressure or induce the selection of *de novo* resistance genes (Thanner, Drissner and Walsh, 2016; Xie, Shen and Zhao, 2018; Kraemer, Ramachandran and Perron, 2019). The presence of antibiotic residues in the environment can reduce the diversity and composition of the microbiome, possibly resulting in a decline in microbes with important environmental roles, such as denitrification or respiration (Cycoń, Mrozik and Piotrowska-Seget, 2019; Kraemer, Ramachandran and Perron, 2019). Additionally, various antibiotics persist for different amounts of time in the soil. For example, β-lactams are susceptible to hydrolysis and therefore degrade within a few days whereas tetracyclines have been found to have a half-life of up to 578 days (Cycoń, Mrozik and Piotrowska-Seget, 2019). This illustrates the persistent selective pressure antibiotic residues may have on the environment.

Manure may also contain other contaminants including biocides and heavy metals such as zinc and copper, which are used as growth promoters, and which may enrich for ARGs

through cross-selection or co-selection mechanisms (Chapman, 2003; Yazdankhah, Rudi and Bernhoft, 2014). Cross resistance occurs when antibiotics have a common mechanism of action to a shared target, whereas co-selection occurs when antibiotic, biocide or heavy metal resistance genes are located on the same MGE, such as a plasmid (Chapman, 2003). Therefore, the use of biocides or heavy metals can inadvertently select for AMR mechanisms.

Manure also has been found to contaminate surface water through runoff from agricultural land or through leaching to groundwater (Hill, Owens and Tchoounwou, 2005; Manyi-Loh *et al.*, 2016; Kivits *et al.*, 2018). Additionally, antibiotics can be transferred into crops through the transportation of water through xylem tissue and by passive absorption (Hu, Zhou and Luo, 2010; Thanner, Drissner and Walsh, 2016). Furthermore, irrigation water is considered a source of ARB contamination in crop production (Thanner, Drissner and Walsh, 2016). As vegetables are commonly eaten raw, this is a possible transfer route for potentially pathogenic bacteria between the environmental microbiome and humans via crops (Araújo *et al.*, 2017). Additionally, the accumulation of ARGs in agricultural soil can lead to an enrichment of ARGs in soil fauna, such as earthworms, illustrating that soil can act as a reservoir and can facilitate ARG dissemination (Ding *et al.*, 2019).

1.7 The Impact of Manure Application on the Soil and Microbiome and Resistome

It is known that the soil is a natural reservoir of known and novel antibiotic resistance genes. It has been found that the environmental resistome and microbiome is influenced by human activity, particularly agricultural practices such as the landspreading of manure. Additionally, a correlation has been found with antibiotic usage and the levels of ARGs and MGEs found in manure amended soils (Binh *et al.*, 2008a; Heuer, Schmitt and Smalla, 2011; Blau *et al.*, 2018; Zhou *et al.*, 2019; Lima, Domingues and Da Silva, 2020).

The fate and maintenance of ARG and ARB from manure to soil is affected by many different contributing factors, such as seasonal variables (e.g. weather conditions), soil properties (eg. soil texture) (Blau *et al.*, 2018), and the manure type used e.g. pig, chicken or cow or treated (Bicudo and Goyal, 2003; Singer *et al.*, 2016). Manure application onto soil can impact the soil microbiome and resistome directly through the introduction of manure derived bacteria and resistance genes into the soil (Lopatto *et al.*, 2019; Macedo *et al.*, 2021). Additionally, manure landspreading can also indirectly influence the soil microbiome and resistome by the introduction of nutrients which may enrich the soil for certain commensals or naturally occurring ARGs (Ding *et al.*, 2014; Udikovic-Kolic *et al.*, 2014). Additionally, manure can contain antibiotic residues, which can create a selection pressure for certain ARGs and ARBs in the soil, therefore influencing the soil environment (Blau *et al.*, 2019).

The landspreading of manure has been found to enrich soil for ARGs conferring resistance to clinically important antibiotics, such the sulphonamide resistance gene *sul1* (Ruuskanen *et al.*, 2016; Lopatto *et al.*, 2019), tetracycline resistance genes *tet(M)* (Ruuskanen *et al.*, 2016) and *tet(W)* (Macedo *et al.*, 2020) and macrolide resistance genes *erm(B)* and *erm(C)* (Lopatto *et al.*, 2019). However, there are conflicting reports of the temporal impact manure landspreading has on soil. In general, it is thought that manure has a short term impact on the soil resistome and microbiome (Fahrenfeld *et al.*, 2014; Lima, Domingues and Da Silva, 2020). Initially after the application of manure there is an increase in ARG diversity and abundance, followed by a decrease in ARG levels (Muurinen *et al.*, 2017; Gou *et al.*, 2018; Macedo *et al.*, 2021). It has been estimated that the ARG levels return to a similar to a pre-manured state around 40 days after application (Macedo *et al.*, 2020). Additionally, Chen *et al.*, (2017) observed that manured soil maintained a higher ARG abundance than untreated soils 60 days following manure application. However, there is some evidence to suggest that the soil resistome remains

perturbed after manure application. Pérez-Valera *et al.*, (2019) found that manure amended soils had a higher amount of tetracycline resistance genes in comparison to control soils 3 months following manure application, illustrating the potential of ARGs to persist for a longer period of time following manure application.

However, like the resistome, the impact on the soil microbial community composition and the temporal influence can vary and there have been conflicting reports. Manure has been found to alter the soil microbiome through the introduction of microbes directly from the manure itself. Lopatto *et al.* (2019) found that manure application introduced 38 manure originating Operational Taxonomic Units (OTUs) into manured soil that were not detected in pre-manured soil; however these OTUs decreased approximately 3 months following manure application. Additionally, manure supplemented with the commonly used veterinary antibiotic, sulfazidime, was found to enhance the presence of the opportunistic pathogens: *Stenotrophomonas* spp. and *Clostridium* spp. (Ding *et al.*, 2014). It has been found that the application of manure to soil has had differential impacts on the alpha diversity of soil communities with increases (Das *et al.*, 2017), decreases (Han *et al.*, 2018) and no effect on soil diversity having been reported (Xie, Shen and Zhao, 2018; Blau *et al.*, 2019; Lopatto *et al.*, 2019; Macedo *et al.*, 2021). The addition of nutrients from the manure onto the soil can enrich the soil for soil commensal bacteria, some of which may be opportunistic pathogens and some that may have intrinsic resistance mechanisms. Udikovic-Kolic *et al.*, (2014) found that manure from animals not treated with antibiotics enhanced the proliferation of β-lactamase genes through the enrichment of the soil microbiota *Pseudomonadaceae* and *Janthinobacterium*, which are naturally β-lactamase producers. Additionally, they found that soil associated cephalothin resistance genes persisted for a longer period (94 days following manure treatment) than manure associated cephalothin resistance genes (38 days following manure treatment).

The dissemination and maintenance of ARBs, ARGs and MGEs was negatively impacted by the soil indigenous microbiota (Chen *et al.*, 2017; Pérez-Valera *et al.*, 2019). It is thought that the short-term impact of manure application on the microbiome and resistome is possibly due to the bacteria originating in manure not being well adapted to the soil (Muurinen *et al.*, 2017; Gou *et al.*, 2018). It has been established that introduced bacterial populations, such as those introduced as a result of manure application, decrease rapidly when the indigenous community is more diverse (Elsas *et al.*, 2012; Moynihan *et al.*, 2015). Chen *et al.*, (2017) demonstrated that irradiated soil treated with manure had a higher abundance of ARGs and a higher bacterial diversity than non-irradiated soil treated with manure, thus illustrating the important role that the soil microbiome plays in mitigating the spread of ARGs and reducing the establishment of manure borne pathogens. Similarly, Pérez-Valera *et al.*, (2019) identified that *tet*(Y) and *tet*(Q) abundances increased in irradiated soils compared to non-irradiated soils following manure application, illustrating the protective function the natural soil bacteria play in preventing the proliferation of manure originating ARGs. The interaction between the indigenous soil microbiome and the manure microbiome is therefore thought to be a limiting factor regarding ARB dissemination. However, the rate at which ARG and ARB decrease occurs is variable and has been shown to vary anywhere from days to up to 3 months (Fahrenfeld *et al.*, 2014; Gou *et al.*, 2018; Lopatto *et al.*, 2019). In general studies investigating the impact of manure application on the soil microbiome and resistome focus on a timescale that is relatively short, around 1 - 4 months (Chen *et al.*, 2017; Lopatto *et al.*, 2019; Pérez-Valera *et al.*, 2019; Macedo *et al.*, 2020, 2021). Studies also tend to investigate the impact of a single application of manure spreading whereas in practice manure tends to be repeatedly spread over a season. Therefore, the cumulative and long-term effects of manure spreading need to be assessed, of which there are few studies. Macedo *et al.*, (2020) predicted that ARG levels recover from manure

landspreading between 29 – 42 days. However, they do not recover between repeated manure spreading sessions and that this was affected by the type of ARG, with *sul1* having a slower decay rate than *tetW* and *ermB.* Additionally, Zhang *et al.*, (2018) demonstrated that soil microbiomes remained perturbed from manure application even after pausing manure application for 13 years, illustrating legacy impact manure application may have on the microbiome of agricultural soil.

1.8 The Comparative Risk of Pig, Cow and Chicken Manure on Agricultural Land

Another factor to consider when investigating the temporal ARG and ARB patterns of agricultural land following manure landspreading is the impact that the manure source may have. Due to differing husbandry practices between livestock, different types and levels of antibiotics being used, different livestock manures may have different resistance profiles and therefore there may be varying risk associated with using manure from one animal or livestock system over another (He *et al.*, 2020). Of particular interest is the AMR profile and pig, cow and chicken manures and the land on which they are applied. In the EU there is an estimated 143 million pigs, 77 million cows (EUROSTAT-Livestock population in numbers, 2020) and 400 million laying hens (EPRS, 2019) producing an estimated 1400 million tonnes of manure annually (Foged *et al*, 2011). It is estimated that wastewater from pig and chicken farms has ARG abundances three to five times higher than hospital waste, whereas cow waste has a similar ARG level. This increased ARG level in pig and chicken farm waste in comparison to human waste is thought to be due to heavy antibiotic use in these livestock animals, therefore resulting in an increased presence of residual antibiotics (He *et al.*, 2020). Additionally, Xu *et al.*, (2020) found that chicken manure had an abundance of ARGs two to four times greater than cow manure. However, the AMR profiles of these manures and the differential impact they have on the environmental resistome when landspread have had conflicting reports.

To date, research to investigate the comparative risk of landspreading with pig, cow and chicken manure is limited. The majority of these studies have been conducted using mesocosm experiments. Zhang *et al.*, (2017) found that while all manured soils had increased abundances of ARGs relative to untreated soils, cow manure and cow manured soil had a lower relative abundance and diversity of ARGs and MGEs than pig and chicken manure and their respective soils. Additionally, pig manure was found to have the highest ARG abundance. They also identified a varying temporal effect of the manure types, with pig and chicken manured soil maintaining a higher diversity of ARGs than untreated soils 130 days after manure spreading. Additionally, by day 130 chicken manured soil maintained higher ARG relative abundance than control soils, whereas pig manure amended soils' ARG relative abundance levels decreased to background levels between day 7 – day 20. Zhang *et al.*, (2017) also found the manures had a different ARG composition and this had a difference on the ARG composition of their respective manured soils. It was found that pig manure had high abundances of tetracycline, sulphonamide, Macrolide – lincosamide – streptogramin B (MLSB) and β-lactam resistance genes which was then found to be enhanced in pig manured soil. A similar pattern was found for cow manure and poultry manure with aminoglycoside and MDR resistance genes, however cow manure had a less pronounced impact than pig and chicken manure. Therefore, this suggests that pig and chicken manure have a more pronounced impact on the soil resistome than cow manure, and therefore may pose an increased risk for AMR transfer from manure into agricultural land. This increased ARG diversity and abundance associated with pig manure in comparison to cow manure has also been observed by Peng *et al.*, (2017). However, contrary to this Han *et al.*, (2018) found that while the application of livestock manure from pigs, poultry and cows increased the number and abundance of ARGs and MGEs in comparison to untreated soils, there was no significant difference between the manure types themselves. However, all manures

resulted in the introduction of tetracycline, MLSB, aminoglycoside, β-lactam and fluoroquinolone resistance genes into the soil. This study also demonstrated a similar long-lasting impact of manure application on the soil resistome as the study of Zhang *et al.*, (2017), as 120 days following manure application manured soils had elevated ARG abundances relative to the control soils. Since both Zhang *et al.*, (2017) and Han *et al.*, (2018) utilised small scale mesocosm experiments, larger field scale studies are needed to fully understand the dynamics of ARG and ARB dissemination from different farmyard manures.

Liu *et al.*, (2021) investigated the cumulative impact landspreading of spreading composted pig, cow and chicken manure had over 3 consecutive years on a field scale. There was no impact on the soil microbiome and resistome from the three different manure sources. However, all manures increased the soil's ARG diversity and abundance directly by introducing manure associated ARGs, enriching the soil for indigenous ARGs and by increasing the abundance of MGEs. These enriched or newly introduced ARGs in manured soil comprised of the following gene classes: β-lactam, MDR, MLSB, aminoglycoside and tetracycline resistance genes. It was also found that landspreading of composted manures increased ARG and 16S rRNA gene abundance continuously over the three years, illustrating that repeat manure spreading annually can cause an accumulation of ARGs and bacteria in agricultural soil. Overall, there is evidence for an increased risk of ARG introduction and enrichment associated with pig and poultry manure over cow manure, especially with tetracycline, aminoglycoside and β-lactam resistance genes. However due to conflicting reports, perhaps due to differences between raw and treated manure, as well as the lack of field scale experiments, more research is required to determine this comparative risk between manures from different livestock on agricultural land in terms of AMR dissemination.

1.9 The Grass Phyllosphere

The phyllosphere, defined as the aerial part of the plant, is a diverse and rich environment of various microorganisms. The phyllosphere is a very dynamic and difficult environment for microorganisms to survive in due to its exposure to many abiotic and biotic factors. Additionally, these factors also impact the diversity and composition of the phyllosphere microbiome. Abiotic factors that affect the microbiome include UV radiation, humidity, nutrient availability, varying temperatures and rainfall (Compant *et al.*, 2019; Sivakumar *et al.*, 2020). Biotic factors include plant immunity and competition from invading plant pathogens that can affect the commensal microbiome or members of the microbiome involved in symbiotic relationships with the host plant (Sivakumar *et al.*, 2020). The phyllosphere, rhizosphere and soil, due to their proximity to each other, are interlinked and share microorganisms (Vorholt, 2012; Sivakumar *et al.*, 2020; Massoni *et al.*, 2021; Yan *et al.*, 2021). Interestingly, the global area of the phyllosphere is double that of the land surface area, making it an important component of One Health (Vorholt, 2012). However, in comparison to other terrestrial environments, such as soil, the phyllosphere is an under-researched area.

From an agricultural perspective, grassland is vital for global food production. It is a source of food for grazing animals in the form of pastureland, or as conserved grass such as hay or silage, and plays an important role in global carbon sequestration. Globally grassland comprises ~70% of agricultural land, therefore being essential for food production (O'Mara, 2012). However, currently, there is little data on the antimicrobial resistance of bacteria in the phyllosphere, with many studies focusing solely on the soil microbiome and resistome (Santamaría, López and Soto, 2011; Jechalke *et al.*, 2013; Van Boeckel *et al.*, 2015; Nõlvak *et al.*, 2016). Owing to the importance of grassland in agriculture, the impact of farming practices on its microbiome and resistome is of key importance for food security and safety. However, there is limited understanding of the

impact of manure application on the phyllosphere of grassland regarding ARG dissemination, or the rate of transfer of ARB from grassland to animals. In general, studies that examine the impact of manure application on grassland focused on the impact of manure application in the soil, rather than on the surface of the grass itself (Santamaría, López and Soto, 2011; Jechalke *et al.*, 2013; G.-C. Ding *et al.*, 2014; Udikovic-Kolic *et al.*, 2014). It has already been shown that plants treated with manure can be a reservoir of ARB, ARGs and MGEs (Hu, Zhou and Luo, 2010; Holvoet *et al.*, 2013; Marti *et al.*, 2013; Zhou *et al.*, 2019). Additionally, it has been found that silage can harbour pathogenic bacteria such as *Listeria monocytogenes* (Nightingale *et al.*, 2004). However, this risk in relation to grassland and forage has yet to be fully assessed. Recently there has been increased research interest in how anthropogenic activity impacts the phyllosphere microbiome and resistome of vegetation, as displayed in Table 1. To date the only study specifically looking at the resistome of the phyllosphere of grass was conducted by Yan *et al.*, (2019) which found 217 ARGs and MGEs detected in the grass phyllosphere of which the most detected were multidrug resistance, tetracycline and aminoglycoside resistance gene classes. However, to date there have been no studies focusing on the impact of manure application on the microbiome and resistome of agricultural grassland. Therefore, the presence of antibiotic-resistant epiphytes and endophytes residing in grassland are possible sources of ARG transfer to animals, which may, in turn, enter the food chain (Y.-J. Zhang *et al.*, 2017; Yan *et al.*, 2019).

Table 1.1: Table displaying a selection of studies investigating the microbiome and resistome of various plant phyllospheres

Plant Phyllosphere Investigated	Aim of Study	Methods Used for Microbiome and/or Resistome Analysis	Reference
Grass	Investigate the resistome of the grass phyllosphere in urban green spaces and to determine important factors influencing the phyllosphere resistome.	HT-qPCR qPCR quantification of 16S rRNA Gene 16S rRNA Gene Amplicon Analysis	(Yan *et al.*, 2019)
Herbaceous plants	The impact biotic, abiotic and biogeographical patterns have on phyllosphere and soil resistomes.	HT-qPCR 16S rRNA Gene Amplicon Analysis	(Yan *et al.*, 2021)
Brassica	The impact struvite application on soil, rhizosphere and phyllosphere resistome.	HT-qPCR 16S rRNA Gene Amplicon Analysis	(Chen *et al.*, 2019)
Galium album	Investigate the impact global warming has on the plant phyllosphere	Total bacterial cell count 16S rRNA Gene Amplicon Analysis	(Aydogan *et al.*, 2018)
Poa pratensis/Poa trivialis, Elymus repens, Alopecurus pratensis, Phalaris arundinacea	Investigate the impact cutting frequency and fertiliser application rate had on yeasts, Micrococcaecae and Listeria.	Culture Isolation on Selective Agar	(Behrendt, Stauber and Müller, 2004)

1.10 Current Surveillance and Monitoring of AMR

In 2015, the WHO outlined the global action plan on antibiotic resistance to prevent the return of a pre-antibiotic era (WHO, 2015). From this WHO action plan, the European Union (EU) One Health Action Plan was implemented in 2017 to address the growing problem on AMR in the EU, by highlighting the need for harmonisation of AMR monitoring in the EU and globally and increasing research and development in the area of AMR (European Commission, 2017). Consequently, there have been efforts to improve global AMR surveillance with the implementation of the Global AMR Surveillance System (WHO-GLASS), which aims to standardise AMR surveillance and detection methods, and collect data regarding antibiotic consumption (GLASS-AMC). However, GLASS-AMR currently largely reports on collections of isolates from human clinical samples, not environmental or livestock. However, this scope aims to be expanded mainly through the GLASS One Health Module which focuses on the occurrence of ESBL producing *E. coli* in human, livestock and environmental samples (GLASS, 2020). Additionally, in the EU monitoring of AMR indicator bacteria of zoonotic origin is carried out in livestock and meat (Commission Regulation, 2020/1729). However, currently there is no legislation for the surveillance of antibiotics or ARB in foods of plant origin. Furthermore, there are no EU standards of minimum acceptable levels of antibiotic resistance in food, therefore the risk of food exposing people to antibiotic resistance via the food chain needs to be evaluated (FSAI, 2015).

Antibiotic resistance surveillance methods outlined in the GLASS initiative largely involve culture-based methods. Bacteria of interest are isolated from a sample then undergo antibiotic susceptibility (AST) testing to clinically utilised antibiotics in human medicine. These culture-based methods can then be paired with molecular methods such as pulse field gel electrophoresis and serotyping to understand the relatedness of isolates

(GLASS, 2020). This approach has resulted in the tracking of AMR in clinical settings (Argimón *et al.*, 2020, GLASS, 2020). These culture-based methods are often coupled with molecular diagnostics such as whole genome sequencing (WGS) which can not only determine the identification of isolates but also allows determination whether the resistance is chromosomal or plasmid mediated, therefore providing an advantage over more traditional approaches. However, AST methods still need to be used in addition to WGS to accurately determine phenotypic resistance and to identify novel resistance mechanisms and accordingly AST is still clinically the gold standard (Ellington *et al.*, 2017; Spencer *et al.*, 2019) (GLASS, 2020).

However, there are limitations to the use of culture in the monitoring and surveillance of AMR in the environment. Environmental bacteria are difficult to isolate and grow under culture conditions designed for human pathogens. Additionally, environmental samples have a large diversity of resistomes and microbiomes, as well as harbouring their own natural resistome. Culture based methods traditionally rely on the isolation of indicator organisms, such as *E. coli* as an indicator of faecal contamination, however this approach does not address the role of environmental bacteria as reservoirs of AMR. Therefore, molecular methods are the preferred approach for large scale monitoring of AMR in the environment; mainly high throughput quantitative PCR (HT-qPCR) and metagenomics (Franklin *et al.*, 2021).

HT-qPCR is a widely used approach for environmental detection of ARGs. It requires low amounts of DNA and allows for the concurrent screening of multiple genes. Additionally, multiple gene arrays used in HT-qPCR are customisable, resulting in the ability to choose the genes that most suit the research aims or screen for all known ARGs (Franklin *et al.*, 2021). Despite HT-qPCR only allowing for resistome analysis it can be coupled with 16S rRNA microbiome analysis to predict the bacterial hosts of detected

genes using network analysis. In comparison, metagenomics involves the sequencing of all DNA in a sample which allows for deeper taxonomic identification of the microbiome in comparison to 16S rRNA amplicon sequencing. Additionally, since HT-qPCR ARG detection relies on primers of known resistance genes, no new resistance mechanism can be detected in comparison to metagenomics which allows for the potential discovery of new resistance genes (Waseem *et al.*, 2019). However, metagenomics requires large volumes of DNA and results in a lower limit of detection of ARGs than HT-qPCR (Waseem *et al.*, 2019; Franklin *et al.*, 2021). Both HT-qPCR paired with 16S rRNA amplicon sequencing approach and metagenomics provide information about the resistome and microbiome of an environment. However, neither technology give an insight into whether members of the microbial communities are viable and if ARGs are expressed. Therefore, a paired approach involving molecular detection methods and metatranscriptomics is needed to fully monitor AMR in the environment (Franklin *et al.*, 2021). However, despite this approach being promising in microbial ecology, metatranscriptomics has limitations including difficulties acquiring enough sample for sequencing, the short half-life of RNA and the lack of metadata available for publicly available datasets (Shakya, Lo and Chain, 2019).

1.11 Future perspectives

The use of clinically important antibiotics in agriculture is thought to be a factor in the antibiotic resistance crisis. However, the magnitude of its involvement has yet to be fully understood (Zalewska *et al.*, 2021); this is due in part to a dearth of information regarding the impact of manure application on the agri-environmental resistome and microbiome (Heuer, Schmitt and Smalla, 2011; Ding *et al.*, 2019; Liu *et al.*, 2021). Due to the importance of grassland in agriculture, the temporal impact of manure application on the grassland and forage resistome and microbiome must be fully evaluated. Future research

must also include the rate of pathogen, ARG and ARB transfer from grassland to animals as these are essential components of agri-food research to fully assess the risk of manure landspreading has on the transfer of ARGs and ARB into the food chain and to humans.

1.12 Thesis Objectives and Scope

The role of agricultural land as a reservoir of AMR has yet to be fully understood. Additionally, the comparative risk posed by manure from different livestock has yet to be fully assessed. To date, studies investigating the landspreading on manure has on the microbiome and resistome of agricultural land have focused on soil, however in recent years the importance of the grass phyllosphere as a possible source of ARB and ARG has been highlighted, and therefore needs to be investigated. Additionally, the occurrence of AMR critically important pathogens in agricultural land needs to be understood to mitigate their spread in the food chain. This project uses culture dependent and independent approaches to achieve the following aims:

- To compare the impacts pig, cow and chicken manure have on the grass phyllosphere and soil microbiome and resistome using molecular methods (HT-qPCR and 16S rRNA sequencing).
- To investigate the temporal impacts manure landspreading has on ARG and microbiome diversities and abundances.
- To investigate the prevalence of antibiotic resistant *Escherichia coli*, *Klebsiella pneumoniae, Acinetobacter baumanii* and *Pseudomonas aeruginosa* using culture dependent methods in manured grassland.

CHAPTER 2

MATERIALS AND METHODS

Chapter 2. Materials and Methods

2.1 Media preparation

2.1.1 Agar

2.1.1.1 Mueller Hinton (MH) agar

Mueller Hinton (MH) agar (30 g) (Oxoid) was dissolved in 1 L of distilled water and the agar was sterilised by autoclaving at 121 °C for 15 minutes.

2.1.1.2 Eosin Methylene Blue (EMB) Agar

Eosin Methylene Blue (EMB) agar (37.5 g) (Oxoid) was dissolved in 1 L of distilled water and sterilised by autoclaving at 121 °C for 15 minutes.

2.1.1.3 Leeds Acinetobacter Agar (LAM)

Duran bottles (500 ml) were autoclaved at 121 °C for 15 minutes. Leeds Acinetobacter Agar (LAM) was made using the established protocol and components (Table 2.1) (Jawad *et al.*, 1994). The agar was dissolved in 500 ml of distilled water and boiled in a microwave with regular agitation until fully dissolved and homogenous.

Table 2.1: Table displaying the components of Leeds Acinetobacter (LAM) agar.

Ingredients	g/Litre
Casein acid hydrolysate (Fisher Scientific)	15
Soya peptone (Duchefa)	5
Sodium chloride (Merck)	5
D-Fructose (Duchefa)	5
Sucrose (Duchefa)	5
D-Mannitol (Duchefa)	5
L-Phenylalanine (Duchefa)	1
Ferric ammonium citrate (Acros Organics, Fisher Scientific)	0.4
Phenol red (Merck)	0.02
Agar (Duchefa)	12

2.1.1.4 Simmons Citrate Agar (SCA)

Simmons Citrate Agar (SCA) (23 g) (Oxoid) and 1% inositol (Alfa Aesar), was dissolved in 1 L distilled water, as described by (Van Kregten, Westerdaal and Willers, 1984), and sterilised by autoclaving at 121 °C for 15 minutes.

2.1.1.5 Pseudomonas Isolation Agar (PIA)

Pseudomonas Isolation Agar (PIA) (24.2 g) (Oxoid) was dissolved in 1 L of distilled water and sterilised by autoclaving at 121 °C for 15 minutes.

2.1.1.6 Luria-Bertani (LB) agar

Luria- Bertani low salt (LB) agar (20 g) (Duchefa) was dissolved in 1 L of distilled water and sterilised by autoclaving at 121 °C for 15 minutes.

2.1.2 Broth

2.1.2.1 LB broth

Luria-Bertani low salt (LB) broth (10 g) (Duchefa) was dissolved in 1 L distilled water and sterilised by autoclaving at 121 °C for 15 minutes.

2.1.2.2 Mueller Hinton Cation Adjusted (MH^{2+}) broth

Mueller Hinton Broth 2 (22 g) (Merck) was dissolved in 1 L of distilled water and sterilised by autoclaving at 115 °C for 10 minutes.

2.1.2.3 Nutrient broth

Nutrient broth (13 g) (Oxoid) was dissolved in 1 L of distilled water and sterilised by autoclaving at 121 °C for 15 minutes.

2.2 Antibiotic Stock Solutions

Colistin (Duchefa), cefotaxime (Duchefa), imipenem (Merck), kanamycin (Merck) and ciprofloxacin (Merck) were all made into stock solutions of 10 mg/L by dissolving 0.1 g of antibiotic in 10 ml of double filtered Milli-Q water. Antibiotic solutions were stored at -20 °C.

2.3 Other Reagents

2.3.1 30% Glycerol

Glycerol (Sigma-Aldrich) was prepared by dissolving 30 ml of glycerol in 70 ml of distilled water. The solution was sterilised by autoclaving at 121 °C for 15 minutes.

2.3.2 Phosphate Buffered Saline (PBS)

Two PBS tablets (Gibco) were dissolved in 1 L of distilled water. The solution was sterilised by autoclaving at 121 °C for 15 minutes.

2.3.3 0.5M Ethylenediaminetetraacetic acid (EDTA) solution at pH 8.0

Ethylenediaminetetraacetic acid (EDTA) (186.1 g) (Sigma-Aldrich) was dissolved in 800ml of distilled water. The solution was dissolved by continuous stirring with a magnetic stirrer. The dissolved solution was adjusted to pH 8.0 using 1 M NaOH (Merck). The solution was brought up to 1 L with distilled water and was then sterilised by autoclaving at 121 °C for 15 minutes.

2.3.4 Clavulanic acid solution

Potassium clavulanate (0.01 g) (Sigma-Aldrich) was dissolved in 10 ml of double filtered MilliQ water by vortexing. The solution was then stored at 4 °C.

2.3.5 Phenylboronic acid solution

Phenylboronic acid (150 mg) (Sigma-Aldrich) was dissolved in 10 ml of double filtered MilliQ water to make a stock solution of 15 µg/ml solution. The solution was then stored at 4 °C.

2.3.6 50X TAE Buffer

Trizma base (242 g) (Sigma-Aldrich) was dissolved in 750 ml of distilled water. Glacial acetic acid (57.1 ml) (Sigma-Aldrich) and 0.5M EDTA pH 8.0 (100 ml) (2.3.3) were added to the solution. The solution was then brought up to 1 L with distilled water. The solution was mixed by inversion 20 times. The solution was stored at room temperature. The 50X TAE solution was diluted to a 1X concentration using distilled water before use. The solution was stored at room temperature.

2.4 General microbiological methods

2.4.1 Overnight cultures

LB broth (3 ml) was aseptically inoculated with a single colony of a bacterial isolate in a 15ml falcon tube and was incubated at 37 °C for 12-18 hours.

2.4.2 Cryopreservation of Bacteria Isolates

For the long-term cryopreservation of bacterial isolates, glycerol stocks were prepared by inoculating 500 µl of 30% glycerol (as described in section 2.3.1) with 500µl of overnight culture (as described in section 2.4.1). The mixture was mixed by inversion and stored at -80 °C.

2.5 Trial Set up

2.5.1 Pot Trial

2.5.1.1 Mesocosm Experiment and Sampling Processes

Six 30L pots were filled with 2 mm sieved soil in a polytunnel in Teagasc Food Research Centre, Ashtown. For each pot, perennial ryegrass (*Lolium perenne*) seeds were planted according to an agriculturally-recommended seeding rate of 30 kg/hectare (Teagasc Greenbook, 2016). Ryegrass seeds were allowed to germinate and grow for 12 weeks. Grass was trimmed to mimic grazing height, to 5 cm tall, with sterile scissors prior to manure application. For manure application, pig manure was applied to three pots by hand using a sterile jug, according to the spreading rate of 40.5 tonnes/ha (Teagasc Greenbook, 2016). Subsamples of manure (50 g) were taken for both cultivation and molecular analysis. Manure samples for molecular analysis were stored at -80 °C and manure samples for cultivation dependent analysis were analysed immediately. The grass and soil were sampled two weeks after the application of pig manure. Soil samples were obtained using a 10 cm hand corer, which was sterilised in between each pot. Six soil cores per pot were sampled and were placed in a sterile plastic bag and were homogenised on the outside of the bag by hand, resulting in one composite sample per pot. Grass samples (100 g per pot) were obtained with sterilised scissors and placed into individual bags. Samples for cultivation dependent analysis were analysed immediately and samples of soil (5 g) were stored at -80 °C for molecular analysis.

2.5.1.2 Sample Processing

2.5.1.2.1 Grass

Each grass sample (100 g) was placed into a sterile 500 ml centrifuge bottle containing 250 ml of sterile PBS (Section 2.3.2). The samples were then sonicated for 5 minutes to isolate the grass microbial biofilm using a modified method from Joyce *et al.*, (2018). Following sonication, the sonication liquid was passed through a sterile sieve to remove any large plant material and 10ml of the sonication liquid was filtered aseptically through a 0.2 μm nitrocellulose membrane (Sartorius, Merck). Using sterile forceps, the filter was then placed into a 50 ml falcon tube containing 20 ml of nutrient broth (Oxoid). The grass samples were then incubated at 37 °C in a shaker at 225 rpm (New Brunswick Scientific C25) for 24 hours for bacterial isolation. The remaining sonication liquid (~240 ml) was immediately frozen at -80 °C for molecular analysis.

2.5.1.2.2 Soil and Manure

One gram each of the manure and soil samples were added to 20 ml of nutrient broth and incubated for 24 hours at 37 °C and 225 rpm.

2.5.1.3 Bacterial Isolation from Manure, Grass and Soil Samples

Following the 24 hour enrichment step, the soil and manure samples were left to stand for 5 minutes to allow solid particles to settle. The enriched soil (n=6), manure (n=1) and grass (n=6) samples then underwent tenfold serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) in sterile PBS. For each enriched manure, grass and soil sample (13 samples in total), 100 μl was used to inoculate various selective agars supplemented with antibiotics. The selective agars included: SCA (Section 2.1.1.4) for the isolation of *Klebsiella* spp., PIA (Section 2.1.1.5) for the isolation of *Pseudomonas* spp., LAM (Section 2.1.1.3) for the isolation of *Acinetobacter* spp., and EMB Agar (Section 2.1.1.2) for the isolation of *Escherichia coli*. Agar plates were supplemented with each respective antibiotic at breakpoint

concentrations (EUCAST, 2018). The antibiotics used were imipenem (16 mg/L), cefotaxime (4 mg/L), colistin (4 mg/L) and ciprofloxacin (1 mg/L). Plates were incubated at 37 °C for 24 - 48 hours. Following incubation, presumptive colonies were picked, subcultured on LB agar (Section 2.1.1.6) and incubated overnight at 37 °C. A maximum of six colonies were picked per agar plate. Glycerol stocks of isolates were stored at -80 °C, as described in section 2.4.2.

2.5.2 Field Trial Set up

2.5.2.1 Field Layout

In the summer of 2019, a field trial was carried out in Johnstown Castle, Co. Wexford, Ireland (52.294117, -6.501027) (Figure 2.1). No animals had been grazed on the field for 7 months prior to the beginning of the trial. In the field, $1m^2$ plots were established in a randomised complete block design. Each plot had a 1.5m buffer zone to avoid cross contamination between treatments. Each plot was designated one of four treatments: untreated with manure (Control), pig manure (SM), cow manure (CM) and chicken manure (PM). Manures were collected from three separate farms and stored outside, out of direct sunlight, for three weeks before spreading. Four biological replicates of each treatment were analysed across 11 sampling points.

Information from each farm regarding antibiotic usage was collected. The chicken farm was a layer farmer and reported no antibiotic usage in the previous year. The pig manure was collected from weaner pigs. These weaner pigs were fed in-feed antibiotics that consisted of amoxicillin (β-lactam) and tilmicosin phosphate (macrolide) for a period of 10 days. Following this, the pigs had medicated feed consisting of amoxicillin and zinc oxide for a period of 10-12 days. Following this, the pigs had medicated feed supplemented with chlortetracycline hydrochloride (tetracycline) for a period of 8 – 10 days. The house from which pig slurry was collected had a mixture of weaners from all

stages of weaning, therefore the slurry pit consisted of slurry from all weaner stages. Dairy cow manure was collected from a slurry tank. Antibiotic usage data for these cows was available during the period they were housed and contributing to the slurry tank, from January 2019 – March 2019. No routing dosing was carried out during this time and animals were treated on an individual basis for digestive upset and mastitis. Antibiotics used during this time were as follows: amoxicillin (β-lactam), tylosin (macrolide), cefalexin monohydrate (β-lactam, cephalosporin), kanamycin monosuplhate (aminoglycoside) and marbofloxacin (fluoroquinolone).

2.5.2.2 Manure Spreading and Sampling Regime

The trial began on the 25th July 2019. Sampling details are listed in Table 2.2. Before manure spreading, grass on the trial area was trimmed to approximately 5 cm in length, mimicking grazing height. From the grass trimmed from the field trial site, three 200 g (fresh weight) biological replicates were taken as background samples (Timepoint BM). Four biological replicates were taken for the background soil samples (10 cm in depth) before manure was spread by collecting with a sterilised corer in a W shape throughout the field site (Timepoint BM). Samples were placed in a sterile plastic bag and homogenised by hand by massaging the outside of the sterile plastic bag. Before pig, cow and chicken manure were spread onto the plots, it was mixed to ensure it was homogenous. Three 200 ml biological samples were then taken of each manure type. Manure was spread on the 25th June 2019, at the start of the field trial. Manure was spread by hand, mimicking splash plate spreading by using a modified watering can (Brennan *et al.*, 2010). For the spreading of chicken manure, due to its pellet like consistency, it was spread by hand evenly over each plot. The amount of manure spread onto each plot was according to application rates outlined in the National Nitrates Action Programme (NAP) regulations (Teagasc Greenbook). Cow slurry was spread at an application rate of 34 t/ha,

pig slurry was applied at 40.5 t/ha and chicken manure was spread at an application rate of 15.5 t/ha.

Following manure spreading soil samples were collected at 10 additional timepoints (T0-T9) as described in Table 2.2. For grass samples a 150-200 g sub sample of grass were collected from each plot using an electric shears that was sterilised with 70 % ethanol in between each plot, resulting in 4 biological replicates for each treatment being collected each timepoint. For timepoint “T0”, directly following manure spreading, grass samples could not be obtained due to not enough material being present to sample. A subset of grass samples was reserved for dry matter (DM) analysis. For timepoints T0 – T9, soil samples were taken randomly in each plot using a sterile soil corer, until 30 individual cores were sampled per biological replicate. Soil cores for each biological replicate were placed in their own sterile plastic bags and homogenised by hand. All samples were stored on ice and transported to the research centre. Sub samples of manure and soil were dried for physiochemical analysis.

Table 2.2: Table displaying sampling timepoints completed during the field trial

Timepoint	Timepoint Description	Date Sampled
Background (BM)	Samples taken the day of manure spreading before any manure applied to the field.	25/06/19
T0	Immediately after manure spreading	25/06/19
T1	1 week following manure spreading	02/07/19
T2	2 weeks following manure spreading	10/07/19
T3	3 weeks following manure spreading	16/07/19
T4	4 weeks following manure spreading	22/07/19
T5	5 weeks following manure spreading	29/07/19
T6	6 weeks following manure spreading	06/08/19
T7	10 weeks following manure spreading	02/09/19
T8	14 weeks following manure spreading	02/10/19
T9	18 weeks following manure spreading	30/10/19

Figure 2.1: Field Trial location in Teagasc, Johnstown Castle.

2.5.2.3 Physiochemical Analysis of Soil and Manure Samples

All physiochemical analysis, except for dry matter (DM) analysis, was conducted by technical staff in Teagasc Environment, Soils and Land Use Research Centre according to in house standard operating procedures (SOPs). All results of physicochemical analysis are located in Appendix 1-5.

2.5.2.3.1 Dry Matter (DM) of Grass

The DM content of grass samples was measured by weighing a 50-100 g sample of grass before and after drying at 55 °C for 1 week.

2.5.2.3.2 Dry Matter of Soil and Manure and and Organic Matter (OM) of Soil.

The DM of soil and manure samples was determined by weighing 20 g of the samples before and after drying at 105 °C for 20 hours (Ashekuzzaman *et al.*, 2019). The organic matter of soils was then determined by ignition of the dried sample at 550 °C in a muffle furnace for 1 h.

2.5.2.3.3 Soil pH

Soil pH was determined by 1:2.5 (w/v) ratio of fresh soil to deionised water solution in an overall volume of 25 ml using a Jenway 3510 pH meter after 1 hour of shaking at 20 rpm.

2.5.2.3.4 Phosphorus, Potassium and Magnesium

Fresh soils were dried at 40 °C for 24-48 h and sieved (< 2mm). Soil underwent extraction with Morgans reagent, and the resulting filtrates were analysed on the Lachat system Lachat QuickChem 8500 series 2 (Dublin Analytical [LDT]).

2.5.2.3.5 Carbon and Nitrogen Analysis

The carbon and nitrogen content of soil and manures were analysed using ground samples by the high temperature combustion method using the LECO TrueSpec CN analyser.

2.5.2.3.6 Trace Element Analysis (Copper, Zinc, Calcium, Manganese, Sodium)

Soil and manure samples underwent analysis for Copper (Cu), Zinc (Zn), Calcium (Ca), Manganese (Mn) and Sodium (Na) analysis using the Agilent 5100 synchronous vertical dual-view inductively coupled plasma optical emission spectrometer (Agilent 5100 ICP-OES) following the microwave-assisted acid digestion method.

2.5.2.4 Sample Processing and Bacterial Isolation

Sample processing of grass, soil and manure samples were the same as in Section 2.5.1.2. Bacterial isolation methods were the same as section 2.5.1.3, except for the antibiotics used for selection. The antibiotics used were ciprofloxacin (1 mg/L), cefotaxime (4 mg/L), colistin (4 mg/L) and kanamycin (16 mg/L).

2.6 Culture Methods

2.6.1 MALDI-TOF identification of isolates

Isolates were sent to Dr. David Drissner's laboratory in Albstadt- Sigmaringen University for MALDI-TOF analysis. Bacterial isolates were prepared according to the following protocol. Colony material of pure cultures was transferred by direct smearing in duplicate onto spots of the MALDI-TOF mass spectrometry (MS) target (MTP ground steel, Bruker Daltonics) with tooth-picks. To the dried spots, 1μL matrix solution (10 mg α-cyano-4-hydroxycinnamic acid, Bruker Daltonics) dissolved in 1mL acetonitrile-water-trifluoroacetic acid (50 : 47.5 :2.5 (vol/vol/vol), Sigma-Aldrich) was added, and this solution was air-dried. Sample spectra were acquired using a microflex LT MALDI-TOF mass spectrometer (Bruker Daltonics) and the flexControl software (ver. 3.4; Bruker Daltonics). Spectra were classified using the Bruker Taxonomy main spectra database (MBT Compass ver. 4.1. with 8468 spectra present, Bruker Daltonics). Bacterial identification was reported to the species level if the score value was above 2.00 or to the genus level if the score was between 1.70 and 1.99. Any isolates that were not

successfully identified using by MALDI-TOF spectroscopy were identified using 16S rRNA PCR as described in Section 2.7.2.1.1.

2.6.2 Antibiotic Susceptibility Testing

A list of bacterial control strains used is listed in Table 2.3. Strains ATCC 25922, ATCC 27853, NCTC 13846 were according to EUCAST (2020) guidelines.

Table 2.3: Table of control strains used for antimicrobial susceptibility testing

Bacterial Species	Strain	Notes	Source
Escherichia coli	ATCC 25922	Control strain (Susceptible strain)	ATCC Collection
Pseudomonas aeruginosa	ATCC 27853	Control strain (Susceptible strain)	ATCC Collection
Escherichia coli	NCTC 13846	*mcr-1* positive strain (colistin resistant)	NCTC Collection
Klebsiella pneumoniae	NCTC 13439	$bla_{\text{VIM-1}}$ and *qnrS1* positive strain (carbapenem and quinolone resistant)	NCTC Collection
Klebsiella pneumoniae	NCTC 13443	$bla_{\text{NDM-1}}$ positive strain (carbapenem resistant)	NCTC Collection
Klebsiella pneumoniae	NCTC 13442	$bla_{\text{OXA-48}}$ positive strain (carbapenem resistant)	NCTC Collection
Klebsiella pneumoniae	NCTC 13438	$bla_{\text{KPC-3}}$ positive strain (carbapenem resistant)	NCTC Collection

2.6.2.1 Disk Diffusion Test

2.6.2.1.1 General resistance screening

Presumptive isolates from the pot trial mesocosm experiment (Section 2.5.1) were tested for resistance to cefotaxime (5 µg), imipenem (10 µg), amikacin (30 µg), kanamycin (30 µg), tetracycline (30 µg) and ciprofloxacin (5 µg) according to EUCAST (2018) guidelines. As there are no EUCAST breakpoints for kanamycin and tetracycline CLSI (2018) guidelines and breakpoints were used. All antibiotic disks were purchased from Oxoid.

Field trial isolates that were confirmed by MALDI-TOF MS (Section 2.6.1) as *E. coli, K. pneumoniae* or *Acinetobacter* spp. underwent antimicrobial susceptibility testing using the disk diffusion method (EUCAST, 2020) (CLSI, 2020). All antibiotic disks were purchased from Oxoid.

E. coli and *K. pneumoniae* were tested for resistance to cefotaxime (5 µg), imipenem (10 µg), amikacin (30 µg), kanamycin (30 µg), tetracycline (30 µg) and ciprofloxacin (5 µg). All antibiotics except kanamycin and tetracycline were according to EUCAST (2020) guidelines for the Enterobacterales. As there are no EUCAST breakpoints for kanamycin and tetracycline CLSI (2020) guidelines and breakpoints were used.

Acinetobacter spp. were tested for resistance to imipenem (10 µg), amikacin (30 µg), kanamycin (30 µg), ciprofloxacin (5 µg), ceftazidime (30 µg) and tigecycline (15 µg). Imipenem, amikacin, kanamycin and ciprofloxacin testing was according to EUCAST (2020) guidelines for *Acinoetbacter* spp. Ceftazidime testing was according to CLSI (2020) guidelines for *Acinetobacter* spp. as there were no breakpoints for these in the EUCAST guidelines. Tigecycline breakpoints were from CLSI (2020) for Enterobacterales, due to the lack of breakpoint guidelines for *Acinetobacter* spp. in EUCAST (2020) and CLSI (2020).

2.6.2.1.2 Extended Spectrum β-lactamase (ESBL) phenotypic identification

To screen for ESBL production third generation cephalosporin resistant isolates underwent combination disk test (CDT) according to EUCAST guidelines (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Miscellaneous/Guidance_document_Confirmation_of_ESBL.pdf). Briefly, overnight cultures of isolates were diluted to 0.5 McFarland concentration and 100 µl of cultures were spread on MH agar plates. Aseptically, two ceftazidime (10 µg) disks were added to the agar plate, at least 10 mm apart and 10 µl potassium clavulanate (1 µg/ ml) (Section 2.3.4) was added to one of the disks. Plates were incubated overnight at 37 °C. An increase in the zone of inhibition of $\geq$ 5 mm indicated ESBL production (Watt, Louie and Simor, 2000).

2.6.2.1.3 AmpC β-lactamase phenotypic identification

For the detection of AmpC β-Lactamases the AmpC Disk test as described by (Gupta, Tak and Mathur, 2014), was performed on third generation cephalosporin (cefotaxime or ceftazidime) resistant isolates. Overnight cultures of isolates were diluted to 0.5 McFarland concentration and 100 µl of cultures were spread on Muller Hinton (MH) agar plates. Aspetically, two cefoxitin (30 µg) disks were added to the agar plate at least 10 mm apart and 300 µg of phenylboronic acid (Section 2.3.5) was added to one of the disks. Plates were incubated overnight at 37 °C. An increase in the zone of inhibition of $\geq$5 mm indicated AmpC production.

2.6.2.1.4 Metallo-β-lactamase (MBL) disk testing

For the detection of metallo-β-lactamase (MBL) production, imipenem resistant isolates underwent imipenem-EDTA double disk synergy testing (Sachdeva, Sharma and Sharma, 2017). Overnight cultures of isolates were diluted to 0.5 McFarland concentration and 100 µl of cultures were spread on MH agar plates. Aseptically, two imipenem (10 µg)

disks were added to the agar plate at least 10 mm apart and 10 µl of 0.5M EDTA (Section 2.3.3) solution added to one of the disks. Plates were incubated overnight at 37 °C. An increase in the zone of inhibition of ≥ 7mm indicated MBL production.

2.6.2.2 Broth microdilution

Isolates that were resistant to imipenem, or were selected on colistin, underwent minimum inhibitory concentration determination by serial broth microdilution according to EUCAST (2020) guidelines. Briefly, in a sterile 96 well plate, a starting concentration of 128 mg/L colistin was used and was serially diluted two-fold to a final concentration of 0.25 mg/L. The remaining two columns were used as a positive control for bacterial growth (no antibiotic) and a negative control (no bacteria added). MH^{2+} broth (Section 2.1.2.2) was used for diluting the antibiotic and the cultures. Overnight cultures were diluted to 0.5M McFarland concentration and 5 µl of culture was used to inoculate each plate. Each isolate was performed in duplicate. The plates were incubated overnight at 37 °C and the lowest concentration at which there was no visible growth by eye was determined as the MIC of the sample. Only results that were identical for each duplicate were accepted. Any samples that had differing duplicate results were repeated until results were obtained.

2.7 Molecular Methods

2.7.1 DNA Extraction Methods

2.7.1.1 Pot Trial

DNA was extracted from a composite manure sample, six soil samples and six grass samples. The six soil and grass samples were taken from a composite sample from each biological replicate. The purity of the extracted DNA was analysed using the Nanodrop spectrophotometer (DeNovix DS-11) and the concentration of the DNA was assessed

using the Qubit High Sensitivity (HS) assay (ThermoFisher) and the Qubit 1.0 fluorometer (ThermoFisher).

2.7.1.1.1 Soil and Manure

Prior to DNA extraction the manure sample was centrifuged at 16,000 x g for 3 minutes, due to the high water content of the manure. The supernatant was removed and the resulting pellet was used for DNA extractions. DNA was extracted from 0.25 g of soil and 0.25 g of the manure pellet using the DNeasy PowerSoil Kit (Qiagen) according to the manufacturer's instructions.

2.7.1.1.2 Grass

For the extraction of DNA from grass, 200 ml of sonication liquid (Section 2.5.1.2.1) from each biological replicate was filtered through 0.2 μm sterile polycarbonate membranes (Whatman, USA). DNA extraction from the resulting filters was performed using the DNeasy PowerWater kit (Qiagen) according to the manufacturer's instructions.

2.7.1.2 Field Trial

DNA was extracted from three biological replicates for soil and manure samples, resulting in 164 and 12 samples respectively. For grass samples, due to low DNA concentrations obtained, biological replicates of grass samples were pooled and DNA extracted from the composite samples, resulting in 37 grass samples. The purity of the extracted DNA was analysed using the Nanodrop spectrophotometer (DeNovix DS-11) and the concentration of the DNA was assessed using the Qubit Broad Range (BR) assay (ThermoFisher) and the Qubit 1.0 fluorometer (ThermoFisher).

2.7.1.2.1 Soil and Manure

DNA was extracted from 0.25 g of soil and 0.25 g of the manure using the DNeasy PowerSoil Kit (Qiagen) according to the manufacturer's instructions.

2.7.1.2.2 Grass

Composite samples were made by pooling 200 ml of sonication liquid from three biological replicates leading to a total of 600 ml of sonication liquid filtered through a 0.2 µm sterile polycarbonate membranes (Whatman, USA). DNA extraction from the resulting filters was performed using the DNeasy PowerWater kit (Qiagen) according to the manufacturer's instructions.

2.7.1.2.3 Bacteria

Bacterial total DNA was extracted from isolates successfully identified as *E. coli* (46 isolates) or *A. baumanii* (69 isolates). Total bacterial DNA was isolated from bacterial overnight cultures using the NucleoSpin Microbial DNA Mini kit for DNA from microorganisms (Machery Nagel) according to manufacturer's instructions.

2.7.2 PCR

2.7.2.1 Colony PCR

To prepare bacterial isolates for PCR, a single colony was suspended in 50 µl double filtered Milli-Q water in a sterile 0.25 ml PCR tube. The colony suspension was then boiled in a Thermocycler (Eppendorf Mastercycler gradient) at 95 °C for 10 minutes.

2.7.2.1.1 Bacterial Identification (16S rRNA PCR)

For bacterial identification, a 1465p fragment of the 16S rRNA gene was amplified by PCR by targeting nucleotide 27 to 1492 of the 16S rRNA gene. The fragment was amplified using the primer set 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) (Weisburg *et al.,* 1991; Heuer *et al.,* 1997). Thermocycling conditions were as follows: 4 min at 96 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 57 °C and 1 min at 72 °C, and a final extension step at 72 °C for 10 min (dos Santos *et al.,* 2019). Amplicons were purified using CleanNA beads and underwent Sanger sequencing in the forward direction (Eurofins Genomics). Resulting sequences

were characterised using BLASTn (http://www.ncbi.nlm.nih.gov/BLAST/). Sequences with ≥ 97% identity were accepted at genus level and samples with ≥ 99% identity were accepted at species level (Woo *et al.*, 2008; Sabat *et al.*, 2017).

2.7.2.1.2 Targeted PCRs

Targeted PCR for resistance genes was carried out on *Lactococcus* spp. (three isolates), *Alcaligenes* spp. (one isolate), *Hafnia paralvei* (two isolates), *Aeromonas* spp. (one isolate) and *Stenotrophomonas maltophilia* (one isolate) from the pot trial work. From field trial work, targeted PCRs were carried out on resistant isolates that whole genome sequence (WGS) data was not available. This resulted in the analysis of one *Klebsiella pneumoniae* isolate, one *Acinetobacter indicus* and two *Acinetobacter calcoaceticus* isolates.

2.7.2.1.2.1 Aminoglycoside Resistance

Isolates that were phenotypically resistant to amikacin or kanamycin were analysed using a multiplex PCR for the presence of 16S rRNA methylase genes (Doi and Arakawa, 2007; Taylor *et al.*, 2018) (Table 2.4) and for other aminoglycoside resistance genes (Table 2.5) (Vakulenko *et al.*, 2003; Dec *et al.*, 2017).

Table 2.4: Primer sets and thermocycling conditions for 16S rRNA methylase aminoglycoside resistance genes.

Multiplex Group	Target Gene	Amplicon Size	Primer Sequences (5' - 3')	Thermocycling Conditions
1	*armA*	269 bp	(f) AAAGTACAATCAGGGGCAGTT (r) TCGTCGTCTTTAACTTCCCAA	Initial denaturation at 96 °C for 5 min; followed by 30 cycles of 96 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; followed by a final elongation step at 72 °C for 5 min.
	rmtA	634 bp	(f) CTAGCGTCCATCCTTTCCTC (r) TTGCTTCCATGCCCTTGCC	
	rmtB	173 bp	(f) GCTTTCTGCGGGCGATGTAA (r) ATGCAATGCCGCGCTCGTAT	
	rmtC	711 bp	(f) CGAAGAAGTAACAGCCAAAG (r) ATCCCAACATCTCTCCCACT	
	rmtD	401 bp	(f) CGGCACGCGATTGGGAAGC (r) CGGAAACGATGCGACGAT	
2	*rmtE*	518 bp	(f) TGGTTGCAGAGGTTCTGTCGAGC (r) CGGCGTAACAGACACGGCATCA	Initial denaturation at 96 °C for 5 min; followed by 30 cycles of 96 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min;

Multiplex Group	Target Gene	Amplicon Size	Primer Sequences (5’ - 3’)	Thermocycling Conditions
	rmtF	338 bp	(f) ATTCATCTGGGCTGCGTGCGAC (r) ATTCATCTGGGCTGCGTGCGAC	followed by a final elongation step at 72 °C for 5 min.
	rmtG	420 bp	(f) CGTGTATGCGCGTCTGTTGGGT (r) ACGGTGCGTTCGATTCGCCATT	
	rmtH	259 bp	(f) ACAAAAAGCCCAAGCAGGCGGT (r) CGGTGCAGCATCAGCGGGTTTA	
	npmA	195 bp	(f) GGTCAGTTTGATCGTGTGCA (r) AGCTGCAATAACAAACACCACA	

Table 2.5: Primer sequences and thermocycling conditions for non – 16S methylase aminoglycoside resistance genes

Target Gene	Amplicon Size	Primer Sequences (5’ - 3’)	Annealing Temperature	Thermocycling Conditions
aac(6’)-Ie-aph(2″)-Ia	348	(f) CAG AGC CTT GGG AAG ATG AAG (r) CCT CGT GTA ATT CAT GTT CTG GC	57℃	Initial denaturation at 94 ℃ for 5 min, 30 cycles of 94 ℃ for 45 s, 50–64 ℃ (according to the annealing temperature for the individual primers) for 45 s, 72 ℃ for 75 s and a final extension step at 72 ℃ for 8 minutes.
aph3IIIa	523	(f) GGC TAA AAT GAG AAT ATC ACC GG (r) CTT TAA AAA ATC ATA CAG CTC GCG		
ant(4’)-Ia	295	(f) CAA ACT GCT AAA TCG GTA GAA GCC (r) GGA AAG TTG ACC AGA CAT TAC GAA CT		
aph(2″)-Ic	444	(f) CCA CAA TGA TAA TGA CTC AGT TCC C (r) CCA CAG CTT CCG ATA GCA AGA G		
aph(2″)-Id	641	(f) GTG GTT TTT ACA GGA ATG CCA TC	57℃	Initial denaturation at 94 ℃ for 5 min, 30 cycles of 94 ℃

Target Gene	Amplicon Size	Primer Sequences (5’ - 3’)	Annealing Temperature	Thermocycling Conditions
		(r) CCC TCT TCA TAC CAA TCC ATA TAA CC		for 45 s, 50–64 °C (according to the annealing temperature for the individual primers) for 45 s, 72 °C for 75 s and a final extension step at 72 °C for 8 minutes
ant(6)-Ia	563	(f) CGG GAG AAT GGG AGA CTT TG (r) CTG TGG CTC CAC AAT CTG AT	56°C	
aac(6’)-Ii	410	(f) TGGCCGGAAGAATATGGAGA (r) GCATTTGGTAAGACACCTACG	55°C	
aadA	282	(f) ATC CTT CGG CGC GAT TTT G (r) GCA GCG CAA TGA CAT TCT TG		
aadE	1100	(f) ATG GAA TTA TTC CCA CCT GA (r) TCA AAA CCC CTA TTA AAG CC	51°C	

2.7.2.1.2.2 Fluoroquinolone Resistance

Isolates that were phenotypically resistant to ciprofloxacin underwent multiplex PCR for plasmid mediated fluoroquinolone resistance genes, as shown in Table 2.6 (Ciesielczuk *et al.*, 2013). Singleplex PCR for the QRDR of the *gyrA* and *parC* genes were carried out on *Acinetobacter* spp. and *Lactoccoccus* spp. according to the primer sets listed in Table 2.7.

Table 2.6: Primer sets and thermocycling conditions for plasmid mediated fluoroquinolone resistance gene PCRs.

Target Gene	Amplicon Size	Primer Sequences (5' – 3')	Thermocycling Conditions
qnrA	630 bp	(f) CAGCAAGAGGATTTCTCACG (r) AATCCGGCAGCACTATTACTC	Initial denaturation at 95 °C for 15 min; 30 cycles of 94 °C for 30 s, optimized annealing temperature for 90 s and 72 °C for 90 s; followed by a final extension at 72 °C for 10 min.
*qnr*B	488 bp	(f) GGCTGTCAGTTCTATGATCG (r) GAGCAACGATGCCTGGTAG	
qnrS	428 bp	(f) GCAAGTTCATTGAACAGGGT (r) TCTAAACCGTCGAGTTCGGCG	
qepA	218 bp	(f) GCAGGTCCAGCAGCGGGTAG (r) CTTCCTGCCCGAGTATCGTG	
aac(6')-Ib-cr	260 bp	(f)TTGGAAGCGGGGACGGAM (r)ACACGGCTGGACCATA	

Table 2.7: Primer sets and thermocycling conditions for mutations in the QRDR gyrA and parC genes for Acinetobacter spp. *and Lactococcus* spp.

Target Gene	Amplicon Size	Primer Sequences (5' – 3')	Thermocycling Conditions	Reference
QRDR *gyrA Acinetobacter* spp.	343 bp	(f) AAATCTGCCCGTGTCGTTGGT (r) GCCATACCTACGGCGATACC	30 cycles at 94℃ for 1 min, 58℃ for 1 min, and 72℃ for 1 min	(Vila *et al.*, 1997)
QRDR *parC Acinetobacter* spp.	327 bp	(f) AAACCTGTTCAGCGCCGCATT (r) AAAGTTGTCTTGCCATTCACT	30 cycles at 94 ℃ for 1 min, 59 ℃ for 1 min, and 72 ℃ for 1 min	(Vila *et al.*, 1997)
QRDR *gyrA Lactococcus* spp.	208 bp	(f) GTACAACGCCTGATAAGCC (r) TGCTTCGGTATAACGTTGAG	One cycle of denaturation at 95 ℃ for 5 min, followed by 30 cycles of annealing at 95 ℃ for 30 s, 55 ℃ for 30 s and 72 ℃ for 1 min, and a final cycle extension at 72 ℃ for 5 min.	(Maki *et al.*, 2008)
QRDR parC Lactococcus spp.	212 bp	(f) AAGGATGGGAATACTTTTGA (r) TTCGGTATAACGCATAGCA		

2.7.2.1.2.3 Tetracycline Resistance

Isolates that were phenotypically identified as tetracycline resistant were tested by PCR for the following tetracycline resistance *genes: tetA, tetB, tetJ, tetM, tetO, tetW, tet32, tetL, tetQ, tetX, tet40, tet44.* Primer sequences and amplicon sizes are detailed in Table 2.8 and 2.9.

Table 2.8: Primers and thermocycling conditions for tetL, tetO and tetX PCR.

Target Gene	Amplicon Size	Primer Sequences (5' – 3')	Thermocycling Conditions	Reference
tetL	229	(f) TGGTGGAATGATAGCCCATT (r) CAGGAATGACAGCACGCTAA	3 min at 93 °C, 30 cycles of 1 min of denaturation at 93 °C, 1 min of annealing at 62 °C, and 4 min of extension at 65 °C, followed by one cycle of 3 min of elongation at 65 °C.	(Malhotra-Kumar *et al.*, 2005)
tetQ	904	(f) TTATACTTCCTCCGGCATCG (r) ATCGGTTCGAGAATGTCCAC	Initial denaturation for 5 mins at 94 °C followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min.	
tetX	468	(f) CAATAATTGGTGGTGGACCC (r) TTCTTACCTTGGACATCCCG		

Table 2.9: Primer sequences and thermocycling conditions for tetA, tetB, tetJ, tetM, tetO, tetW, tet32, tet40, tet44.

Target Gene	Amplicon Size	Primer Sequences (5' – 3')	Annealing Temperature	Thermocycling Conditions	Reference
tetA	164 bp	(f) GCGCGATCTGGTTCACTCG (r) AGTCGACAGYRGCGCCGGC	61°C	Initial denaturation at 94 °C for 5 min, followed by cycling at 94° for 30 s, 30 s of annealing temperature for primer (Table 8), 30 s of extension at 72 °C, with final extension at 72 °C for 7 min	(Aminov *et al.*, 2004)
tetB	206 bp	(f) TACGTGAATTTATTGCTTCGG (r) ATACAGCATCCAAAGCGCAC			
tetJ	184 bp	(f) CGAAAACAGACTCGCCAATC (r) TCCATAATGAGGTGGGGC			
tetM	171 bp	(f) ACAGAAAGCTTATTATATAAC (r) TGGCGTGTCTATGATGTTCAC	55°C		
tetO	171bp	(f) ACGGARAGTTTATTGTATACC (r) TGGCGTATCTATAATGTTGAC	60°C		
tetW	168	(f) GAGAGCCTGCTATATGCCAGC (r) GGGCGTATCCACAATGTTAAC	64°C		
tet32	277	(f) TCGACCTACAGCGTGTTTACC	62°C		

Target Gene	Amplicon Size	Primer Sequences (5’ – 3’)	Annealing Temperature	Thermocycling Conditions	Reference
		(r) CTAATAGTTCATCGCTTCCGG			
tet40	446	(f) CGGAGGAAGAGGACAAACCC (r) TAAGCCGCTGCCGATAAGAC	56 ℃	Initial denaturation at 94 ℃ for 4 min; 35 cycles of 94 ℃ for 5 s, different annealing temperatures (listed in Table 1) for 45 s, 72 ℃ for 1 min; final extension at 72 ℃ for 6 min.	(Kang *et al.*, 2018)
tet44	1927	(f) AAAATAATCAACATTGGTATTCTTGCTCA (r)TAGTAACTTAATTTTCTTTTTTATTAAACATATGGCG	56℃		

2.7.2.1.2.4 Third Generation Cephalosporin Resistance and ESBL

Isolates that were phenotypically resistant to a third generation cephalosporin (cefotaxime or ceftazidime) underwent PCR for ESBL genes. Isolates were tested for *bla*$_{CTX-M}$ (Group 1, 2, 8, 9 and 25) (Woodford, Fagan and Ellington, 2006) *bla*$_{TEM}$ and *bla*$_{SHV}$ (Dallenne *et al.*, 2010). Primer sequence and amplicon sizes are detailed in Table 2.10.

Table 2.10: Primer sets and thermocycling conditions for ESBL PCR

Target Gene	Amplicon Size	Primer Sequences (5' – 3')	Thermocycling Conditions
bla$_{CTX-M}$ Group 1	425 bp	(f) AAA AAT CAC TGC GCC AGT TC (r) AGC TTA TTC ATC GCC ACG TT	Initial denaturation at 94 °C for 5 min; 30 cycles of 94 °C for 25 s, 52 °C for 40 s and 72 °C for 50 s; and a final elongation at 72 °C for 6 min.
bla$_{CTX-M}$ Group 2	552 bp	(f) CGA CGC TAC CCC TGC TAT T (r) CCA GCG TCA GAT TTT TCA GG	
bla$_{CTX-M}$ Group 8	666 bp	(f) TCG CGT TAA GCG GAT GAT GC (r) AAC CCA CGA TGT GGG TAG C	
bla$_{CTX-M}$ Group 9	205 bp	(f) CAA AGA GAG TGC AAC GGA TG (r) ATT GGA AAG CGT TCA TCA CC	
bla$_{CTX-M}$ Group 25	327 bp	(f) GCA CGA TGA CAT TCG GG (r) AAC CCA CGA TGT GGG TAG C	
bla$_{TEM}$	800 bp	(f) CATTTCCGTGTCGCCCTTATTC (r) CGTTCATCCATAGTTGCCTGAC	Initial denaturation step of 10 min at 94 °C followed by 30 cycles of 40 s at 94 °C, 40 s at 60 °C and 1min at 72 °C final extension for 7 min at 72 °C.
bla$_{SHV}$	713 bp	(f) AGCCGCTTGAGCAAATTAAAC (r) ATCCCGCAGATAAATCACCAC	

2.7.2.1.2.5 Carbapenem Resistance

Isolates that were phenotypically identified as imipenem resistant underwent multiplex PCR for the following carbapenemases: *bla*$_{GES,}$ *bla*$_{GIM}$, *bla*$_{IMI}$, *bla*$_{IMP}$, *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-23}$, *bla*$_{OXA-40}$, *bla*$_{OXA-48}$, *bla*$_{OXA-51}$, *bla*$_{OXA-58}$, *bla*$_{VIM}$ (Table 2.11) (Cerezales *et al.*, 2021).

Table 2.11: Primer sets and thermocycling details for carbapenemase multiplex PCR

Multiplex Group	Target Gene	Amplicon Size	Primer Sequences 5' – 3')	Thermocycling Conditions
Group 1	*VIM*	202 bp	(f) GATGGTGTTTGGTCGCATATC (r) CGTCATGAAAGTGCGTGGAG	Initial denaturation step of 3 min at 94 °C followed by 30 cycles of 30 sec at 94 °C 15 sec at 58 °C 1 min at 72 °C final extension for 5 min at 72
	OXA-48	611 bp	(f) GGTAGCAAAGGAATGGCAAGAA (r) CGACCCACCAGCCAATCTTA	
	OXA-23	718 bp	(f) TCTGGTTGTACGGTTCAGCA (r) GCATTTCTGACCGCATTTCC	
	KPC	312 bp	(f) CGCCAATTTGTTGCTGAAGG (r) CAGGTTCCGGTTTTGTCTCC	
	NDM	517 bp	(f) GTTTGATCGTCAGGGATGGC (r) CTCATCACGATCATGCTGGC	
	OXA-40	413 bp	(f) AGTTTCTCTCAGTGCATGTTCA (r) CCCGCTTTACTTCTTTCTGCA	
Group 2	*OXA-58*	303 bp	(f) ATCAAGAATTGGCACGTCGT (r) CCACATACCAACCCACTTGC	
	IMP	587 bp	f) GAAGGCGTTTATGTTCATAC	

Multiplex Group	Target Gene	Amplicon Size	Primer Sequences 5' – 3')	Thermocycling Conditions
Group 2			(r) GTACGTTTCAAGAGTGATGC	
	GIM	508 bp	(f) TTATCCTGGGCGACTGACAG (r) CAGCGGTCGGTTGCATTAAT	
	GES	416 bp	(f) CTCAGATCGGTGTTGCGATC (r) TGTATCTCTGAGGTCGCCAG	
	OXA-51	704 bp	(f) TGTGGTAAGCACTTGATGGG (r) ATTGCCATAACCAACACGCT	
	IMI	206 bp	(f) AGACTCGATCGTTGGGAGTT (r) CAATCGCTTGGTACGCTAGC	

2.7.2.1.2.6 Colistin Resistance

Isolates that were identified as colistin resistant from broth microdilution assays (Section 2.6.2.4) underwent PCR for plasmid mediated colistin resistance genes. Isolates underwent multiplex PCR for the genes: *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4* and *mcr-5* (Rebelo *et al.*, 2018) and multiplex PCR for the genes *mcr-6*, *mcr-7*, *mcr-8 and mcr-9* (Table 10) (Borowiak *et al.*, 2020). Primer sequence and amplicon sizes are detailed in Table 2.12.

Table 2.12: Primer sets and thermocycling conditions for multiplex PCR mcr 1-5 genes

Multiplex Group	Target Gene	Amplicon Size	Primer Sequences (5' – 3')	Thermocycling Conditions
1	*mcr-1*	320	(f) AGTCCGTTTGTTCTTGTGGC (r) AGATCCTTGGTCTCGGCTTG	Initial denaturation at 94 °C for 15 min, followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 90 s and elongation at 72 °C for 60 s, and a final cycle of elongation at 72 °C for 10 min.
	mcr-2	715	(f) CAAGTGTGTTGGTCGCAGTT (r) TCTAGCCCGACAAGCATACC	
	mcr-3	929	(f) AAATAAAAATTGTTCCGCTTATG (r) AATGGAGATCCCCGTTTTT	
	mcr-4	1116	(f) TCACTTTCATCACTGCGTTG (r) TTGGTCCATGACTACCAATG	
	mcr-5	1644	(f) ATGCGGTTGTCTGCATTTATC	

Multiplex Group	Target Gene	Amplicon Size	Primer Sequences (5’ – 3’)	Thermocycling Conditions
			(r) TCATTGTGGTTGTCCTTTTCTG	
2	*mcr-6*	252	(f) AGCTATGTCAATCCCGTGAT (r) ATTGGCTAGGTTGTCAATC	The PCR conditions were optimized as follows: Initial denaturation for 3 min at 95 °C, 30 cycles denaturation for 30 s at 95 °C, primer annealing for 30 s at 55 °C and elongation for 60 s at 72 °C followed by a final elongation step for 10 min at 72 °C.
	mcr-7	551	(f) GCCCTTCTTTTCGTTGTT (r) GGTTGGTCTCTTTCTCGT	
	mcr-8	856	(f) TCAACAATTCTACAAAGCGTG (r) AATGCTGCGCGAATGAAG	
	mcr-9	1011	(f) TTCCCTTTGTTCTGGTTG (r) GCAGGTAATAAGTCGGTC	

2.7.3 HT-qPCR

2.7.3.1 Sample Preparation

DNA was extracted as described in Section 2.7.1. DNA was normalised to 10 ng/µl in 100 µl of double filtered Milli-Q water. Normalised DNA was sent to Resistomap (Finland) for analysis using a HT-qPCR array. The HT-qPCR array was composed of 216 genes, comprising 2 primers for the 16S rRNA gene (AY1, AY600), 28 primers for MGEs/integrons, 3 taxonomic genes and 183 ARGs (Appendix 6). DNA from composite grass samples from 7 timepoints, soil samples from 9 timepoints and composite manure samples were analysed (Appendix 7). Two no template controls (NTC) and one positive control (pEK499) were also included in the array.

2.7.3.2 Data analysis

2.7.3.2.1 Data clean up

Data analysis was performed using RStudio (v4.0.2). Gene amplifications outside the range of 1.75-2.2 were removed from the dataset. The cycle threshold (Ct) value of 27 was used as the detection limit and any gene amplifications with a Ct value greater than 27 were removed from the dataset. Additionally, any gene amplifications that had less than two of the three technical replicates were removed from the dataset.

Following these filtering steps, the dataset was inspected for potential false positives, by comparison with results for the negative and positive controls. The primer set *orf37-IS26* (AY307) and *tetPA* (AY575) were detected in the negative control samples. The positive results for these genes in the samples were processed as described previously (Muurinen *et al.*, 2017). Briefly, Ct values in samples that were higher than the negative controls were determined false positives and were removed from the dataset. For *tetPA* this resulted in removal of 20 gene results and 4 gene results for *orf-IS26*. For any positive

genes remaining that had a lower Ct value than AY307 and AY575, the Ct values were modified using the following formula:

$$Step\ 1: Ct\ value\ of\ false\ positive\ genes - Ct\ value\ of\ samples$$

$$Step\ 2: New\ Ct\ value\ for\ samples = Ct\ threshold\ cut\ off\ (27) - values\ from\ Step\ 1$$

In the positive control, 82 genes were detected and these positive genes were cross checked with the known genes in pEK499 (Woodford *et al.*, 2009) to determine if any genes detected were false positives. Of the 82 genes detected, 46 were not in pEK499 and were assigned as false positives. These false positive genes were processed the same as the negative control false positive results. The removal of false positive genes resulted in the removal of 776 data points.

2.7.3.2.2 Comparative Delta Delta Ct Method

The remaining HT-qPCR results were processed by the Comparative Delta Delta Ct method (Schmittgen and Livak, 2008). Delta Ct, Delta Delta Ct, gene relative abundance and fold change was calculated according to the following equations:

$$\Delta Ct = Ct(ARG/MGE) - Ct(16S)$$

$$\Delta\Delta Ct = \Delta Ct(Treated\ Sample) - \Delta Ct(Reference\ Sample)$$

$$Relative\ Abudance = 2^{-\Delta Ct}$$

For the calculation of delta Ct values, the values from the 16S rRNA primers "16S1 and "16S2" were used. The Ct values for the 16S rRNA primer "16S1" were used for calculations except in samples "SS.T0" and "SP.T3" where the 16S rRNA primer "16S2" values were used due to poor results efficiency and Ct values obtained for "16S1".

2.7.3.2.3 Statistical Analysis and Visualisations

Plots were constructed using the packages ggplot2 (v.3.3.5) (Wickham, 2016) and ggpubr (0.4.0) (Kassambara, 2020). Dissimilarity matrices and non-metric multidimensional scaling (NMDS) ordination were carried out on gene relative abundances vegdist and metaMDS functions, respectively, using the vegan package (2.5-7) (Oksanen, 2020). Statistical testing of relative abundances was conducted using the package stats (v4.1.0) (R Core Team, 2021). Shapiro wilk normality testing was used to assess normality of the data. Kruskal Wallis testing and Dunn test were used to identify significant differences in gene relative abundances. To determine the relationship between the microbiome and resistome, the mantel test and Procrustes analysis using Spearmans rank correlations were performed using the *mantel* function and the *procrustes* and *protest* functions respectively from the vegan package (v2.5-7) (Oksanen, 2020).

2.7.4 16S rRNA microbiome analysis

2.7.4.1 Pot Trial

2.7.4.1.1 Sample preparation

The extracted DNA from one manure sample, six soil samples and six grass samples were used for the microbiome analysis. A negative control sample (NTC) and a mock community standard (ZymoBIOMICS) were included during each sequencing run. The V4 region of the 16S rRNA gene was amplified using the primer set 515F (GTGCCAGCMGCCGCGGTAA) - 806R (GGACTACHVGGGTWTCTAAT) (Caporaso *et al.*, 2011). The amplicons were sent to the Centre for Genomic Research in the University of Liverpool for library preparation according to in house protocols. The prepared libraries underwent 2 x 250bp paired end sequencing on the Illumina Miseq. https://support.illumina.com/documents/documentation/chemistry_documentation/16S/16S-metagenomic-library-prep-guide-15044223-b.pdf). Primers were removed from

sequences using cutadapt through the Galaxy platform (Galaxy Version 1.16.5) (Martin, 2011). Sequences were processed using mothur through the Galaxy platform (Schloss *et al.*, 2009; Hiltemann *et al.*, 2018).

2.7.4.1.2 Data Analysis and Visualisation

Data visualisation and statistical analysis was performed using R (v4.0.2) (RStudio Team, 2020). Data cleaning and rarefying was carried out in phyloseq (v1.36.0) (McMurdie and Holmes, 2013). Alpha diversity indices and relative phyla abundance were determined using phyloseq (v1.36.0) (McMurdie and Holmes, 2013) and statistical tests were performed using the package stats (v4.1.0) (R Core Team, 2021) using Benjamini-Hochberg corrected p-values. Sequence summaries were produced using the microbiome package (v.14.0) (Lahti *et al,* 2012-2019). Plots were constructed using the packages ggplot2 (v.3.3.5) (Wickham, 2016) and ggpubr (0.4.0) (Kassambara, 2020). Rarefaction curves were produced using the vegan package (2.5-7). Differential OTU testing was conducted using DESeq2 package (v1.32.0) (Love *et al*, 2014). An adjusted pvalue of <0.05 with Benjamini-Hochberg correction for false discovery rate (FDR) was deemed a significant result.

2.7.4.2 Field Trial

2.7.4.2.1 Library Preparation and Sequence Processing

16S rRNA amplicon sequencing was performed on extracted DNA from manure, soil and grass. For manure and soil samples three biological replicates were used. For grass samples, composite samples, as described above, were used. In total there were 3 chicken manure samples, 3 pig manure samples, 3 cow manure samples, 93 soil samples and 37 grass samples. A negative control sample (NTC) and mock community DNA Standard (ZymoBIOMICS) were included during each sequencing run to determine the sequencing error for each plate. The sequencing error rate for Plate 1 was 1.76051×10^{-4} and Plate 2

was: 7.67098 x 10^{-5}. The V4 region of the 16S gene was amplified using the primer set 515F (GTGCCAGCMGCCGCGGTAA) - 806R (GGACTACHVGGGTWTCTAAT) (Caporaso *et al.*, 2011). The prepared libraries were sent to the Teagasc Food Research Centre, Moorepark for 2 x 250bp paired end sequencing was performed according to standard Illumina protocols on the Illumina Miseq V2 (500 cycles) (https://support.illumina.com/documents/documentation/chemistry_documentation/16S/16S-metagenomic-library-prep-guide-15044223-b.pdf). Primers were removed from sequences using cutadapt through the Galaxy platform (Galaxy Version 1.16.5) (Martin, 2011). Sequences were processed using mothur through the Galaxy platform (Schloss *et al.*, 2009; Hiltemann *et al.*, 2018).

2.7.4.2.2 Data analysis and Visualisation

Data visualisation and statistical analysis was performed using R (v4.0.2) (RStudio Team, 2020). Data cleaning and rarefying was carried out in phyloseq (v1.36.0) (McMurdie and Holmes, 2013). Alpha diversity indices and relative phyla abundance were determined using phyloseq (v1.36.0) (McMurdie and Holmes, 2013) and statistical tests were performed using the package stats (v4.1.0) (R Core Team, 2021). Sequence summaries were produced using the microbiome package (v.14.0) (Lahti *et al*, 2012-2019). Plots were constructed using the packages ggplot (v.3.3.5) (Wickham, 2016), ggpubr (v0.4.0) (Kassambara, 2020) and ggforce (v0.3.2.9) (Pedersen, 2021). Rarefaction curves were produced using the vegan package (v2.5-7) (Oksanen, 2020). Dissimilarity matrices and NMDS ordination were carried out on Hellinger-transformed rarefied OTU relative abundances with singletons removed using the *vegdist* and *metaMDS* functions from the vegan package, respectively. Homogenity of variance testing was performed on dissimilarity metrices using the betadisper and permutest functions in vegan package (2.5-7) (Oksanen, 2020) to ensure distance matrices met the correct assumptions for

PERMANOVA testing. The effect of variables on the ordination were determined using a PERMANOVA with the *adonis* function in the vegan package (v2.5-7). An adjusted p-value of <0.05 with Benjamini-Hochberg correction for false discovery rate (FDR) was deemed a significant result.

To determine the relationship between the microbiome and resistome, the mantel test using Spearmans rank correlations were performed using the *mantel* function from the vegan package (v2.5-7) (Oksanen, 2020). For soil samples, the mantel test dissimilarity matrices were constructed from the average relative abundances of biological replicates as there were no biological replicates in the HT-qPCR array. Additionally for grass samples timepoints T2, T4 and T6 and soil samples timepoints T2 and T4 were not included in the analysis as there was no corresponding resistome data.

Differential OTU testing was conducted using DESeq2 package (v1.32.0) on non-rarefied data with singletons removed (Love *et al*, 2014). An adjusted pvalue of <0.01 with Benjamini-Hochberg correction for false discovery rate (FDR) was deemed a significant result. Venn diagrams were constructed with the list of differentially abundant OTUs from DESeq2 analysis using the ggvenn (v0.1.9) (Yan, 2021) and ggVennDiagram (1.2.0) packages (Gao, 2021). Heatmaps were then constructed of these OTUs at family level using the *plot_heatmap* function in phyloseq (v1.36.0) to investigate the temporal pattern of these families (McMurdie and Holmes, 2013).

2.7.5 Whole Genome Sequencing of Bacterial Isolates

One-hundred and fifteen isolates were sent for whole genome sequencing (WGS), consisting of 46 *E. coli* and 69 *A. baumanii*.

2.7.5.1 Sample Preparation

DNA was extracted as described in section 2.7.1. Three *E. coli* samples (SS T3 PIA CEF 6, SM EMB CIPRO 5, SM EMB CIPRO 3) had DNA send to MicrobesNG and the remainder were then sent to Novogene for sequencing on the Illumina NovaSeq PE150 platform for 2 x 150 paired end sequencing.

2.7.5.2 Sequence Processing and Data Analysis

Each read pair (sample) was subjected to adapter removal and quality trimming using TrimGalore v.0.6.6. (Krueger, 2012) using default settings. Adapter removal during the TrimGalore pipeline was powered by CutAdapt v.3.0 (Martin, 2011) and FastQC v. 0.11.9 (Andrews et al., 2015). Each sample was assembled using Unicycler v.0.4.7 (Wick et al., 2017) using default paired-end settings. Unicycler used SPAdes v.3.14.1. (Bankevich *et al.*, 2012) to assemble reads and used Bowtie2 v.2.4.2. (Langmead and Salzberg, 2012), Pilon v.1.23 (Walker *et al.*, 2014), BLAST v.2.11 (Altschul *et al.*, 1990, Camacho et al., 2009), and samtools v.1.11 (Li et al., 2009) to further complete the assembly. Each assembly was quality assessed using CheckM (Parks *et al.*, 2015) using the *Acinetobacter* database and sequence typed using MLST v.2.19.0 (Jolley and Maiden, 2010; Seemann, 2014) using default settings**.** Instances where a sequence type (ST) could not be completely identified, the approximated alleles were used to approximate a ST using the "search by locus combinations" option for *Acinetobacter* spp. using PubMLST (Seemann.T (2014); Jolley, Bray and Maiden, 2018). Each assembly was separated into "chromosomes" and "plasmids" (hereafter referred to as "mobilomes" as complete plasmids were not always guaranteed and were treated collectively as an

extrachromosomal entity) using Platon v.1.5.0 (Schwengers *et al.*, 2020) These partitioned assemblies were analysed alongside their concatenated "whole genome" assemblies. Assemblies that had a reported completeness percentage ≤ 95% were retained for further analyses.

Each isolate was assessed for antimicrobial resistance using ABRicate v.1.0.1 (Seemann, 2014) with the associated ResFinder database (Bortolaia et al., 2020) using a minimum identity stringency score (--minid) of 0.5. Resistances associated with single nucleotide polymorphisms (SNPs) were determined using PointFinder v.1 (Zankari et al., 2017) under default settings using the associated *P. aeruginosa, E. coli, Klebsiella* spp., and *Salmonella* spp. databases as templates. As *Acinetobacter* spp. are not represented in the list of PointFinder formatted databases, *P. aeruginosa* was selected as the closest relative (both are members of Order *Pseudomonadales*) and *E. coli, Salmonella* spp., *and Klebsiella ssp.* were also selected due to their phylogenetic proximity (all of which are members of Class *Gammaproteobacteria*).

CHAPTER 3

The Impact of Pig Manure Application on the Microbiome and the Occurrence of Antibiotic Resistant Opportunistic Pathogens in Grassland

Chapter 3. The Impact of Pig Manure Application on the Microbiome and the Occurrence of Antibiotic Resistant Opportunistic Pathogens in Grassland

3.1 Introduction

The rising threat of antibiotic resistance (AMR) is amongst the foremost challenges facing human health. Since the revolutionary discovery of salvarsan in 1910 (Zaffiri, Gardner and Toledo-Pereyra, 2012), many other classes of antibiotics have been discovered (Aminov, 2010). However, alongside development of these drugs, resistance has developed and antibiotic resistant infections have now become a global occurrence (WHO (World Health Organisation), 2017; Chokshi *et al.*, 2019). Antibiotic resistance is considered a One Health issue, involving interactions between human, animal and environmental microbiomes (Trinh *et al.*, 2018). The occurrence of AMR in the food chain in animal products, as well as fruits and vegetables, has been well documented (Verraes *et al.*, 2013; Al-Kharousi *et al.*, 2016; Österberg *et al.*, 2016; Davis *et al.*, 2018; Hölzel, Tetens and Schwaiger, 2018). In recent years there has been increased interest in the role that the environment, particularly agro-ecosystems, play in the maintenance and dissemination of AMR (Binh *et al.*, 2008a; Heuer, Schmitt and Smalla, 2011; Muurinen *et al.*, 2017; Kraemer, Ramachandran and Perron, 2019). Soil contains bacteria that are naturally resistant to some clinically important antibiotics by encoding intrinsic resistance mechanisms (Clardy, Fischbach and Currie, 2009; José L Martínez, 2012). Additionally, land management practices, such as those in agriculture can impact the soil microbiome. Of particular interest is the impact animal manure application has on the soil microbiome and resistome. Manure is an important organic fertiliser; however, it may be a potential hotspot of ARB due to the use of antibiotics in animal husbandry and animal welfare. The use, overuse or misuse of these antibiotics in animals can result in a selective pressure in the animal gut for ARB which can then be transferred to the environment via manure land-spreading or by direct deposition by grazing animals (Chen *et al.*, 2019; Kraemer,

Ramachandran and Perron, 2019). Manure may also contain residual amounts of antibiotics that can lead to an enrichment of AMR genes by providing a selection pressure in the environment (Lee *et al.*, 2018). Manure application can perturb the microbiome, increasing certain members of the soil microbiota that naturally contain ARGs (Udikovic-Kolic *et al.*, 2014).

While the transfer of ARB from manure to soil has been investigated and soil has been identified as a potential reservoir of AMR, the roles of plant microbiomes eaten by food-producing animals, as vectors, reservoirs or locations of AMR enrichment are poorly understood. In particular the roles of the microbiome and resistome of grassland has been the focus of very few studies (Grady *et al.*, 2019; Yan *et al.*, 2019, 2021). Globally grassland comprises approximately 70% of agricultural land and plays a pivotal role in food security as a food source for grazing animals (O'Mara, 2012). The presence of ARB within grassland may lead to their transfer to food-producing animals which may be a possible route of transmission to the food chain. Therefore, due to the importance grassland plays in agriculture, the potential of the grass phyllosphere to act as a reservoir of ARB needs to be assessed. To examine the microbiome and resistome of grass and soil amended with manure we utilised culture dependent approaches, combined with amplicon sequencing, to characterise the impact on microbial community structure, and also the occurrence and antibiotic susceptibility of opportunistic pathogens that are of clinical importance.

The aim of this study was to gain an insight into the bacterial communities of the soil and phyllosphere of grassland and to investigate the impact that the application of manure has on the microbiome and antibiotic resistant opportunistic pathogens present in soil and grass.

3.2 Results

3.2.1 Identification of Isolates

From the pot trial experiment, in total 98 bacterial isolates were collected two weeks post manure application: 13 isolates from soil, 82 isolates from grass and 3 isolates from manure (Figure 3.1). As explained in the materials and methods a threshold of 97 % was used for genus level identification (Section 2.7.2.1.1.), resulting in 14 isolates not being identified. These isolates were therefore removed from the analysis, leaving 83 isolates: 12 soil isolates, 68 grass isolates and 3 manure isolates. The percent identities, species level identification and sequence length of the isolates are found in Appendix 8. Soil isolates were identified within the genera: *Alcaligenes* (n=1), *Achromobacter* (n=5), *Pseudomonas* (n=2), *Bacillus* (n=1), *Acinetobacter* (n=2) and *Lactoccocus* (n=1). Isolates from grass were identified as: *Hafnia* (n=2), *Stenotrophomonas* (n=21), *Pseudomonas* (n=33), *Lactococcus* (n= 2), *Achromobacter* (n=3), *Aeromonas* (n=1) and *Enterococcus* (n=5) and *Acinetobacter* (n=1). Manure samples contained *Providencia* (n= 2) and *Bacillus* (n=1). Isolates were assigned at species level at 99% identity, resulting in 31 isolates which had multiple species identified above the 99% threshold and 41 isolates which were successfully identified at species level. These isolates were composed of *Bacillus cereus* (n=1), *Acinetobacter calcoaceticus* (n=3), *Lactococcus garvieae* (n=1), *Stenotrophomonas maltophilia* (n=19), *Pseudomonas aeruginosa* (n =12), *Enterococcus hirae* (n =3), *Pseudomonas fluorescens* (n =1) and *Providencia rettgeri* (n =1) (Appendix 8).

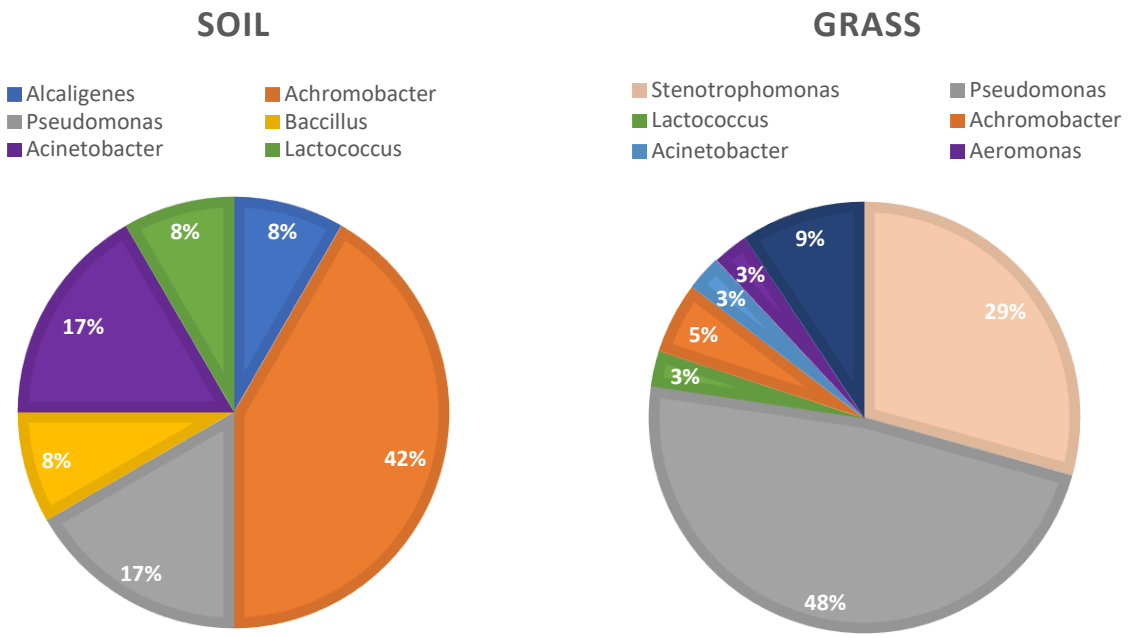

Figure 3.1: Pie charts illustrating the percentage of each genus of bacteria successfully cultured from soil and grass

3.2.2 Antibiotic Susceptibility Profiles of Isolates

The antibiotic resistance profiles of the 83 isolates, as described in section 2.6.2.1, are summarised in Table 3.1. Due to many environmental bacteria harbouring intrinsic resistance mechanisms for clinically important antibiotics these resistance results were not reported as resistant and were indicated as intrinsic resistance in Table 3.1. Additionally, some bacteria do not have EUCAST (2020) or CLSI (2020) guidelines for the antibiotics tested, indicated by grey in Table 3.1, therefore these results were not included in the number of resistant isolates reported.

In total there were 0 cefotaxime, 2 imipenem, 20 kanamycin, 6 amikacin and 6 ciprofloxacin resistant isolates from soil and grass samples. Additionally for gram positive isolates, there was no linezolid or vancomycin resistant isolates detected. AmpC β-lactamase detection resulted in the identification of 33 isolates as presumptive AmpC β-lactamase producers, which consisted of *Pseudomonas* spp. and *Stenotrophomonas*

spp. (Appendix 10). Metallo β-lactamase (MBL) testing identified 20 isolates as positive for MBL production and ESBL testing resulted in 3 positive isolates, all of which were *Stenotrophomonas* spp. Microdilution assays to determine colistin resistance were performed on all isolates and resulted in 6 isolates identified as resistant (MIC ≥ 4 mg/L) out of the 83 tested. (Table 3.2). Full details of antibiotic susceptibility testing are described in Appendix 9, 10 and 11.

Table 3.1: Table summarising disk test results for bacteria isolated for soil, grass and pig manure. The table contains the number of resistant isolates from each genus identified. Only gram-positive bacteria were tested for linezolid and vancomycin resistance. [1]

Bacteria	Total isolates tested	Cefotaxime	Imipenem	Kanamycin	Amikacin	Ciprofloxacin	Linezolid	Vancomycin
Soil	**Number of Isolates**							
Alcaligenes	1	NG	NG	NG	NG	NG	N/A	N/A
Achromobacter	5	IR	0	NG	NG	NG	N/A	N/A
Pseudomonas	2	IR	0	0	0	0	N/A	N/A
Bacillus	1	NG	0	NG	NG	0	0	NG
Acinetobacter	2	IR	0	1	1	0	N/A	N/A
Lactococcus	1	NG	1	2	3	2	N/A	N/A
Grass								
Hafnia	2	0	0	0	0	0	N/A	N/A
Stenotrophomonas	21	IR	IR	IR	IR	NG	N/A	N/A
Pseudomonas	33	IR	0	16	1	3	N/A	N/A
Lactococcus	2	IR	0	1	1	0	0	0
Achromobacter	3	IR	0	NG	NG	NG	N/A	N/A

[1] *IR indicates bacteria that harbour intrinsic resistance mechanisms to the antibiotic therefore the result was not reported as resistant. NG indicates that there were no guidelines available for the antibiotic for that particular isolate. N/A indicates testing for linezolid and vancomycin was not required for gram – negative bacteria due to their lack of clinical effect on gram negative bacteria.*

Bacteria	Total isolates tested	Cefotaxime	Imipenem	Kanamycin	Amikacin	Ciprofloxacin	Linezolid	Vancomycin
Aeromonas	1	IR	IR	IR	IR	0	N/A	N/A
Enterococcus	5	NG	0	IR	IR	0	0	0
Acinetobacter	1	IR	0	0	0	0	N/A	N/A
Pig Manure								
Providencia	2	0	0	0	1	0	N/A	N/A
Bacillus	1	NG	1	NG	NG	0	0	0
Total	82	0	2	20	6	6	0	0

Table 3.2: Number of colistin resistant isolates detected from each sample type.

Bacteria	**Colistin Resistant (MIC ≥ 4mg/L)**
Soil	**Number of Isolates**
N/A	N/A
Grass	
Hafnia paralevi	2
Stenotrophomonas spp.	1
Aeromonas spp.	1
Manure	
Providencia spp.	2

3.2.3 Targeted PCRs

Targeted PCRs, as described in section 2.7.2.1.2, were performed on isolates resistant to one or more clinically relevant antibiotics that were not intrinsically resistant, listed in Table 3.3: 1 *Alcaligenes* spp. isolate (Isolate 1), 2 *Lactococcus* spp. isolates (Isolate 31, 33), 1 *Lactoccus garvieae* isolate (Isolate number 11), 2 *Hafnia paralvei* and 1 *Aeromonas* spp. isolate, 1 *Stenotrophomonas maltophilia* isolate. All isolates were negative for mobile colistin resistance genes (*mcr1-9,* Section 2.7.2.1.2.6), third generation cephalosporin resistance genes (*bla*$_{\text{CTX-M}}$ Group 1,2,8,9,25, *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$, Section 2.7.2.1.2.4) and carbapenem resistance (*bla*$_{\text{GES,}}$ *bla*$_{\text{GIM}}$, *bla*$_{\text{IMI}}$, *bla*$_{\text{IMP}}$, *bla*$_{\text{KPC}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-40}}$, *bla*$_{\text{OXA-48}}$, *bla*$_{\text{OXA-51}}$, *bla*$_{\text{OXA-58}}$, *bla*$_{\text{VIM}}$, Section 2.7.2.1.2.5) genes by PCR. Three targeted PCRs were positive, as listed in Table 3.3. For aminoglycoside resistance the PCR reactions for 16S rRNA methylases (*armA, rmtA, rmtB, rmtC, rmtD, rmtE, rmtF, rmtG, rmtH, npmA,* Section 2.7.2.1.2.1) and non 16S methylase genes (*aac(6')-Ie-aph(2")-Ia, aph3IIIa, ant(4')-Ia, aph(2")-Ic, aph(2")-Id, ant(6)-Ia, aadA,*

aadE, Section 2.7.1.2.1) were negative but the gene *aac(6')-Ii* was identified in one *Lactococcus* spp. isolate (Isolate Number 33). The gene sequence had 99% identity to *aac(6')-Ii* in *Enterococcus faecium*. Additionally, another *Lactococcus garvieae* isolate (Isolate Number 11) was identified as positive for mutations in the QRDR of the *gyrA* (TCT-TTT, Ser-81 to Phe) and *parC* genes (TCG-TTG, Ser-81 to Ile) conferring fluoroquinolone resistance (Section 2.7.2.1.2.2).

Table 3.3: Table summarising the resistance phenotype of selected isolates for targeted PCRs and the results of targeted PCRs. CTX = cefotaxime, AK = amikacin, KAN = kanamycin, IMP = imipenem, COL = colistin

Isolate Code	**Isolate Name**	**Isolate ID**	**Resistance phenotype investigated by PCR**	**Results of Targeted PCR**
1	S1 CIPRO 1/10 EMB 1	*Alcaligenes* spp.	CTX	Negative
11	S6 CIPRO 1/10 LAM 2	*Lactoccocus garvieae*	CTX, AK, CIPRO	QRDR gyrA and parC positive
31	G2 IMI PIA 1	*Lactoccocus* spp.	CTX, KAN, AK	Negative
33	G2 IMI 1/10 PIA 3	*Lactoccocus* spp.	CTX, KAN, AK, IMP	*aac(6')-Ii positive*
60	G4 COL -5 LAM 2	*Aeromonas* spp.	COL	Negative
14	G1 COL -5 EMB 1	*Hafnia paralvei*	COL	Negative
15	G1 COL-5 EMB 4	*Hafnia paralevei*	COL	Negative
32	G2 IMI PIA 2	*Stenotrophomonas maltophilia*	COL	Negative

3.2.4 Microbiome 16S rRNA Gene Sequence Description and Rarefaction Curves

Following trimming there were a total number of 1,103,469 reads obtained from the samples and an average number of 84,882 reads obtained from each sample, as described in section 2.7.4.1. The sequencing depths for each sample are shown in Appendix 12. Rarefaction curves constructed from the data illustrated that most samples reached a plateau, indicating that a sufficient sampling effort was reached (Figure 3.2). Inspection of the rarefaction curves and rarefying of the data to a depth of 24,583 resulted in the removal of one sample, "Soil Pot 3" from the analysis, resulting in 12 samples remaining in the analysis. The remaining rarefied 12 samples consisted of a total of 294,996 reads with 2,903 OTUs at 97% similarity with 17 phyla.

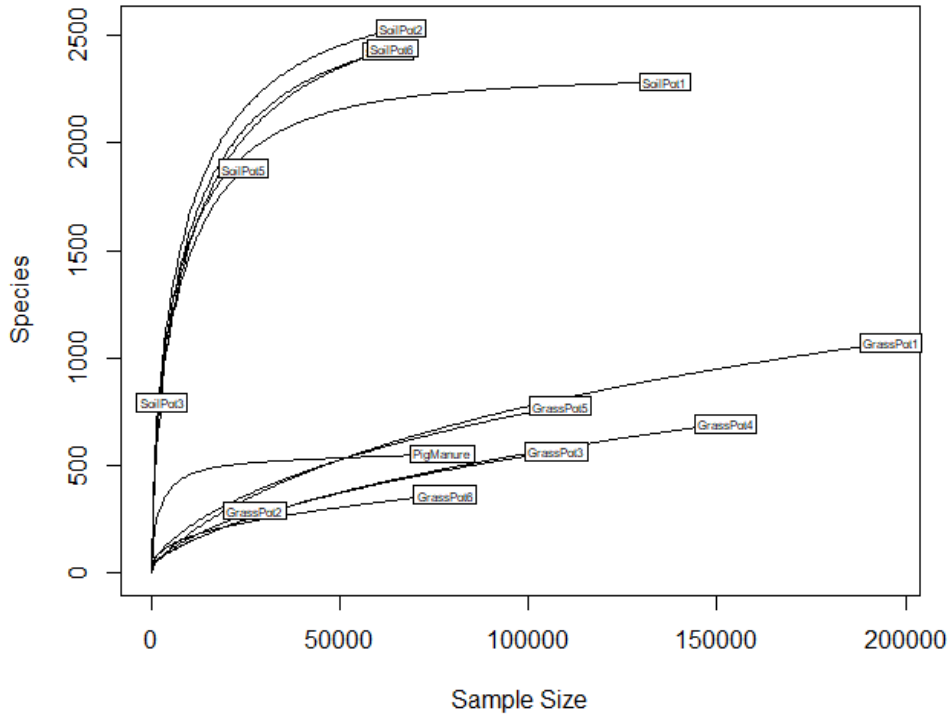

Figure 3.2: Rarefaction curve illustrating the number of OTUs at cut off level of 97% for all samples.

3.2.5 Microbial Composition of Grass, Manure and Soil Samples

The relative abundance of the top 50 phyla and top 30 families for each sample type is shown in Figure 3.3. Grass samples (control and treated) were mainly comprised of the phyla: *Proteobacteria* (71.6% ; 63.6%) and *Firmicutes* (25.8 ; 29.8%), and the families: *Enterobacteriaceae* (39.2% ; 37.32%), *Pseudomonadaceae* (33.1% ; 26.2%) and *Clostridiaceae_1* (14.58% ; 8.26%). Pig manure was mainly found to be composed of the phyla*: Bacteroidetes* (43.56%), *Firmicutes* (21.8%) and Unclassified Bacteria (34.4%). At the family level, manure was found to be predominantly comprised of Unclassified Bacteria (34.9%), *Bacteroidetes_unclassifed* (21.3%), *Porphyromonadaceae* (22.08%) and *Clostridiaceae_1* (13.81%). The soil samples (control and treated) were mainly composed of the phyla *Proteobacteria* (36.3%;27.08%), *Actinobacteria* (39.8%;43.4%) and Bacteria Unclassified (13.2% ; 11.98%) and the families: *Xanthomonadaceae* (22.6%, 14.15%), *Micrococcaceae* (21.9%; 25.5%), *Intransporangiaceae* (14.1% ; 11.03%). Details of sample composition at genus level is displayed in Appendix 13; however, many genera were unclassified.

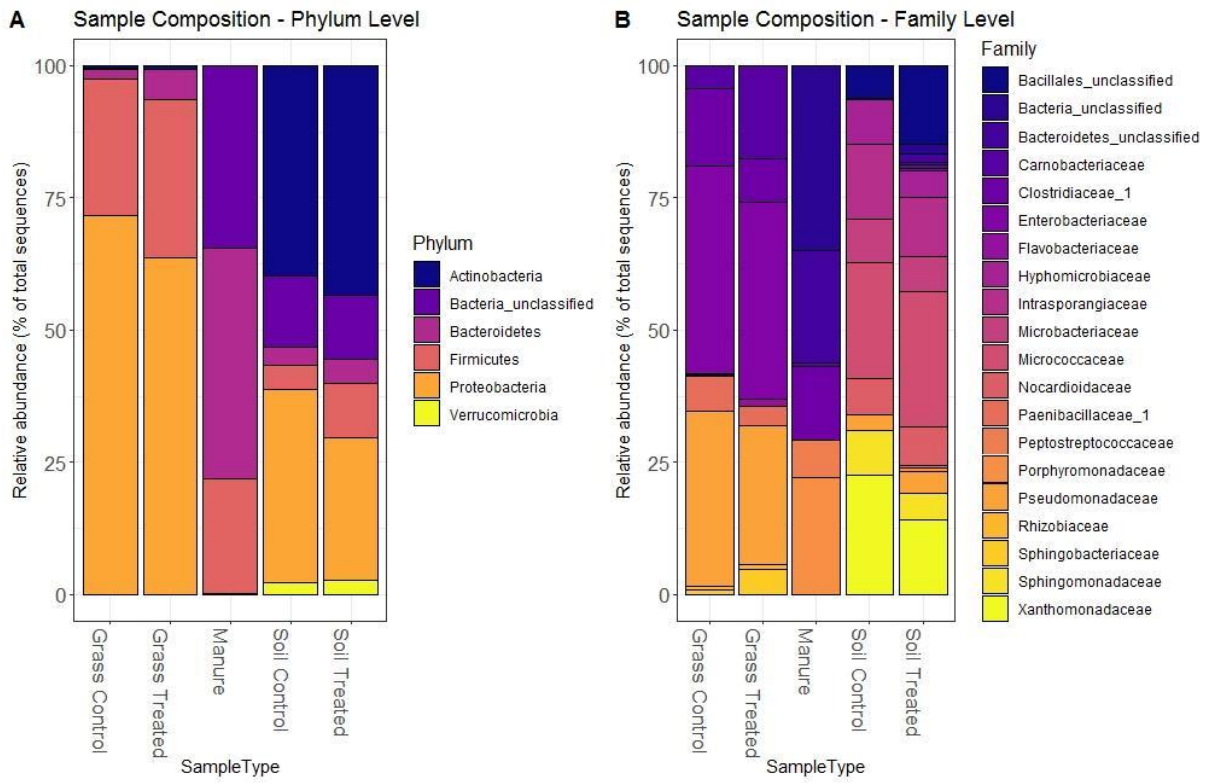

Figure 3.3: Bar plot of the relative abundance of: (A) The top 50 Phyla and (B) the top 30 Families in treated and control grass and soil samples and pig manure

3.2.6 Alpha Diversity

The alpha diversity of the manure, soil and grass samples were calculated using the Chao1 richness index and the Shannon diversity index (Figure 3.4, Section 2.7.4.1.2). Richness measures the number of species in an ecosystem whereas diversity refers to the species richness and distribution (evenness) of species in an ecosystem (Morris *et al.*, 2014). The impact of treatment on the alpha diversity (Shannon and Chao1) was investigated using the Mann Whitney U test to compare the differences between control and treated grass samples, as well as the differences between the control and treated soil samples. It was found for both Chao1 and Shannon alpha diversity indices that there were no significant differences ($p > 0.05$) between the alpha diversities of control and treated grass samples

or between control and treated soil samples. The impact of sample type on alpha diversity was then investigated using Kruskal Wallis testing and was found to have a significant impact ($p < 0.05$) on the alpha diversity. To further investigate this significance, Mann Whitney U testing was used to investigate the differences in alpha diversity between each sample type: grass samples (treated and control grass combined) vs soil samples (treated and control soil combined). The alpha diversities between soil and grass alpha diversities were found to be significant in both Chao1 and Shannon diversity indices ($p < 0.05$).

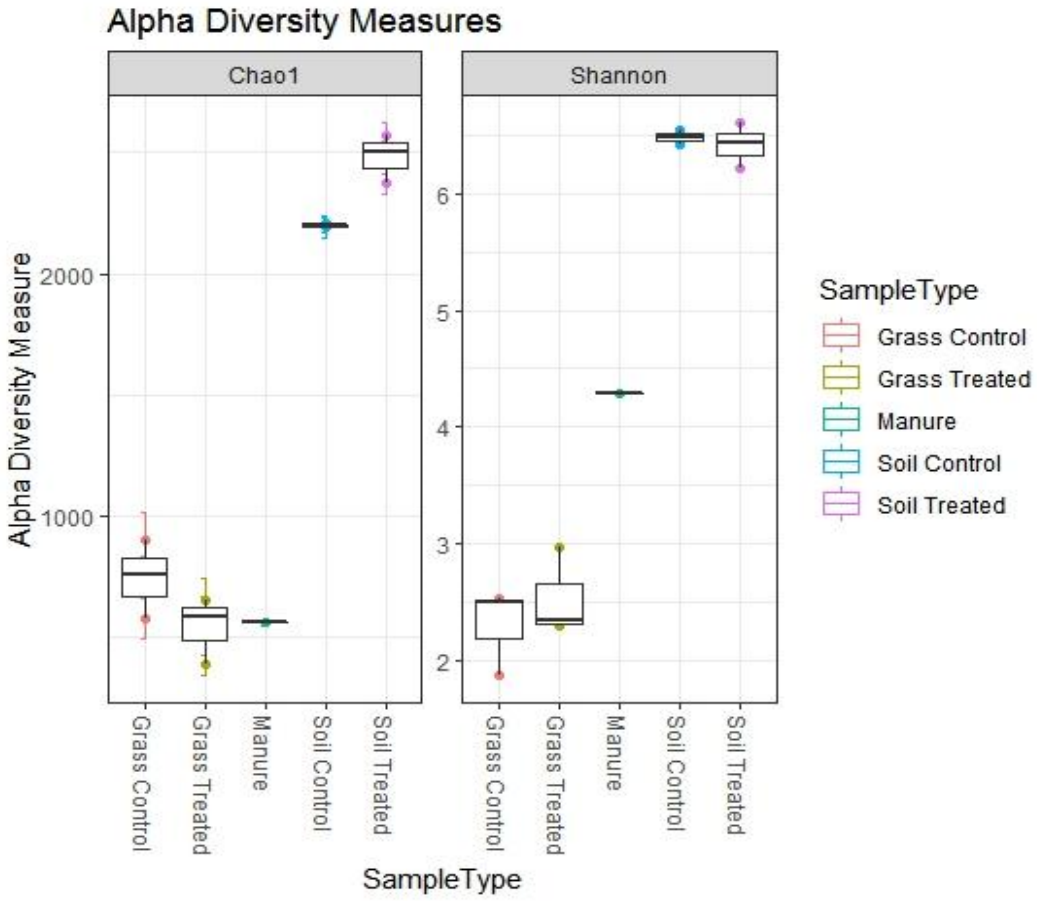

Figure 3.4: Alpha Diversity Measures of Manure, Soil and Grass Samples.

Alpha diversity for manure, grass and soil samples measured by Chao1 and Shannon indices. There was no significant difference between treated and control samples, however there was a significant difference between the alpha diversities of soil and grass samples.

3.2.7 Beta Diversity of Samples

The DESeq2 package was used to identify bacterial taxa that were statistically different between treated and control grass samples and between treated and control soil samples (Section 2.7.4.1.2). There were no significant compositional differences ($p < 0.05$) between treated and control grass samples. In soil samples there were no taxa that were significantly decreased in treated samples in comparison to the controls. However, numerous taxa were significantly enriched ($p < 0.05$) in the treated soil in comparison to the control soil (Appendix 14, Figure 3.5). These included the phyla: *Bacteroidetes, Proteobacteria, Firmicutes, Verrucomicrobia, Tenericutes* and *Actinobacteria.* At the family level it was observed that the families such as the *Carnobacteriacea, Peptococcaceae_1, Clostridiaceae_Incertae_Sedis_XI, Clostridiaceae_Incertae_Sedis_XIII, Enterococcaceae* and *Pseudomonadaceae* were significantly increased in abundance in manured soil in comparison to the controls. At the genus level, many taxonomic groups were unclassified. However, genera such as *Pseudomonas, Trichococcus* and *Enterococcus* were identified as being more abundant in manured soil (Figure 3.5).

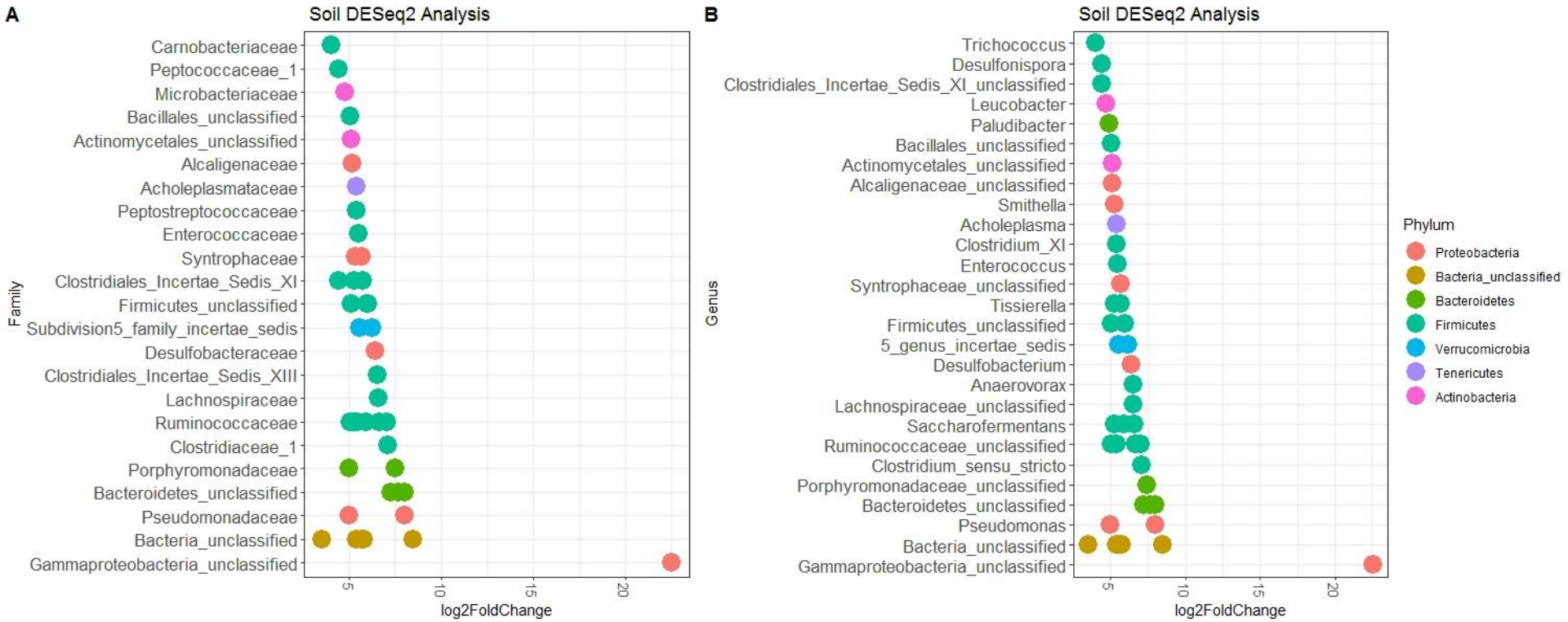

Figure 3.5: DESeq2 analysis visualising the log fold change of bacteria at phylum and family level between treated and control soils samples ($p < 0.05$) at A) Family level and B) Genus level. The log2fold change indicates which bacterial phyla, genera or families had an increased abundance in the treated soil samples in comparison to the controls. No OTUs were found to decrease in abundance.

3.3 Discussion

Despite culturing only allowing for approximately ~1% of the environmental microbiome to be grown in the laboratory environment, culturing bacteria allows for the isolation of pathogens of interest that may be in low abundance in the environment (Stefani *et al.*, 2015) and for phenotypic analysis of these isolates. This study aimed to characterise the bacterial microbiomes of the soil and grass phyllosphere and to investigate the impact manure application has on these microbiomes, using culture dependent and independent approaches.

In this study environmental bacteria were isolated from both grass and soil samples, some of which were opportunistic pathogens of clinical interest. These include *Stenotrophomonas maltophilia* (Brooke, 2012) and some members of the *Pseudomonas* spp. (de Bentzmann and Plésiat, 2011), *Achromobacter* spp. (Marion-Sanchez *et al.*, 2019) and *Lactococcus garvieae* (Russo *et al.*, 2012; Westberg *et al.*, 2020).

Of particular interest was the isolation of 3 Lactococcus spp. isolates, 1 from soil which was identified as *L. garvieae* (Isolate 11) and 2 from grass (Isolates 31 and 33), which were only identified at genus level as their percentage identities were below the 99% threshold for species level (98.86% and 98.23% respectively) (Appendix 3). *Lactoccus garvieae* is a zoonotic pathogen which is mainly associated with lactococcosis in fish (Vendrell *et al.*, 2006). However, there have been reports of *L. garvieae* presenting as opportunistic pathogens in humans (Choksi and Dadani, 2017; Tariq *et al.*, 2020). All *Lactococcus* spp. that were identified as resistant to the one or more aminoglycosides (kanamycin and amikacin) and cefotaxime, with isolate 33 also exhibiting imipenem resistance and isolate 11 also exhibiting ciprofloxacin resistance. Isolate 33 was negative for MBL production according to phenotypic tests, therefore this carbapenem resistance may be due to mutations in their penicillin binding proteins (PBPs) or acquisition of new

PBPs as has been found in other gram-positive bacteria (Papp-Wallace *et al.,* 2011). Additionally, the three *Lactococcus* isolates were negative for ESBL and AmpC production according to phenotypic tests although exhibited cefotaxime resistance. This resistance to third generation cephalosporins may be due to acquisition or alteration in their PBPs as has been seen in other gram-positive bacteria (Jubeh, Breijyeh and Karaman, 2020). The identification by targeted PCR of the aminoglycoside resistance gene *aac(6')-li gene* in isolate number 33 was an unusual finding as this gene is normally found on the chromosome of *E. faecium,* conferring low level of aminoglycoside resistance (Costa *et al.,* 1993). The presence of this gene could be due to mobilisation of this gene onto a plasmid. For isolates 11 and 31 aminoglycoside resistance may be due to the expression of efflux pumps, potentially the efflux pump LmrA which has been found in *Lactoccous* spp., including *L.garvieae* (Castro *et al.,* 2017), and has been found to cause reduced susceptibility to aminoglycosides (Poelarends, Mazurkiewicz and Konings, 2002; Poole, 2005). Additionally isolate number 11 had mutations in the QRDRs of the *gyrA* and *parC* genes therefore conferring the observed fluoroquinolone resistance. This confirms a similar result previously identified in *L. garvieae* isolated from Maki *et al.,* (2008). In Europe, the quinolone oxolinic acid and the fluoroquinolone flumequine, are used in aquaculture (Rico *et al.,* 2019). Therefore, the incidence of these fluoroquinolone resistant *Lactococci* is veterinary relevant and their occurrence must be monitored to avoid dissemination into the ecosystem, particularly into water systems.

Six isolates were identified as colistin resistant by having an MIC ≥ 2mg/L. Two isolates (Isolates 14 and 15) were identified as *Hafnia paralevi* which is known to be intrinsically resistant to colistin (Jayol *et al.,* 2017). Isolates 96 and 97 were identified as *Providencia* spp. which are also known to be intrinsically colistin resistant (Gogry *et al.,* 2021). Additionally, isolate 60 was identified as *Aeromonas salmonicida* and had a colistin MIC

of > 128 mg/L. *Aeromonas* spp. are thought to be susceptible to colistin except for *A. janadaei and A. hydrophilia. Aeromonas* spp. have been associated with the spread of *mcr-3* to *Enterobacteriaceae* (Yin *et al.*, 2017) and have also been found to harbour *mcr-5* (Ma *et al.*, 2018). Despite this isolate not having *mcr-1 – mcr-9* genes detected it is possible this isolate harbours a new variant of the *mcr* gene or has other resistance mechanism such as the addition of L-Ara-4N to the lipopolysaccharide layer (LPS) (Gonzalez-Avila *et al.*, 2021). Therefore whole genome sequencing of this isolate would be required to fully understand the colistin resistance mechanism at play. *Aeromonas salmonicida* is mostly associated with aquatic disease and human infection is rare and has been associated with ingestion of contaminated water or food (Martins, Marquez and Yano, 2002; Salehi *et al.*, 2019). Isolate 32 was identified as *Stenotrophomonas maltophilia* which can be intrinsically colistin resistant (Gonzalez-Avila *et al.*, 2021) and can also be resistant through acquisition of the *mcr* genes (Li *et al.*, 2019). Isolate 32 was negative for all tested *mcr* genes therefore it is probable that this resistance is due to an intrinsic mechanism, similar to the *Aeromonas* isolate previously described. Nevertheless, the identification of colistin resistant *Aeromonas* and *S. maltophilia* isolates from grass indicates the potential of the grass phyllosphere to act a reservoir of opportunistic pathogens with clinically relevant AMR phenotypes.

Antibiotic susceptibility showed all detected presumptive AmpC β-lactamases producers were *Pseudomonas* spp. and *S. maltophilia*. The positive AmpC β-lactamase result in *Pseudomonas* spp. was not unexpected as both pathogenic and environmental strains of *Pseudomonas* spp. are known to contain chromosomal AmpC β-lactamase genes (Jacoby, 2009). The positive AmpC β-lactamase result in *S. maltophilia* isolates is less common as *S. maltophlia* is not known to harbour a chromosomal AmpC β-lactamase gene (Jacoby, 2009). However, the *S. maltophlia* L2 β-lactamase gene is seen as homologous

to the AmpR-AmpC system observed in members of the Enterobacteriaceae, as L2 is also mediated by the regulator AmpR. The combination of L1 and L2 β-lactamase genes in *S. maltophilia* give these bacteria natural resistance to nearly all β-lactams. In addition, the boronic acid compound, bicyclic boronate, has been found to have inhibitory effects on the L2 β-lactamase (Calvopiña *et al.*, 2017).This could result in a false positive result in *Stenotrophomonas* spp. for AmpC β-lactamase production. Therefore these AmpC results were not further investigated (Chang *et al.*, 2015). All MBL positive isolates were *Stenotrophomonas* spp. which contain a chromosomal MBL (L1 enzyme). Additionally, ESBL disk testing resulted in 3 positive isolates however these were determined as false positives, as all isolates were *Stenotrophomonas* spp. which contain the L2 β-lactamase, a member of Ambler class A β-lactamase which is susceptible to clavulanic acid (Crossman *et al.*, 2008; Brooke, 2012).

In recent years there have been many studies with the aim of characterising the environmental microbiome and an increased interest in the impact of anthropogenic pollutants on the environmental microbiome. Of particular interest is the influence agricultural processes, such as the landspreading of manure, have on the agricultural land microbial communities (Udikovic-Kolic *et al.*, 2014; Han *et al.*, 2018; Chen *et al.*, 2019). In this study 16S rRNA amplicon sequencing was used to investigate the impact of pig manure application on the bacterial communities of grass and soil. The low richness of the perennial ryegrass phyllosphere microbiome observed in this study has also been reported for the phyllosphere microbiome of other plants (Bodenhausen, Horton and Bergelson, 2013; Grady *et al.*, 2019). Additionally, the significantly higher alpha diversity in soil than the phyllosphere has also been reported (Yan *et al.*, 2019, 2021).

The composition of soil and manure were similar to previous findings (Leclercq *et al.*, 2016; Lopatto *et al.*, 2019), with soils in this study being dominated by *Proteobacteria*,

Acinetobacteria, Firmicutes and Unclassified Bacteria and manure being dominated by *Firmicutes, Bacteroidetes* and Unclassified Bacteria. DESeq2 analysis revealed statistically significant differences in the abundances of OTUs between control and treated soil samples. There were no bacterial families that were found to decrease in the treated samples in comparison to the controls. Notably, some families of bacteria that contain members known to cause human infections increased significantly ($p < 0.05$) in soil treated with manure, including: *Enterococcaceae, Peptostreptococcaceae, Clostridiaceae_Incertae_Sedis_XI* and *Pseudomonadaceae*. However, at genus level it was noted that many genera were unclassified, therefore deeper sequencing is required to fully understand the compositional differences at genera level. This enrichment of potentially pathogenic bacteria following manure spreading has been observed in other studies (Marti *et al.*, 2013). However, it has been shown that the application of manure can cause short term alterations on the soil microbiome (Leclercq *et al.*, 2016; Muurinen *et al.*, 2017), therefore it is possible that the changes observed in treated soil samples in this study would decrease over time.

In regard to the microbiome of grass samples, to date there has been little research on the microbiome of its phyllosphere, in comparison to microbiome studies of other plants (Williams and Marco, 2014; Singh *et al.*, 2019). Over recent years there has been increased interest in the phyllosphere as a reservoir of potentially pathogenic or antibiotic resistant bacteria. In this study, it was found that the phyllosphere microbiome of both control and treated grass mainly consisted of the phyla *Proteobacteria, Firmicutes* and *Bacteroidetes,* which mirrors other findings (Grady *et al.*, 2019; Yan *et al.*, 2019). At family level both control and manure amended grass samples contained a high percentage of *Enterobacteriaceae, Clostridiaceae_1, Pseudomonadaceae* and *Micrcoccaceae.* These families are known to contain both commensal and pathogenic bacteria, therefore like the

soil samples, deeper sequencing is required to fully understand the grass microbiome at genus level. DESeq2 analysis found that there were no statistically differential OTUs between control and treated grass identified. To date, no research has been done to investigate the effect manure treatment has on the microbiome of the phyllosphere of grass. However, one study by (Zhou *et al.*, 2019) showed that manure treatment impacted the resistome of the phyllosphere of rice and wheat and that this was linked to microbiome changes. The phyllosphere is known to be a harsh environment for microbes to survive in due to its exposure to weather fluctuations, UV radiation and plant pathogens (Compant *et al.*, 2019; Sivakumar *et al.*, 2020). Therefore, this could explain the identification of no significantly altered OTUs in manured grass as the manure originating bacteria may not have the ability to tolerate and survive the harsh phyllosphere environment. Regardless these results illustrate the diversity of the grass phyllosphere microbiome and the potential of the grass phyllosphere to be a reservoir of potentially pathogenic bacteria which may in turn enter the food chain. They also highlight the difference between the impact of manure on the grass phyllosphere and soil microbiomes.

3.4 Conclusion

The soil and grass microbiomes are reservoirs of naturally antibiotic resistant bacteria that may act as opportunistic pathogens in a clinical setting such as *S. maltophilia* and *Achromobacter* spp.. We identified that soil and grass contained potentially pathogenic members of the *Lactococcus* family which are important zoonotic pathogens, particularly in fish. Additionally, these *Lactoccocus* spp., were resistant to the fluoroquinolones which are used in Europe in aquaculture and additionally to aminoglycosides which are important antibiotics in human medicine. Through 16S rRNA sequencing used to investigate the microbiome, it was found that the application of manure on the soil microbiome was found to perturb the soil microbiome but not the grass phyllosphere.

Therefore, temporal studies and resistome analysis is needed to fully understand the impact manure application has on the agricultural environment.

CHAPTER 4

An Investigation into the Differential Impact of Pig, Cow and Chicken Manure on Microbiome and Resistome of Agricultural Grassland

Chapter 4: An Investigation into the Differential Impact of Pig, Cow and Chicken Manure on Microbiome and Resistome of Agricultural Grassland

4.1 Introduction

The dissemination of antibiotic resistant bacteria into the environment is of great concern from a public health perspective. Despite the environment being a natural reservoir of bacteria, antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs), the indigenous microbiome and resistome can be perturbed by anthropogenic pollution. (Larsson and Flach, 2021). Of recent interest is the impact of manure landspreading on the environmental microbiome and resistome (Lima, Domingues and Da Silva, 2020). This practice is essential for waste management and recycling nutrients back into soils, which facilitates crop growth (Manyi-Loh *et al.*, 2016). However, despite the benefits of manure landspreading, the practice can perturb the indigenous soil microbial communities (G. C. Ding *et al.*, 2014; Udikovic-Kolic *et al.*, 2014; Lopatto *et al.*, 2019; Macedo *et al.*, 2021). Manure landspreading has been found to have a short-term impact, around 4 - 12 weeks on the microbiome and resistome (Muurinen *et al.*, 2017; Gou *et al.*, 2018; Pérez-Valera *et al.*, 2019; Macedo *et al.*, 2021). However, factors such as soil type (Blau *et al.*, 2018), manure origin (Bicudo and Goyal, 2003; Singer *et al.*, 2016) and previous manure use on the land (Zhang *et al.*, 2018; C. Wang *et al.*, 2020) can have differential impacts on the microbiome and resistome, therefore the temporal impact of manure landspreading from various livestock sources needs to be fully understood.

Manure application can not only impact the soil but recent studies have demonstrated the increased ARG and MGE load of manured crops, such as lettuce (Blau *et al.*, 2019; Sun *et al.*, 2021), rice and wheat (Zhou *et al.*, 2019). One area of plant microbiomes that has gained recent attention is the aerial part of the plant, the phyllosphere. Globally, the total surface area of the phyllosphere is approximately twice that of land (Vorholt, 2012) and

recent findings have found that the phyllosphere can possess a diverse microbiome and resistome (Yan *et al.*, 2019; Sun *et al.*, 2021). However, despite the identification of the phyllosphere as a reservoir of ARGs there has been little research investigating the impact manure spreading has on the phyllosphere microbiome and resistome. It has been shown that manure application can increase ARG abundance in rice and wheat phyllosphere samples (Zhou *et al.*, 2019), but this must be further studied for other plants of agricultural importance. Of particular interest in this study is the impact of manure application on the microbiome and resistome of the grass phyllosphere, due to its important role in global food security (O'Mara, 2012). However, to date, there has been no research investigating the impact of manure application on the grass phyllosphere microbiome and resistome.

Many studies investigating the impact agricultural practices have on the environmental microbiome and resistome have used a sequencing-based approach rather than traditional culture-based approaches (Lopatto *et al.*, 2019; Macedo *et al.*, 2021; Sun *et al.*, 2021; Wang *et al.*, 2022). A combined approach using amplicon sequencing and HT-qPCR is commonly used (F. Wang *et al.*, 2020; Gupta *et al.*, 2021; Xiang *et al.*, 2021), however in recent years a metagenomics-based approach has also been used for both microbiome and resistome analysis (Xiao *et al.*, 2016; Qian *et al.*, 2021). For resistome analysis, microarray technology has also been used however, despite it allowing for the screening of hundreds of target genes it is considered less specific and less sensitive (Waseem *et al.*, 2019). Each method approach has its own benefits and limitations. Metagenomics provides non target screening of environmental samples; allowing for the analysis of a myriad of microbial communities in comparison to amplicon sequencing which is limited to the target housekeeping gene used in the initial PCR step. Metagenomic resistome analysis is not limited to a pre-chosen primer set such as the HT-qPCR array approach, therefore allowing for detection of a wider range of ARGs and MGEs as well as novel

resistance genes (Franklin *et al.*, 2021). Additionally, the delta delta CT approach used for HT-qPCR array analysis assumes that all target genes have a similar amplification efficiency to the housekeeping gene used to normalise the data, which is not the case (Schmittgen and Livak, 2008). Therefore, metagenomics avoids this PCR bias. However, due to high costs and computationally demanding data analysis associated with metagenomics, amplicon sequencing may be preferred in some cases. Additionally, due to the sequencing depth needed in the analysis of certain environment, such as soil, HT-qPCR may be preferred for resistome analysis rather than metagenomics due to its increased sensitivity (Waseem *et al.*, 2019). In this study a 16S rRNA amplicon sequencing paired with HT-qPCR array-based approach was used.

The aims of this study are to 1) Characterise the microbiome and resistome of the soil and grass phyllosphere 2) Identify the differential impact of pig, cow and chicken manure on the soil and grass phyllosphere microbiome and resistome 3) Understand the temporal impact landspreading of pig, cow and chicken manure has on the grassland microbiome and resistome.

4.2 Results

4.2.1 Diversity and composition of ARGs and MGEs detected across samples

In total 178 ARGs of the 214 ARGs selected in the HT-qPCR array were detected. One hundred and sixty-seven genes were detected in grass phyllosphere samples from 14 different gene classes, 85 in soil samples from 11 gene classes, 104 in pig manure from 12 gene classes, 114 in cow manure from 13 gene classes and 63 in chicken manure from 10 gene classes (Figure 4.1, Table 4.1).

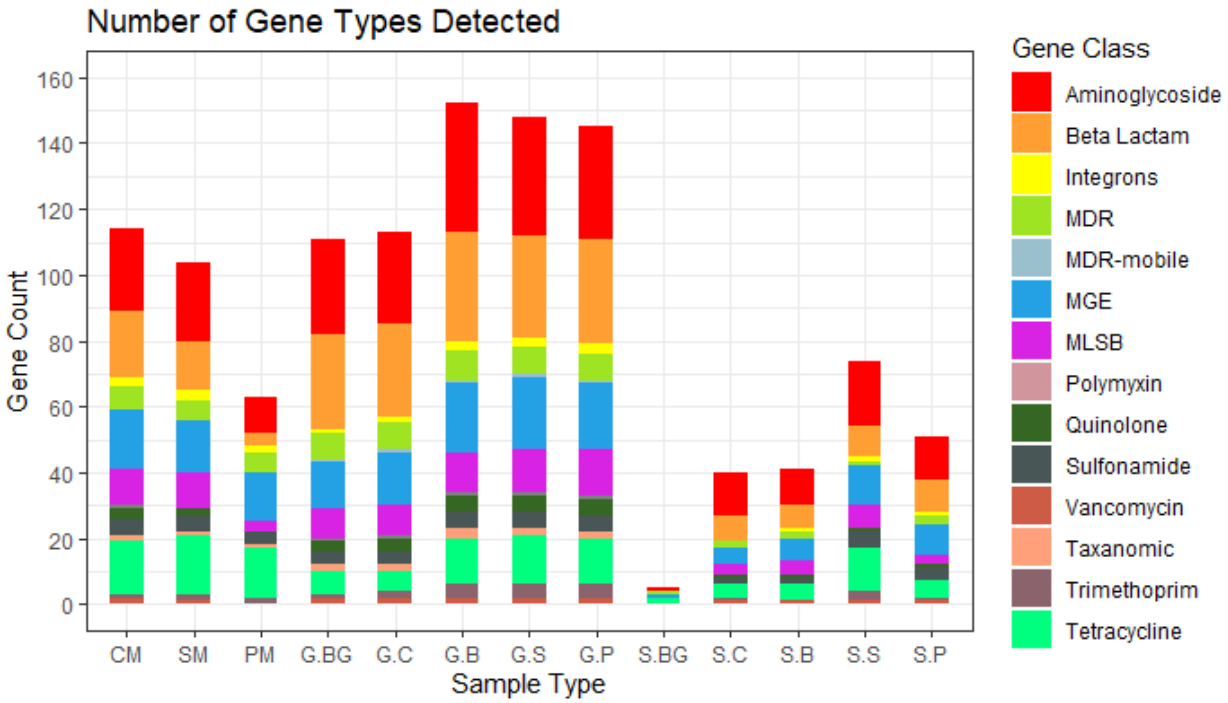

Figure 4.1: Barplot displaying the number of target genes detected for each sample type and treatment in the HT-qPCR array. Sample names are indicated by the sample codes: CM =Cow Manure, SM = Pig Manure, PM = Chicken Manure, G.BG = Background Grass, G.C= Control Grass, G.B = Cow Manured Grass, G.S = Pig Manured Grass, G.P= Chicken Manured Grass, S.BG = Background Soil, S.C = Control Soil, S.B = Cow Manured Soil, S.S = Pig Manured Soil, S.P = Pig Manured Soil.

Table 4.1: Table displaying the presence or absence of gene classes detected in each sample type using the HT-qPCR array. This data represents one pig manure sample, one cow manure sample, one chicken manure sample, 25 grass samples and 32 soil samples. [2]

Gene Classes	AG	VA	β-L	INT	TAX	MDR	MDR-MOB	MGE	MLSB	PYX	QN	SUL	TET	TR	Sample Type
Sample															
Pig Manure															**Manure**
Cow Manure															
Chicken Manure															
Grass Background															**Grass**
Grass Control															
Grass Cow															
Grass Chicken															
Grass Pig															
Soil Background															**Soil**
Soil Control															
Soil Cow															
Soil chicken															
Soil Pig															

[2] *Red indicates the gene class was absent, green indicates the gene class was present. AG = Aminoglycoside, VA = vancomycin, β-L = β-lactam, INT = integron, TAX= taxonomic genes, MDR= multidrug resistance, MGE = mobile genetic element, MDR-MOB = multidrug resistance mobile genes, MLSB = macrolide-lincosamide-streptogramin B, PYX = polymyxin, QN = quinolone, SUL = sulphonamide, TET = tetracycline, TR = trimethoprim.*

Of the manure samples, cow manure had the highest number of ARG classes detected (n=13) and chicken manure had the lowest (n=10) (Table 4.1). The most prominent gene class in the cow, pig manure and chicken manure were the same: resistance to the aminoglycosides (21.93%, 23.08% and 17.5%), respectively. The next most abundant number of ARGs in the pig and chicken manure were the MGEs (15.38%, 23.8%) and tetracycline resistance genes (17.31%, 23.8%); whereas cow manure was slightly different with the next most prominent gene classes detected being the aminoglycoside resistance (21.9%), β-lactam resistance (17.54%) and the MGEs (15.79%) (Appendix 15). For manure samples the overall gene classes detected were similar, except for cow manure which had polymyxin resistance genes present and chicken manure lacked vancomycin and quinolone resistance genes (Table 4.1).

The manure amended soil and grass phyllosphere samples had higher numbers of ARGs detected than the background (S.BM and G.BM) and control samples (S.C and G.C) However, the cow manure amended soil only differed from the control by only one gene (Figure 4.1). In soil samples aminoglycosides (27.75%), β-lactams (12.97%), MGEs (15.27%) and tetracyclines (16.91%) were the most common gene classes detected. For soil samples, background soil samples contained very few gene classes in comparison to the control and manure treated samples. It was found that of the soil samples, only manure treated soil samples had integrons detected (Table 4.1). In grass phyllosphere samples the most common gene classes detected were the aminoglycosides (26.82%), β-lactams (23.02%), MGEs (13.50%) and MLSB (7.17%) (Appendix 16). All grass phyllosphere samples, consisted of the same classes, except for background grass phyllosphere samples which did not contain MDR-mobile genes (Table 4.1).

Through statistical testing of NMDS ordinations, as described in section 2.7.3.2.3, sample type was a significant factor in resistome composition (PERMANOVA, $p < 0.05$, $R^2 =$

0.22, “permutest” ANOVA, $p < 0.05$, $F = 6.14$) (Appendix 17 -A), accounting for 22% of the variance in the data. However, treatment was not found to be a significant factor in terms of resistome composition for either grass (PERMANOVA, $p > 0.05$, $R^2 = 0.16$, “permutest”, ANOVA, $p > 0.05$, $F = 0.67$) or soil samples (PERMANOVA, $p > 0.05$, $R^2 = 0.14$, “permutest” ANOVA, $p > 0.05$, $F = 2.9$) (Appendix 17 -B, C).

4.2.2 Abundance of ARGs and MGEs

There were significant differences between the manure samples in terms of ARG and MGE relative abundance (Figure 4.2-A and B). Cow manure had a significantly lower relative ARG abundance than chicken and pig manure (Kruskal-Wallis Test, $p < 0.05$), whereas there were no significant differences between chicken and pig manure ARG abundance (Kruskal-Wallis Test, $p > 0.05$) (Figure 4.2-A). For MGE gene abundance pig manure had significantly higher relative abundance than cow manure (Kruskal-Wallis Test, $p < 0.05$), but not chicken manure (Kruskal-Wallis Test, $p > 0.05$) (Figure 4.2-B). At gene class level, chicken manure had significantly higher relative abundance in the MDR gene class than cow manure and pig manure (Dunn Test $p < 0.05$) (Figure 4.2-C). Pig manure had significantly higher tetracycline resistance in comparison to the other two manure types (Dunn Test $p < 0.05$) (Figure 4.2-D).

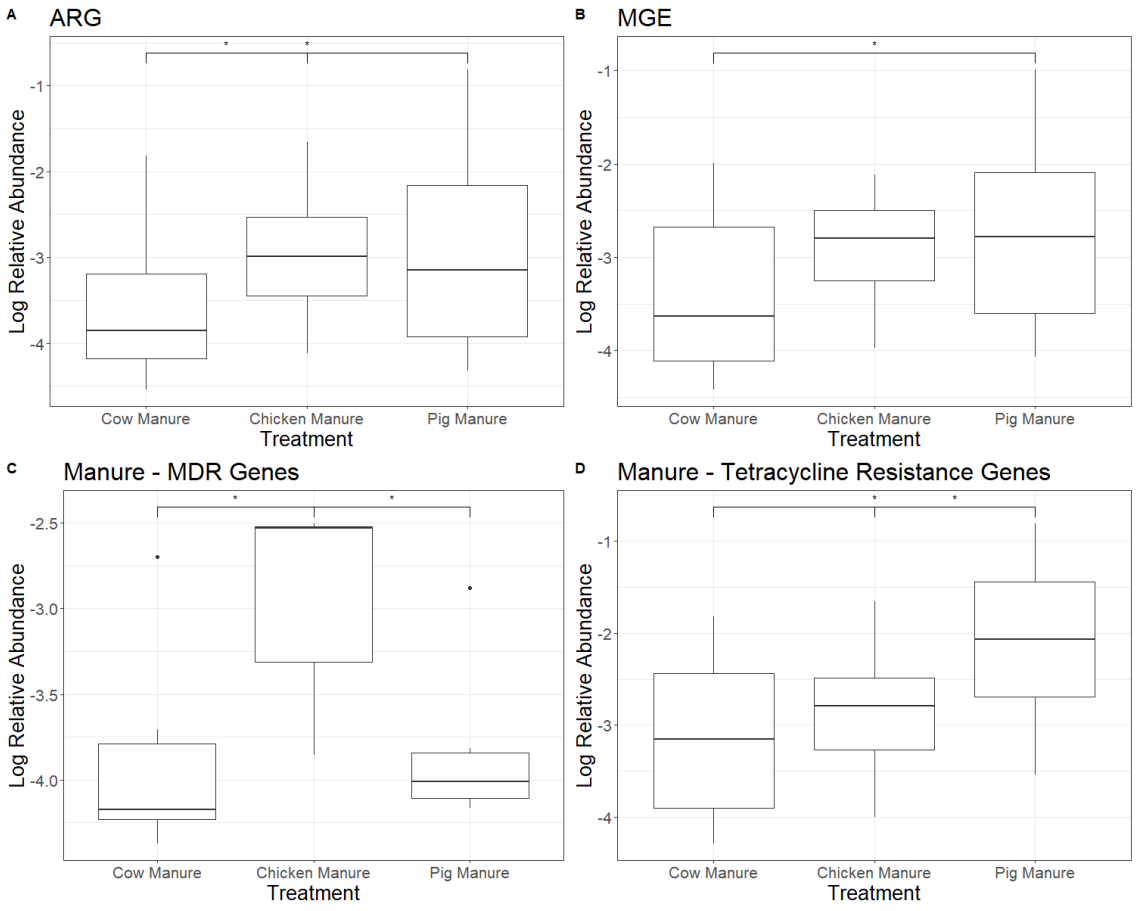

*Figure 4.2: Boxplots illustrating the log relative abundance of (A) ARG and (B) MGE relative abundance of manure samples, and the relative abundance of statistically differing antibiotic resistance genes classes (C) and (D). * Indicates significance between groups ($p < 0.05$).*

For grass phyllosphere and soil relative abundance testing background samples were removed as it did not consist of multiple samples and was determined an outlier. In grass phyllosphere samples, treatment had a significant impact on ARG relative abundances (Kruskal-Wallis Test, $p < 0.05$) but not on MGE relative abundance (Kruskal-Wallis Test, $p > 0.05$) (Figure 4.3-A, Figure 4.3-B). Grass control samples had significantly higher ARG relative abundance than chicken and cow manured grass (Kruskal-Wallis Test, $p < 0.05$). Pig manured grass had significantly higher ARG relative abundance than cow manured, chicken manured, and control grass phyllosphere samples (Kruskal-Wallis Test, $p < 0.05$). At gene class level, pig manured grass had significantly higher relative

abundance of β-lactamase genes in comparison to cow manured grass and chicken manured grass (Figure 4.3-C) and also had a significantly higher relative abundances of aminoglycoside resistance genes in comparison to the chicken manure treated grass and cow manure treated grass (Dunn Test $p < 0.05$), but not the control grass (Dunn Test $p > 0.05$) (Figure 4.3-D). Pig manure also had higher abundances of sulphonamide resistance genes than control and chicken manured grass (Dunn Test $p < 0.05$) (Figure 4.3-E). Pig manured grass had higher MLSB resistance genes than cow and chicken grass samples but not control (Dunn Test $p < 0.05$) (Figure 4.3 – F).

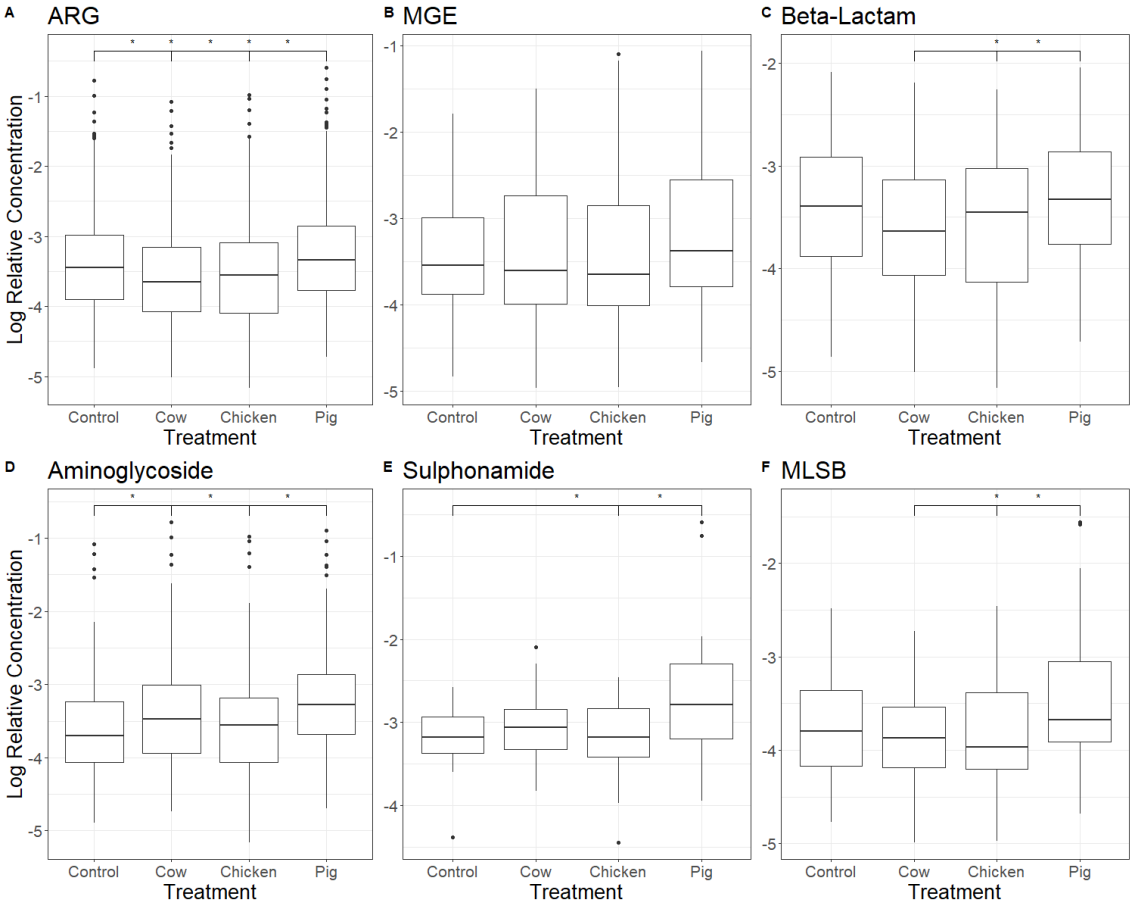

*Figure 4.3: Bar charts illustrating the relative abundance of (A) ARGs (B) MGEs (C) β-lactam Resistance Genes (D) Aminoglycoside Resistance Genes (E) Sulphonamide Resistance Genes (F) MLSB Resistance Genes in Grass. * Indicates significance between groups ($p < 0.05$).*

Treatment had no effect on the overall soil ARG and MGE relative abundance in soil (Kruskal-Wallis Test, $p > 0.05$) (Figure 4.4-A, B). However, at gene class level cow manured soil had significantly higher relative abundance of β-lactam resistance genes than pig manured soil (Dunn Test $p < 0.05$) (Figure 4.4-C). Additionally, pig manured soil had a significantly higher relative abundance of sulphonamide resistance genes than chicken manured soil (Dunn Test $p < 0.05$) (Figure 4.4-D).

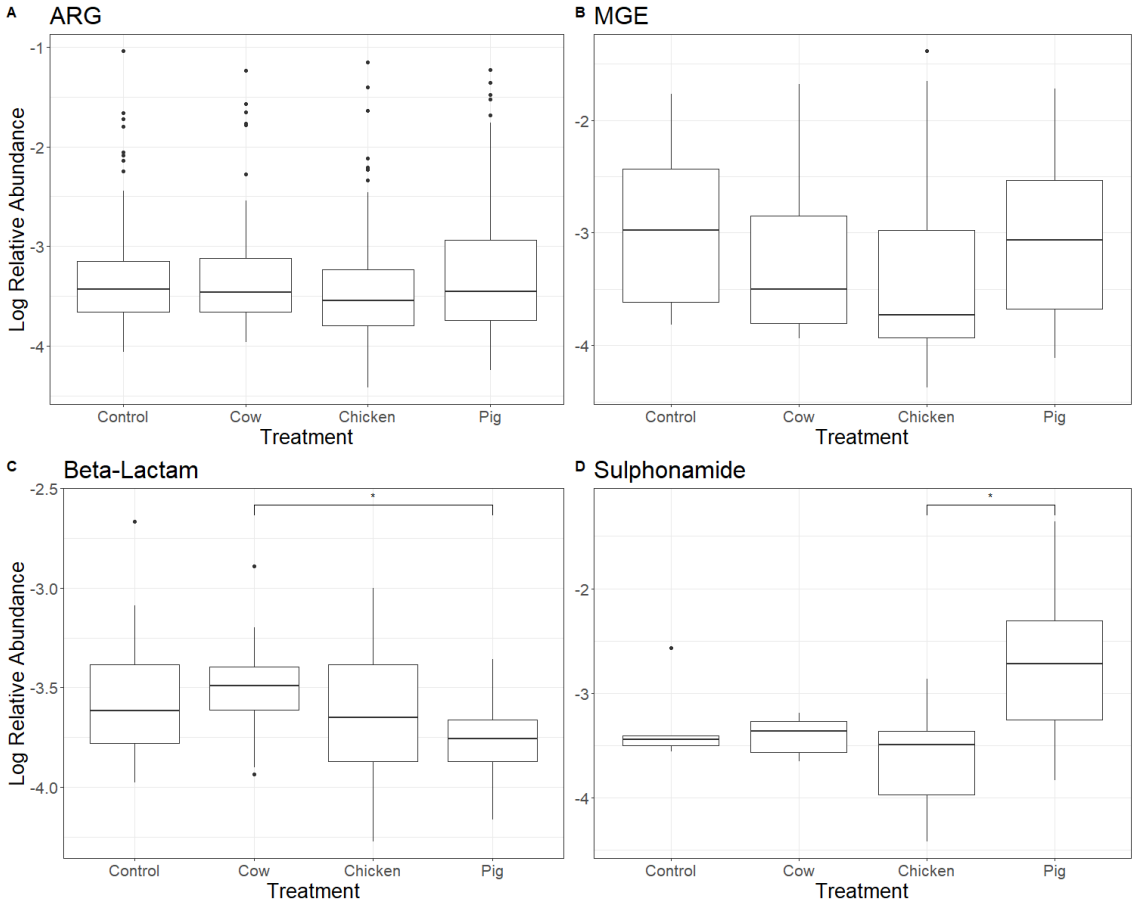

*Figure 4.4: Boxplots illustrating the log relative abundance of (A) Beta Lactamase Genes (B) Sulphonamide Resistance Genes in Soil Samples. * Indicates significance between groups ($p < 0.05$).*

4.2.3 Identification of Core and Accessory Resistome and The Fate of the Unique ARGs in Manure Treated Samples

Venn Diagrams were constructed to identify which genes were present in all respective sample groups (core resistome) and which genes were unique to each treatment (accessory resistome) (Appendix 18, Section 2.7.3.2.3). There were 50 genes core to all manure samples. There were 25, 4 and 10 genes unique to cow, chicken and pig manure respectively (Appendix 18 -A, Appendix 19)

There were 101 genes core to all grass phyllosphere samples (Appendix 18-B, Appendix 20) and 5 genes in common to all soil samples (Appendix 18-C, Appendix 21). The ARGs/MGEs present in pig, cow and chicken manure amended grass phyllosphere samples and all manures but were absent from background or control grass phyllosphere samples comprised of the seven genes: *intI2_2, aadA2_1 tetW, tet39, ISAba3, dfra17* and *sul1_2*. Of the grass phyllosphere samples, grass treated with pig manure had four unique genes detected which were not present in the other grass samples: *ermF, ermT_1, tetO_2, qnrS_1* with all four also being detected in pig manure. The chicken manured grass had 7 unique genes: *msrA_1, blaZ, ermC_2, tetK, mecA, msrC_1, bla*$_{OKP}$, none of which were detected in chicken manure. Cow manured grass phyllosphere samples had six unique genes consisting of ARGs, MGEs and one taxonomic gene which indicates the presence of *Klebsiella pneumoniae*: *tetC_2, K. pneumoniae, ermD, maR_3, aacA/aphD, tnpA_5,* with *tnpA_5 al*so being detected in cow manure (Appendix 22).

For pig manured grass there were 24 genes that were shared between pig treated grass and pig manure, (Appendix 23-A), 13 shared between cow manure and cow manure treated grass (Appendix 23-B) and 13 genes shared between chicken manure and chicken manured grass (Appendix 23-C). The fate of these manure originating genes was tracked to investigate at what point during the field trial they stopped being detected (Figure 4.5-

A). For grass phyllosphere samples the genes introduced from the manure onto the grass were no longer detected after 18 weeks (T9), with the exception of *tnpa4* in cow manured grass, which was detected 18 weeks following manure application (T9).

All manure amended soil samples and their respective manures contained *tnpA_2, sul2_1* and *aadA2_3* which were absent in control and background soils (Appendix 18-C). Of the manured soil samples, pig manured soil had 28 unique genes (*ant6-ib, aph(3'')-ia, tetQ, tetPA, blaTEM_1,tnpA_1, tet32, ermT_1, ermA, tet36_1, aadA5_2, dfra17, tetO_2, IS613, Tn3, ermX_1, Tp614, tetW, tet39, dfrA1_1, sul2_2, tnpA_4, aadD, aph4-ia, aacC2, aph3-iii, aadA6, ermF*) of which all were also found in pig manure, chicken manured soil had 4 unique genes (*tolC_2, IS6/257, ermC_2, ampC_6*), of which *tolC_2, IS6/257 and ampC_6* were also found in chicken manure. Cow manured soil samples contained no unique genes (Appendix 24).

For pig manured soil there were 39 genes that were shared between pig treated soil and pig manure (Appendix 23 – D) , nine shared between cow manure and cow manure treated soil (Appendix 23 – E) and nine genes shared between chicken manure and chicken manured soil (Appendix 23 – F)). In soil samples, most genes were no longer detected 10 weeks following manure application (T7). The genes *intl13* and t*npA2* were detected consistently for 16 weeks and 18 weeks, respectively, after manure application in pig manure amended soils (Figure 4.5- B).

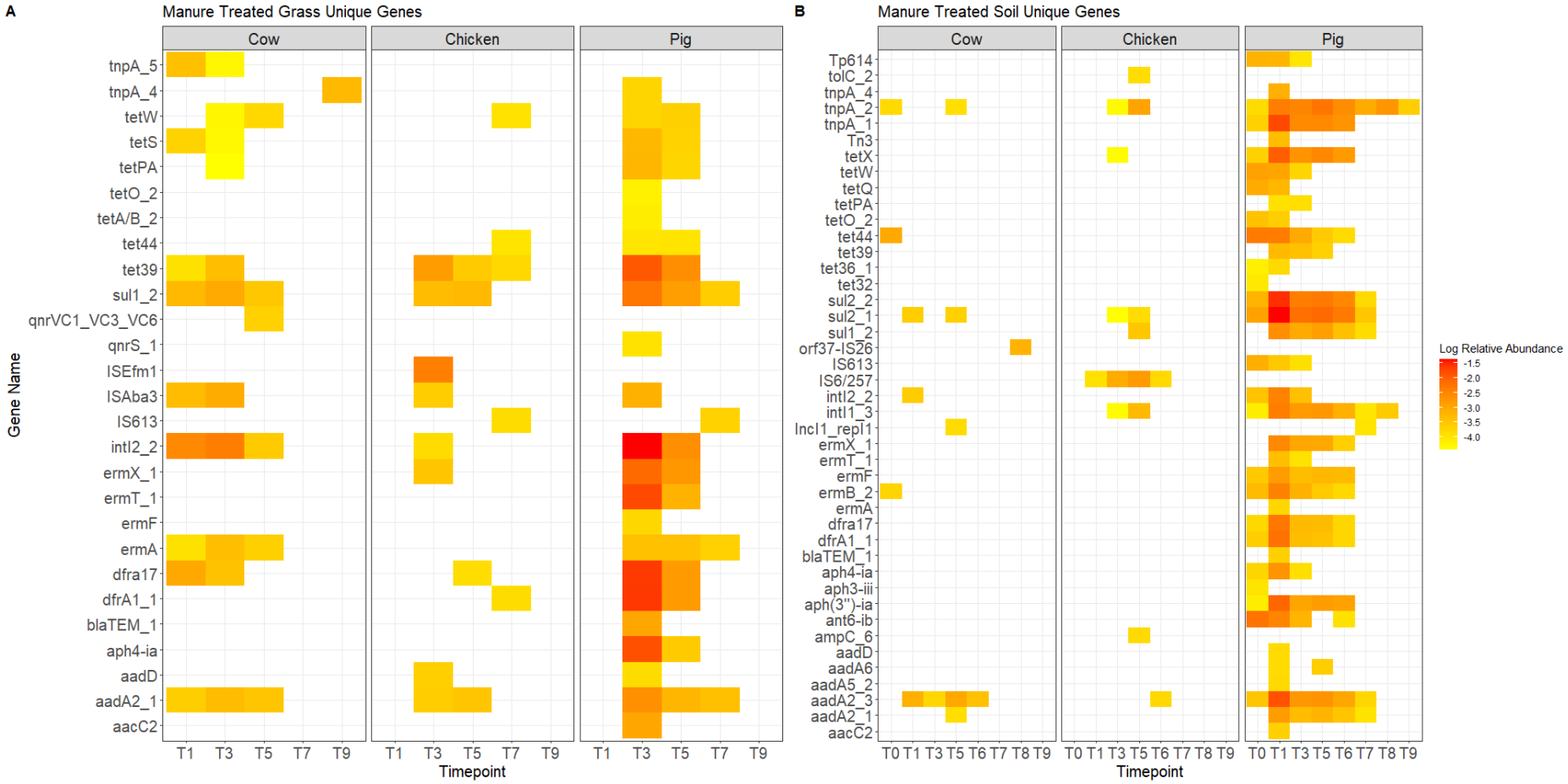

Figure 4.5: Heatmap displaying the log relative abundances of the genes introduced into the grass (A) and soil (B) from manure application. Timepoints are indicated by codes : T1= Timepoint1, T3= Timepoint 3, T5 = Timepoint 5, T6 = Timepoint 6, T7 = Timepoint 7, T8 = Timepoint 8, T9 = Timepoint 9.

4.2.4 Description of Sequences

Following trimming and removal of positive and negative controls, as described in Section 2.7.4.2.1, there were a total number of 12,377,074 reads obtained from the samples and an average number of 73,237 reads per sample. The sequencing depths for each sample are shown in Appendix 25. Rarefaction curves constructed from the data illustrated that most samples reached a plateau, indicating that a sufficient sampling effort was reached (Appendix 26). As explained in the materials and methods in Section 2.7.4.2.2, inspection of the rarefaction curves and rarefying of the data to a depth of 30,000 reads resulted in the removal of 12 samples from the analysis, resulting in 157 samples remaining in the analysis. Following rarefaction, the dataset contained 4,710,000 reads and 16,570 OTUs at 97% similarity.

4.2.5 Microbial Composition and Alpha Diversity

Sample compositions at phylum level and family level are visualised in Figure 4.6 (A and B). Genus level is displayed in Appendix 27. Cow manure was mainly composed of the phyla *Firmicutes* (32%) and *Proteobacteria* (29.8%) and the families: *Microbacteriaceae* (29.9%), *Comamondaceae* (21.65%) and *Planococcaceae* (19.16%). Chicken manure was mainly composed the phyla *Firmicutes* (55%) and the *Proteobacteria* (40.9%) and the families *Microbacteriaceae* (33.11%) and *Pseudomonadaceae* (16.12%). Pig manure was dominated by phyla *Proteobacteria* (39.8%) and *Actinobacteria* (34%) and the families *Planococcaceae* (56.24%) and *Moraxellaceae* (39.77%). Grass phyllosphere samples were mainly composed of the phyla the *Proteobacteria* (66.3%) and the families the *Enterobacteriaceae* (20.8%), *Rhizobiaceae* (12.61%) and *Pseudomonadaceae* (11.81%). Soil samples were mainly composed of the phyla the *Proteobacteria* (34%) and the *Acidobacteria* (24.5%) and the families the *Spartobacteria_family_incertae_sedis* (20.5%),

Acidobacteria_Gp16_family_incertae_sedis (15.64%) and *Bradyrhizobiaceae* (12.97%). Genus level compositions can be found in Appendix 27. Alpha diversity was calculated using Chao1 and Shannon diversity measurements, as described in Section 2.7.4.2.2, and results are displayed in Figure 4.6 – C and D. Manure treatment had no significant difference on Shannon or Chao1 diversity measures for grass phyllosphere samples and soil samples (Kruskal Wallis Test $p > 0.05$). Sample type had a significant impact on both Shannon and Chao1 diversity measures (Kruskal Wallis Test $p < 0.05$). Soil for both measures was significantly higher than cow manure, pig manure and grass (Kruskal Wallis Test $p < 0.05$).

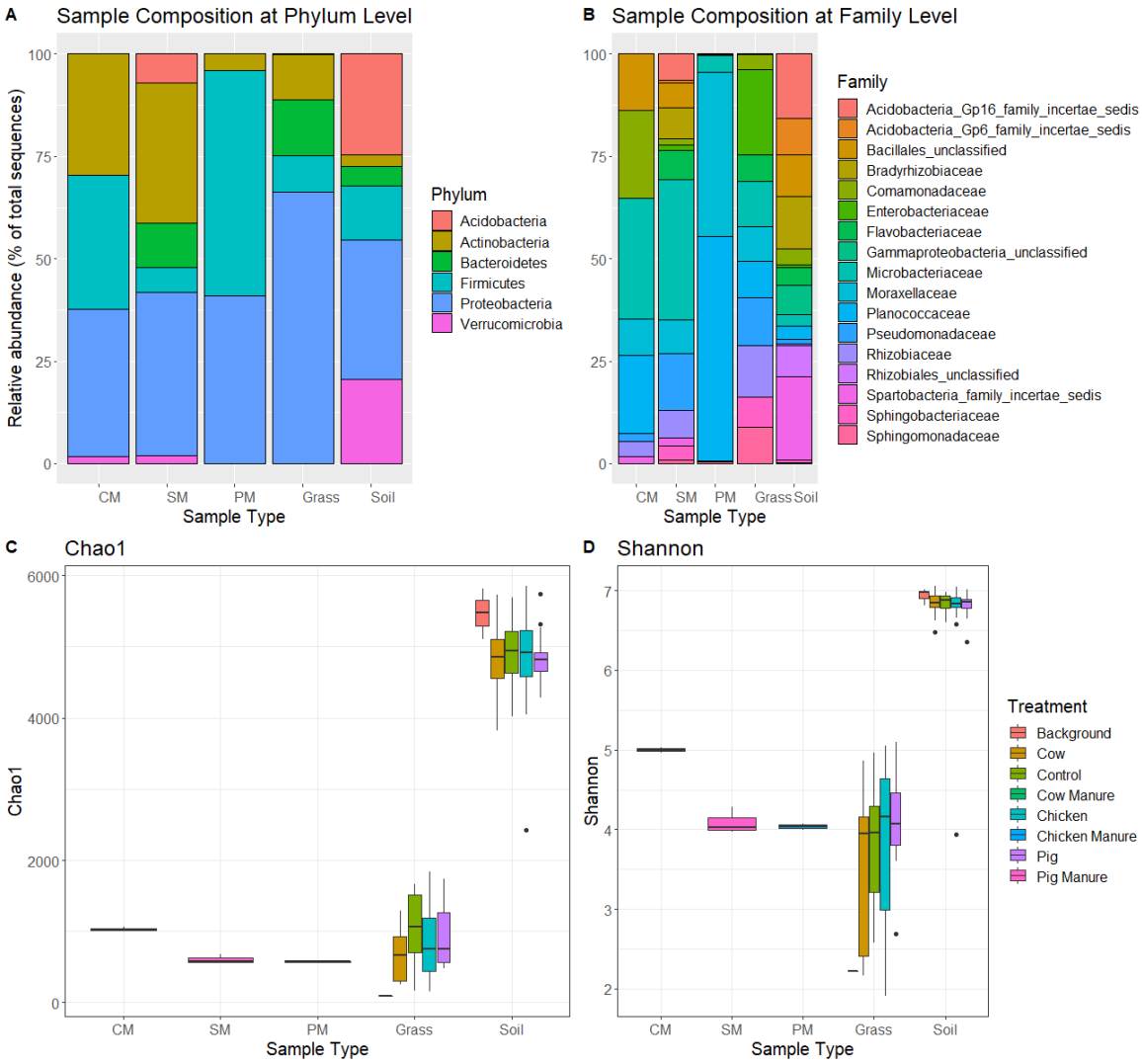

Figure 4.6: (A) Sample composition at family level of the top 20 phyla. (B) Sample composition at family level of the top 20 families. (C) Alpha diversity using the Chao1 index (D) Alpha Diversity using the Shannon index. Sample codes are as follows: CM = cow manure, SM = pig manure, PM = chicken manure.

4.2.6 β-Diversity of the Microbiome

Through NMDS ordination and PERMANOVA analysis (Section 2.7.4.2.2) , sample type (Soil, Grass, Cow Manure, Pig Manure and Chicken Manure) was found to have a significant impact on microbiome composition ($p < 0.05$) and accounted for 55% of the variation in the data (PERMANOVA, $p < 0.05$, $R^2 = 0.55$). However, the permutest function to test for the non-homogenous dispersion of the data indicated a positive result ("permutest" ANOVA $p < 0.05$, $F = 50.021$), but as indicated by the separate clustering on the NMDS plot (Figure 4.7), sample type had an obvious impact on the microbial composition.

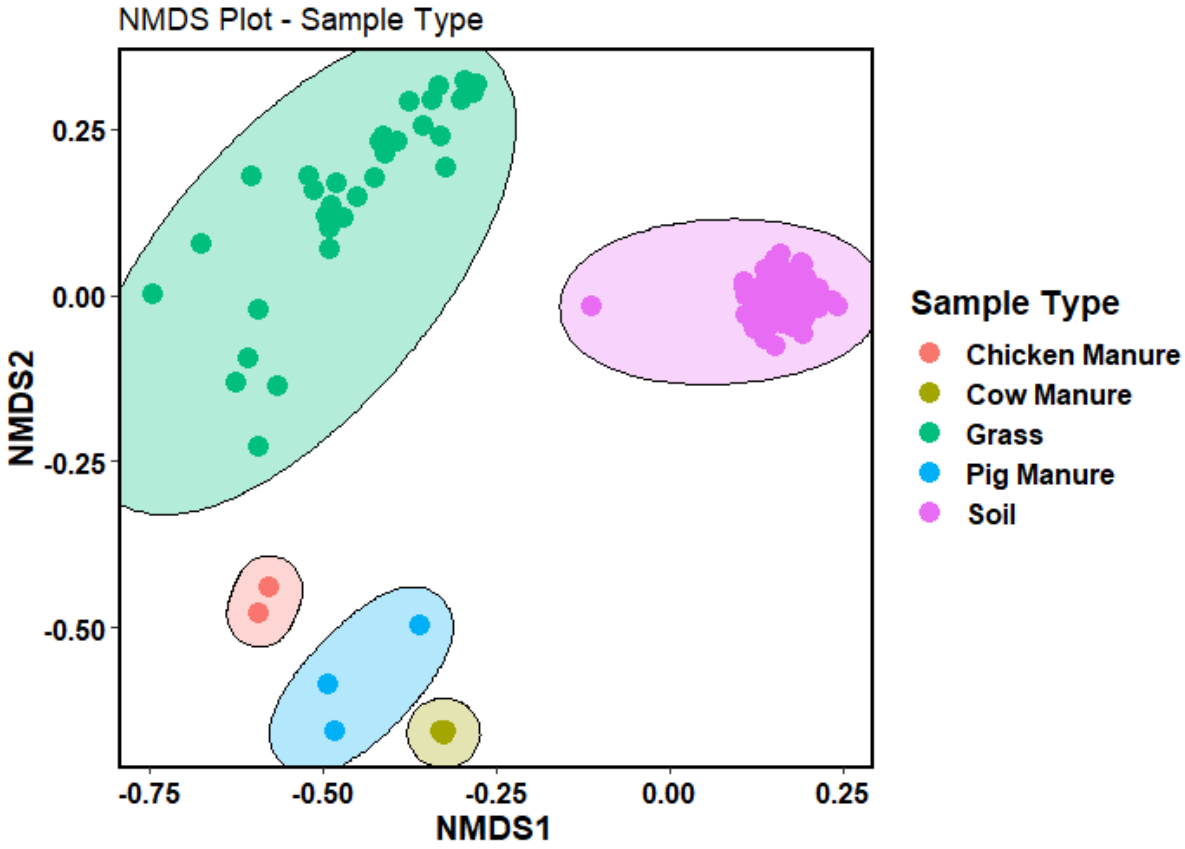

Figure 4.7: Non-metric multidimensional scaling (NMDS) ordination plot of 16S rRNA amplicon data. K=2, stress value = 0.06485712 using Bray – Curtis distances.

Soil and grass phyllosphere samples were analysed separately for treatment and timepoint effects. Treatment had a significant impact on the soil microbial composition, however only accounted for 5% variance in the data (PERMANOVA $p < 0.05$, $R^2 = 0.05$), therefore this effect was considered minimal. Additionally, these results may be affected by homogeneity of variance testing which resulted in a positive result ("permutest" ANOVA $p < 0.05$, $F = 2.84$). However, due to background soil samples ("S.BM.R1", "S.BM.R2", "S.BM.R3") only consisting of one timepoint the PERMANOVA and homogeneity of variance testing were repeated on the four remaining treatments (Control Soil, Pig Manured Soil, Chicken Manured Soil and Cow Manured Soil) to promote a more balanced statistical design. As a result, the homogeneity of variance testing was not significant ("permutest" ANOVA $p > 0.05$, $F = 1.33$) and treatment effect still had a small effect on microbiome composition (PERMANOVA, $p < 0.05$, $R^2 = 0.04$). Pairwise PERMANOVA testing of the treatments indicated that pig manured soil and chicken manured soil had significantly different microbial compositions than control soil (PERMANOVA $p < 0.05$). Additionally, chicken manured soil and pig manured soil had significantly different microbial compositions from each other (PERMANOVA $p < 0.05$). The combined effect of treatment and timepoint was significant, explaining 23% of the variation in the data (PERMANOVA $p < 0.05$, $R^2 = 0.23$). For grass sample analysis, the background timepoint (G.BM) was removed from statistical analysis as there was no replicates for this sample. Treatment was not found to have a significant impact on the grass phyllosphere microbiome composition (PERMANOVA $p > 0.05$, $R^2 = 0.14$; "permutest" ANOVA, $p > 0.05$, $F = 0.9$). Therefore overall, sample type was deemed to have an impact on the microbiome and manure treatment had a small yet significant impact on soil β-diversity but not on grass sample β-diversity.

To investigate how manure application impacts the grass and soil microbiome at OTU level, DESeq2 was used to calculate significantly varying OTUs between manure treated samples and control samples (Section 2.7.4.2.2). In grass and soil samples there were differentially abundant OTUs identified in each manure treatment. Cow manured grass vs control grass resulted in 50 differentially abundant OTUs across four different phyla (Appendix 28-A). Pig manured grass vs control grass resulted in 16 differentially abundant OTUs across 4 different phyla (Appendix 28-B). Chicken manured grass vs control grass resulted in 13 differentially abundant OTUs across 4 different phyla (Appendix 28-C). For soil samples, cow manured soil vs control soil resulted in 37 differentially abundant OTUs across 4 phyla (Appendix 28 – D). Chicken manured soil vs control soil resulted in 84 differentially abundant OTUS across 4 phyla (Appendix 28 – E). Pig manured soil vs control soil resulted in 105 differentially abundant OTUs across 4 phyla (Appendix 28 – F). Chicken manured soil vs control soil resulted in 84 differentially abundant OTUS across 4 phyla (Appendix 28 – E). Heatmaps were then constructed of the OTUs at family level to investigate the temporal patterns of the families. (Figure 4.8, Figure 4.9).

Heatmaps of the relative abundances of differentially abundant OTUs revealed that bacterial families such as the *Pseudomonadaceae*, *Enterococcaceae*, *Peptrostreptococcacea, Staphylococcaceae* and the *Halomonadaceae* were found in high abundance in cow manure and cow manured grass but not found or found in low abundance in control grass. These families that were enriched in cow manured grass were largely no longer detected from ten weeks after the manure was applied (Timepoint 7) (Fig. 4.8-A*)*. Additionally, some members of the microbiota were decreased in abundance in manure treated grass in comparison with the control grass across timepoints, such as *Rhizobiaceae* and *Sphinogobacteriaceae* (Appendix 28 – A, Fig 4.8-A).

From DESeq2 analysis it was found that in chicken manured grass there were bacterial families which were found in a decreased abundance in comparison with the control in the families: *Protebacteria_unclassified, Microbacteriaceae* and *Sphingobacteriaceae* (Appendix 28-B). There was an increase in *Carnobacteriaceae, Alcalgienaceae, Cornyebacteriaceae, Brucellaceae* and *Betaproteobaceri_unclassified. Carnobacteriaceae* and *Corynebacteriaceae* were no longer detected after 6 weeks following manure application (Timepoint 6 onwards) (Fig 4.8-B, Appendix 28- B). *Betaproteobacteria_unclassified* was detected up until 14 weeks following manure application (Timepoint 8) and *Alcaligenaceae* and *Brucellaceae* until the final sampling timepoint at 18 weeks following manure application (Timepoint 9) (Figure 4.8-B).

For pig manured grass, temporal analysis using heatmaps revealed that at family level *Aerococcaceae* was the only family that was found in pig manure and pig manured grass and not in background or control soils and was detected at Timepoints 2-6 and again at Timepoint 9 (Fig 4.8-C). In pig manured grass *Burkholderiaceae, Sphingomonadaceae* and *Sphingobacteriaceae* were decreased in abundance (Appendix 28– C). Families that were increased in abundance in pig manured grass were detected up until 6 weeks following manure application (*Carnobacteriaceae*) and 10 weeks following manure application (*Corynebacteriaceae* and *Moraxellaceae*) (Fig 4.8-C).

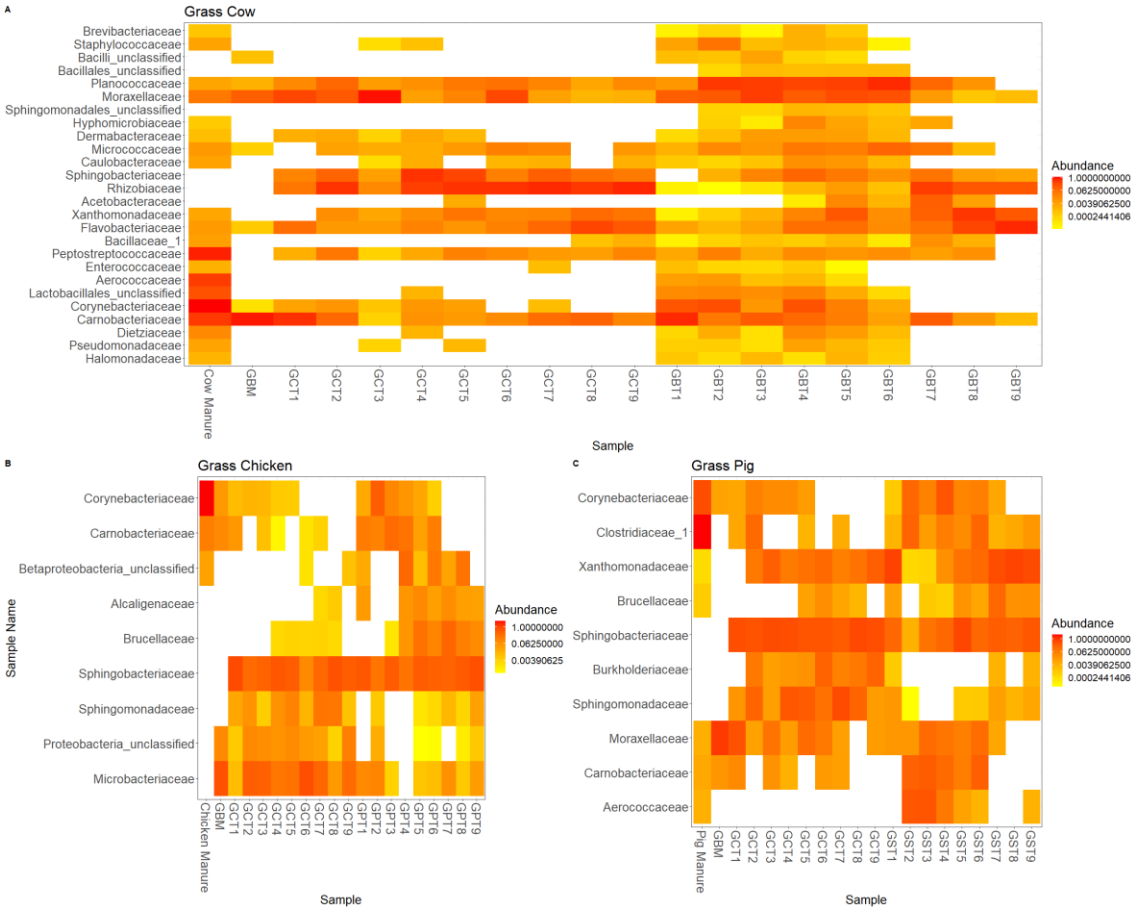

Figure 4.8: Heatmaps of the relative abundances of differentially abundant OTUs determined by DESeq2 analysis (p <0.01) in (A) Cow manure amended Grass (B) Chicken manure amended grass (C) Pig manure amended Grass. Sample codes indicate treatment (GC = grass control, GB = grass amended with cow manure, GP = grass amended with chicken manure and GS = grass amended with pig manure). Timepoints are indicated by codes (BM= background, T1= Timepoint1, T2= Timepoint 2, T3= Timepoint 3, T4 = Timepoint 4, T5 = Timepoint 5, T6 = Timepoint 6, T7 = Timepoint 7, T8 = Timepoint 8, T9 = Timepoint 9.

Soil amended with cow manure contained bacterial families such as the *Lactobacillales_unclassified* and *Porphyromonadaceae* which were not present in control soils but found in high abundance in cow manured soils. These families were detected until 10 weeks following manure application (Timepoint 7). Other families such as *Corynebacteriaceae, Carnobacteriaceae, Planococcaceae,* and *Dietaziaceae* were found in low abundance in control soils and in high abundance in cow manure and cow manured soils and were detected up to 14 or 18 weeks following manure application, with

the exception of *Planococcaceae* which was no longer detected after 5 weeks following cow manure application (Figure 4.9-A).

Soil amended with chicken manure has families that were found in higher relative abundance in chicken manure but were found in low abundance or not present in control soils (Figure 4.9-B). Of these families, *Dermabacteraceae* was not detected in background or control soils and was detected until the end of the trial; 18 weeks post manure application. Other families that were enriched in chicken manured soil and were in low abundance in background and control soils such as *Staphylococcaceae, Corneybacteriaceae, Brevibacteriaceae, Promicromonosporaceae , Comamondaceae* and *Glycomycetacae* were detected until 14 – 18 weeks following manure application (Figure 4.9– B). According to DESeq2 results, eight OTUs were significantly less abundant in chicken manured soils: *Pasteuriaceae, Subdivision3_family_incertae_sedis,Planctomycetaceae,Spartobacteria_family_incertae_sedis, Chitinophagaceae, Bacteroidetes_unclassified* and *Sphinobacteriaceae* (Appendix 28-D).

For pig manured soil samples, the families *Lachnospiraceae, Aerococcaceae, Acidminococcaceae, Bacteroidaceae, Porpyromonadaceae, Gammaproteobacteria_unclassified* and *Streptococcaceae* were only found in pig manure and pig manured soil and therefore were determined to be found in high abundances in pig manured soil due to their introduction from pig manure (Figure 4.9 – C). The majority of these families were no longer detected after 6 weeks following manure application (after Timepoint 6). The families *Dietziaceae, Pseudomonadaceae, Carnobacteriaceae, Enterococcaceae, Corynebacteriaceae* and *Lactobacillaceae* were other families that were found in high abundance in pig manure and in pig manured soils but found in low abundances in control soil. These families were detected up until

between 6 – 10 weeks following manure application (Timepoint 6 and Timepoint 7) (Figure 4.9-C, Appendix 28 - F).

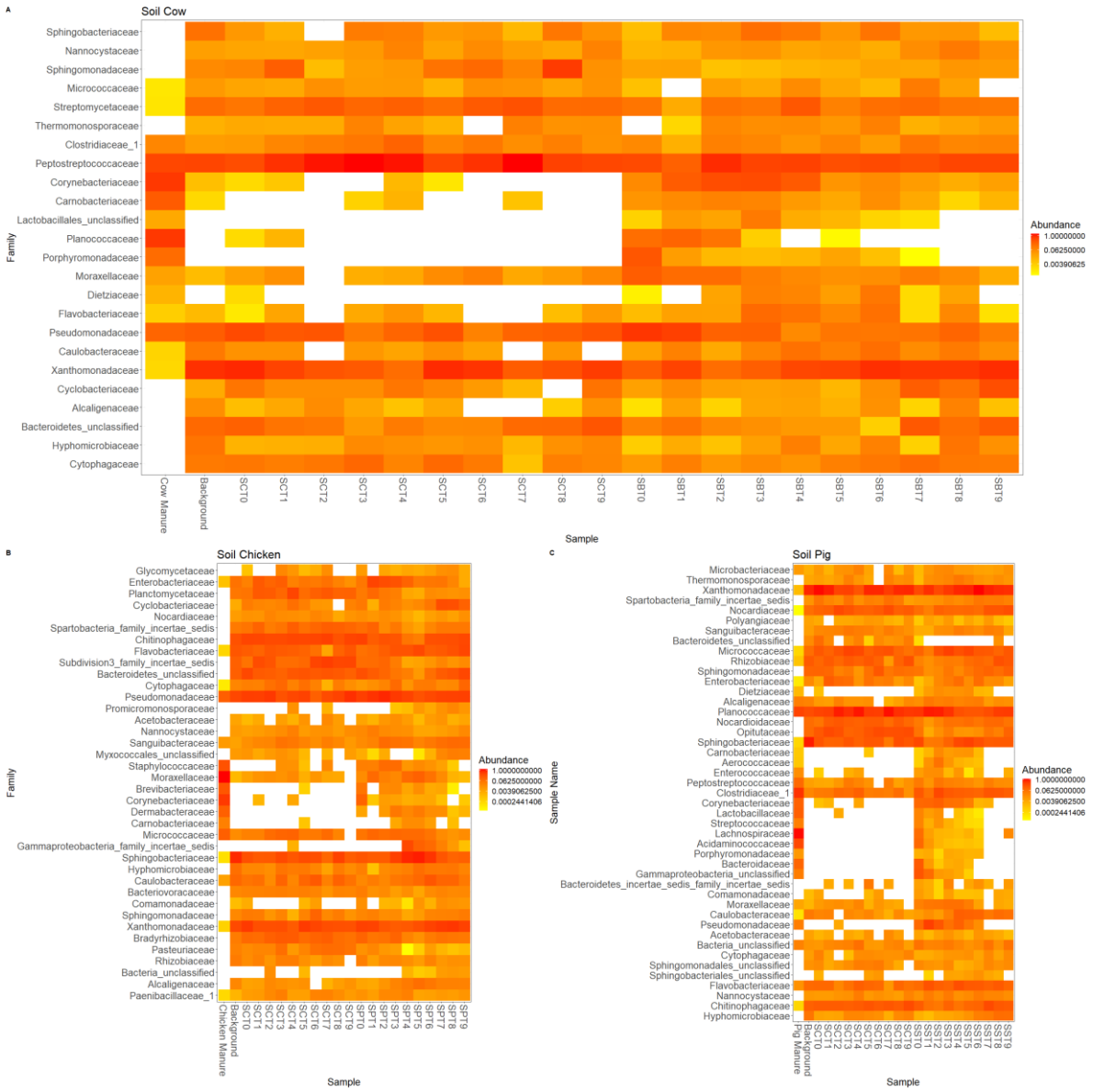

Figure 4.9: Heatmaps of the relative abundances of differentially abundant OTUs determined by DESeq2 analysis (p <0.01) in (A) Cow manure amended soil(B) Chicken manure amended soil (C) Pig manure amended soil. Sample codes indicate treatment (SC = soil control, SB = soil amended with cow manure, SP = soil amended with chicken manure and SS = soil amended with pig manure). Timepoints are indicated by codes (BM= background, T1= Timepoint1, T2= Timepoint 2, T3= Timepoint 3, T4 = Timepoint 4, T5 = Timepoint 5, T6 = Timepoint 6, T7 = Timepoint 7, T8 = Timepoint 8, T9 = Timepoint 9.

4.2.7 Relationship of ARGs and MGEs with the Bacterial Community

In grass phyllosphere samples the microbiome and resistome were found to be strongly correlated based on Bray-Curtis distances according to the mantel test (Spearman, $r = 0.59$, $p < 0.05$). The soil microbiome and resistome were found to have weak but significant correlation according to the mantel test (Spearman, $r = 0.2399$, $p < 0.05$).

4.3 Discussion

An aim of this study was to investigate the differing resistomes and microbiome of cow, pig and chicken manure. Manure has been shown in previous studies to be a hotspot of antimicrobial resistance genes and genes associated with horizontal gene transfer (HGT), such as plasmids and integrons (Binh *et al.*, 2008a; Xu *et al.*, 2020; Yang *et al.*, 2020). The resistome composition of pig, cow and chicken manure was found to be largely similar, with the three manures being dominated by aminoglycoside resistance genes, tetracycline resistance genes and MGEs. Pig manure had the highest abundance of ARGs and cow manure had a significantly lower relative abundance of ARGs than the other two manure types, which has also been reported by Zhang *et al.*, (2017). Chicken manure harboured higher abundances of MDR resistance genes and pig manure contained higher abundances of MGEs and tetracycline resistance genes, which has also been reported by Zhang *et al.*, (2017). This high abundance of tetracycline resistance genes in pig manure could be related to the tetracycline use in the pigs from which the manure was sampled.

The environment is known to naturally harbour a diverse resistome (Forsberg *et al.*, 2012). Most studies investigating the impact of manure application on the microbiome and resistome focus on soil (Santamaría, López and Soto, 2011; Jechalke *et al.*, 2013; Boeckel *et al.*, 2015; Nõlvak *et al.*, 2016), however the phyllosphere of plants has been shown to harbour a diverse microbiome and resistome (Yan *et al.*, 2019, 2020; Zhou *et al.*, 2019). Due to its importance as a food source for livestock as well as its physical

contact with livestock, the impact manure spreading has on the grass phyllosphere was of particular interest in this study. Manure, soil and grass phyllosphere samples were found to all contain a diverse range of resistance genes to clinically important antibiotics, with grass containing 167 genes across 14 gene classes; the highest diversity of genes detected in the samples. Similarly, Yan *et al.*, (2019) investigated the resistome of grass and soil and also found that the grass phyllosphere was rich in ARGs.

A main focus of this study was the impact manure application had on the resistome of soil and grass and the differential impact the three different manure types had on the resistome over time. For grass in particular this aspect of the study was vital as there is, to date, no data regarding the impact agricultural practices, such as manure application, have on the grass phyllosphere microbiome and resistome. Through NMDS plots and PERMANOVA testing, manure treatment was not found to have a significant impact on the overall resistome composition, which is in contrast to what other studies have found for soil and for the episphere of other plants (Han *et al.*, 2018; Liu *et al.*, 2021; Sun *et al.*, 2021). However, it was found that manured soil and manured grass had a higher diversity of resistance genes detected, which has previously been reported for soil (F. Wang *et al.*, 2020; Liu *et al.*, 2021) but to date, not for the grass phyllosphere. A differential impact was found in terms of overall ARG abundance with pig manured grass harboured higher ARG abundance than control grass phyllosphere samples, as well as cow manured and chicken manured grass, which is reflective of the relative abundance patterns of the manures themselves. Interestingly, cow manured and chicken manured grass had lower ARG relative abundance but higher ARG diversity than control grass (Figure 4.1, Figure 4.3-A). Pig manured grass contained higher relative abundances of β-lactam, MLSB and aminoglycoside resistance genes compared to other manure amended grass samples. In comparison to grass phyllosphere samples, manure treatment had no significant impact

on ARG or MGE relative abundance in soils but at gene class level pig manured soil had higher abundances of sulphonamide resistance genes than chicken manured soil, and cow manured soil had higher abundance of β-lactam resistance genes than pig manure.

Manure landspreading has been shown to alter the resistome of agricultural soils by the introduction of manure derived ARGs and MGEs and by enriching the natural resistome for indigenous ARGs (Chen *et al.*, 2017; Gou *et al.*, 2018; F. Wang *et al.*, 2020). In this study, both the grass and soil resistome were disturbed due to the enrichment of indigenous ARGs and also through the direct introduction of manure originating ARGs and MGEs. Both manure amended soil and grass phyllosphere samples resulted in the direct introduction of ARGs, integrons and MGEs from manure application. Manure application introduced genes belonging to 9 gene classes into soil and 10 gene classes into grass, with pig manure introducing the greatest number of genes into soil and grass in comparison to the cow and chicken manure. Notably, the enrichment of soil for sulphonamide, tetracycline and macrolide resistance genes has been reported previously (Ruuskanen *et al.*, 2016; Lopatto *et al.*, 2019; Macedo *et al.*, 2020). In soil samples, only manured soil and manures had integrons detected, illustrating how the application of manure can introduce genes associated with ARG mobilisation into agricultural land. Additionally, all manure treated grass and soil samples had an observed higher number of MGEs detected than control and background samples, illustrating not only the introduction of these genes from manure but also the enrichment of the natural mobilome. This increase in MGEs due to manure application in soil has been previously reported (Nõlvak *et al.*, 2016; Han *et al.*, 2018; Wolters *et al.*, 2018)**,** but to date, not in the grass phyllosphere resistome. However this impact of manure landspreading has been observed in the phyllosphere of rice and wheat crops (Zhou *et al.*, 2019). The increase in genes associated with mobilisation indicates that manured soil and grass may act as hotspots for

HGT, resulting in increased dissemination of ARGs and also their persistence in agricultural land due to their possible integration into indigenous members of the environmental microbiome (Heuer, Schmitt and Smalla, 2011).

One aspect of manure landspreading which has not been fully ratified is the temporal impact manure landspreading has on agricultural land. It has been found that following manure application there is an increase in ARG diversity and abundance, followed by a decrease in ARG abundance (Muurinen *et al.*, 2017; Gou *et al.*, 2018; Macedo *et al.*, 2021). This decrease has been associated with outcompeting of the manure originating bacteria harbouring these genes by the indigenous soil community as the manure bacteria were not well adapted to the soil (Chen *et al.*, 2017; Pérez-Valera *et al.*, 2019). The impact that manure application has is thought to be short term, however, there have been conflicting reports regarding the exact time it takes for the soil to return to a pre-manured state (Fahrenfeld *et al.*, 2014; Lima, Domingues and Da Silva, 2020). Muurinen *et al.*, (2017) found that the majority of manure associated ARGs and MGEs decreased 6 weeks following manure application. Similarly, Macedo *et al.*, (2020), found it took a period of 40 days for ARG levels to return to a pre-manured state. However, a longer impact on the soil resistome has been reported by Chen *et al.*, (2017) who observed that manured soil maintained a higher ARG abundance than untreated soils 60 days following manure application. To investigate the temporal effects of manure, soil and grass were analysed for 18 weeks post manure application and compared this with non-manured soil and grass over the same timeframe. It was found that a manure application resulted in a short-term impact on the soil and grass resistome, as the majority of these manure introduced genes were no longer detected 10 weeks following manure application with the exception of *tnpa4* in cow manured grass, and *intl13* and t*npA2* in pig manure amended soils which were detected 10 – 18 weeks following manure application. In large, these results support

current landspreading guidelines in Ireland for splash plate manure spreading, as livestock can be reintroduced to land 6 weeks following manure application and by this timepoint in this study most of the manure introduced genes were in low relative abundance or below the detection limit. In terms of the differential impact of different manure types it was found that pig manure in both soil and grass resulted in the greatest introduction of manure unique ARGs, illustrating the potential increased risk of antimicrobial resistance (AMR) dissemination associated with pig manure application.

In relation to the impact on the soil and grass microbiomes, manure treatment did not impact soil or grass alpha diversity in this study. The alpha diversity measures for both richness (Chao1) and diversity (Shannon) were similar to previous studies on soil (Xie *et al.*, 2018; Macedo *et al.*, 2021). For grass, richness was similar to Yan *et al.*, (2019) however the measured diversity in this study was lower. As the phyllosphere is known to be impacted by abiotic factors such as climate, the geographical differences between this study and the study performed by (Yan *et al.*, 2019) (Ireland vs Australia) as well as the different environments the grass originated from (park vs agricultural land) may account for differences in results between the two studies. Soil was found to have a significantly higher alpha diversity and richness than grass phyllosphere samples which has also been reported previously (Yan *et al.*, 2019). Sample composition for soil was similar to previous studies, with soil samples being dominated by the phyla: *Verrucomicobia, Acidobacteria* and *Proteobacteria* (Lopatto *et al.*, 2019; Macedo *et al.*, 2021; Sukhum *et al.*, 2021). The manure sample's composition differed from each other, however this corresponded with other reports, with cow manure being composed mainly of *Firmicutes, Proteobacteria* and *Actinobacteria* (Boukerb *et al.*, 2021; Sukhum *et al.*, 2021), pig manure being largely dominated by the phyla *Proteobacteria, Firmicutes, Actinobacteria* and *Bacteroidetes* (Lim *et al.*, 2018; Boukerb *et al.*, 2021), while chicken manure was

dominated by *Proteobacteria* and *Firmicutes* (Boukerb *et al.*, 2021; Peng *et al.*, 2021). In this study it was found that the grass phyllosphere microbiome was mainly composed of the phyla *Protebacteria, Firmicutes, Bacteroidetes* and *Actinobacteria* which corresponds to what has previously been reported (Yan *et al.*, 2019, 2020).

Similar to the impact manure landspreading has on the resistome of agricultural land, manure can alter the environmental microbiome through the introduction of bacteria from the manure (Lopatto *et al.*, 2019) and also through the enrichment of the natural bacterial microbiota (Udikovic-Kolic *et al.*, 2014). It has previously been shown that manure treatment impact the soil bacterial community composition (Udikovic-Kolic *et al.*, 2014; F. Wang *et al.*, 2020; Sun *et al.*, 2021). Through NMDS plots and PERMANOVA testing, manure treatment was found to have a small yet significant effect on the soil β-diversity but this was not the same for grass. As identified by pairwise PERMANOVA testing, pig manured soil and chicken manured soil microbiomes were significantly different to control samples, whereas cow manured soils did not significantly differ from control soils. This result corresponds with what has been previously reported for pig and chicken manured soils but not for cow manured soils (Han *et al.*, 2018). Despite manure treatment not having an overall significant impact on the grass β diversity, both grass and soil samples had OTUs that differed in abundance between manure treated samples in comparison to control samples. DESeq2 analysis revealed that of the grass phyllosphere samples treatments, cow manured grass resulted in the greatest number of differentially abundant bacteria, while in soil pig manured grass had the most impact. Manure application on grass resulted in a decrease in OTUs that were bacteria associated with plant pathogen protection, such as *Burkholderiaceae* and *Sphingomonadaceae* (Chapelle *et al.*, 2016). However, overall, in both grass and soil manure application resulted in an increase of both plant and soil commensal bacteria and also of some families that can be

potential pathogens such as *Enterococcaceae, Staphylococcaceae, Aerococcaceae, Xanthomondaceae* and *Enterobacteriaceae.* This increase in opportunistic pathogens in manured soil has been previously reported by Ding *et al.*, (2014) who reported increases in *Stenotrophomonas* spp. and *Clostridium* spp. Additionally, heatmap construction of the differentially abundant families found that manured grass and soil samples had enriched bacterial families that were in high abundance in manure samples and either not found or found in low abundance in control samples, illustrating how manure alters the grassland microbiota through the direct introduction of bacteria from manure into grassland, but also the enrichment of the indigenous microbiota. This enrichment of the natural microbiome is attributed to the addition of nutrients from manure into the soil (Udikovic-Kolic *et al.*, 2014). In this study, the majority of manure originating bacteria were no longer detected after 6 weeks post manure application and enriched members of the natural microbiota were no longer detected by 10 weeks post manure application. Manure appeared to have a more pronounced impact on the soil microbiome, with the majority of the manure introduced bacteria persisting until week 10 in cow manured soil and week 18 in chicken manured soil and week 6 in pig manured soil. Additionally, the enrichment of the indigenous microbiota lasted between 14 – 18 weeks in cow manure treated soil and chicken manure treated soil, and between 6 - 10 weeks in pig manured soil. This reduced temporal effect of manure treatment on the grass phyllosphere samples in comparison to soil may reflect the dynamic and difficult nature of the phyllosphere environment due to its exposure to both abiotic and biotic factors, such as UV radiation, temperature fluxes and invading plant pathogens (Compant *et al.*, 2019; Sivakumar *et al.*, 2020). Other studies investigating the time dependent impact of manure estimate that the temporal effect of manure landspreading lasts for approximately 2 months in soil (Fahrenfeld *et al.*, 2014; Muurinen *et al.*, 2017; Gou *et al.*, 2018), therefore corresponding to the results found in this study. Additionally, according to the mantel test, the

microbiome and resistome were found to be correlated in both soil and grass. The temporal trends of manure application on both the resistome and microbiome were similar, with the majority of manure introduced ARGs, MGEs and bacteria no longer detected between week 6 -10, indicating that the decline of the resistome is possibly due to the die off of the manure bacteria that may be hosting these ARGs and MGEs. This supports previous studies which show that the impact manure landspreading has on the soil microbiome is short term due to manure originating bacteria not being well adapted to the soil and therefore being outcompeted by the indigenous microbiome (Chen *et al.*, 2017; Muurinen *et al.*, 2017; Gou *et al.*, 2018; Pérez-Valera *et al.*, 2019). Additionally, these results demonstrate, to date the first time, the ability of manure application to alter the grass phyllosphere microbiome.

4.4 Conclusion

In summary, this study demonstrates the impact temporal manure treatment from different livestock sources has on the microbiome and resistome of soil and the grass phyllosphere using 16S rRNA amplicon sequencing and HT-qPCR. Overall, this study highlights the potential role of the grass phyllosphere as a diverse reservoir of MGEs and ARGs to clinically important antibiotics. The presence of clinically important ARGs in grassland may lead to their transfer to livestock through ingestion or through direct contact. Therefore, the potential rate of transfer between grass ARGs and bacteria to livestock needs be assessed to fully elucidate the role the grass phyllosphere plays in the dissemination antimicrobial resistance. Despite manure treatment not having an overall significant effect on soil resistome and grass microbiome compositions, and only having a small effect on soil microbial composition, manure was found to both introduce and enrich the soil and grass for ARGs, MGEs and both beneficial and potentially pathogenic members of the bacterial microbiome. Manure application was found to result in short

term alteration in the microbiome and resistome of grass and soil. Key aspects of this study were the temporal and differential impact cow, pig and chicken manure had on the microbiome and resistome of grass and soil. Overall, there was no significant difference in overall resistome diversity between the manure types; however, it identified a potential increased risk with pig manure use, as pig manure harboured higher overall abundances of ARGs than cow and pig manure. Additionally, pig manure introduced the largest amount of ARGs and MGEs into grass and soil, as well as resulting in the largest differentially abundant OTUs in soil microbiomes. Therefore, further research is required to fully elucidate the risk associated with manure from various livestock and also the role of phyllosphere microbes in the maintenance and dissemination of AMR on plants.

CHAPTER 5

Characterisation of Antimicrobial Resistant World Health Organisation Priority Pathogens in Manure Amended Agricultural Grassland

Chapter 5: Characterisation of Antimicrobial Resistant World Health Organisation Priority Pathogens in Manure Amended Agricultural Grassland

5.1 Introduction

The One Health concept is critically important to understand the dissemination of antimicrobial resistance (AMR). One Health involves understanding the link between humans, animals and the environment and in the case of AMR, is particularly relevant due to the ubiquitous nature of ARB (Robinson *et al.*, 2016). Antimicrobial resistance naturally occurs in the environment. Environmental bacteria and fungi are natural producers of antibiotics and use them as a defensive mechanism to survive in competitive environments, such as the soil. In response to this antibiotic production, bacteria have developed antimicrobial resistance mechanisms to survive in the presence of the antibiotics (José L Martínez, 2012). Antibiotics have been harnessed by humans for clinical use, however, due to the misuse and overuse of these agents novel AMR determinants have developed and the spread of AMR has increased (Ventola, 2015).

The role of the environment in the spread of AMR and the impact antibiotic use has on the occurrence, spread and persistence of AMR in environmental bacteria is of interest; particularly in environments associated with human use such as agricultural land (Binh *et al.*, 2008b; Hu *et al.*, 2016; Chen *et al.*, 2017, 2019; Blau *et al.*, 2018; Checcucci *et al.*, 2020; Liu *et al.*, 2021) and waters receiving wastewater effluent (Pantanella *et al.*, 2020; Sabri *et al.*, 2020). In recent years, the impact manure application has on the occurrence of AMR on agricultural land has received attention. This is due to the AMR selection pressure exerted by manure application and the associated introduction of ARGs, MGEs and antibiotic residues into the soil (Larsson and Flach, 2021). Additionally, many clinically relevant ARGs have environmental origins, such as $bla_{\text{CTX-M}}$ in *Kluyvera* spp. and *qnrA* and $bla_{\text{OXA-58}}$ in *Shewanella algae* (Nazic, Poirel and Nordmann, 2005; Poirel

et al., 2005; Tamang *et al.*, 2008; Hidalgo *et al.*, 2019). Additionally, bacteria that are clinically important nosocomial pathogens can also occur in the environment such as the World Health Organisation (WHO) priority pathogens: *Klebsiella pneumoniae, Acinetobacter baumannii, Escherichia coli* and *Pseudomonas aeruginosa.* In particular, carbapenem resistant strains of these species and extended spectrum β-lactamase (ESBL) producing *Enterobacterales* are of critical priority.

Escherichia coli and *K. pneumoniae* are members of the *Enterobacterales* family. Due to its presence in the gastrointestinal tract of warm-blooded animals, *E. coli* is generally considered to be a commensal organism. However, pathogenic forms of *E. coli,* such as enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) have been isolated from both humans and animals (Gerba, 2015; Lupindu, 2017). Additionally, *E. coli* has been found to become naturalised in soil (Ishii *et al.*, 2006; Brennan *et al.*, 2010), therefore the characterisation of these environmental strains is vital in understanding AMR from a One Health perspective. *Klebsiella pneumoniae* and *P. aeruginosa* are both considered ubiquitous in the environment and have been identified in soil, water and plants (Deredjian *et al.*, 2014; Martin and Bachman, 2018). *Acinetobacter baumannii* is an opportunistic pathogen of hospital patients and has been isolated from agricultural soils, manure and crops (Ferreira *et al.*, 2011; Hrenovic *et al.*, 2014). However, the exact ecological niche occupied by *A. baumannii* as well as the distribution and diversity in the environment of *A. baumannii* needs to be fully evaluated (Towner, 2009).

The aims of this study were to isolate antibiotic resistant *E. coli, A. baumannii, K. pneumoniae* and *P. aeruginosa* from cow, pig and chicken manure and grass and soil prior to and after the application of the manures and to characterise the antimicrobial susceptibility profiles and genes present in these isolates to a panel of critically important antibiotics through phenotypic and genotypic testing. Additionally, through whole

genome sequencing (WGS) of the *E. coli* and *A. baumannii* isolates, the multilocus sequence type of the isolates aimed to be determined as well as the presence of antibiotic resistance genes (ARGs) and plasmid replicon types in the isolates to further characterise their antibiotic resistance profiles.

5.2 Results

5.2.1 Isolation and identification of AMR priority pathogens

As part of this study, 745 bacteria were successfully isolated and identified. The bacteria were selected on selective media supplemented with antibiotics at breakpoint concentrations using EUCAST (2020) and CLSI (2020) guidelines to select for antibiotic resistant strains. The antibiotics used were ciprofloxacin (1mg/L), cefotaxime (4 mg/L), colistin (4 mg/L) and kanamycin (16 mg/L). The selective agars included: SCA (Section 2.1.1.4) for the isolation of *Klebsiella* spp., PIA (Section 2.1.1.5) for the isolation of *Pseudomonas* spp., LAM (Section 2.1.1.3) for the isolation of *Acinetobacter* spp., and EMB Agar (Section 2.1.1.2) for the isolation of *Escherichia coli*. Overall, 720 isolates were identified to species level using MALDI-TOF and the 26 isolates not identified using MALDI-TOF were identified through their 16S rRNA sequences. In total, 383 bacteria were identified from grass, 326 from soil, 21 from cow manure, 7 from chicken manure and 9 from pig manure. Overall, 25 genera of bacteria were identified (Figure 5.1, Appendix 29). Of particular interest in this study was the isolation of 46 *E. coli*, 8 *K. pneumoniae* and 255 *Acinetobacter* spp., 67 of which were identified as *A. baumannii*, 185 as *A. calcoaceticus* and 3 as *A. indicus.*

Escherichia coli were isolated from pig manure (n=7), cow manure (n=14), soil (n=11) and grass (n=14) (Appendix 29, Appendix 30). Of the *E. coli* isolated from soil, six were found in background soil samples that were sampled from the field before any manure

was applied, three from non-manured soil (control samples) 10 weeks following manure application (T7) and one isolate from pig manured soil three weeks post manure application (T3). All fourteen *E. coli* isolated from grass were isolated from manured grass. Three isolates were from pig manured grass 1 week post manure application, one isolate from poultry manured grass 3 weeks post manure application and nine isolates from cow manured grass 5 weeks post manure application.

All eight *K. pneumoniae* were isolated from grass samples with seven isolates originating from non-manured grass (control samples) at week one of the field trial (n=5) and at week five week of the field trial (n=2). The remaining *K. pneumoniae* isolate came from cow manured grass at week one of the field trial (Appendix 29, Appendix 30).

Acinetobacter spp. were isolated from grass (n= 129) and soil (n= 126) (Appendix 29, Appendix 30). The species were distributed according to sample type: 63 grass isolates were *A. baumannii,* 63 were *A. calcoaceticus* and three were *A. indicus.* Of the *Acinetobacter* spp. isolated from grass, 10 were isolated from background grass samples, 32 were isolated from control grass samples with isolates being found one week (n=14), three weeks (n=1), five weeks (n=1), 10 weeks (n=5), 14 weeks (n=8) and 18 weeks (n=3). Twenty-three *Acinetobacter* spp. isolates originated from pig manured grass at 1 week (n=5), 3 weeks (n=1), five weeks (n=1), 10 weeks (n=11), 14 weeks (n=4) and 18 weeks (n=1) post pig manure application. Forty-four *Acinetobact*er spp. isolates came from chicken manured grass and were isolated 1 one week (n=14), three weeks (n=8), five weeks (n=2), 10 weeks (n=9), 14 weeks (n=7) and 18 weeks (n=4) weeks following chicken manure application. Twenty *Acinetobacter* spp. were isolated from cow manured grass at one week (n=3), three weeks (n=9), 10 weeks (n=2) and 14 weeks (n=6) following cow manure application. In soil samples four isolates were *A. baumannii* and 122 were *A. calcoaceticus*. One isolate was isolated from background soil. Forty *Acinetobacter* spp.

isolates were found in non-manured soil (control soil) 1 week (n=12), three weeks (n=3), five weeks (n=11), 10 weeks (n=7) and 14 weeks (n=7). Twenty-three *Acinetobacter* spp. isolates were found in pig manured soil and were found one week (n=5), five weeks (n=4), 10 weeks (n=9), 14 weeks (n=3) and 18 weeks (n=2) following pig manure application. Forty-three *Acinetobacter* spp. isolates were found in chicken manured soil and were found one week (n=6), three weeks (n= 7), five weeks (n=6), 10 weeks (n=10), 14 weeks (n=8) and 18 weeks (n=6) following chicken manure application. Nineteen *Acinetobacter* spp. isolates were found in cow manured soil and were found one week (n=4), three weeks (n= 1), 10 weeks (n=4), 14 weeks (n=9) and 18 weeks (n=1) following cow manure application. No *P. aeruginosa* isolates were identified. The *E. coli, K. pneumoniae* and *Acinetobacter* spp. isolates were further analysed using antimicrobial susceptibility testing against a range of clinically relevant antibiotics.

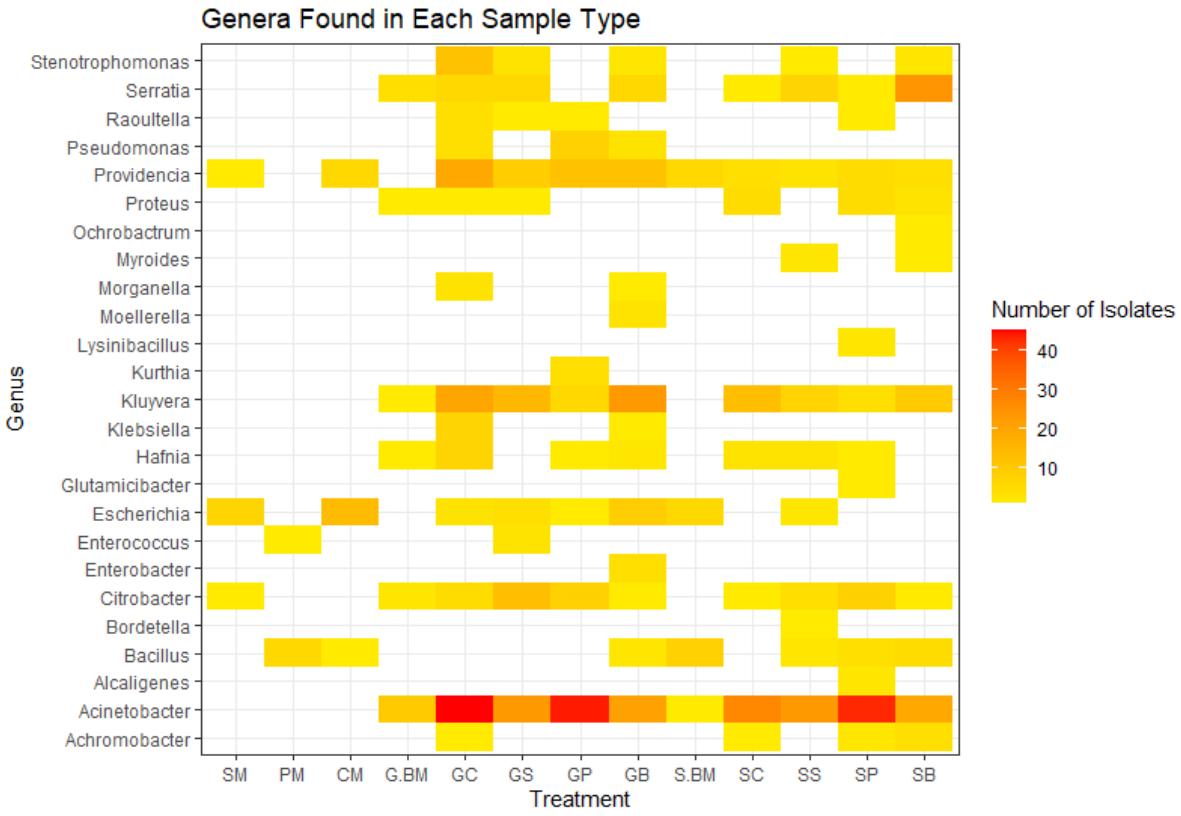

Figure 5.1: Heatmap illustrating the number of each genus identified in each sample type and treatment. Sample codes are as follows: SM = pig manure, PM= chicken manure, CM = cow manure, G.BM = Background Grass, GC = control grass, GS = pig manured grass, GP = chicken manured grass, GB = cow manured grass, S.BM = background soil, SC = control soil, SS = pig manured soil, SP = chicken manured soil, SB = cow manured soil.

5.2.2 Antibiotic Susceptibility Testing and Targeted PCR

Overall, the antibiotic susceptibility profiles of 309 *E. coli*, *K. pneumoniae* and *Acinetobacter* spp. were analysed against clinically relevant antibiotics (Appendix 30). Of the 46 *E. coli* identified, 35 were tetracycline resistant, 9 were cefotaxime resistant, 24 were kanamycin resistant, 0 were amikacin or imipenem resistant and 16 were ciprofloxacin resistant. Of these 46 isolates, 31 isolates were found to be multidrug resistant with 19 conferring resistance to two antibiotic classes and 12 were resistant to three antibiotic classes. *Escherichia coli* isolates displaying resistance to two classes of antibiotics were resistant to tetracycline and kanamycin (n=16) or tetracycline and ciprofloxacin (n=3). Isolates demonstrating resistance to three classes of antibiotics were

resistant to tetracycline, kanamycin and ciprofloxacin (n=3) or tetracycline, cefotaxime ciprofloxacin (n=9). The nine cefotaxime resistant *E. coli* were isolated from cow manure and tested negative for AmpC β-lactamase production and positive for ESBL production according to double disk synergy testing (Appendix 31). Only one isolate of *K. pneumoniae* was resistant to any of the antibiotics tested: tetracycline. *Acinetobacter* spp. isolates were susceptible to the antibiotics tested with the exception of two isolates (*A. baumannii*) that were tigecycline resistant and one isolate (*A. indicus*) that was kanamycin resistant. All three were isolated from grass samples. Colistin MIC determination by broth microdilution was carried out on 16 isolates: four *E. coli* isolates, eight *K. pneumoniae* isolates and four *A. baumannii* isolates. These isolates were selected to colistin MIC determination as they were selected on colistin supplemented agar plates. This resulted in 15 isolates being identified as colistin resistant (MIC ≥4 mg/L) and one *A. baumannii* was susceptible (Table 5.1). The colistin resistant isolates underwent targeted PCR for plasmid mediated colistin resistance genes (*mcr1-9*, Section 2.7.2.1.2.6) and all isolates were negative for these genes.

Table 5.1: Table displaying colistin MIC results from broth microdilution testing.

Isolate Name	Sample Origin	Colistin MIC (mg/L)	Bacteria ID
SC T7 EMB COL 2	Soil	4	*E. coli*
SC T7 EMB COL 3		4	
SC T7 EMB COL 4		8	
GP T3 EMB COL 2	Grass	4	
GC T5 SC COL 2		4	*K. pneumoniae*
GC T5 SC COL 3		32	
GC T1 PIA COL 4		128	
GC T1 LAM COL 1		32	
GC T1 LAM COL 2		16	
GC T1 LAM COL 3		4	
GC T1 LAM COL 4		64	
GC T1 LAM COL 6		64	
GP T3 LAM COL 2		2	*A. baumannii*
GP T3 LAM COL 3		4	
GP T3 EMB COL 1		4	
GB T3 LAM COL 1		4	

5.2.3 Whole Genome Sequencing

Due to the results from antibiotic susceptibility testing and their clinical importance *E. coli* and *A. baumannii* samples underwent whole genome sequencing (WGS), resulting in the sequencing of 46 *E. coli* and 67 *A. baumannii*. Isolates were analysed for the presence of multi locus sequence types (MLST), plasmid replicon types and antibiotic resistance genes (ARGs). *Escherichia coli* isolates also underwent PointFinder analysis to identify

point mutations in genes. A summary of WGS data is found in Table 5.2 and Appendix 32.

5.2.3.1 MLST Typing

Multi-locus sequence types were assigned according to the Pasteur naming scheme. For *E. coli*, a total of 11 different MLST types were identified: ST10 (n=2), ST58 (n=17), ST106 (n=1), ST156 (n=4), ST189 (n=2), ST446 (n = 4), ST453 (n=1), ST617 (n=2), ST1126 (n=3), ST1248 (n=1) and ST1431 (n= 9) (Table 5.2, Appendix 32). ST1431 and ST145 were the dominant MLST types identified in cow and pig manure respectively. ST58 was the dominant MLST type obtained from grass and soil and additionally was detected across all sample types. Additionally, ST58 was only in common between cow manure and cow manured grass whereas it was only identified in non manure-amended soil.

All 67 MALDI-TOF identified *A. baumannii* isolates were also identified as *A. baumannii* by WGS. For the 67 *A. baumannii* isolates MLST types were identified for 35 of the isolates but could not be identified for the remaining 32 isolates. Multi-locus sequence types included ST44 (n=15), ST462 (n=10), ST584 (n=2), ST1027 (n= 8), ST1190 (n=1) (Table 5.2, Appendix 32). The *A. baumannii* isolated from soil could not have their MLST classification successfully identified and 28 grass isolates could not be classified, perhaps due to their environmental origin.

5.2.3.2 Plasmid Replicon Typing

No plasmid replicons were detected in the *A. baumannii* isolates. However, this is due to the bias in the composition of the databases towards *E. coli* rather than an absolute identification of no replicon types. *Escherichia coli* samples had 23 different plasmid replicon types detected (Table 5.2, Appendix 32). Additionally, plasmids were detected in all 46 *E. coli* samples with 44 (95.65%) isolates harbouring more than one replicon,

illustrating the horizontal gene transfer (HGT) of resistance genes in these samples. In total, 9,12,11 and 11 plasmid replicons were identified in cow manure, pig manure, grass and soil, respectively. The majority of *E. coli* isolates (n=44) had more than one plasmid replicon detected by WGS with isolates harbouring 2 (n=13), 3 (n=6), 4 (n=2), 5 (n=9), 6 (n=4) and 7 (n=10) plasmid replicons detected. The most common plasmid replicon type detected in the *E. coli* isolates were ColRNAI_1 (n=38), IncFII_1 (n=27) and IncFIB_1 (n=25).

5.2.3.3 Antimicrobial Resistance Gene Detection

In *E. coli*, 31 genes were detected belonging to 9 different classes of antibiotic resistance genes: Aminoglycosides resistance genes, Chloramphenicol resistance genes, MDR resistance genes, MLSB resistance genes, Quinolone resistance genes, β-lactam resistance genes, Sulphonamide resistance genes, Tetracycline resistance genes and Trimethoprim resistance genes (Table 5.2, Appendix 32). All *E. coli* isolates contained the multidrug efflux pump *mdf(A)_1*.

Phenotypic resistance to tetracyclines were supported by the detection of *tetA* or *tetB* genes in 33 out of the 35 *E. coli* identified as tetracycline resistant (n=35) or as having reduced tetracycline susceptibility (n=1). Of the ESBL producing *E. coli* isolated from cow manure, eight contained $bla_{\text{CTX-M-15}}$. The remaining ESBL positive isolate (CM EMB CIPRO 1) was only found to harbour the *E. coli* efflux pump *mdf(A)-1*. For the 16 isolates displaying ciprofloxacin resistance phenotypes, eight isolates were shown by WGS to harbour the fluoroquinolone resistant gene *qnrS_1* and four ciprofloxacin susceptible isolates also contained this gene. All ciprofloxacin resistant isolates had mutations in the QRDR of one or more of the genes: *gyrA, parC* and *parE* which confers reduced susceptibility or resistance to the fluoroquinolones (Hopkins, Davies and Threlfall, 2005). The point mutations in the *gyrA* gene consisted of the following point mutations which

resulted in amino acid changes: p.D87N (D to N), p.S83L (S to L) and p.D87H (D to H). Mutations in the *parC* genes consisted of the amino acid altering point mutations p.S80I (S to I) and p.S57T (S to T). Mutations in the *parE* gene consisted of the amino acid altering point mutation p.S458A (S to A) (Appendix 29). Additionally, one ciprofloxacin susceptible isolate (CM EMB KAN 1) had a mutation in the *parC* gene (p.S57T). For aminoglycoside resistance nine genes were detected in *E. coli* isolates: the N-Acetyltransferase *aac(3)-IVa_1,* the O-Adenylyltransferase *ant(3'')-Ia_1,* the adenylyltransferase *aadA5_1* and the O-Phosphotransferases *aph(3'')-Ib_2, aph(3'')-Ib_5, aph(3')-Ia_1, aph(3')-Ia_10, aph(4)-Ia*_1 and *aph(6)-Id_1*. Of these aminoglycoside resistance genes only *aph(3')-Ia-I* and *aph(3')-Ia-10* confer resistance to kanamycin. Of the 24 isolates displaying phenotypic kanamycin resistance, 17 contained *aph(3'-IA-1)* and 3 contained the *aph(3')-Ia-10.* Four isolates that were identified as phenotypically kanamycin resistant (SM EMB CIPRO 1 – 4) had no kanamycin resistance genes detected (Appendix 32).

Outside of the antibiotic classes to which resistance was phenotypically detected for, resistance genes to other antibiotic classes were identified in the samples through WGS. Twenty-nine *E. coli* isolates had resistance to ampicillin (bla_{TEM-1}) and the dihydrofolate reductase inhibitor, trimethoprim (*dfra5_1, dfrA1_10, dfrA17_1* and *dfrA14_5*). *Escherichia coli* isolated from pig manure and pig manured grass contained MLSB resistance genes *erm(B*) and *mph(A)_2. Escherichia coli* isolated from pig manure had chloramphenicol resistance genes *catA1* and *catB3* and *E. coli* from pig manure, cow manure and pig and cow manured grass had sulphonamide resistance genes *sul1_5* or *sul2_2.* No colistin resistance genes were detected in samples that were phenotypically resistant according to broth microdilution results for colistin (Appendix 32).

Two *A. baumannii* isolates were phenotypically identified as tigecycline resistant however WGS analysis did not identify the presence of any tigecycline resistance genes in these isolates. All *A. baumannii* isolates identified contained the Ambler Class A β-lactamase *bla*ADC-25_1. All *A. baumannii* isolates contained one of the 11 Class D β-lactamases (OXA β-lactamases enzymes) detected in the samples. The OXA genes detected in the samples consisted of OXA106 (n=18), OXA125 (n=2), OXA180 (n=14), OXA 203 (n=7), OXA262 (n=11), OXA326 (n=2), OXA322 (n=1), OXA332 (n=2), OXA354 (n=1), OXA430 (n=1) and OXA70 (n=8) (Table 5.2, Appendix 32).

Table 5.2: Table summarising whole genome sequencing (WGS) data for E. coli and A. baumannii isolates.

Sample Type	Bacteria ID	Number of Isolates	Antibiotic Resistance Genes	Plasmid Replicons	MLST	Point Finder
Cow Manure	*E. coli*	14	*aac(3)-IVa_1 (n=3)* *aph(3'')-Ib_2 (n=3)* *aph(3'')-Ib_5 (n=10)* *aph(3')-Ia_1 (n=2)* *aph(3')-Ia_10 (n=3)* *aph(4)-Ia_1 (n=3)* *aph(6)-Id_1 (n=13)* *$bla_{CTX-M-15\_1}$ (n=8)* *$bla_{TEM-104\_1}$ (n=1)* *bla_{TEM-1B_1} (n=9)* *dfrA5_1 (n=2)* *floR_2 (n=4)* *mdf(A)_1 (n=14)* *qnrS1_1 (n= 8)* *sul1_5 (n=1)* *sul2_2 (n=8)* *sul2_3 (n=1)* *tet(A)_6 (n= 9)*	Col(BS512)_1(n=1) Col156_1 (n=1) Col440II_1 (n=3) Col8282_1 (n=1) ColRNAI_1 (n=14) ColpVC_1 (n=1) IncFIB(AP001918)_1 (n=5) IncFII_1 (n=2) IncY_1 (n=7)	ST106 (n=1) ST1126 (n=2) ST58 (n=1) ST1431 (n= 10)	*gyrA* (n=18) *parC* (n=10), *parE* (n=9)
	A. baumannii	0	*N/A*	N/A	N/A	N/A
Chicken Manure	*E. coli*	0	*N/A*	N/A	N/A	N/A
	A. baumannii	0	*N/A*	N/A	N/A	N/A

Sample Type	Bacteria ID	Number of Isolates	Antibiotic Resistance Genes	Plasmid Replicons	MLST	Point Finder
Pig Manure	*E. coli*	7	*ant(3'')-Ia_1 (n=2)* *aph(6)-Id_1 (n=3)* *aph(3'')-Ib_2 (n=2)* *aph(3'')-Ib_5 (n=1)* *aph(3')-Ia_1 (n=1)* *bla*$_{TEM-1A\_1}$ *(n=2)* *bla*$_{TEM-1B\_1}$ *(n=4)* *bla*$_{TEM-190\_1}$ *(n=1)* *tet(B)_1 (n=4)* *tet(A)_6 (n=1)* *dfrA5_1 (n=1)* *dfrA1_10 (n=6)* *sul1_5 (n=6)* *catA1_1 (n=4)* *catB3_2 (n=4)* *erm(B)_18 (n=4)* *floR_2 (n=1)* *mdf(A)_1 (n=7)* *mph(A)_2 (n=4)*	Col(BS512)_1 (n=1) Col156_1 (n=1) Col440I_1 (n=4) Col8282_1 (n=1) ColRNAI_1 (n=6) ColpVC_1 (n=1) IncFIB(AP001918)_1 (n=3) IncFIC(FII)_1 (n=2) IncFII(pECLA)_1_pECLA (n=4) IncFII_1 (n=6) IncI1_1_Alpha (n=4) p0111_1 (n=4)	ST617 (n=2) ST156 (n=4) ST58(n=1)	*gyrA*(n=8) *parC* (n=4) *parE* (n=1)
	A. baumannii	0	*N/A*	N/A	N/A	N/A

Sample Type	Bacteria ID	Number of Isolates	Antibiotic Resistance Genes	Plasmid Replicons	MLST	Point Finder
Soil	*E. coli*	11	*aph(3'')-Ib_5 (n=6)* *aph(3')-Ia_1 (n=6)* *aph(6)-Id_1 (n=6)* *blaTEM-104_1 (n=2)* *blaTEM-190_1 (n=4)* *dfrA5_1 (n=6)* *floR_2 (n=6)* *mdf(A)_1 (n=11)* *tet(A)_6 (n=6)*	Col(BS512)_1 (n=6) Col156_1 (n=6) Col440II_1 (n=1) Col8282_1 (n=6) ColRNAI_1 (n=7) ColpVC_1 (n=6) IncFIA(HI1)_1_HI1 (n=4) IncFIA_1 (n=1) IncFIB(AP001918)_1 (n=7) IncFIB(pB171)_1_pB171 (n=4) IncFII_1 (n=6)	ST1248 (n=1) ST58 (n= 6) ST446 (n=4)	*N/A*
	A. baumannii	5	*bla*$_{ADC-25}$*_1 (n=5)* *bla*$_{OXA-326}$ (n=2) *bla*$_{OXA-332}$ (n=1) *bla*$_{OXA-354}$ (n=2)	None detected	Cannot be determined	None detected

Sample Type	Bacteria ID	Number of Isolates	Antibiotic Resistance Genes	Plasmid Replicons	MLST	Point Finder
Grass	*E. coli*	14	*aadA5_1 (n=2)* *ant(3'')-Ia_1 (n=3)* *aph(3'')-Ib_5 (n=9)* *aph(3')-Ia_1 (n=9)* *aph(6)-Id_1 (n=11)* *bla$_{TEM-1A_1}$ (n=5)* *bla$_{TEM-1B_1}$ (n=9)* *dfrA14_5 (n=2)* *dfrA17_1 (n=2)* *dfrA1_10 (n=1)* *dfrA5_1 (n=9)* *erm(B)_18 (n=2)* *mdf(A)_1 (n=14)* *mph(A)_2 (n=4)* *qnrS1_1 (n=4)* *sul1_5 (n=3)* *sul2_2 (n=2)* *sul2_3 (n=9)* *tet(A)_6 (n=5)* *tet(B)_2 (n=9)*	Col156_1 (n=9) Col440II_1 (n=11) ColRNAI_1 (n=11) IncFIB(AP001918)_1 (n=10) IncFIB(pKPHS1)_1_pKPHS1 (n=2) IncFII_1 (n=13) IncN_1 (n=2) IncX1_1 (n=2) IncX2_1 (n=2) IncX3_1 (n=2) IncX5_1 (n=2)	ST10 (n=2) ST58 (n=9) ST189 (n=2) ST453 (n=1)	*gyrA* (n=2) *parC* (n=2)
	A. baumannii	64	*bla$_{ADC-25_1}$ (n=67)* *bla$_{OXA-106_1}$* (n=18) *bla$_{OXA-125_1}$* (n=2) *bla$_{OXA-180_1}$* (n=14) *bla$_{OXA-203_1}$* (n=7) *bla$_{OXA-262_1}$* (n=11) *bla$_{OXA-322_1}$* (n=1) *bla$_{OXA-430_1}$* (n=1_ *bla$_{OXA-70_1}$* (n=8)	None detected	ST44 (n=15) ST462 (n=10) ST584 (n=2) ST1027 (n=7) ST1190 (n=1)	None detected

5.3 Discussion

In this study, *E. coli, K. pneumoniae* and *A. baumannii* were successfully isolated from samples, while *P. aeruginosa* was not detected. In addition, the use of selective agar for the isolation of these pathogens also resulted in the co-culturing of 21 genera of environmental bacteria. The isolation of such a wide range of additional bacteria is important to note as in non-clinical environments the further speciation of the isolates is vital in order to characterise the bacteria as susceptible or resistant to the tested antibiotics.

Klebsiella pneumoniae has been previously isolated from environmental samples such as soil, water and plants (Deredjian *et al.*, 2014; Huang *et al.*, 2016; Martin and Bachman, 2018) and has been associated with plant growth promotion (Riggs *et al.*, 2001) and nitrogen fixation (Iniguez, Dong and Triplett, 2004). Therefore, the identification of grass associated *K. pneumoniae* was not unexpected. Despite the *K. pneumoniae* isolated in this study being largely antibiotic susceptible, the potential of this opportunistic pathogen to reside in the grass phyllosphere is a potential route for pathogenic strains and antimicrobial resistant strains to transfer to humans and animals. A study by Huang *et al.*, (2016) showed a maize associated pathogenic strain of *K. pneumoniae* being additionally infectious to murine models and also a human clinical strain being pathogenic to maize crops, illustrating the potential diverse pathogenicity of *K. pneumoniae* isolates. Therefore, the presence of *K. pneumoniae* in the grass phyllosphere is an important finding for potential AMR dissemination.

The isolation of *E. coli* from cow manure (Martínez-Vázquez *et al.*, 2021), pig manure (Duggett *et al.*, 2020; Trongjit and Chuanchuen, 2021) and soil (Ishii *et al.*, 2006; Montealegre *et al.*, 2018) has previously been reported. As *E. coli* has primarily been associated with the gastrointestinal tract of mammals it has been used as an indicator of faecal contamination. However, the presence and persistence of naturalised *E. coli* in soil

has jeopardised the use of *E. coli* as a faecal indictor (Ishii *et al.*, 2006). To date, the presence of *E. coli* on grass has not been documented, however, *E. coli* has been documented to survive and colonise on other plants, such as lettuce (Dublan *et al.*, 2014; Williams and Marco, 2014). Therefore, plants, like the soil, can be considered a secondary reservoir of *E. co*li (Dublan et al., 2014). In this study *E. coli* were only isolated from manure contaminated grass. The ST types detected in pig manure and pig manured grass differed, whereas ST58 was in common between cow manure and cow manured grass. Therefore, these *E. coli* may have been found in the grass due to introduction from the manure itself or, in the case of the pig manured grass, were present before manuring and become of a detectable abundance due to the nutrients introduced by the manure stimulating their proliferation. Nevertheless, the identification of these *E. coli* isolates in the grass phyllosphere provides potential evidence of a novel reservoir for this bacterium in the environment. Considering the ubiquity of grassland in agriculture and the close contact the phyllosphere has to livestock illustrates the importance in fully elucidating this habitat plays in the maintenance and spread of *E. coli* in the environment.

To date, there have been few WGS based studies of environmental *E. coli* therefore there is a dearth of information regarding MLST types of environmental strains. The MLST results showed that different samples had differing MLST types detected, however ST58, which was the most common sequence type detected in the samples, has also been found globally in a wide range of hosts such as companion animals (Dierikx *et al.*, 2012), livestock and human clinical isolates (Leverstein-van Hall *et al.*, 2011; Paramita *et al.*, 2020). Other MLST types detected in the samples, such as ST453 and ST10 in pig and chicken amended grass samples have been detected in both livestock and humans and have been found to be associated with clinically important antibiotic resistance genes such as $bla_{\text{CTXM-15}}$ (Gerhold *et al.*, 2016; Pietsch *et al.*, 2017; Abraham *et al.*, 2018). In this

study these ST types had phenotypic resistance to tetracycline (n=2) and ciprofloxacin (n=1) and through WGS were determined to have ARGs to multiple other classes of antibiotics detected, illustrating the potential of the grass phyllosphere to harbour *E. coli* with ARGs to priority antibiotics.

The *E. coli* isolates in this study contained a diverse range of plasmid and ARGs. Overall, 23 plasmid replicon types were detected in *E. coli* with ColRNAI_1, IncFII_1 and IncFIB replicon types being the most commonly detected in the isolates. Additionally, the detection of multiple plasmids in 95.65% of samples indicates the potential of HGT in livestock associated and environmental strains of *E. coli.* The majority of the *E. coli* in this study were multidrug resistant with tetracycline and kanamycin resistance being the most common resistance phenotype detected. In Ireland, tetracyclines are the most commonly used veterinary antibiotic (HPRA, 2018) and there have been many studies illustrating the abundance of tetracycline resistant bacteria in manured agricultural soil (Schmitt *et al.*, 2006; Kang *et al.*, 2018). The antimicrobial susceptibility profiles paired with WGS identification of resistance genes illustrated the presence of ARGs in manure, soil and grass *E. coli* isolates. In the *E. coli* isolates ARGs were identified that are commonly associated with plasmids such as the fluoroquinolone resistance gene *qnrS1* and the N-Acetyltransferases, O-Adenyltransferases and O-Phosphotransferases conferring aminoglycoside resistance. Of particular interest was the detection of the third generation cephalosporinase $bla_{\text{CTXM-15}}$ in cow manure isolates, which is often associated with plasmid mediated resistance (Carattoli, 2009). The isolates harbouring $bla_{\text{CTXM-15}}$ were all the MLST type ST1431 and contained the plasmid replicon types ColRNA_1 and IncY_1. The plasmid replicon IncY_1 has been associated with the carriage of $bla_{\text{CTXM-15}}$ in *E. coli* and *K. pneumoniae* (Saidani *et al.*, 2019) as well as resistance genes to other antibiotics such as the colistin resistance gene, *mcr-1* (C. Zhang *et al.*, 2017; Han

et al., 2020). Of interest is also the lack of *bla*$_{\text{CTXM-15}}$ in *E. coli* isolated from manured soil and grass. This indicates that while this ARG was present in the manure it was either not transferred, not maintained or out-competed in the soil and grass *E. coli*. The importance of this study is in the identification of the transfer of the AMR pathogens and ARGs to the next phase of the food chain (grass) or environment (soil) and not only the detection of these in manure and the presumption of transfer. However, to fully characterise these plasmids and to determine the exact location of the resistance genes detected in these isolates a hybrid assembly approach of extracted plasmids using long read and short read technologies would be required (Ashton *et al.*, 2015; Berbers *et al.*, 2020). The *E. coli* isolates also harboured chromosomally associated resistance mechanisms such as point mutations in the QRDR region of the *gyrA*, *parC* and *parE* genes, conferring fluoroquinolone resistance.

Acinetobacter baumannii has previously been detected in soil (Byrne-Bailey *et al.*, 2009) and crops (Berlau *et al.*, 1999; Soliman *et al.*, 2021), however its exact environmental niche is unknown (Towner, 2009). Despite its largely unknown natural reservoir it has become an opportunistic pathogen of extreme clinical concern in human health, particularly in terms of carbapenem resistance (Wong *et al.*, 2017). This study demonstrates the detection of *A. baumannii* in soil but also in the grass phyllosphere, illustrating a possible novel environmental habitat of this bacterium and a potential reservoir of pathogenic or antimicrobial resistant strains of this bacterium. The *A. baumanniii* isolates examined in this study were not deemed to be of high clinical concern due to the phenotypic antimicrobial susceptibility data revealing that these isolates were largely susceptible to clinically important antibiotics used to treat *A. baumannii* infections. Additionally, whole genome sequencing revealed that the isolates had no plasmids detected. The carriage of plasmids can exert a fitness cost on bacteria therefore

perhaps the absence of plasmids in these isolates and their antimicrobial susceptibility is due to a lack of selection pressure for ARGs on these isolates. It is known that *A. baumannii* can contain the chromosomal β-lactamases; the Ambler Class D β-lactamases the OXA β-lactamases and the Ambler Class A β-lactamase, *blaADC-25_1*, as seen in the *A. baumannii* in this study. Thus, illustrating the potential of *A. baumannii* can act as an environmental reservoir for clinically important genes.

Colistin resistance was detected in 4 *E. coli* isolates, 8 *K. pneumoniae* isolates and 4 *A. baumannii*. However, none of these isolates (analysed using PCR or WGS) harboured mobile colistin resistant genes. Additionally, PointFinder analysis on the *E. coli* isolates did not identify any mutations in the *pmrA* or *pmrB* genes that can confer colistin resistance (Olaitan, Morand and Rolain, 2014). The colistin MICs for the isolates within each species varied from 4 mg/L to 128 mg/L for *K. pneumoniae*, 2mg/L to 4mg/L for *A. baumannii* and 4 mg/L to 8 mg/L for the *E. coli*. Thus, there is not a common phenotype present in these isolates but the data rather suggests more than one novel mechanism of resistance across these isolates. The exact identification of these potentially novel mechanisms will be performed in future studies rather than within this project, due to time constraints.

5.4 Conclusion

In summary, the results from this study show that priority pathogens harbouring antibiotic resistance genes to clinically relevant antibiotics can be isolated from manure, soil and grass samples. The bacteria *E. coli, K. pneumoniae* and *A. baumannii* were readily isolated from soil and grass samples and *E. coli* was additionally isolated from manure samples. Additionally, both *E. coli* and *A. baumannii* were isolated from both manure treated and untreated soil. With *E. coli* being isolated from only manure treated grass and *A. baumannii* being isolated from both manure treated and untreated grass. Notably, the

isolation of these clinically important pathogens from the phyllosphere of grassland provides a novel potential secondary reservoir for these bacteria in the environment as well as providing a potential route for these bacteria into the food chain. The *E. coli* isolates harboured a diverse plasmidome as well the majority of isolates displaying a multi drug resistant phenotype. Additionally, WGS found that resistance genes to other high priority antibiotics were found in the samples as well as a multitude of ARGs that are often plasmid mediated. Grass and soil associated *A. baumannii* isolates harboured chromosomal mechanisms of resistance to β-lactamases and therefore illustrated the potential of the environment, particularly the grass phyllosphere, as a natural reservoir of ARGs. Additionally, numerous *E. coli and A. baumannii* isolates demonstrated phenotypic resistance to antibiotics but did not have known resistance mechanism detected, illustrating the potential of environmental bacteria to harbour not only clinically relevant ARGS but also novel resistance mechanisms. Overall, the results of this study illustrate the important role that the grass phyllosphere plays in harbouring the clinically important pathogens: *E. coli, K. pneumoniae* and *A. baumannii*. However, more research is required to fully elucidate the role agricultural grassland plays in AMR dissemination into the food chain.

Chapter 6

Final Discussion

The danger posed by antibiotic resistance is undoubtably one of the greatest threats to global health (Aslam *et al.*, 2018). In addition to the issue of AMR in clinical settings there has been an abundance of reports in the literature regarding the environment as a potential reservoir of AMR (Berg, Eberl and Hartmann, 2005; Binh *et al.*, 2008a; Berendonk *et al.*, 2015). Therefore, due to the multifaceted nature of antimicrobial resistance, a One Health approach is required to fully understand how AMR disseminates (Robinson *et al.*, 2016). The natural environment is a known reservoir of antibiotics, as well as antibiotic resistance mechanisms (José L. Martínez, 2012). Additionally, environmental microbes can act as opportunistic pathogens in a clinical setting and often due to their intrinsic resistance to many antibiotics can pose a difficulty to treat (de Bentzmann and Plésiat, 2011; Brooke, 2012). It has been shown that the environment can not only harbour natural resistance mechanisms but can also be affected by anthropogenic pollution, such as discharge from hospitals, wastewater treatment plants, or manure landspreading onto agricultural land (Gatica and Cytryn, 2013; Lima, Domingues and Da Silva, 2020; Sabri *et al.*, 2020) which can result in the introduction of pathogens and ARGs. Due to the many niches occupied by microorganisms in the environment it is important to evaluate the potential of all of these to harbour ARB.

One niche that has been underexplored is the phyllosphere, in particular the phyllosphere of grass. Since the phyllosphere is in physical contact with livestock and humans it is vital to understand it's involvement in the dissemination of AMR. Globally, the surface area of the phyllosphere of plants constitutes a surface area double than that of land surface area, therefore it is a large section of the environment of which little is understood about its involvement in the One Health approach to AMR (Vorholt, 2012). Of extreme importance is the involvement of the grassland phyllosphere as it is the start of the food

chain, as well as being ubiquitous globally and playing a vital role in agriculture. Overall, the aims of this thesis was to evaluate the potential of the grass phyllosphere as a reservoir of antimicrobial resistance genes and of the WHO priority pathogens: resistant *E. coli, K. pneumoniae, A. baumannii* and *P. aeruginosa*. Additionally, this thesis aimed to elucidate the potential differential impact, as well as the temporal impact, landspreading of pig, cow and chicken manures have on the microbiome and resistome of soil and the grass phyllosphere.

In Chapters 3 and 4 culture independent methods were used to investigate the impact manure had on the microbiome, and additionally, in Chapter 4, the resistome. As the landspreading of manure is an essential agricultural practice for the recycling of organic waste and the fertilisation of soils within the circular economy, it is vital to understand the temporal impact of this on agricultural land. Additionally, as there are varying antibiotic usage regimes in different livestock types, the risk of each of these manure sources when land spread needs to be assessed and compared within the same experimental parameters. The majority of studies investigating the impact manure application has on the microbiome and resistome of agricultural land focuses on soil (Santamaría, López and Soto, 2011; Jechalke *et al.*, 2013; Boeckel *et al.*, 2015; Nõlvak *et al.*, 2016), therefore this study aims to provide the first insight into the impact manure application has on the grass phyllosphere of agricultural land. As was found in both pot based and field based experiments, the application of manure had no significant effect on the microbiome alpha diversity of grass and soil. Additionally, in Chapter 4 by NMDS ordination and PERMANOVA analysis, manure treatment had no significant effect on the overall β-diversity of the grass microbiome, however it had a small yet significant effect on the soil microbiome composition with pig manured soil and chicken manured soil being significantly to control soil. At OTU level, manure application was found to

perturb the soil in both Chapter 3 and 4, and the grass microbiome in Chapter 4, through the introduction of manure derived bacteria into the environments, or through the enrichment of the indigenous microbiome. Differing results, in terms of the impact of manure application on the differential abundance of OTUs as identified using DESeq2 analysis in Chapter 3 and Chapter 4, may be due to differences in polytunnel and field trial environments; with the field trial experiment representing a more realistic environment. Of note in Chapter 4, manure application on grass resulted in a decrease in OTUs associated with plant pathogen protection, such as *Burkholderiaceae* and *Sphingomonadaceae*. In grass and soil, manure application resulted in an increase in abundance of both plant and soil commensal bacteria and also of some families that can be potential human pathogens such as *Enterococcaceae, Staphylococcaceae, Aerococcaceae, Xanthomondaceae* and *Enterobacteriaceae.*

Both the grass phyllosphere and soil contained a diverse resistome containing ARGs conferring resistance to clinically important antibiotics, as well as containing genes associated with mobilisation of ARGs prior to the addition of manure and also in an increased diversity and abundance post manure spreading. Manuring of both soil and grass resulted in an increased diversity of ARGs and MGE, but NMDS and PERMANOVA testing demonstrated it did not alter the overall resistome composition. Both grass and soil microbiomes and resistomes were found to be correlated according to mantel testing, indicating that fluctuations in the resistome may be due to changes in the bacterial communities.

Assessment of the temporal impact that manure landspreading has on agricultural land is critical to ensure a reduced risk of microbial contamination on forage for livestock. Current landspreading practice in Ireland for splash plate manure spreading, which was replicated in this study, recommends the reintroduction of grazing livestock onto land at

least six weeks following manure landspreading. The results from Chapter 4 illustrate that the majority of manure associated ARGs were no longer detected 6 weeks post manure application. However, the impact on the microbiome had a more prolonged effect on soil with cow manured soil resulting in a continued enrichment of the indigenous microbiota to at least 18 weeks after manure application. To fully understand the dynamics manure landspreading has on the microbiome and resistome of agricultural land future work should include investigations into the impact the repeat and cumulative manure spreading has over multiple seasons on the microbiome and resistome. Such studies have recently been published in relation to soil (Zhang *et al.*, 2018; Macedo *et al.*, 2020), however they do not include this impact on the phyllosphere. The data from the microbiome and resistome analysis in Chapter 4 indicates there may be an increased risk in ARG dissemination with pig manure landspreading in soil and on grass. In both grass and soil, pig manured samples contained an increased abundance of resistance genes to important antibiotic classes relative to the other manured samples: sulphonamides, β-lactams, MLSBs and the aminoglycosides. Additionally, pig manure resulted in the greatest introduction of manure originating ARGs into soil. However, overall, the temporal patterns of the microbiome and resistome for all manure types were similar indicating that the temporal dynamics rather than the manure type had more of an influence on microbiome and resistome patterns at the end of the field trials.

Chapter 3 and Chapter 5 included both culture-based methods paired with molecular methods such as WGS and PCR to investigate the phenotypic and molecular mechanisms of resistance in environmental bacteria. In Chapter 3 the known opportunistic pathogens such as *S. maltophilia, Achromobacter* spp. and *Lactococcus garvieae* were isolated from soil and grass. *Lactococcus garvieae* is a known pathogen of concern in aquaculture and harboured resistance mechanism to antibiotics of veterinary relevance such as the

fluoroquinolones (point mutations in the QRDR of the *gyrA* and *parC* genes) and the aminoglycosides (*aac(6')-Ii*). This illustrates a reservoir for this pathogen in the environment and a route for it to disseminate into water systems. Of particular importance is the analysis of the WHO priority pathogens *E. coli, K. pneumoniae, A. baumanii* and *P. aeruginosa* to clinically important antibiotics, in Chapter 5. It was found that the grass phyllosphere harboured *E. coli, K. pneumoniae* and *A. baumannii* and soil contained both *E. coli* and *A. baumannii* prior to manure application, therefore illustrating the potential of the grass phyllosphere to be a novel reservoir for these pathogens. Additionally, *E. coli* and *A. baumannii* isolates identified contained a myriad of resistance genes conferring resistance to clinically important antibiotics. These resistance mechanisms were both intrinsic e.g. point mutations in QRDR of *gyrA, parC* and *parE* genes for fluoroquinolone resistance and acquired resistance e.g. *qnrS* gene for fluoroquinolone resistance and the N-Acetyltransferases for aminoglycoside resistance. Additionally, the results from Chapter 5 revealed the diverse range of plasmids found in *E. coli* isolates as well as the detection of plasmid associated resistance genes such as the fluoroquinolone resistance gene *qnrS1*. This illustrates that the grass phyllosphere and soil environments have the potential to disseminate AMR by both vertical and horizontal transmission. Future work to fully understand the role of the environment, particularly of the phyllosphere of grass and other plants, should include in depth analysis and characterisation of the environmental mobilome due to the importance of plasmids and other MGEs in the dissemination of antimicrobial resistance. Additionally, further work to characterise the virulence, antibiotic resistance and the relatedness of environmental strains of *E. coli, K. pneumoniae, A. baumanii* to clinical isolates is required to fully evaluate their risk to human health.

Overall, the results from this thesis show the soil but most notably, the grass phyllosphere, as a reservoir of ARGs, MGEs and the WHO priority pathogens, as well as other opportunistic pathogens that can harbour resistance mechanisms to antibiotics crucial for human health. Manuring of grass and soil resulted in changes in these environments through enrichment of their natural microbiota and resistome and through the introduction of manure originating bacteria, ARGs and MGEs. Of concern is the presence of potential pathogens and ARGs in the grass phyllosphere, as their presence in grassland may lead to their transfer to livestock through ingestion or through direct contact. Additional research is needed to understand the involvement of grass phyllosphere, as well as the phyllosheres of other crops, in AMR dissemination. The potential rate of transfer between grass ARGs and bacteria to livestock needs be assessed to fully elucidate the role the grass phyllosphere plays in the dissemination of antimicrobial resistance, as well as characterisation of the phyllosphere mobilome. This data is required to mitigate the spread of AMR from this newly identified environmental source into the food chain.

Chapter 7

Bibliography

Chapter 7: Bibliography

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CHAPTER 8

Appendix

Chapter 8: Appendix

Appendix 1: Dry matter results for grass samples.

Sample	Wet Weight of Grass	Dry Weight of Grass	Dry Matter %	Average
BM	122.22	41.03	34%	34%
GC T1 R1	N/A	N/A	N/A	52%
GC T1 R2	N/A	N/A	N/A	
GC T1 R3	72.52	37.83	52%	
GC T1 R4	N/A	N/A	N/A	
GS T1 R1	N/A	N/A	N/A	50%
GS T1 R2	N/A	N/A	N/A	
GS T1 R3	64.58	32.33	50%	
GS T1 R4	N/A	N/A	N/A	
GB T1 R1	N/A	N/A	N/A	54%
GB T1 R2	80.1	43	54%	
GB T1 R3	N/A	N/A	N/A	
GB T1 R4	N/A	N/A	N/A	
GP T1 R1	N/A	N/A	N/A	60%
GP T1 R2	N/A	N/A	N/A	
GP T1 R3	55.76	33.62	60%	
GP T1 R4	N/A	N/A	N/A	
GC T2 R1	78.58	31.34	40%	39%
GC T2 R2	N/A	N/A	N/A	
GC T2 R3	N/A	N/A	N/A	
GC T2 R4	65.44	24.43	37%	

Sample	Wet Weight of Grass	Dry Weight of Grass	Dry Matter %	Average
GS T2 R1	N/A	N/A	N/A	38%
GS T2 R2	N/A	N/A	N/A	
GS T2 R3	35.13	13.36	38%	
GS T2 R4	35.33	13.28	38%	
GB T2 R1	N/A	N/A	N/A	43%
GB T2 R2	76.52	35.94	47%	
GB T2 R3	43	16.83	39%	
GB T2 R4	N/A	N/A	N/A	
GP T2 R1	47.65	18.02	38%	64%
GP T2 R2	25.39	31.28	123%	
GP T2 R3	80.71	25.64	32%	
GP T2 R4	N/A	N/A	N/A	
GC T3 R1	102.65	33.88	33%	33%
GC T3 R2	N/A	N/A	N/A	
GC T3 R3	83.72	27.57	33%	
GC T3 R4	81.54	26.35	32%	
GS T3 R1	100	23.79	24%	25%
GS T3 R2	150.24	37.33	25%	
GS T3 R3	99.43	26.36	27%	
GS T3 R4	191.14	44.14	23%	
GB T3 R1	N/A	N/A	N/A	28%
GB T3 R2	N/A	N/A	N/A	
GB T3 R3	103.32	31.52	31%	
GB T3 R4	171.38	43.84	26%	
GP T3 R1	76.4	47.73	44%	44%
GP T3 R2	N/A	N/A	N/A	

Sample	Wet Weight of Grass	Dry Weight of Grass	Dry Matter %	Average
GP T3 R3	N/A	N/A	N/A	
GP T3 R4	N/A	N/A	N/A	
GC T4 R1	88.66	36.73	41%	24%
GC T4 R2	117.79	22.39	19%	
GC T4 R3	97.75	17.85	18%	
GC T4 R4	88.33	15.51	18%	
GS T4 R1	104.67	9.34	9%	15%
GS T4 R2	107.28	20.38	19%	
GS T4 R3	108.57	17.69	16%	
GS T4 R4	109.05	17.1	16%	
GB T4 R1	110.26	20.27	18%	18%
GB T4 R2	30.06	6.86	23%	
GB T4 R3	134.13	18.75	14%	
GB T4 R4	77.6	13.28	17%	
GP T4 R1	39.17	17.47	45%	22%
GP T4 R2	111.78	17.03	15%	
GP T4 R3	103.61	15.78	15%	
GP T4 R4	108.2	15.47	14%	
GC T5 R1	52.94	19.66	37%	32%
GC T5 R2	N/A	N/A	N/A	
GC T5 R3	86.41	28.59	33%	
GC T5 R4	102.44	26.95	26%	
GB T5 R1	93.27	21.78	23%	28%
GB T5 R2	23.78	8.66	36%	
GB T5 R3	108.32	25.28	23%	
GB T5 R4	34.93	10.47	30%	

Sample	Wet Weight of Grass	Dry Weight of Grass	Dry Matter %	Average
GP T5 R1	102.85	24.35	24%	23%
GP T5 R2	104.21	24.08	23%	
GP T5 R3	N/A	N/A	N/A	
GP T5 R4	11.68	23.81	204%	
GS T5 R1	11.39	26.59	233%	23%
GS T5 R2	90.87	22.83	25%	
GS T5 R3	N/A	N/A	N/A	
GS T5 R4	136.28	28.28	21%	
GC T6 R1	86.2	23.56	27%	27%
GC T6 R2	135.79	35.02	26%	
GC T6 R3	125.24	34.86	28%	
GC T6 R4	85.21	24.23	28%	
GB T6 R1	115.65	31.73	27%	29%
GB T6 R2	20.84	7.31	35%	
GB T6 R3	39.68	11.51	29%	
GB T6 R4	117.79	28.66	24%	
GP T6 R1	151.24	33.68	22%	21%
GP T6 R2	148.07	27.29	18%	
GP T6 R3	137.47	30.85	22%	
GP T6 R4	163.42	32.5	20%	
GS T6 R1	137.39	21.63	16%	20%
GS T6 R2	106.14	25.24	24%	
GS T6 R3	122.64	19.29	16%	
GS T6 R4	102.65	24.5	24%	
GC T7 R1	105.11	24.37	23%	24%
GC T7 R2	105.17	25.96	25%	

Sample	Wet Weight of Grass	Dry Weight of Grass	Dry Matter %	Average
GC T7 R3	104.54	25.6	24%	
GC T7 R4	100.94	22.43	22%	
GB T7 R1	99.97	22.14	22%	21%
GB T7 R2	99.23	21.1	21%	
GB T7 R3	110.89	24.49	22%	
GB T7 R4	101.33	20.74	20%	
GPT7 R1	107.77	23.46	22%	20%
GPT7 R2	95.53	19.51	20%	
GPT7 R3	108.5	21.38	20%	
GPT7 R4	99.38	18.11	18%	
GS T7 R1	107.1	24.5	23%	20%
GS T7 R2	106.2	19.31	18%	
GS T7 R3	108.41	22.11	20%	
GS T7 R4	106.41	20.42	19%	
GC T8 R1	103.18	32.02	31%	28%
GC T8 R2	100.58	25.4	25%	
GC T8 R3	86.77	24.8	29%	
GC T8 R4	103.01	26.81	26%	
GB T8 R1	94.84	29.81	31%	25%
GB T8 R2	100.23	21.04	21%	
GB T8 R3	102.45	25.36	25%	
GB T8 R4	101.67	22.58	22%	
GP T8 R1	100.09	30.73	31%	26%
GP T8 R2	70.248	17.24	25%	
GP T8 R3	101.48	28.25	28%	
GP T8 R4	102.96	21.18	21%	

Sample	Wet Weight of Grass	Dry Weight of Grass	Dry Matter %	Average
GS T8 R1	107.3	30.91	29%	25%
GS T8 R2	106.61	23.2	22%	
GS T8 R3	103.82	28.23	27%	
GS T8 R4	102.7	21.01	20%	
GC T9 R1	23.82	9.22	39%	35%
GC T9 R2	80.42	24.87	31%	
GC T9 R3	28.24	11.67	41%	
GC T9 R4	58.98	17.46	30%	
GB T9 R1	21.66	17.25	80%	51%
GB T9 R2	42.03	15.53	37%	
GB T9 R3	N/A	N/A	N/A	
GB T9 R4	39.96	14.01	35%	
GP T9 R1	13.91	5.51	40%	35%
GP T9 R2	25.63	7.53	29%	
GP T9 R3	43.45	15.49	36%	
GP T9 R4	29.1	10.7	37%	
GS T9 R1	114.37	35.08	31%	30%
GS T9 R2	45.45	15.57	34%	
GS T9 R3	100.07	29.48	29%	
GS T9 R4	74.94	19.07	25%	

Appendix 2: Dry matter results for soil samples.

Sample (Replicates)	Weight of foil cup (g)	Weight of wet soil (g)	Weight of Dry Soil and Container (g) 24 hours	Weight of Dry Soil (g)	Dry Matter %	Average
S.NM R1	3.27	20.2	18.15	14.88	74%	74%
S. NM. R2	3.26	20.1	18.23	14.97	74%	
S. NM. R3	3.25	20.1	18.47	15.22	76%	
S. NM. R4	3.53	20.39	18.47	14.94	73%	
SC. T0. R1	3.2	*no soil left for test*	*no soil left for test*	*no soil left for test*	*N/A*	
SC. T0. R2	3.23	20.28	18.25	15.02	74%	74%
SC. T0. R3	3.21	20.04	17.89	14.68	73%	
SC. T0. R4	3.2	20.1	18.21	15.01	75%	
SB.T0.R1	3.24	20.5	18.87	15.63	76%	76%
SB.T0.R2	3.22	20.1	18.78	15.56	77%	
SB.T0.R3	3.21	20.01	18.97	15.76	79%	
SB.T0.R4	3.21	20.04	17.61	14.4	72%	
SP.T0.R1	3.25	20.306	18.58	15.33	75%	75%
SP.T0.R2	3.21	20.2	18.06	14.85	74%	
SP.T0.R3	3.53	20.13	18.96	15.43	77%	
SP.T0.R4	3.57	20.12	18.45	14.88	74%	
SS.T0.R1	3.52	20.1	16.06	12.54	62%	72%
SS.T0.R2	3.11	20.09	18.34	15.23	76%	
SS.T0.R3	3.1	20.19	18	14.9	74%	
SS.T0.R4	3.57	20.06	19.09	15.52	77%	
SC T1 R1	3.58	20.08	18.81	15.23	76%	75%
SC T1 R2	3.26	19.98	18.69	15.43	77%	
SC T1 R3	3.12	20	17.88	14.76	74%	

Sample (Replicates)	Weight of foil cup (g)	Weight of wet soil (g)	Weight of Dry Soil and Container (g) 24 hours	Weight of Dry Soil (g)	Dry Matter %	Average
SC T1 R4	3.28	20.1	18.2	14.92	74%	
SS T1 R1	3.21	20.09	18.29	15.08	75%	74%
SS T1 R2	3.27	19.99	18.24	14.97	75%	
SS T1 R3	3.23	20.04	17.63	14.4	72%	
SS T1 R4	3.56	20.09	18.18	14.62	73%	
SB T1 R1	3.11	20.04	18.83	15.72	78%	77%
SB T1 R2	3.24	20	18.81	15.57	78%	
SB T1 R3	3.23	20.04	18	14.77	74%	
SB T1 R4	3.29	20.01	18.79	15.5	77%	
SP T1 R1	3.24	20.04	19.39	16.15	81%	78%
SP T1 R2	3.23	20	18.98	15.75	79%	
SP T1 R3	3.23	20.03	18.81	15.58	78%	
SP T1 R4	3.53	20.04	18.43	14.9	74%	
SC T2 R1	3.21	20.05	19.76	16.55	83%	81%
SC T2 R2	3.52	19.99	20.07	16.55	83%	
SC T2 R3	3.11	19.99	19.17	16.06	80%	
SC T2 R4	3.25	20	19.11	15.86	79%	
SS T2 R1	3.56	20.03	19.87	16.31	81%	81%
SS T2 R2	3.27	20.02	19.65	16.38	82%	
SS T2 R3	3.28	20	19.48	16.2	81%	
SS T2 R4	3.24	20	18.81	15.57	78%	
SB T2 R1	3.13	20.01	19.64	16.51	83%	78%
SB T2 R2	3.24	20.04	16.89	13.65	68%	
SB T2 R3	3.23	20.01	19.64	16.41	82%	

Sample (Replicates)	Weight of foil cup (g)	Weight of wet soil (g)	Weight of Dry Soil and Container (g) 24 hours	Weight of Dry Soil (g)	Dry Matter %	Average
SB T2 R4	3.22	20.03	18.99	15.77	79%	
SP T2 R1	3.22	20.05	20.13	16.91	84%	83%
SP T2 R2	3.23	20.02	19.76	16.53	83%	
SP T2 R3	3.54	20.06	20.06	16.52	82%	
SP T2 R4	3.26	20.04	20.04	16.78	84%	
SC T3 R1	3.53	20	20.42	16.89	84%	84%
SC T3 R2	3.2	20	19.97	16.77	84%	
SC T3 R3	3.11	20.02	20.2	17.09	85%	
SC T3 R4	3.56	20.01	20.12	16.56	83%	
SS T3 R1	3.52	20.06	20.4	16.88	84%	83%
SS T3 R2	3.53	20	20.08	16.55	83%	
SS T3 R3	3.61	20.06	20.49	16.88	84%	
SS T3 R4	3.56	20.01	20.15	16.59	83%	
SB T3 R1	3.51	20.06	17.64	14.13	70%	75%
SB T3 R2	3.53	20.04	20.33	16.8	84%	
SB T3 R3	3.44	20.06	20.02	16.58	83%	
SB T3 R4	3.59	20.01	16.6	13.01	65%	
SP T3 R1	3.11	20.05	20.13	17.02	85%	84%
SP T3 R2	3.13	20.04	20.09	16.96	85%	
SP T3 R3	3.46	20.01	20.5	17.04	85%	
SP T3 R4	3.59	20.02	20.21	16.62	83%	
SC T4 R1	3.53	20	19.27	15.74	79%	78%
SC T4 R2	3.23	20	18.92	15.69	78%	
SC T4 R3	3.45	20.04	19.12	15.67	78%	

Sample (Replicates)	Weight of foil cup (g)	Weight of wet soil (g)	Weight of Dry Soil and Container (g) 24 hours	Weight of Dry Soil (g)	Dry Matter %	Average
SC T4 R4	3.58	20.04	19	15.42	77%	
SS T4 R1	3.56	20	19.29	15.73	79%	79%
SS T4 R2	3.56	20.04	19.44	15.88	79%	
SS T4 R3	3.54	20	19.41	15.87	79%	
SS T4 R4	3.12	20.03	18.52	15.4	77%	
SB T4 R1	3.59	20.02	19.3	15.71	78%	78%
SB T4 R2	3.58	20.01	19.1	15.52	78%	
SB T4 R3	3.12	20.03	19.13	16.01	80%	
SB T4 R4	3.51	20.05	19.13	15.62	78%	
SP T4 R1	3.44	20.02	19.86	16.42	82%	78%
SP T4 R2	3.54	20.01	19.32	15.78	79%	
SP T4 R3	3.14	20	18.86	15.72	79%	
SP T4 R4	3.53	20	18.36	14.83	74%	
SC T5 R1	3.53	20	19.27	15.74	79%	78%
SC T5 R2	3.23	20	18.92	15.69	78%	
SC T5 R3	3.45	20.04	19.12	15.67	78%	
SC T5 R4	3.58	20.04	19	15.42	77%	
SS T5 R1	3.56	20	19.29	15.73	79%	79%
SS T5 R2	3.56	20.04	19.44	15.88	79%	
SS T5 R3	3.54	20	19.41	15.87	79%	
SS T5 R4	3.12	20.03	18.52	15.4	77%	
SB T5 R1	3.59	20.02	19.3	15.71	78%	78%
SB T5 R2	3.58	20.01	19.1	15.52	78%	
SB T5 R3	3.12	20.03	19.13	16.01	80%	

Sample (Replicates)	Weight of foil cup (g)	Weight of wet soil (g)	Weight of Dry Soil and Container (g) 24 hours	Weight of Dry Soil (g)	Dry Matter %	Average
SB T5 R4	3.51	20.05	19.13	15.62	78%	
SP T5 R1	3.44	20.02	19.86	16.42	82%	79%
SP T5 R2	3.54	20.01	19.32	15.78	79%	
SP T5 R3	3.14	20	18.86	15.72	79%	
SP T5 R4	3.53	20	18.36	14.83	74%	
SC T6 R1	3.58	20.01	19.42	15.84	79%	78%
SC T6 R2	3.12	20.05	18.02	14.9	74%	
SC T6 R3	3.46	19.99	19.16	15.7	79%	
SC T6 R4	3.59	20.04	19.24	15.65	78%	
SS T6 R1	3.53	20.03	19.85	16.32	81%	78%
SS T6 R2	3.22	20.03	18.89	15.67	78%	
SS T6 R3	3.53	20.03	18.88	15.35	77%	
SS T6 R4	3.14	20	18.67	15.53	78%	
SB T6 R1	3.57	20.04	19.36	15.79	79%	77%
SB T6 R2	3.57	20.03	19.14	15.57	78%	
SB T6 R3	3.12	20.03	18.55	15.43	77%	
SB T6 R4	3.56	20.02	18.19	14.63	73%	
SP T6 R1	3.51	20	19.22	15.71	79%	77%
SP T6 R2	3.43	20.04	18.82	15.39	77%	
SP T6 R3	3.53	20.02	18.99	15.46	77%	
SP T6 R4	3.53	20.02	18.69	15.16	76%	
SC T7 R1	3.55	20.06	18.77	15.22	76%	75%
SC T7 R2	3.27	20.06	18.5	15.23	76%	
SC T7 R3	3.59	20.07	18.21	14.62	73%	

Sample (Replicates)	Weight of foil cup (g)	Weight of wet soil (g)	Weight of Dry Soil and Container (g) 24 hours	Weight of Dry Soil (g)	Dry Matter %	Average
SC T7 R4	3.21	20.02	18.09	14.88	74%	
SS T7 R1	3.24	20.08	18.78	15.54	77%	77%
SS T7 R2	3.24	20.1	18.18	14.94	74%	
SS T7 R3	3.23	20.08	18.98	15.75	78%	
SS T7 R4	3.14	20.1	18.54	15.4	77%	
SB T7 R1	3.53	20.08	18.89	15.36	76%	75%
SB T7 R2	3.11	20.15	18.42	15.31	76%	
SB T7 R3	3.24	20.16	18.23	14.99	74%	
SB T7 R4	3.25	20.05	18.3	15.05	75%	
SP T7 R1	3.22	20.11	18.87	15.65	78%	77%
SP T7 R2	3.27	20.01	18.52	15.25	76%	
SP T7 R3	3.25	20.11	19.11	15.86	79%	
SP T7 R4	3.28	20.05	18.28	15	75%	
SC T8 R1	3.11	19.99	17.25	14.14	71%	71%
SC T8 R2	3.22	20.05	17.23	14.01	70%	
SC T8 R3	3.53	20.09	17.58	14.05	70%	
SC T8 R4	3.56	20.02	18.47	14.91	74%	
SS T8 R1	3.53	20.08	18.19	14.66	73%	73%
SS T8 R2	3.14	20	17.93	14.79	74%	
SS T8 R3	3.55	20.11	18.12	14.57	72%	
SS T8 R4	3.53	20.02	18.44	14.91	74%	
SB T8 R1	3.14	20.03	17.4	14.26	71%	72%
SB T8 R2	3.51	20.15	18.21	14.7	73%	
SB T8 R3	3.58	20.16	18.57	14.99	74%	

Sample (Replicates)	Weight of foil cup (g)	Weight of wet soil (g)	Weight of Dry Soil and Container (g) 24 hours	Weight of Dry Soil (g)	Dry Matter %	Average
SB T8 R4	3.57	20.07	17.7	14.13	70%	
SP T8 R1	3.43	20.18	18	14.57	72%	74%
SP T8 R2	3.52	20.23	18.13	14.61	72%	
SP T8 R3	3.57	20.03	18.85	15.28	76%	
SP T8 R4	3.46	20.07	17.6	15.39	77%	
SC T9 R1	3.23	20.11	18.24	14.37	71%	73%
SC T9 R2	3.22	20.1	17.85	15.02	75%	
SC T9 R3	3.53	20.12	18.44	14.32	71%	
SC T9 R4	3.25	20.09	17.4	15.19	76%	
SS T9 R1	3.22	20.06	17.83	14.18	71%	72%
SS T9 R2	3.25	20.01	17.3	14.58	73%	
SS T9 R3	3.23	20.19	18.48	14.07	70%	
SS T9 R4	3.22	20.1	17.41	15.26	76%	
SB T9 R1	3.27	20.17	18.1	14.14	70%	72%
SB T9 R2	3.21	20.22	17.67	14.89	74%	
SB T9 R3	3.57	20.03	18.14	14.1	70%	
SB T9 R4	3.29	20.09	17.54	14.85	74%	
SP T9 R1	3.35	20.15	17.56	14.19	70%	71%
SP T9 R2	3.1	20.05	16.99	14.46	72%	
SP T9 R3	3.52	20.04	18.36	13.47	67%	
SP T9 R4	3.12	20.05	17.39	15.24	76%	

Appendix 3: Dry matter results for manure samples.

Slurry Dry Matter	Replicate	Weight of foil cup (g)	Weight of wet slurry (g)	Weight of Dry Slurry (g) 24 hours	Weight of Dry Slurry (1hr after initial measurement)	Dry Matter %	AVG
Pig Manure	S1	3.2	25.2	4.2	4.153	3.75	3.51 %
	S2	3.2	25.1	4.2	4.125	3.68	
	S3	3.2	24.8	4.1	3.974	3.12	
Chicken Manure	P1	3.1	20.4	20.5	20.399	85.216	85.66 %
	P2	3.2	20.2	20.7	20.687	86.569	
	P3	3.2	21	21.7	21.094	85.2095	
Pig Manure	B1	3.5	20.7	4.9	4.923	8.273	8.39 %
	B2	3.5	21	5	4.978	8.445	
	B3	3.5	20.9	5.1	4.974	8.471	

Appendix 4: Nutrient results for soil.

Sample Name	C (Soil) - JC	Cu (Soil) - JC	K - Morgan (Soil) - JC	Mg - Morgan (Soil) - JC	N (Soil) - JC	OM (Soil) - JC	P - Morgan (Soil) - JC	Zn (Soil) - JC	pH (Soil) - JC
	%	**mg/kg**	**mg/l**	**mg/l**	**%**	**%**	**mg/l**	**mg/kg**	
S NM	N/A	No sample	N/A	N/A	N/A	N/A	N/A	No sample	N/A
SC T0 R1	2.8	12.38	46.5	92.5	0.264	6.9	13.4	72.06	6.91
SC T0 R2	3.51	12.23	51.8	118	0.336	8.6	18.1	61.84	6.73
SC T0 R3	2.91	12.71	34.7	108	3.279	6.4	12.3	58.39	6.91
SC T0 R4	2.86	12.99	35.5	92.7	0.279	6.4	11.3	75.56	6.86
SS T0 R1	3.05	14.78	147	134	0.316	7.2	23.2	81.75	6.88
SS T0 R2	3.17	16.21	132	169	0.317	7.4	23.7	81.32	6.98
SS T0 R3	3.17	15.39	142	147	0.36	7.1	24.3	87.19	6.89
SS T0 R4	3.12	14.5	123	110	0.305	6.8	16	76.46	6.85
SB T0 R1	3.16	11.81	138	161	0.33	7.6	20.5	62.11	6.82
SB T0 R2	2.94	14.79	81.6	190	0.292	7.3	7.36	64.78	6.01
SB T0 R3	2.7	13.86	91.9	137	0.266	6.4	11.5	61.4	6.66
SB T0 R4	3.07	14.4	143	169	0.298	6.7	14.8	69.25	6.52
SP T0 R1	3.21	10.22	152	139	0.325	7.1	18.1	69.19	6.29
SP T0 R2	3.7	14.38	155	202	0.362	8.4	21.2	68.68	6.8
SP T0 R3	2.86	13.14	56.6	136	0.288	6.7	15.9	61.24	6.79
SP T0 R4	3.01	13.16	59.5	100	0.293	6.7	14.5	74.85	6.78
SNM R2	2.71	13.09	49.5	124	0.278	6.7	10.6	64.91	6.61
SNM R3	2.71	14.09	40.6	113	0.279	6.2	9.51	70.3	6.64
SNM R4	2.91	12.58	42.9	128	0.297	6.9	10.3	62.01	6.52

Sample Name	C (Soil) - JC	Cu (Soil) - JC	K - Morgan (Soil) - JC	Mg - Morgan (Soil) - JC	N (Soil) - JC	OM (Soil) - JC	P - Morgan (Soil) - JC	Zn (Soil) - JC	pH (Soil) - JC
SC T1 R1	3.12	11.55	44.5	147	0.302	6.8	11.8	62	6.48
SC T1 R2	3.14	13.64	50	174	0.309	6.6	6.82	64.76	6.06
SC T1 R3	3.21	14.4	43.1	97.7	0.298	7.6	17.6	60.37	7.23
SC T1 R4	3.35	14.04	64.7	133	0.343	7.4	12.5	72.76	6.53
SS T1 R1	2.97	14.63	146	194	0.306	7.6	18.4	81.01	6.11
SS T1 R2	3.46	13.66	118	129	0.365	7.7	20.5	70.1	6.88
SS T1 R3	3.91	14.22	106	136	0.352	7.9	12.1	70.72	6.05
SS T1 R4	3.81	16.98	116	170	0.382	7.9	23	93.39	6.28
SB T1 R1	2.79	10.79	45.3	147	0.283	6.5	14.8	52.98	6.59
SB T1 R2	3.03	12.91	114	159	0.314	7.1	11.7	66.15	6.2
SB T1 R3	3.12	20.41	87.4	138	0.31	7.4	12.6	64.31	6.08
SB T1 R4	3.01	12.37	129	131	0.298	7.6	18.7	61.03	6.79
SP T1 R1	2.82	9.15	121	172	0.308	6.5	23.6	56.84	6.48
SP T1 R2	3.1	12.93	125	166	0.32	7.2	20.9	64.61	6.96
SP T1 R3	3.41	17.49	102	179	0.349	8.2	21.3	77.11	6.95
SP T1 R4	3.07	13.01	88.2	138	0.314	6.6	19.6	70.07	6.7
SC T2 R1	3.05	10.51	49.9	123	0.313	7.4	8.95	58.83	6.21
SC T2 R2	3.65	12.67	72.9	157	0.359	8.3	24	62.38	6.94
SC T2 R3	2.74	12.6	46.4	128	0.285	7.6	11.9	58.28	6.58
SC T2 R4	2.85	11.42	55.9	141	0.3	6.8	16.3	61.27	6.33
SS T2 R1	3.17	12.31	136	149	0.317	7.6	35.4	76.28	6.8
SS T2 R2	3.43	17.09	165	203	0.341	8.9	52.9	99.76	6.71
SS T2 R3	3.41	13.93	152	143	0.342	7.7	30.4	65.35	6.91

Sample Name	C (Soil) - JC	Cu (Soil) - JC	K - Morgan (Soil) - JC	Mg - Morgan (Soil) - JC	N (Soil) - JC	OM (Soil) - JC	P - Morgan (Soil) - JC	Zn (Soil) - JC	pH (Soil) - JC
SS T2 R4	3.13	15.29	123	129	0.324	7.8	17.6	83.94	6.03
SB T2 R1	2.85	7.53	294	150	0.312	7.4	12.5	48.6	6.46
SB T2 R2	3.45	12.51	113	181	0.352	8.6	20.3	58.84	6.54
SB T2 R3	3.02	10.9	214	129	0.32	6.9	14.6	48.25	6.56
SB T2 R4	3.28	13.11	140	154	0.343	7.9	24.5	81.59	6.58
SP T2 R1	3.15	9.01	182	119	0.342	7.6	50.6	50.91	7.09
SP T2 R2	3.33	10.59	193	178	0.359	7.7	38.8	60.98	6.76
SP T2 R3	3.19	14.61	146	149	0.325	7.7	76.4	64.81	6.94
SP T2 R4	3.04	12.72	146	139	0.311	7.6	52.2	63.08	7.12
SC T3 R1	3.28	9.06	73.8	90.8	0.331	7.8	20.4	49.28	7.23
SC T3 R2	2.99	10.41	57	109	0.295	8	24.4	52.31	7.14
SC T3 R3	3.43	10.54	45.5	115	0.357	7.8	27.4	53.68	7
SC T3 R4	3.31	11.62	52	95.1	0.319	7.1	20	59.38	7.03
SS T3 R1	2.76	13.38	179	145	0.301	6.6	19.8	65.05	6.36
SS T3 R2	3.98	13.87	147	138	0.385	8.5	28.2	75.21	6.96
SS T3 R3	3	14.93	129	125	0.315	7.5	33.7	81.09	6.89
SS T3 R4	3.15	14.33	111	117	0.318	7.4	25.5	74.89	6.95
SB T3 R1	2.76	9.58	238	148	0.292	6.5	18.2	55.13	6.78
SB T3 R2	3.35	12.64	134	145	0.35	7.3	22.3	63.99	6.8
SB T3 R3	3.22	14.2	153	158	0.32	7.6	21.1	65.46	6.6
SB T3 R4	3.04	12.37	136	136	0.307	6.9	16.9	68.49	6.57
SP T3 R1	2.99	10.82	209	212	0.328	7.3	63.6	59.91	6.72
SP T3 R2	2.63	12.5	139	183	0.332	6.6	26.3	63.34	6.51

Sample Name	C (Soil) - JC	Cu (Soil) - JC	K - Morgan (Soil) - JC	Mg - Morgan (Soil) - JC	N (Soil) - JC	OM (Soil) - JC	P - Morgan (Soil) - JC	Zn (Soil) - JC	pH (Soil) - JC
SP T3 R3	3.76	10.3	134	179	0.305	7.4	32.6	52.49	6.75
SP T3 R4	3.26	14.31	116	168	0.355	8.2	27.3	75.23	6.38
SC T4 R1	3.47	12.62	53.7	198	0.297	7.58	10.6	62.34	6.26
SC T4 R2	3.93	13.13	45.7	167	0.327	8.96	26.3	71.74	6.87
SC T4 R3	3.34	13.75	49.1	114	0.246	7.69	23.6	57.37	7.51
SC T4 R4	3.41	12.7	49.2	114	0.249	7.04	8.87	85.64	6.19
SS T4 R1	3	13.72	110	232	0.247	7.77	17.7	75.66	6.12
SS T4 R2	3.7	17.84	170	232	0.313	8.61	56.8	99.39	6.82
SS T4 R3	2.8	13.13	89.6	136	0.221	6.71	16	78.43	6.55
SS T4 R4	3.63	14.74	120	171	0.307	8.53	23.2	80.82	6.16
SB T4 R1	2.4	9.09	104	148	0.2	7.13	13	48.5	6.39
SB T4 R2	4.47	15.58	97	176	0.335	9.36	16	67.92	6.78
SB T4 R3	3.61	12.81	186	193	0.296	8.19	19.8	57.69	6.52
SB T4 R4	3.02	15.14	150	169	0.243	8.49	16.6	75.63	6.18
SP T4 R1	2.94	9.84	154	114	0.232	7.56	25.2	55.96	6.7
SP T4 R2	3.7	12.46	138	136	0.296	8.55	34.8	63.13	6.6
SP T4 R3	4.07	14.17	118	140	0.316	7.81	48.3	63.65	7.27
SP T4 R4	4.38	14.95	141	188	0.378	8.43	44.2	78.9	6.28
SC T5 R1	3.36	11.22	59.3	140	0.259	7.67	10.8	68.22	6.33
SC T5 R2	3.21	12.98	46.7	157	0.244	8.15	13.3	60.12	6.91
SC T5 R3	2.85	10.17	35.7	142	0.229	7.03	12.9	51.25	6.62
SC T5 R4	3.72	11.74	94.9	96.8	0.29	8.34	21	65.4	6.98
SS T5 R1	3.25	13.99	142	195	0.259	8.64	24.4	76.84	6.44

Sample Name	C (Soil) - JC	Cu (Soil) - JC	K - Morgan (Soil) - JC	Mg - Morgan (Soil) - JC	N (Soil) - JC	OM (Soil) - JC	P - Morgan (Soil) - JC	Zn (Soil) - JC	pH (Soil) - JC
SS T5 R2	4.14	14.99	179	228	0.311	8.95	30.5	79.37	6.51
SS T5 R3	2.84	14.13	141	184	0.228	8.2	24.9	77.04	6.32
SS T5 R4	3.16	14.36	111	109	0.231	7.6	21.3	73.01	7.26
SB T5 R1	3.1	9.99	155	154	0.222	7.27	13	60.84	6.53
SB T5 R2	3.94	14	170	265	0.329	8.64	20.3	64.7	6.32
SB T5 R3	3.22	12.46	126	166	0.262	8.04	16.3	55.52	6.48
SB T5 R4	4.36	12.63	120	149	0.372	8.46	20.4	63.75	6.51
SP T5 R1	3.14	9.29	165	193	0.259	7.6	40.6	51.71	6.64
SP T5 R2	5.01	12.96	192	171	0.409	8.87	47.1	67.73	6.8
SP T5 R3	4.23	11.04	145	146	0.336	8.55	50.2	56.06	6.99
SP T5 R4	3.5	12.2	150	123	0.289	7.86	43.2	60.61	6.92
SC T6 R1	3.22	10.15	94.9	134	0.286	7.27	15.2	57.89	6.67
SC T6 R2	2.97	11.61	62.4	105	0.267	7.17	8.5	57.87	6.59
SC T6 R3	3.63	14.44	37.7	112	0.309	7.92	19.5	61.25	6.84
SC T6 R4	3.22	17.11	41.1	126	0.273	7.22	13	62.43	6.41
SS T6 R1	3.35	12.34	86.8	156	0.299	6.92	15.2	62.99	6.31
SS T6 R2	3.14	12.76	234	142	0.288	7.9	13	66.96	6.31
SS T6 R3	3.62	13.3	204	145	0.332	7.86	16.9	69.28	6.12
SS T6 R4	3.71	18	102	167	0.337	9.11	33.5	122.68	6.72
SB T6 R1	3.24	9.8	223	152	0.282	7.41	14.7	50.51	6.4
SB T6 R2	3.46	13.98	158	200	0.306	8.7	41.4	60.23	7.03
SB T6 R3	3.53	11.17	127	144	0.317	8.16	14	58.08	6.23
SB T6 R4	3.18	14.29	103	179	0.285	7.77	23.8	68.35	6.61

Sample Name	C (Soil) - JC	Cu (Soil) - JC	K - Morgan (Soil) - JC	Mg - Morgan (Soil) - JC	N (Soil) - JC	OM (Soil) - JC	P - Morgan (Soil) - JC	Zn (Soil) - JC	pH (Soil) - JC
SP T6 R1	3.59	10.4	182	190	0.331	9.07	71.3	61.54	6.52
SP T6 R2	3.66	10.61	132	148	0.336	8.2	30.4	55.77	6.25
SP T6 R3	3.59	14.43	109	165	0.32	8.5	42.3	66.19	7.11
SP T6 R4	2.96	12.46	176	162	0.264	8.21	71.6	63.14	6.9
SC T7 R1	3.23	9.92	44	102	0.284	6.7	10.4	52.07	7.04
SC T7 R2	3.38	10.99	80.6	175	0.292	7.4	7.68	51.67	6.14
SC T7 R3	3.49	13.27	42.7	118	0.288	8	17.3	57.91	7.46
SC T7 R4	3.5	12.32	83.4	106	0.312	7.7	19.6	61.32	7.05
SS T7 R1	3.22	13.34	102	140	0.291	7.5	26.3	77.99	7.07
SS T7 R2	3.83	16.17	97.3	240	0.359	9.3	23.7	84.03	6.38
SS T7 R3	3.56	14.82	92.7	199	0.32	8	22	70.73	6.55
SS T7 R4	3.88	16.12	104	181	0.355	8.8	29.7	92.44	6.65
SB T7 R1	3.58	10.11	156	147	0.318	7.4	15.9	54.13	6.79
SB T7 R2	3.8	12.92	292	196	0.363	9.2	26.4	61.01	6.7
SB T7 R3	2.97	12.19	110	131	0.257	7.2	21	52.36	7.07
SB T7 R4	3.8	13.11	138	165	0.336	8.9	25.5	70.49	6.81
SP T7 R1	4.79	11.22	190	184	0.434	8.3	71.5	56.66	7.23
SP T7 R2	3.82	13	126	260	0.35	9.1	62.7	64.82	6.67
SP T7 R3	3.04	12.19	136	179	0.292	7.6	51.8	56.95	6.67
SP T7 R4	2.9	13.77	130	183	0.271	8.1	69.8	71.97	7.14
SC T8 R1	3.09	11.67	43.7	129	0.267	6.8	6.78	80.62	6.17
SC T8 R2	3.17	12.95	56	154	0.281	7.83	13.1	72.02	6.24
SC T8 R3	2.74	14.08	57.2	172	0.261	7.74	14.3	68.88	6.17

Sample Name	C (Soil) - JC	Cu (Soil) - JC	K - Morgan (Soil) - JC	Mg - Morgan (Soil) - JC	N (Soil) - JC	OM (Soil) - JC	P - Morgan (Soil) - JC	Zn (Soil) - JC	pH (Soil) - JC
SC T8 R4	2.92	10.48	49.4	132	0.251	6.78	11.1	57.13	6.09
SS T8 R1	2.77	10.34	88.4	152	0.253	6.76	11.4	59.37	6.48
SS T8 R2	3.17	12.81	73.7	143	0.272	7.46	13.9	67.86	6.41
SS T8 R3	2.55	13.7	66.9	153	0.221	6.81	10.5	69.29	6.22
SS T8 R4	3.16	13.18	84	101	0.259	6.71	17.7	68.72	6.86
SB T8 R1	2.9	8.8	124	141	0.272	6.22	7.28	50.97	6.27
SB T8 R2	2.9	11.21	208	153	0.262	7.39	10.2	54.84	6.25
SB T8 R3	2.87	10.34	81.5	121	0.248	6.76	14.7	76.27	7.18
SB T8 R4	3.41	12.98	114	155	0.259	7.01	12.6	63.4	6.34
SP T8 R1	2.96	13.15	106	175	0.272	6.95	20.1	60.46	6.43
SP T8 R2	2.98	13.2	69.8	227	0.263	7.64	24.4	69.36	6.6
SP T8 R3	3.18	11.02	108	196	0.242	6.98	24.4	54.1	6.53
SP T8 R4	2.98	13.7	90.5	153	0.256	7.46	26.2	80.13	6.55
SC T9 R1	3.33	10.09	43.8	92.7	0.323	6.36	8.67	66.12	6.87
SC T9 R2	3.29	12.53	107	178	0.283	8.01	9.8	68.77	5.9
SC T9 R3	3.34	12.72	45.7	108	0.286	6.86	12.6	52.97	7.15
SC T9 R4	2.86	11.87	41.8	95.3	0.245	6.09	8.96	59.17	6.56
SS T9 R1	2.69	11.02	79	107	0.256	6.43	10.5	62	6.73
SS T9 R2	3.57	14.89	87.2	211	0.296	7.87	23.4	93.2	6.67
SS T9 R3	2.57	11.77	75.3	146	0.256	6.32	7.99	59.83	6.31
SS T9 R4	2.61	11.78	66	130	0.249	6.82	10.8	64.43	6.22
SB T9 R1	3.03	9.84	97.9	92.8	0.265	6.54	11.3	49.01	6.92
SB T9 R2	3.23	12.11	70.1	180	0.293	7.07	12.7	65.22	6.73

Sample Name	C (Soil) - JC	Cu (Soil) - JC	K - Morgan (Soil) - JC	Mg - Morgan (Soil) - JC	N (Soil) - JC	OM (Soil) - JC	P - Morgan (Soil) - JC	Zn (Soil) - JC	pH (Soil) - JC
SB T9 R3	2.66	12.4	54.4	146	0.255	6.08	9.23	59.65	6.45
SB T9 R4	2.66	13.45	99.5	162	0.247	6.59	10.4	62.34	6.65
SP T9 R1	2.75	10.2	127	127	0.248	6.69	21	55.44	6.86
SP T9 R2	2.89	12.3	105	174	0.285	7.93	45.4	60.79	6.83
SP T9 R3	3.42	12.6	57.9	159	0.323	7.49	43.6	57.22	7.21
SP T9 R4	3.16	11.58	62	135	0.298	6.9	32.8	60.08	6.94

Appendix 5: Nutrient results for manure samples.

External Reference	C (Soil) - JC	Ca (Crop) - JC	Cu (Crop) - JC	K (Crop) - JC	Mg (Crop) - JC	Mn (Crop) - JC	N (Crop) - JC	Na (Crop) - JC	P (Crop) - JC	Zn mg/kg
Pig Manure	1.81	0.077	21.2	0.272	0.032	11	0.378	0.06	0.039	102.57
Pig Manure	1.82	0.079	21.43	0.277	0.034	12	0.374	0.061	0.042	108.44
Cow Manure	2.93	0.213	12.62	0.393	0.072	19.29	0.328	0.035	0.057	14.51
Cow Manure	3.09	0.212	12.44	0.386	0.072	19.19	0.329	0.034	0.057	13.75
Chicken Manure	32.3	10.53%	N/A	2.07%	N/A	N/A	4.08	N/A	N/A	344.74
Chicken Manure	32.40%	N/A	N/A	N/A	N/A	N/A	342.00%	N/A	N/A	N/A

Appendix 6: Gene and Primer list for HT-qPCR array

Gene Code	Gene Name	Gene Classification	Forward Primer	Reverse Primer
AY1	16S rRNA1	Housekeeping	GGGTTGCGCTCGTTGC	ATGGYTGTCGTCAGCTCGTG
AY2	aacC2	Aminoglycoside	ACGGCATTCTCGATTGCTTT	CCGAGCTTCACGTAAGCATTT
AY4	aacA/aphD	Aminoglycoside	AGAGCCTTGGGAAGATGAAGTTT	TTGATCCATACCATAGACTATCTCATCA
AY6	aac(6')-II	Aminoglycoside	CGACCCGACTCCGAACAA	GCACGAATCCTGCCTTCTCA
AY8	aac(6')-Ib_1	Aminoglycoside	CGTCGCCGAGCAACTTG	CGGTACCTTGCCTCTCAAACC
AY9	aadA2_1	Aminoglycoside	ACGGCTCCGCAGTGGAT	GGCCACAGTAACCAACAAATCA
AY13	aadD	Aminoglycoside	CCGACAACATTTCTACCATCCTT	ACCGAAGCGCTCGTCGTATA
AY328	aadA5_2	Aminoglycoside	ATCACGATCTTGCGATTTTGCT	CTGCGGATGGGCCTAGAAG
AY330	aph(2')-Ib	Aminoglycoside	TGAGCAGTATCATAAGTTGAGTGAAAAG	GACAGAACAATCAATCTCTATGGAATG
AY331	aadA2_3	Aminoglycoside	CAATGACATTCTTGCGGGTATC	GACCTACCAAGGCAACGCTATG
AY385	aac(6')-Iy	Aminoglycoside	GCCTCAATCCGCCACGATTA	ACGCGCTCTGTTTCCTCAAA
AY386	aac(6')I1	Aminoglycoside	GGGAATTATCGGAATAGCTCTTGG	TTGGGCTGTTCTTCCTAGCTAA
AY388	aac3-IVa	Aminoglycoside	CCAACACGACGCTGCATC	GCTGTCGCCACAATGTCG
AY389	aph6-ia	Aminoglycoside	CGCTGGGAGCTGAAGAGG	AGCATCGTGCTGCTCTCC
AY390	spcN	Aminoglycoside	GCTATGTGCTGGTGGACTGG	GGAACCACTCGACGAACTCG
AY391	aac(3)-ib	Aminoglycoside	CAGCGAGACGTTCATCGC	CACGCTTCAGGTGGCTAATC
AY392	aac(3)-id_ie	Aminoglycoside	AGATAGTTATGCCCGCAACAAG	ACGCGCTGCGCCTATA
AY393	aac(3)-iid_iia	Aminoglycoside	CGATGGTCGCGGTTGGTC	TCGGCGTAGTGCAATGCG
AY394	aac(3)-xa_1	Aminoglycoside	GCAAGCGGTTCGTGACGTA	TCAGGTGCTCCTCGATCCAG
AY396	aac(6)-ig	Aminoglycoside	GCGATGTTAGAAGCCTCAATTCG	CACACTTCGGCCTGTCGAA
AY397	aac(6)-iic	Aminoglycoside	CAGTCTTTGGCTAATCCATCACAG	AACGAACCCGGCCTTCTC
AY398	aac(6)-ij	Aminoglycoside	ATGCCTGTATCTGAATCCCTGATG	GGCAATCGCTTGTTGAGTATCTG
AY399	aac(6)-im	Aminoglycoside	CGTGAGCATTATACAGAGCAATGG	CCATTTCCGTTCGTAGATATTGGC

Appendix 6: Gene and Primer list for HT-qPCR array

Gene Code	Gene Name	Gene Classification	Forward Primer	Reverse Primer
AY400	aac(6)-ir	Aminoglycoside	GCTATAACGATCAGCAGCAAGC	CGCGATGCATGGCATGAC
AY401	aac(6)-is_iu_ix	Aminoglycoside	AAGCTTACTCTGGCCTGATCATG	TGCCTGAACGTCGATATTCAGG
AY402	aac(6)-iv_ih	Aminoglycoside	TTGGCTTATACCGACACCCA	CCCGTTGCGATACCTGAAC
AY403	aac(6)-iw	Aminoglycoside	TGCGTCAGTTACTTACACGAAC	CCTGATGCATTGCATGACTGA
AY404	aac(6)-iz	Aminoglycoside	TGCGCCATGACTACGTGAAC	GACTGTCCGAAGCCAGTTCG
AY406	aac6-aph2	Aminoglycoside	CCAAGAGCAATAAGGGCATACCAA	GCCACACTATCATAACCACTACCG
AY407	aacA43	Aminoglycoside	CTTGGCCTACATTAGATTCAGCTC	GCTCTCAATCTTTGATAGGAGCAG
AY408	aadA10	Aminoglycoside	ACAGGCACTCAACGTCATCG	CGCGGAGAACTCTGCTTTGA
AY409	aadA16	Aminoglycoside	ACGGTGGCCTGAAGCC	GAATTGCAGTTCCCGTCTGG
AY411	aadA6	Aminoglycoside	CCATCGAGCGTCATCTGGAA	CCCGTCTGGCCGGATAAC
AY412	aadA7	Aminoglycoside	CACTCCGCGCCTTGGA	TGTGGCGGGCTCGAAG
AY413	aadB	Aminoglycoside	CCTGCTTGGTGGGCAGAC	CGGCACGCAAGACCTCAA
AY414	ant4-ib	Aminoglycoside	GATGGCCGCTGACACATG	TCAACATTGCGCCATAGTGG
AY415	ant6-ia	Aminoglycoside	TCGCCATGAGCTGCTGA	CCTATCATACTCCGGATAGGCATA
AY416	ant6-ib	Aminoglycoside	AGAACATCCGACAGCACGTTC	CCAACCTTCCATGAAATCATTCGC
AY417	aph_viii	Aminoglycoside	TCGGTATCCCGGTTGTGAG	ACACGAGGTACGGGAATCC
AY418	aph(3")-ia	Aminoglycoside	TAACAGCGATCGCGTATTTCG	TCCGACTCGTCCAACATCAATA
AY419	aph3-ib	Aminoglycoside	AACAGGTTTGGGAGGCGATG	CGCAACAAGCCTCTCCTGAA
AY420	aph3-iii	Aminoglycoside	CAGAAGGCAATGTCATACCACTTG	GACAGCCGCTTAGCCGAA
AY421	aph3-viia	Aminoglycoside	CTCTCTCATGGAGATATGAGCGCTA	AATCCGGTTCAAGTCCCAACATG
AY422	aph3-via	Aminoglycoside	TCTCATGGCGATATCACGGATAG	TTTCCTCCGATGCATCCTCTC
AY423	aph4-ia	Aminoglycoside	CGCTCCCGATTCCGGAA	CACAGTTTGCCAGTGATACACA
AY424	aph4-ib	Aminoglycoside	GGGAACACCGTGCTCACC	GTTGGTCCCGTGCAGGTC
AY426	apmA	Aminoglycoside	GGCGCACATGCATTCATCA	CTATACTCCAGTCCCACCATTTGA
AY427	armA	Aminoglycoside	TCTTCGACGAATGAAAGAGTCG	GCTAATGGATTGAAGCCACAACC

Appendix 6: Gene and Primer list for HT-qPCR array

Gene Code	Gene Name	Gene Classification	Forward Primer	Reverse Primer
AY101	blaMOX/blaCMY	Beta Lactam	CTATGTCAATGTGCCGAAGCA	GGCTTGTCCTCTTTCGAATAGC
AY103	blaPAO	Beta Lactam	CGCCGTACAACCGGTGAT	GAAGTAATGCGGTTCTCCTTTCA
AY105	blaVEB	Beta Lactam	CCCGATGCAAAGCGTTATG	GAAAGATTCCCTTTATCTATCTCAGACAA
AY107	blaROB	Beta Lactam	GCAAAGGCATGACGATTGC	CGCGCTGTTGTCGCTAAA
AY108	blaOXY	Beta Lactam	CGTTCAGGCGGCAGGTT	GCCGCGATATAAGATTTGAGAATT
AY111	cphA_1	Beta Lactam	GCGAGCTGCACAAGCTGAT	CGGCCCAGTCGCTCTTC
AY117	ampC/blaDHA	Beta Lactam	TGGCCGCAGCAGAAAGA	CCGTTTTATGCACCCAGGAA
AY125	blaGES	Beta Lactam	GCAATGTGCTCAACGTTCAAG	GTGCCTGAGTCAATTCTTTCAAAG
AY126	blaSFO	Beta Lactam	CCGCCGCCATCCAGTA	GGGCCGCCAAGATGCT
AY127	blaTLA	Beta Lactam	ACACTTTGCCATTGCTGTTTATGT	TGCAAATTTCGGCAATAATCTTT
AY128	blaZ	Beta Lactam	GGAGATAAAGTAACAAATCCAGTTAGATATGA	TGCTTAATTTTCCATTTGCGATAAG
AY129	blaVIM	Beta Lactam	GCACTTCTCGCGGAGATTG	CGACGGTGATGCGTACGTT
AY133	mecA	Beta Lactam	GGTTACGGACAAGGTGAAATACTGAT	TGTCTTTTAATAAGTGAGGTGCGTTAATA
AY134	blaCTX-M_5	Beta Lactam	GCGATAACGTGGCGATGAAT	GTCGAGACGGAACGTTTCGT
AY147	blaCTX-M_8	Beta Lactam	CGTCACGCTGTTGTTAGGAA	CGCTCATCAGCACGATAAAG
AY152	blaNDM	Beta Lactam	GGCCACACCAGTGACAATATCA	CAGGCAGCCACCAAAAGC
AY336	blaACC	Beta Lactam	CACACAGCTGATGGCTTATCTAAAA	AATAAACGCGATGGGTTCCA
AY431	blaSME	Beta Lactam	GAGGAAGACTTTGATGGGAGGATTG	CGCTATATTGCAATGCAGCAGAAG
AY432	blaCTX-M	Beta Lactam	CGTACCGAGCCGACGTTAA	CAACCCAGGAAGCAGGCA
AY433	blaFOX	Beta Lactam	CCTACGGCTATTCGAAGGAAGATAAG	CCGGATTGGCCTGGAAGC
AY437	blaPER	Beta Lactam	GCAAATGAAGCGCAGATGC	GACCACAGTACCAGCTGGTA

Appendix 6: Gene and Primer list for HT-qPCR array

Gene Code	Gene Name	Gene Classification	Forward Primer	Reverse Primer
AY438	blaSHV11	Beta Lactam	TTGACCGCTGGGAAACGG	TCCGGTCTTATCGGCGATAAAC
AY440	blaKPC	Beta Lactam	GCCGCCAATTTGTTGCTGAA	GCCGGTCGTGTTTCCCTTT
AY442	bl1acc	Beta Lactam	TGTTATCCGTGATTACCTGTCTGG	CTCAGCGAGCCAACTTCAAATA
AY445	blaB	Beta Lactam	CGTGCCGGAGGTCTTGAATA	GGGATAGTAAACCTGAAACTCGGA
AY446	blaCARB	Beta Lactam	TGATTTGAGGGATACGACAACTCC	CTGTAATACTCCGAGCACCAA
AY449	blaIMI	Beta Lactam	ACATCTACACCTGCAGCAGTAG	AATCGCTTGGTACGCTAGCA
AY451	blaLEN	Beta Lactam	TGTTCGCCTGTGTGTTATCTCC	GCAGCACTTTAAAGGTGCTCAC
AY452	blaMIR	Beta Lactam	CGGTCTGCCGTTACAGGTG	AAAGACCCGCGTCGTCATG
AY199	acrB_1	MDR	AGTCGGTGTTCGCCGTTAAC	CAAGGAAACGAACGCAATACC
AY201	acrF	MDR	GCGGCCAGGCACAAAA	TACGCTCTTCCCACGGTTTC
AY207	acrA_1	MDR	GGTCTATCACCCTACGCGCTATC	GCGCGCACGAACATACC
AY211	mdtE	MDR	CGTCGGCGCACTCGTT	TCCAGACGTTGTACGGTAACCA
AY215	mexA	MDR	AGGACAACGCTATGCAACGAA	CCGGAAAGGGCCGAAAT
AY224	oprD	MDR	ATGAAGTGGAGCGCCATTG	GGCCACGGCGAACTGA
AY353	tolC_2	MDR	CAGGCAGAGAACCTGATGCA	CGCAATTCCGGGTTGCT
AY360	marR_3	MDR	GCTGTTGATGACATTGCTCACA	CGGCGTACTGGTGAAGCTAAC
AY482	oqxA	MDR	GAGTCAACCTACCTCCACTATCA	GCTGCGAGTTATCCAGCAG
AY489	qacF/H	MDR-mobile	CTGAAGTCTAGCCATGGATTCACTAG	CAAGCAATAGCTGCCACAAGC
AY297	Tp614	MGE	GGAAATCAACGGCATCCAGTT	CATCCATGCGCTTTTGTCTCT
AY298	IS613	MGE	AGGTTCGGACTCAATGCAACA	TTCAGCACATACCGCCTTGAT
AY299	tnpA_1	MGE	GCCGCACTGTCGATTTTTATC	GCGGGATCTGCCACTTCTT
AY300	tnpA_2	MGE	CCGATCACGGAAAGCTCAAG	GGCTCGCATGACTTCGAATC
AY301	tnpA_3	MGE	GGGCGGGTCGATTGAAA	GTGGGCGGGATCTGCTT
AY302	tnpA_4	MGE	CATCATCGGACGGACAGAATT	GTCGGAGATGTGGGTGTAGAAAGT

Appendix 6: Gene and Primer list for HT-qPCR array

Gene Code	Gene Name	Gene Classification	Forward Primer	Reverse Primer
AY303	tnpA_5	MGE	GAAACCGATGCTACAATATCCAATTT	CAGCACCGTTTGCAGTGTAAG
AY306	trfA	MGE	ACGAAGAAATGGTTGTCCTGTTC	CGTCAGCTTGCGGTACTTCTC
AY307	orf37-IS26	MGE	GCCGGGTTGTGCAAATAGAC	TGGCAATCTGTCGCTGCTG
AY309	ISPps	MGE	CACACTGCAAAAACGCATCCT	TGTCTTTGGCGTCACAGTTCTC
AY311	ISAba3	MGE	TCAGAGGCAGCGGTATACGA	GGTTGATTCAGTTAAAGTACGTAAAACTTT
AY312	ISEfm1	MGE	AGGTGTCCATGACGTGAAAGTG	TCCTTTGTCCCCTAGGATATTGG
AY313	IS1111	MGE	GTCTTAAGGTGGGCTGCGTG	CCCCGAATCTCATTGATCAGC
AY314	IS1133	MGE	GCAGCGTCGGGTTGGA	ACGCGTTCGAACAACTGTAATG
AY316	IncN_rep	MGE	AGTTCACCACCTACTCGCTCCG	CAAGTTCTTCTGTTGGGATTCCG
AY318	IncP_oriT	MGE	CAGCCTCGCAGAGCAGGAT	CAGCCGGGCAGGATAGGTGAAGT
AY319	IncQ_oriT	MGE	TTCGCGCTCGTTGTTCTTCGAGC	GCCGTTAGGCCAGTTTCTCG
AY324	pAKD1	MGE	GGTAAGATTACCGATAAACT	GTTCGTGAAGAAGATGTA
AY503	IncHI2-smr0018	MGE	ATAATGATTCACCGGGGTAG	CTTCAGGCTATCGTTTCG
AY504	IncI1_repI1	MGE	CGAAAGCCGGACGGCAGAA	TCGTCGTTCCGCCAAGTTCGT
AY515	IS6/257	MGE	ATATCGTGCCATTGATGCAGAG	ACCATTGCTACCTTCGTTGAAG
AY521	lncF_FIC	MGE	GTGAACTGGCAGATGAGGAAGG	TTCTCCTCGTCGCCAAACTAGAT
AY523	Tn3	MGE	GCTGAGGTGTTCAGCTACATCC	GCTGAGGTAGTCACAGGCATTC
AY526	traN	MGE	GCTTGGCGGTCAGCAATT	TTAGGAATAACAATCGCTACACCTTTA
AY527	trbC	MGE	CGGYATWCCGSCSACRCTGCG	GCCACCTGYSBGCAGTCMCC
AY44	ermD/K	MLSB	GAGCCGCAAGCCCCTTT	GTGTTTCATTTGACGCGGAGTAA
AY54	erm36	MLSB	GGCGGACCGACTTGCAT	TCTGCGTTGACGACGGTTAC

Appendix 6: Gene and Primer list for HT-qPCR array

Gene Code	Gene Name	Gene Classification	Forward Primer	Reverse Primer
AY57	ermT_1	MLSB	GTTCACTAGCACTATTTTTAATGACAGAAGT	GAAGGGTGTCTTTTTAATACAATTAACGA
AY58	msrC_1	MLSB	TCAGACCGGATCGGTTGTC	CCTATTTTTTGGAGTCTTCTCTCTAATGTT
AY66	msrA_1	MLSB	CTGCTAACACAAGTACGATTCCAAAT	TCAAGTAAAGTTGTCTTACCTACACCATT
AY68	ermX_1	MLSB	GCTCAGTGGTCCCCATGGT	ATCCCCCCGTCAACGTTT
AY72	vgaB_1	MLSB	TAAAAGAGAATAAGGCGCAAGGA	TGTTTAGTAGCATGTTGCATTTTCC
AY83	ermY	MLSB	TTGTCTTTGAAAGTGAAGCAACAGT	TAACGCTAGAGAACGATTTGTATTGAG
AY90	ermA/ermTR	MLSB	ACATTTTACCAAGGAACTTGTGGAA	GTGGCATGACATAAACCTTCATCA
AY528	ereA	MLSB	GATAATTCTGCTGGCGCACA	GCAGGCGTGGTCACAAC
AY532	ermA	MLSB	TCGTTGAGAAGGGATTTGCGA	TTGCATGCTTCAAAGCCTGTC
AY533	ermB_2	MLSB	GAACACTAGGGTTGTTCTTGCA	CTGGAACATCTGTGGTATGGC
AY534	ermD	MLSB	TTTCCGGACAGCATTTGATGC	TCCACTGCCAATACCTTACCG
AY535	ermF	MLSB	TCTGATGCCCGAAATGTTCAAG	TGAAGGACAATTGAACCTCCCA
AY541	vga(A)LC_1	MLSB	GTGAAGATGTCTCGGGTACAATTG	GAAATACCAGGATTCCCATGCAC
AY544	ermE	MLSB	GTCACGCAGCTGGAGTTCG	CGGTGAAGCACAGCTCGAC
AY545	ermC_2	MLSB	CCCTTGAATTAGTACAGAGGTG	GCAAACTCGTATTCCACGA
AY548	ermO	MLSB	GAGTACGCCCGCAAACG	GCGTTCGATCCGGAGGA
AY466	mcr1	Other	CACATCGACGGCGTATTCTG	CAACGAGCATACCGACATCG
AY467	mcr2	Other	CGGCGTACTTTAAGCGTTATGATG	GCATTTGGCATACCATGCAGATAG
AY455	norA	Quinolone	ATCGCCGTTTGGTGGTACG	TCCACCAATCCCTGGTCCTAAA
AY456	qepA	Quinolone	GGGCATCGCGCTGTTC	GCGCATCGGTGAAGCC
AY457	qnrB4	Quinolone	TCACCACCCGCACCTG	GGATATCTAAATCGCCCAGTTCC

Appendix 6: Gene and Primer list for HT-qPCR array

Gene Code	Gene Name	Gene Classification	Forward Primer	Reverse Primer
AY459	qnrD	Quinolone	CGCTGGAATGGCACTGTGA	GCTCTCCATCCAACTTCACTCC
AY460	qnrS_1	Quinolone	CCACTTTGATGTCGCAGATCTTC	CCCTCTCCATATTGGCATAGGAAA
AY461	qnrS2	Quinolone	TCCCGAGCAAACTTTGCCAA	GGTGAGTCCCTATCCAGCGA
AY462	qnrVC1_VC3_VC6	Quinolone	CTCACATCAGGACTTGCAAGAA	ATGAAGCATCTCGAAGATCAGC
AY245	sul1_2	Sulfonamide	GCCGATGAGATCAGACGTATTG	CGCATAGCGCTGGGTTTC
AY365	sul2_2	Sulfonamide	TCATCTGCCAAACTCGTCGTTA	GTCAAAGAACGCCGCAATGT
AY249	tet36_1	Tetracycline	AGAATACTCAGCAGAGGTCAGTTCCT	TGGTAGGTCGATAACCCGAAAAT
AY250	tet32	Tetracycline	CCATTACTTCGGACAACGGTAGA	CAATCTCTGTGAGGGCATTTAACA
AY258	tetK	Tetracycline	CAGCAGTCATTGGAAAATTATCTGATTATA	CCTTGTACTAACCTACCAAAAATCAAAATA
AY259	tetQ	Tetracycline	CGCCTCAGAAGTAAGTTCATACACTAAG	TCGTTCATGCGGATATTATCAGAAT
AY260	tetH	Tetracycline	TTTGGGTCATCTTACCAGCATTAA	TTGCGCATTATCATCGACAGA
AY263	tetW	Tetracycline	ATGAACATTCCCACCGTTATCTTT	ATATCGGCGGAGAGCTTATCC
AY264	tetO_2	Tetracycline	CAACATTAACGGAAAGTTTATTGTATACCA	TTGACGCTCCAAATTCATTGTATC
AY267	tetX	Tetracycline	AAATTTGTTACCGACACGGAAGTT	CATAGCTGAAAAAATCCAGGACAGTT
AY268	tetC_2	Tetracycline	ACTGGTAAGGTAAACGCCATTGTC	ATGCATAAACCAGCCATTGAGTAAG
AY269	tetS	Tetracycline	TTAAGGACAAACTTTCTGACGACATC	TGTCTCCCATTGTTCTGGTTCA
AY273	tetE	Tetracycline	TTGGCGCTGTATGCAATGAT	CGACGACCTATGCGATCTGA
AY276	tetT	Tetracycline	CCATATAGAGGTTCCACCAAATCC	TGACCCTATTGGTAGTGGTTCTATTG
AY367	tetL_2	Tetracycline	ATGGTTGTAGTTGCGCGCTATAT	ATCGCTGGACCGACTCCTT

Appendix 6: Gene and Primer list for HT-qPCR array

Gene Code	Gene Name	Gene Classification	Forward Primer	Reverse Primer
AY568	tet39	Tetracycline	TATAGCGGGTCCGGTAATAGGTG	CCATAACGATCCTGCCCATAGATAAC
AY571	tetD	Tetracycline	AATTGCACTGCCTGCATTGC	GACAGATTGCCAGCAGCAGA
AY572	tetG	Tetracycline	TCGCGTTCCTGCTTGCC	CCGCGAGCGACAAACCA
AY573	tetJ	Tetracycline	CAGCGCCCATACGCCATTTA	CCTACTTCAGTAGTGTGCCAAGC
AY575	tetPA	Tetracycline	GGAAACCTTAGTTCAGTGACTTGG	CCCATTTAACCACGCACTGAA
AY576	tet44	Tetracycline	CTCATGTAGATGCAGGAAAGACG	GTAACTGCTGCCTGAATTGTGA
AY577	tetR	Tetracycline	CCGTCAATGCGCTGATGAC	GCCAATCCATCGACAATCACC
AY284	dfrA1_1	Trimethoprim	GGAATGGCCCTGATATTCCA	AGTCTTGCGTCCAACCAACAG
AY285	dfrA12	Trimethoprim	CCTCTACCGAACCGTCACACA	GCGACAGCGTTGAAACAACTAC
AY578	dfrA10	Trimethoprim	CTTCAACTATCACAGAGCACGAAG	TCTACCGGTACATACACATCAGC
AY581	dfra17	Trimethoprim	CGGGAACGGCCCTGATATTCC	CGTGTTGCGACCGCATACTTTC
AY588	dfrA7	Trimethoprim	GTAATCGGTAGTGGTCCTGA	ATCAGGACCACTACCGATTAC
AY589	dfrA8	Trimethoprim	GGTCGCACCTGCATCGTTA	AGCGCCACCAATGACGTAG
AY159	vanB_1	Vancomycin	TTGTCGGCGAAGTGGATCA	AGCCTTTTTCCGGCTCGTT
AY595	vanA	Vancomycin	GGGCTGTGAGGTCGGTTG	TTCAGTACAATGCGGCCGTTA
AY98	ampC_1	Beta Lactam	AACAAAAGATCCCCGGTATGG	ACGCCCGTAAATGTTTTGCT
AY100	ampC_2	Beta Lactam	TCCGGTGACGCGACAGA	CAGCACGCCGGTGAAAGT
AY106	blaOKP	Beta Lactam	GCCGCCATCACCATGAG	GGTGACGTTGTCACCGATCTG
AY110	blaOXA10_1	Beta Lactam	CGCAATTATCGGCCTAGAAACT	TTGGCTTTCCGTCCCATTT
AY119	ampC_3	Beta Lactam	CCGCCCAGAGCAAGGACTA	GCTCGACTTCACGCCGTAAG
AY121	blaCTX-M_1	Beta Lactam	GGAGGCGTGACGGCTTTT	TTCAGTGCGATCCAGACGAA
AY122	blaCTX-M_2	Beta Lactam	GCCGCGGTGCTGAAGA	ATCGGATTATAGTTAACCAGGTCAGATTT
AY123	blaCTX-M_3	Beta Lactam	CGATACCACCACGCCGTTA	GCATTGCCCAACGTCAGATT
AY124	blaCTX-M_4	Beta Lactam	CTTGGCGTTGCGCTGAT	CGTTCATCGGCACGGTAGA

Appendix 6: Gene and Primer list for HT-qPCR array

Gene Code	Gene Name	Gene Classification	Forward Primer	Reverse Primer
AY130	blaIMP_1	Beta Lactam	AACACGGTTTGGTGGTTCTTGTA	GCGCTCCACAAACCAATTG
AY136	blaTEM_1	Beta Lactam	AGCATCTTACGGATGGCATGA	TCCTCCGATCGTTGTCAGAAGT
AY137	blaCTX-M_6	Beta Lactam	CACAGTTGGTGACGTGGCTTAA	CTCCGCTGCCGGTTTTATC
AY141	blaIMP_2	Beta Lactam	AAGGCAGCATTTCCTCTCATTTT	GGATAGATCGAGAATTAAGCCACTCT
AY143	ampC_4	Beta Lactam	GCAGCGAAGCGTCAGTCA	AGATCCGTGGCCGCATAA
AY144	ampC_5	Beta Lactam	CAGCCGCTGATGAAAAAATATG	CAGCGAGCCCACTTCGA
AY149	blaCTX-M_7	Beta Lactam	CGATGTGCAGTACCAGTAA	GCAATGGGATTGTAGTTAA
AY150	blaIMP_3	Beta Lactam	GGAATAGAGTGGCTTAATTC	GGTTTAACAAAACAACCACC
AY153	blaKPC_2	Beta Lactam	GCCGCCGTGCAATACAGT	GCCGCCCAACTCCTTCA
AY154	bla-SME	Beta Lactam	AACGGCTTCATTTTTGTTTAG	GCTTCCGCAATAGTTTTATCA
AY337	ampC_6	Beta Lactam	GCAGCACGCCCCGTAA	TGTACCCATGATGCGCGTACT
AY290	intI1_3	Integrons	GCCTTGATGTTACCCGAGAG	GATCGGTCGAATGCGTGT
AY292	intI3_1	Integrons	GCCACCACTTGTTTGAGGA	GGATGTCTGTGCCTGCTTG
AY294	intI2_2	Integrons	TGCTTTTCCCACCCTTACC	GACGGCTACCCTCTGTTATCTC
AY233	qacA/qacB	MDR	TGGCAATAGGAGCTATGGTGTTT	AAGGTAACACTATTTTCGGTCCAAATC
AY238	qac	MDR	GGAGATTTAGCTCATGTAGCTGAAGAA	AAGCTGTTTTATCCCCGTAGCTTTA
AY48	ereB_2	MLSB	GCTTTATTTCAGGAGGCGGAAT	TTTTAAATGCCACAGCACAGAATC
AY71	vgaA_1	MLSB	CGAGTATTGTGGAAAGCAGCTAGTT	CCCGTACCGTTAGAGCCGATA
AY93	pikR1	MLSB	TCGACATGCGTGACGAGATT	CCGCGAATTAGGCCAGAA
AY195	mcr3	Other	CCAATCAAAATGAGGCGTTAGCATAT	TAACGAAATTGGCTGGAACAATCTC
AY196	mcr4	Other	ATTGGGATAGTCGCCTTTTT	TTACAGCCAGAATCATTATCA

Appendix 6: Gene and Primer list for HT-qPCR array

Gene Code	Gene Name	Gene Classification	Forward Primer	Reverse Primer
AY242	sul1_1	Sulfonamide	CGCACCGGAAACATCGCTGCAC	TGAAGTTCCGCCGCAAGGCTCG
AY243	sul2_1	Sulfonamide	TCCGGTGGAGGCCGGTATCTGG	CGGGAATGCCATCTGCCTTGAG
AY244	sul3_1	Sulfonamide	TCCGTTCAGCGAATTGGTGCAG	TTCGTTCACGCCTTACACCAGC
AY473	A. baumannii	Taxanomic	TCTTGGTGGTCACTTGAAGC	ACTCTTGTGGTTGTGGAGCA
AY478	K. pneumoniae	Taxanomic	ACGGCCGAATATGACGAATTC	AGAGTGATCTGCTCATGAA
AY479	P. aeruginosa	Taxanomic	AGCGTTCGTCCTGCACAAGT	TCCACCATGCTCAGGGAGAT
AY248	tetA_1	Tetracycline	GCTGTTTGTTCTGCCGGAAA	GGTTAAGTTCCTTGAACGCAAACT
AY252	tetD_1	Tetracycline	TGCCGCGTTTGATTACACA	CACCAGTGATCCCGGAGATAA
AY262	tetA/B_2	Tetracycline	GCCCAGTGCTGTTGTTGTCAT	TGAAAGCAAACGGCCTAAATACA
AY270	tetV	Tetracycline	GCGGGAACGACGATGTATATC	CCGCTATCTCACGACCATGAT
AY272	tet38_1	Tetracycline	TTAATGTGGCGGTATCTGTAGGTATT	TTGCCTGGGAAATTTAATGCTTT
AY376	tetU_2	Tetracycline	AACAGCGGGTTAAGTGTGCAA	ATGGTATCATTCAGTTTTCCGACAAT
AY600	16S rRNA2	Housekeeping	CCTACGGGAGGCAGCAG	ATTACCGCGGCTGCTGGC
AY601	blaOXA48	Beta Lactam	TGTTTTTGGTGGCATCGAT	GTAAMRATGCTTGGTTCGC
AY602	armA	Aminoglycoside	TGCATCAAATATGGGGGTCT	TGAAGCCACAACCAAAATCT
AY603	rmtB	Aminoglycoside	GCTGTGATATCCACCAGGGA	AAGCTTAAAAATCAGCGCCA

Appendix 7: Table detailing the samples used in the HT-qPCR array. BM = background samples,T0= Timepoint 0, T1 = Timepoint 1, T5= Timepoint 5, T6= Timepoint 6, 7= Timepoint 7, T8 = Timepoint 8, T9 = Timepoint 9

Soil Samples	Grass Samples	Manure Samples	Controls
S.BM	G.BM	Pig Manure	Positive Control pEK499
SC.T0	GC.T1	Cow Manure	NTC
SS.T0	GS.T1	Chicken Manure	NTC
SP.T0	GP.T1		
SB.T0	GB.T1		
SC.T1	GC.T3		
SS.T1	GS.T3		
SP.T1	GP.T3		
SB.T1	GB.T3		
SS.T5	GC.T5		
SB.T5	GS.T5		
SP.T5	GP.T5		
SC.T6	GB.T5		
SS.T6	GC.T7		
SP.T6	GS.T7		
SB.T6	GP.T7		
SC.T7	GB.T7		
SS.T7	GC.T8		
SP.T7	GS.T8		
SB.T7	GP.T8		
SC.T8	GB.T8		
SB.T8	GC.T9		
SS.T8	GS.T9		
SP.T8	GP.T9		
SC.T9	GB.T9		
SS.T9			
SB.T9			
SP.T9			

Appendix 8: Table displaying the results for 16S rRNA Identification of bacterial isolates in Section 3.2.1. NA indicates there was no result and samples highlighted in red indicate samples that were removed from analysis due to their genus identification percentage identity being under the threshold of 97%.

Isolate Code	Isolate Name	Barcode	Identification (Genus) (<98-98.9)	Percentage Genus (%)	Species (< 99).	Percentage Species	Query Length
1	S1 CIPRO 1/10 EMB 1	CJU267_16062673_16062673	*Alcaligenes*	98.99	*NA*	*NA*	894
2	S1 CIPRO 1/10 EMB 4	CJU266_16062666_16062666	*Achromobacter*	98.12	*NA*	*NA*	853
3	S2 CEF-4 EMB 1	CJU268_16062680_16062680	*Pseudomonas*	99.74	*brassiacearum (99.74), flurorescens (99.66)*	*multiple results*	1181
4	S2 CIPRO N PIA 2	CJU263_16062635_16062635	*Bacillus*	99.73	*cereus*	*99.73*	1111
5	S2 CIPRO N PIA 4	CJU264_16062642_16062642	*Achromobacter*	99.91	*spanius (99.82), marplatensis (99.82), xylosoxidans (99.82 and Acc.length 7013095)*	*multiple results*	1095
6	S3 CIPRO 1/10 PIA 1	DZE835_26828351_26828351	*Achromobacter*	99.84	*spanius (99.84), kerstersii (99.84), xylosoxidans (99.84)*	*multiple results*	635
7	S3 CIPRO 1/10 EMB 2	DZE836_26828368_26828368	*Achromobacter*	98.34	*N/A*	*N/A*	1071
8	S6 CEF -4 EMB 3	CJU260_16062604_16062604	*Pseudomonas*	99.84	*corrugata (99.84), brassicacearum (99.84), thivervalensis (99.84)*	*multiple results*	646
9	S6 CEF -4 EMB 4	CJU261_16062611_16062611	*Acinetobacter*	99	*calcoaceticus*	*99.55*	892
10	S6 CEF -5 EMB 4	CJU265_16062659_16062659	*Acinetobacter*	99.84	*calcoaceticus*	*99.84*	628
11	S6 CIPRO 1/10 LAM 2	DZE837_26828375_26828375	*Lactoccocus*	99.54	*garvieae*	*99.54*	875

Isolate Code	Isolate Name	Barcode	Identification (Genus) (<98-98.9)	Percentage Genus (%)	Species (< 99).	Percentage Species	Query Length
12	S6 CIPRO 1/10 LAM 3	CJU224	*Bacillus*	NA	*NA*	*NA*	
13	S5 CIPRO 1/10 PIA 3	DZE838_26828382_26828382	*Achromobacter*	99.71	*marplatensis (99.51), spanius (99.41)*	*multiple results*	1030
14	G1 COL -5 EMB 1	DZE840_26828405_26828405	*Hafnia*	99.31	*paralvei (99.22), alvei (99.22).*	multiple results	1156
15	G1 COL-5 EMB 4	DZE845_26828450_26828450	*Hafnia*	99.32	*paralvei (99.32), alvei(99.32)*	multiple results	596
16	G1 IMI N PIA 1	CJU242_16062420_16062420	*Stenotrophomonas*	99.65	*maltophilia*	99.77	861
17	G1 CEF -5 PIA 1	CJU245_16062451_16062451	*Pseudomonas*	98.77	*aeruginosa (98.86), flurorscens (98.86)*	NA	1139
18	G1 CEF-5 PIA 2	CJU246_16062468_16062468	*Pseudomonas*	99.58	*aeruginosa*	99.58	1176
19	G1 CEF 10-5 PIA 3	CJU249_16062499_16062499	*Pseudmononas*	96.87	*aeruginosa*	97.39	1117
20	G1 CEF -5 PIA 4	CJU253_16062536_16062536	*Pseudomonas*	99.65	*aeruginosa*	99.65	577
21	G1 CEF-5 LAM 2	CJU248_16062482_16062482	*Pseudmomonas*	99.83	*aeruginosa*	99.83	580
22	G1 CEF 10-5 LAM 3	DZE842_26828429_26828429	*Pseudomonas*	99.32	*aeruginosa*	99.32	594
23	G1 CEF 10-5 LAM 4	DZE841_26828412_26828412	*Pseudmomonas*	99	*aeruginosa*	99.41	1196
24	G2 CEF 10-5 SC 1	CJU239_16062390_16062390	*Pseudomonas*	99.58	*aeruginosa*	99.15	594
25	G2 CEF 10-5 SC 2	CJU240_16062406_16062406	*Pseudomonas*	94.77	*aeruginosa*	92.64	219
26	G2 CEF 10-5 SC 3	CJU241_16062413_16062413	*Pseudomonas*	99.32	*aeruginosa*	99.16	592

Isolate Code	Isolate Name	Barcode	Identification (Genus) (<98-98.9)	Percentage Genus (%)	Species (< 99).	Percentage Species	Query Length
27	G2 CEF 10-4 SC 1	CJU243_16062437_16062437	*Pseudomonas*	99.24	*aeruginosa*	99.24	1183
28	G2 CEF 10-4 SC 2	CJU244_16062444_16062444	*Psuedomonas*	100	*aeruginosa*	100	572
29	G2 CIPRO 1/10 PIA 2	EFP117_28491171_28491171	*Stenotrophomonas*	99.78	*maltophilia (99.78)*	99.78	912
30	G2 CIPRO 1/10 PIA 4	EFP116_28491164_28491164	*Pseudomonas*	96.39	*aeruginosa*	95.58	1054
31	G2 IMI PIA 1	CJU233_16062338_16062338	*Lactoccus*	98.86	*garvieae*	98.86	1140
32	G2 IMI PIA 2	CJU234_16062345_16062345	*Stenotrophomonas*	99.56	*maltophilia*	99.56	1138
33	G2 IMI 1/10 PIA 3	CJU235_16062352_16062352	*Lactoccus*	97.87	*garvieae*	98.23	283
34	G2 IMI 1/10 LAM 1	CJU238_16062383_16062383	*Stenotrophomonas*	99.66	*maltophilia*	99.78	899
35	G2IMI 1/10 LAM 3	CJU236_16062369_16062369	*Stenotrophomonas*	99.45	*maltophilia*	99.45	906
36	G2 IMI 1/10 LAM 4	CJU237_16062376_16062376	*Stenotrophomonas*	99.18	*maltophilia*	99.26	1221
37	G3 CIPRO 1/10 PIA 1	DZE871_26828719_26828719	*Achromobacter*	99	*spanius (99.26), marplantensis (99.26)*	multiple results	1084
38	G3 CIPRO 1/10 PIA 2	DZE870_26828702_26828702	*Achromobacter*	98.99	*spanius (98.99), marplantensis (98.99)*	multiple results	1093
39	G3 CIPRO 1/10 PIA 3	DZE872_26828726_26828726	*Achromobacter*	96.51	*xylosoxidans (97.01), marplatensis (96.61)*	multiple results	1015
40	G3 IMI 1/10 LAM 1	DZE877_26828771_26828771	*Stenotrophomonas*	98.81	*maltophilia*	99.01	922
41	G3 IMI 1/10 LAM 3	DZE876_26828764_26828764	*Stenotrophomonas*	98.42	*maltophilia*	98.64	886

Isolate Code	Isolate Name	Barcode	Identification (Genus) (<98-98.9)	Percentage Genus (%)	Species (< 99).	Percentage Species	Query Length
42	G3 CIPRO 1/10 LAMY 4	DZE875_26828757_26828757	*Acinetobacter*	99.58	*calcoaceticus*	99.41	1185
43	G3 IMI 1/10 EMB 1	DZE874_26828740_26828740	*Stenotrophomonas*	99.45	*maltophilia*	99.45	905
44	G3 IMI 1/10 EMB 2	DZE843_26828436_26828436	*Stenotrophomonas*	98.95	*maltophilia*	99.19	859
45	G3 IMI 1/10 EMB 4	DZE848_26828481_26828481	*Stenotrophomonas*	99.33	*maltophilia*	99.18	1211
46	G3 CEF 10-4 SC 1	EFP118_28491188_28491188	*Pseudomonas*	97.46	*koreensis (97.45), flurorescens (97.45)*		432
47	G3 CEF 10-4 SC 2	DZE847_26828474_26828474	*Pseudomonas*	99.8	*putida (99.61), koreensis (99.80)*	multiple results	515
48	G4 IMI N EMB 1	DZE846_26828467_26828467	*Stenotrophomonas*	99.67	*maltophilia*	99.67	912
49	G4 IMI N EMB 4	DZE849_26828498_26828498	*Stenotrophomonas*	99.08	*maltophilia*	99.2	876
50	G4 IMI N PIA 1	DZE889_26828894_26828894	*Stenotrophomonas*	94.51	*maltophilia*	94.51	425
51	G4 CEF 10-4 PIA 2	DZE896_26828962_26828962	*Pseudomonas*	100	*aeruginosa*	100	242
52	G4 CEF 10-4 PIA 3	DZE895_26828955_26828955	*Pseudomonas*	97.89	*aeruginosa*	98.95	95
53	G4 CEF 10-4 PIA 4	DZE894_26828948_26828948	*Pseudomonas*	99.72	*aeruginosa*	99.72	365
54	G4 CEF 10-4 PIA 5	DZE899_26828993_26828993	*Pseudomonas*	99.62	*aeruginosa*	99.62	796
55	G4 CIPRO N PIA 1	DZE850_26828504_26828504	*Stenotrophomonas*	99.74	*maltophilia*	99.74	762
56	G4 CEF 10-4 SC 1	DZE879_26828795_26828795	*Pseudomonas*	99.35	*moraviensis (99.03), koreensis (99.03)*	multiple results	636

Isolate Code	Isolate Name	Barcode	Identification (Genus) (<98-98.9)	Percentage Genus (%)	Species (< 99).	Percentage Species	Query Length
57	G4 CEF 10-4 SC 2	DZE884_26828849_26828849	*Pseudomonas*	99.26	*moraviensis (99.08), koreeensis (99.08_*	multiple results	556
58	G4 CEF 10-4 SC 3	DZE883_26828832_26828832	*Pseudomonas*	98.93	*baetica (98.93), koreensis (98.93)*	multiple results	1030
59	G4 CEF 10-4 SC 4	DZE882_26828825_26828825	*Pseudomonas*	95.31	*moraviensis (95.90), putida (95.22)*	multiple results	1085
60	G4 COL -5 LAM 2	DZE881_26828818_26828818	*Aeromonas*	99.63	*salmonicida (99.75), enchelelia (99.75)*	multiple results	816
61	G4 COL-5 LAM 4	DZE880_26828801_26828801	*Aeromonas*	96.24	*salmonicida*	96.32	1173
62	G5 CEF 10-4 SC 1	DZE857_26828573_26828573	*Pseudomonas*	99.16	*koreensis (99.16), reinekei (99.16), fluorescens (99.16)*	multiple results	1078
63	G5 CEF 10-4 SC 2	DZE898_26828986_26828986	*Pseudomonas*	99.83	*putida (99.82), koreensis (99.83)*	mulitple	1159
64	G5 CEF 10-4 SC 3	DZE856_26828566_26828566	*Pseudomonas*	99.62	*koreensis (99.62), reinekei (99.62), flurorescens (99.62)*	mulitple	1062
65	G5 CEF 10-4 SC 4	DZE907_26829075_26829075	*Pseudomonas*	99.05	*fluorecens (98.74) koreensis (98.74)*	multiple results	317
66	G5 CEF 10-5 SC 1	DZE897_26828979_26828979	*Pseudomonas*	98.65	*moraviensis (98.43), koreensis (98.20)*	multple	454
67	G5 CEF 10-5 SC 2	DZE902_26829020_26829020	*Pseudomonas*	98.94	*koreensis (98.60), flurorescens (98.43)*	multiiple	573
68	G5 CEF-4 EMB 1	EFP121_28491218_28491218	*Pseudomonas*	98.91	*moraviensis (98.91), koreensis (98.75)*	multiiple	640
69	G5 CEF-4 EMB 3	DZE900_26829006_26829006	*Pseudomonas*	98.49	*moraviensis (98.49), koreensis (98.11)*	multiiple	264
70	G5 IMI N EMB 1	DZE855_26828559_26828559	*Stenotrophomonas*	99.08	*maltophilia*	99.08	867
71	G5 IMI N EMB 2	DZE860_26828603_26828603	*Stenotrophomonas*	99.1	*maltophilia*	99.1	1113

Isolate Code	Isolate Name	Barcode	Identification (Genus) (<98-98.9)	Percentage Genus (%)	Species (< 99).	Percentage Species	Query Length
72	G5 IMI N EMB 3	DZE859_26828597_26828597	*Stenotrophomonas*	99.3	*maltophilia*	99.3	1199
73	G5 IMI N EMB 4	DZE858_26828580_26828580	*Stenotrophomonas*	99.35	*maltophilia*	99.09	767
74	G5 CIPRO 1/10 EMB 1	DZE908_26829082_26829082	*Enterococcus*	97.46	*gallinarum (97.31), casseliflavus (97.31)*	multiple results	674
75	G5 CIPRO 1/10 EMB 4	DZE903_26829037_26829037	*Enterococcus*	97.2	*casseliflavus (97.20), gallinarum (97.20)*	multiple results	683
76	G5 CEF-4 PIA 1	DZE905_26829051_26829051	*Enterococcus*	99.91	*hirae*	99.91	1098
77	G5 CEF -4 PIA 2	DZE901_26829013_26829013	*Enterococcus*	99.55	*hirae*	99.55	672
78	G5 CEF -4 PIA 3	DZE904_26829044_26829044	*Enterococcus*	99.9	*hirae*	100	1045
79	G5 CEF -4 PIA 4	DZE852_26828528_26828528	*Stenotrophomonas*	99.08	*maltophilia*	99.13	870
80	G5 IMP N LAM 3	DZE851_26828511_26828511	*Stenotrophomonas*	98.83	*maltophilia*	99.18	875
81	G5 IMP N LAM 4	DZE854_26828542_26828542	*Stenotrophomonas*	98.34	*maltophilia*	98.24	1026
82	G5 CEF-4 LAM 2	DZE853_26828535_26828535	*Pseudomonas*	99.83	*cleamanca (99.83), koreensis (99.83)*	multiple results	604
83	G6 CEF 10-5 PIA 1	DZE862_26828627_26828627	*Pseudmonas*	99.65	*thodesia (99.65), fluorescens (99.65)*	multiple results	573
84	G6 CIPRO N PIA 1	DZE861_26828610_26828610	*Stenotrophomonas*	98.9	*maltophilia*	98.81	1090
85	G6 CIPRO N PIA 6	EFP120_28491201_28491201	*Enterococcus*	99.74	*gallinarum(99.75) casseliflavus (99.74)*	multiple results	1146
86	G6 CIPRO N PIA 5	DZE866_26828665_26828665	*Pseudomonas*	97.48	*aeruginosa*	97.06	238

Isolate Code	Isolate Name	Barcode	Identification (Genus) (<98-98.9)	Percentage Genus (%)	Species (< 99).	Percentage Species	Query Length
87	G6 CEF 10-4 LAM 8	DZE914_26829143_26829143	*Pseudomonas*	99.31	*moraviensis (99.31), fluorescens (99.31), koreensis (99.14)*	multiple results	583
88	G6 CIPRO 1/10 EMB 4	DZE865_26828658_26828658	*Achromobacter*	98.88	*spanius (98.64), kerstersii (98.76), marplatensis (98.76)*	multiple results	809
89	G6 CEF LAM 2	DZE909_26829099_26829099	*Pseudomonas*	98	*koreensis*	98	699
90	G6 CEF -4 SC 2	DZE864_26828641_26828641	*Enterococcus*	98.56	*hirae*	98.56	832
91	G6 CEF 10-4 LAM 7	DZE912_26829129_26829129	*Pseudomonas*	94	*koreensis (94.26), thivervalensis (94.40)*	multiple results	414
92	G6 CEF 10-5 PIA 2	DZE913_26829136_26829136	*Pseudomonas*	99.83	*rhodesiae (99.83), flurorescens (99.83)*	multiple results	608
93	G6 CEF -5 EMB 2	DZE869_26828696_26828696	*Pseudmononas*	98.5	*putida*	98.84	862
94	G6 CEF 10-5 PIA 3	DZE906_26829068_26829068	*Enterococcus*	99.64	*gallinarum (99.64), casseliflavus (99.64)*	multiple results	567
95	G6 CEF LAM 4	DZE911_26829112_26829112	*Pseudomonas*	99.03	*fluorescens*	99.03	1031
96	PIA COL 1/10,000 3	EFP119	*Providencia*	99.72	*Burodogranariea (99.17%), alcalifaciens (99.07%)*	Multiple results	1079
97	PIA CEF 1/10,00- 3	EFP124	*Providencia*	98.83	*rettgeri*	98.44	1032
98	LAM 1/10,000 CEF 3	EFP123	*Bacillus*	99.91	*proteolyticus (99.91) thuringiensis (99.91)*	multiple results	1081

Appendix 9: Disk Diffusion Results for Section 3.2.2 Antibiotic Susceptibility of Isolates. CTX =cefotaxime, IPM = imipenem, KAN = kanamycin, AK =amikacin, CIP = ciprofloxacin, LZD = linezolid, VANCO = vancomycin. N/A indicates that disk testing for that antibiotic was not relevant for the isolate. Cells highlighted in grey indicates that there were no guidelines for that bacterial species for the antibiotic due to the isolate not being clinically relevant. Cells highlighted in orange indicates the bacteria were intrinsically resistant to an antibiotic according to EUCAST (2020) expert rules. Cells highlighted in green indicate susceptibility, yellow indicates intermediate susceptibility and red indicates resistance.

Isolate Code	Isolate Name	CTX	IPM	KAN	AK	CIP	LZD	VANCO	
									Genus ID
1	S1 CIPRO 1/10 EMB 1	0	25	17	20	23	N/A	N/A	*Alcaligenes*
2	S1 CIPRO 1/10 EMB 4	0	30	21.5	24	22	N/A	N/A	*Achromobacter*
5	S2 CIPRO N PIA 4	0	33	16.5	21.5	26.5	N/A	N/A	
6	S3 CIPRO 1/10 PIA 1	0	26.5	18	19	18.8	N/A	N/A	
7	S3 CIPRO 1/10 EMB 2	0	25	20.5	21	17	N/A	N/A	
12	S5 CIPRO 1/10 PIA 3	0	27.5	23.5	23.5	18.5	N/A	N/A	
37	G3 CIPRO 1/10 PIA 1	0	28.5	20	22.5	18	N/A	N/A	
38	G3 CIPRO 1/10 PIA 2	0	26.5	17	20	19	N/A	N/A	

Isolate Code	Isolate Name	CTX	IPM	KAN	AK	CIP	LZD	VANCO	
39	G3 CIPRO 1/10 PIA 3	0	27	20	21	19	N/A	N/A	
88	G6 CIPRO 1/10 EMB 4	0	33	23	24.5	23	N/A	N/A	
16	G1 IMI N PIA 1	0	0	0	22.5	30	N/A	N/A	
29	G2 CIPRO 1/10 PIA 2	0	0	10	25.5	27	N/A	N/A	
32	G2 IMI PIA 2	0	0	0	24.5	24.5	N/A	N/A	
34	G2 IMI 1/10 LAM 1	0	0	0	23.5	22	N/A	N/A	
35	G2IMI 1/10 LAM 3	0	0	0	22.5	25.5	N/A	N/A	
36	G2 IMI 1/10 LAM 4	0	0	0	21.5	25	N/A	N/A	
40	G3 IMI 1/10 LAM 1	0	0	0	27	24	N/A	N/A	*Stenotrophomonas*
41	G3 IMI 1/10 LAM 3	0	0	0	18	22	N/A	N/A	
43	G3 IMI 1/10 EMB 1	0	0	0	28	24	N/A	N/A	
44	G3 IMI 1/10 EMB 2	0	0	0	25.5	24	N/A	N/A	
45	G3 IMI 1/10 EMB 4	0	0	0	29	24.5	N/A	N/A	
48	G4 IMI N EMB 1	0	0	0	18.5	25	N/A	N/A	
49	G4 IMI N EMB 4	0	0	0	18.5	27	N/A	N/A	
55	G4 CIPRO N PIA 1	0	0	10.5	25	28	N/A	N/A	
70	G5 IMI N EMB 1	0	0	9	27	21.5	N/A	N/A	

Isolate Code	Isolate Name	CTX	IPM	KAN	AK	CIP	LZD	VANCO	
71	G5 IMI N EMB 2	0	0	0	24	26	N/A	N/A	
72	G5 IMI N EMB 3	0	0	0	25.5	25.5	N/A	N/A	
73	G5 IMI N EMB 4	0	0	0	25.5	22.5	N/A	N/A	
79	G5 CEF -4 PIA 4	0	0	0	26	25	N/A	N/A	
80	G5 IMP N LAM 3	0	0	0	25	24	N/A	N/A	
81	G5 IMP N LAM 4	0	0	0	25	24	N/A	N/A	
84	G6 CIPRO N PIA 1	19.5	32	20	11	24.5	N/A	N/A	
3	S2 CEF-4 EMB 1	15.5	35	31	35	38	N/A	N/A	
8	S6 CEF -4 EMB 3	0	30	21	31	30	N/A	N/A	
17	G1 CEF -5 PIA 1	0	27	8	22	31	N/A	N/A	
18	G1 CEF-5 PIA 2	0	26	0	22.5	31	N/A	N/A	
19	G1 CEF 10-5 PIA 3	0	27	8	21.5	32	N/A	N/A	
20	G1 CEF -5 PIA 4	0	26	8.5	20.5	31	N/A	N/A	
21	G1 CEF-5 LAM 2	0	26	0	21.5	31	N/A	N/A	
22	G1 CEF 10-5 LAM 3	0	25	0	22.5	30	N/A	N/A	*Pseudomonas* spp
23	G1 CEF 10-5 LAM 4	0	25	8	21	35	N/A	N/A	
24	G2 CEF 10-5 SC 1	0	27	0	21.5	34.5	N/A	N/A	
26	G2 CEF 10-5 SC 3	0	26	0	21	30	N/A	N/A	
27	G2 CEF 10-4 SC 1	0	27.5	9	22.5	34	N/A	N/A	

Isolate Code	Isolate Name	CTX	IPM	KAN	AK	CIP	LZD	VANCO	
28	G2 CEF 10-4 SC 2	0	25.5	8	21	31	N/A	N/A	
46	G3 CEF 10-4 SC 1	0	26	26	24.5	32	N/A	N/A	
47	G3 CEF 10-4 SC 2	0	28.5	29	29	30	N/A	N/A	
51	G4 CEF 10-4 PIA 2	0	29.5	10	23	32	N/A	N/A	
52	G4 CEF 10-4 PIA 3	0	31	10	24.5	36	24	17.5	
53	G4 CEF 10-4 PIA 4	0	30	10	25	33	N/A	N/A	
54	G4 CEF 10-4 PIA 5	0	29	10.5	23.5	35	N/A	N/A	
56	G4 CEF 10-4 SC 1	0	21	25.5	22	25.5	N/A	N/A	
57	G4 CEF 10-4 SC 2	0	24	22	21	27	N/A	N/A	
58	G4 CEF 10-4 SC 3	0	27.5	26	25	30	N/A	N/A	
62	G5 CEF 10-4 SC 1	8.5	25	22.5	24	32	N/A	N/A	
63	G5 CEF 10-4 SC 2	0	26	23.5	24	26.5	N/A	N/A	
64	G5 CEF 10-4 SC 3	9	28	32	33	33	N/A	N/A	
65	G5 CEF 10-4 SC 4	0	23	21.5	23	26	N/A	N/A	
66	G5 CEF 10-5 SC 1	0	29	30	32	32	N/A	N/A	

Isolate Code	Isolate Name	CTX	IPM	KAN	AK	CIP	LZD	VANCO	
67	G5 CEF 10-5 SC 2	0	24	23	23	30	N/A	N/A	
68	G5 CEF-4 EMB 1	10	26	26	28	32	N/A	N/A	
69	G5 CEF-4 EMB 3	0	25	23.5	24	30	N/A	N/A	
82	G5 CEF-4 LAM 2	11	25	27	26	24	N/A	N/A	
83	G6 CEF 10-5 PIA 1	0	24	34	30	32	N/A	N/A	
86	G6 CIPRO N PIA 5	20.5	28	17.5	15	28	N/A	N/A	
87	G6 CEF 10-4 LAM 8	0	22	22.5	25	29	N/A	N/A	
89	G6 CEF LAM 2	0	23.5	24	25	29	N/A	N/A	
92	G6 CEF 10-5 PIA 2	0	28	29	29	39	N/A	N/A	
93	G6 CEF -5 EMB 2	12	30	30	30	30	N/A	N/A	
94	G6 CEF 10-5 PIA 3	0	27.5	10.5	13.5	19.5	N/A	N/A	
95	G6 CEF LAM 4	0	25.5	24.5	25.5	29	N/A	N/A	
9	S6 CEF -4 EMB 4	0	29	12	11	21.5	N/A	N/A	*Acinetobacter*
10	S6 CEF -5 EMB 4	0	30	20	22	22.5	N/A	N/A	
42	G3 CIPRO 1/10 LAMY 4	0	32	20.5	22.5	23	N/A	N/A	
11	S6 CIPRO 1/10 LAM 2	0	30	14.5	15	20	25	17.5	*Lactococcus*
31	G2 IMI PIA 1	0	25	15	15	23.5	23	14	
33	G2 IMI 1/10 PIA 3	0	12.5	0	0	26	24	18	

Isolate Code	Isolate Name	CTX	IPM	KAN	AK	CIP	LZD	VANCO	
74	G5 CIPRO 1/10 EMB 1	0	32	10.5	13.5	21	21	13.5	*Enteroccoccus*
75	G5 CIPRO 1/10 EMB 4	0	30	12	13	19	22	15	
76	G5 CEF-4 PIA 1	0	25	11	11	23	23	14.5	
77	G5 CEF -4 PIA 2	0	25	12.5	10	23	23.5	17.5	
78	G5 CEF -4 PIA 3	0	24	11.5	10	24	23.5	18	
85	G6 CIPRO N PIA 6	0	30	12.5	15	19	25.5	14	
90	G6 CEF -4 SC 2	0	21.5	14.5	13	21	23.5	17.5	
4	S2 CIPRO N PIA 2	0	33	21.5	21	25	22.5	12.5	*Bacillus*
98	LAM 1/10,000 CEF 3	28	23	19.5	18.5	27	24	18	
14	G1 COL -5 EMB 1	23	23	18.8	20	28	N/A	N/A	*Hafnia*
15	G1 COL-5 EMB 4	20.5	22.5	19	20	25	N/A	N/A	
60	G4 COL -5 LAM 2	31	17	22	22	31	N/A	N/A	*Aeromonas*
61	G4 COL-5 LAM 4	30	18	24	22.5	30	N/A	N/A	
96	PIA COL 1/10,000 3	24	20.5	19	17.5	27	N/A	N/A	*Providencia*
97	PIA CEF 1/10,00-3	28	20	19	18.5	25	N/A	N/A	

Appendix 10: Table displaying AmpC β-lactamase, ESBL and Metallo – β-lactamse disk tests for Section 3.2.2. Green indicates that isolates were not an enzyme producer and indicates isolates were an enzyme producer. FOX = cefoxitin, CAZ = ceftazidime, CLAV ACID = clavulanic acid, IPM = imipenem.

Isolate Code	Isolate Name	AmpC Test			ESBL Test			EDTA Test		
		FOX	FOX with BA	Zone diameter difference in the presence of Boronic Acid	CAZ	CAZ with CLAV ACID	Zone diameter difference in the presence of Clauvalanic Acid	IPM	IPM with EDTA	Zone diameter difference in the presence of EDTA
1	S1 CIPRO 1/10 EMB 1	0	0	0	22	22	0	N/A	N/A	N/A
2	S1 CIPRO 1/10 EMB 4	0	0	0	20	20	0	N/A	N/A	N/A
3	S2 CEF-4 EMB 1	0	0	0	22.5	24	1.5	N/A	N/A	N/A
4	S2 CIPRO N PIA 2	16.5	17	0.5	0	0	0	N/A	N/A	N/A
5	S2 CIPRO N PIA 4	0	0	0	27	29.5	2.5	N/A	N/A	N/A
6	S3 CIPRO 1/10 PIA 1	0	0	0	22	21.5	-0.5	N/A	N/A	N/A
7	S3 CIPRO 1/10 EMB 2	0	0	0	19.5	21	1.5	N/A	N/A	N/A

Isolate Code	Isolate Name	AmpC Test			ESBL Test			EDTA Test		
		FOX	FOX with BA	Zone diameter difference in the presence of Boronic Acid	CAZ	CAZ with CLAV ACID	Zone diameter difference in the presence of Clauvalanic Acid	IPM	IPM with EDTA	Zone diameter difference in the presence of EDTA
8	S6 CEF -4 EMB 3	0	20	20	21.5	21.5	0	N/A	N/A	N/A
9	S6 CEF -4 EMB 4	8	11	3	15	16	1	N/A	N/A	N/A
10	S6 CEF -5 EMB 4	10.5	13	2.5	16.5	18	1.5	N/A	N/A	N/A
11	S6 CIPRO 1/10 LAM 2	0	0	0	0	0	0	N/A	N/A	N/A
12	S5 CIPRO 1/10 PIA 3	0	0	0	23.5	25	1.5	N/A	N/A	N/A
13	S6 CIPRO 1/10 LAM 3	14	14	0	0	0	0	N/A	N/A	N/A
14	G1 COL -5 EMB 1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15	G1 COL-5 EMB 4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
16	G1 IMI N PIA 1	0	16	16	18.5	22	3.5	0	27.5	27.5
17	G1 CEF -5 PIA 1	0	0	0	21	22	1	N/A	N/A	N/A
18	G1 CEF-5 PIA 2	0	0	0	20.5	21	0.5	N/A	N/A	N/A
19	G1 CEF 10-5 PIA 3	0	0	0	20.5	22	1.5	N/A	N/A	N/A
20	G1 CEF -5 PIA 4	0	0	0	19.5	22.5	3	N/A	N/A	N/A

Isolate Code	Isolate Name	AmpC Test			ESBL Test			EDTA Test		
		FOX	FOX with BA	Zone diameter difference in the presence of Boronic Acid	CAZ	CAZ with CLAV ACID	Zone diameter difference in the presence of Clauvalanic Acid	IPM	IPM with EDTA	Zone diameter difference in the presence of EDTA
21	G1 CEF-5 LAM 2	0	0	0	21	20.5	-0.5	N/A	N/A	N/A
22	G1 CEF 10-5 LAM 3	0	0	0	21	21	0	N/A	N/A	N/A
23	G1 CEF 10-5 LAM 4	0	0	0	20	20.5	0.5	N/A	N/A	N/A
24	G2 CEF 10-5 SC 1	0	0	0	20.5	20	-0.5	N/A	N/A	N/A
25	G2 CEF 10-5 SC 2	0	0	0	20	20.5	0.5	N/A	N/A	N/A
26	G2 CEF 10-5 SC 3	0	0	0	25	20.5	-4.5	N/A	N/A	N/A
27	G2 CEF 10-4 SC 1	0	0	0	20	21	1	N/A	N/A	N/A
28	G2 CEF 10-4 SC 2	0	0	0	20	20.5	0.5	N/A	N/A	N/A
29	G2 CIPRO 1/10 PIA 2	0	0	0	23	23.5	0.5	0	13	13
30	G2 CIPRO 1/10 PIA 4	0	0	0	0	0	0	N/A	N/A	N/A
31	G2 IMI PIA 1	14.5	15	0.5	0	0	0	N/A	N/A	N/A
32	G2 IMI PIA 2	0	15	15	0	14.5	14.5	0	11.5	11.5
33	G2 IMI 1/10 PIA 3	0	0	0	0	0	0	11	14	3

Isolate Code	Isolate Name	AmpC Test			ESBL Test			EDTA Test		
		FOX	FOX with BA	Zone diameter difference in the presence of Boronic Acid	CAZ	CAZ with CLAV ACID	Zone diameter difference in the presence of Clauvalanic Acid	IPM	IPM with EDTA	Zone diameter difference in the presence of EDTA
34	G2 IMI 1/10 LAM 1	0	15	15	0	15	15	0	13.5	13.5
35	G2IMI 1/10 LAM 3	0	0	0	0	0	0	0	17	17
36	G2 IMI 1/10 LAM 4	0	15	15	0	15	15	0	12.5	12.5
37	G3 CIPRO 1/10 PIA 1	0	0	0	20	20.5	0.5	N/A	N/A	N/A
38	G3 CIPRO 1/10 PIA 2	0	0	0	21	21	0	N/A	N/A	N/A
39	G3 CIPRO 1/10 PIA 3	0	0	0	21.5	23.5	2	N/A	N/A	N/A
40	G3 IMI 1/10 LAM 1	0	15	15	0	0	0	0	14.5	14.5
41	G3 IMI 1/10 LAM 3	9	14.5	5.5	21	22	1	0	14.5	14.5
42	G3 CIPRO 1/10 LAMY 4	0	0	0	16.5	16	-0.5	N/A	N/A	N/A
43	G3 IMI 1/10 EMB 1	0	14	14	0	0	0	0	14	14
44	G3 IMI 1/10 EMB 2	0	15	15	0	0	0	0	15.5	15.5
45	G3 IMI 1/10 EMB 4	0	15.5	15.5	0	0	0	0	4	4

Isolate Code	Isolate Name	AmpC Test			ESBL Test			EDTA Test		
		FOX	FOX with BA	Zone diameter difference in the presence of Boronic Acid	CAZ	CAZ with CLAV ACID	Zone diameter difference in the presence of Clauvalanic Acid	IPM	IPM with EDTA	Zone diameter difference in the presence of EDTA
46	G3 CEF 10-4 SC 1	9	12.5	3.5	24.5	22.5	-2	N/A	N/A	N/A
47	G3 CEF 10-4 SC 2	0	10	10	23.5	25	1.5	N/A	N/A	N/A
48	G4 IMI N EMB 1	0	15	15	0	0	0	0	13.5	13.5
49	G4 IMI N EMB 4	0	14	14	0	0	0	0	15	15
50	G4 IMI N PIA 1	0	15	15	0	0	0	0	14	14
51	G4 CEF 10-4 PIA 2	0	0	0	22.5	22.5	0	N/A	N/A	N/A
52	G4 CEF 10-4 PIA 3	0	0	0	24	25	1	N/A	N/A	N/A
53	G4 CEF 10-4 PIA 4	0	0	0	27	28	1	N/A	N/A	N/A
54	G4 CEF 10-4 PIA 5	0	0	0	20.5	22.5	2	N/A	N/A	N/A
55	G4 CIPRO N PIA 1	0	8.5	8.5	0	0	0	0	15	15
56	G4 CEF 10-4 SC 1	0	11.5	11.5	23	25	2	N/A	N/A	N/A
57	G4 CEF 10-4 SC 2	0	12.5	12.5	26	27	1	N/A	N/A	N/A
58	G4 CEF 10-4 SC 3	0	15	15	27.5	27.5	0	N/A	N/A	N/A

Isolate Code	Isolate Name	AmpC Test			ESBL Test			EDTA Test		
		FOX	FOX with BA	Zone diameter difference in the presence of Boronic Acid	CAZ	CAZ with CLAV ACID	Zone diameter difference in the presence of Clauvalanic Acid	IPM	IPM with EDTA	Zone diameter difference in the presence of EDTA
59	G4 CEF 10-4 SC 4	0	15	15	25	26.5	1.5	N/A	N/A	N/A
60	G4 COL -5 LAM 2	23.5	27	3.5	28	29.5	1.5	N/A	N/A	N/A
61	G4 COL-5 LAM 4	22	25.5	3.5	28.5	30.5	2	N/A	N/A	N/A
62	G5 CEF 10-4 SC 1	0	10.5	10.5	29	29.5	0.5	N/A	N/A	N/A
63	G5 CEF 10-4 SC 2	0	9.5	9.5	25.5	25.5	0	N/A	N/A	N/A
64	G5 CEF 10-4 SC 3	0	10.5	10.5	24.5	24.5	0	N/A	N/A	N/A
65	G5 CEF 10-4 SC 4	0	9	9	23.5	22.5	-1	N/A	N/A	N/A
66	G5 CEF 10-5 SC 1	0	11.5	11.5	23	22.5	-0.5	N/A	N/A	N/A
67	G5 CEF 10-5 SC 2	0	21	21	25	24.5	-0.5	N/A	N/A	N/A
68	G5 CEF-4 EMB 1	0	12.5	12.5	22	23	1	N/A	N/A	N/A
69	G5 CEF-4 EMB 3	0	9	9	21	23.5	2.5	N/A	N/A	N/A
70	G5 IMI N EMB 1	0	0	0	0	0	0	0	14	14
71	G5 IMI N EMB 2	0	0	0	0	0	0	0	14	14

Isolate Code	Isolate Name	AmpC Test			ESBL Test			EDTA Test		
		FOX	FOX with BA	Zone diameter difference in the presence of Boronic Acid	CAZ	CAZ with CLAV ACID	Zone diameter difference in the presence of Clauvalanic Acid	IPM	IPM with EDTA	Zone diameter difference in the presence of EDTA
72	G5 IMI N EMB 3	0	0	0	0	0	0	0	15.5	15.5
73	G5 IMI N EMB 4	0	0	0	0	0	0	0	16.5	16.5
74	G5 CIPRO 1/10 EMB 1	16	16.5	0.5	0	0	0	N/A	N/A	N/A
75	G5 CIPRO 1/10 EMB 4	14	15.5	1.5	0	0	0	N/A	N/A	N/A
76	G5 CEF-4 PIA 1	14	15	1	0	0	0	N/A	N/A	N/A
77	G5 CEF -4 PIA 2	17.5	22	4.5	0	0	0	N/A	N/A	N/A
78	G5 CEF -4 PIA 3	15.5	20	4.5	0	0	0	N/A	N/A	N/A
79	G5 CEF -4 PIA 4	0	0	0	0	0	0	0	15	15
80	G5 IMP N LAM 3	0	0	0	0	0	0	0	10	10
81	G5 IMP N LAM 4	0	0	0	0	0	0	0	14	14
82	G5 CEF-4 LAM 2	0	10	10	21.5	21	-0.5	N/A	N/A	N/A
83	G6 CEF 10-5 PIA 1	0	10.5	10.5	24.5	23	-1.5	N/A	N/A	N/A
84	G6 CIPRO N PIA 1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Isolate Code	Isolate Name	AmpC Test			ESBL Test			EDTA Test		
		FOX	FOX with BA	Zone diameter difference in the presence of Boronic Acid	CAZ	CAZ with CLAV ACID	Zone diameter difference in the presence of Clauvalanic Acid	IPM	IPM with EDTA	Zone diameter difference in the presence of EDTA
85	G6 CIPRO N PIA 6	14.5	15.5	1	0	0	0	N/A	N/A	N/A
86	G6 CIPRO N PIA 5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
87	G6 CEF 10-4 LAM 8	0	16	16	23.5	25	1.5	N/A	N/A	N/A
88	G6 CIPRO 1/10 EMB 4	0	0	0	23	24	1	N/A	N/A	N/A
89	G6 CEF LAM 2	0	15	15	24.5	24.5	0	N/A	N/A	N/A
90	G6 CEF -4 SC 2	0	17	17	0	0	0	N/A	N/A	N/A
91	G6 CEF 10-4 LAM 7	0	10	10	21.5	21.5	0	N/A	N/A	N/A
92	G6 CEF 10-5 PIA 2	0	23	23	24.5	24.5	0	N/A	N/A	N/A
93	G6 CEF -5 EMB 2	0	10	10	25	25	0	N/A	N/A	N/A
94	G6 CEF 10-5 PIA 3	15	15	0	0	0	0	N/A	N/A	N/A
95	G6 CEF LAM 4	0	10	10	22	23.5	1.5	N/A	N/A	N/A
96	PIA COL 1/10,000 3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
97	PIA CEF 1/10,00- 3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Isolate Code	Isolate Name	AmpC Test			ESBL Test			EDTA Test		
		FOX	FOX with BA	Zone diameter difference in the presence of Boronic Acid	CAZ	CAZ with CLAV ACID	Zone diameter difference in the presence of Clauvalanic Acid	IPM	IPM with EDTA	Zone diameter difference in the presence of EDTA
98	LAM 1/10,000 CEF 3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

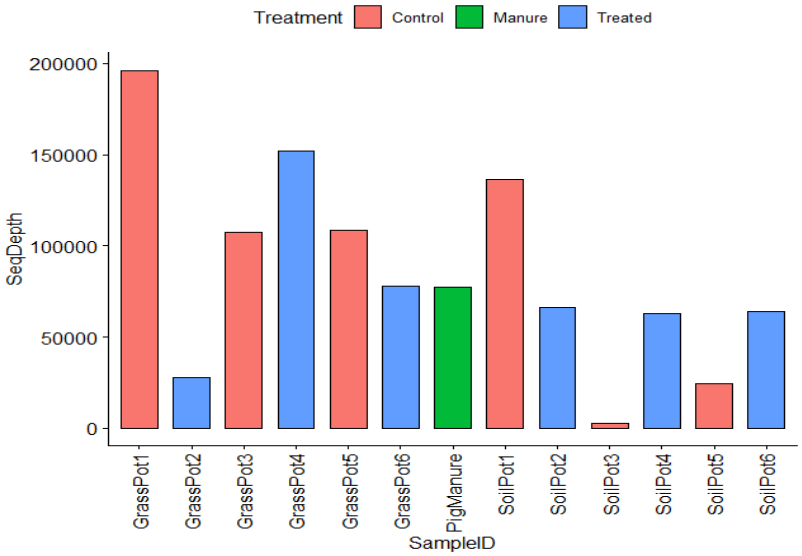
Appendix 11: Table displaying minimum inhibitory concentration (MIC) results for colistin for Section 3.2.1. Green indicates the isolate was susceptible and red indicates the isolate was resistant. N/A indicates that colistin MIC was not determined for this isolate as these isolates were gram positive bacteria and outside of the spectrum of activity for colistin.

Isolate Code	**Isolate Name**	**Colisitin MIC (mg/L)**
1	S1 CIPRO 1/10 EMB 1	2
2	S1 CIPRO 1/10 EMB 4	0.5
3	S2 CEF-4 EMB 1	1
4	S2 CIPRO N PIA 2	0.5
5	S2 CIPRO N PIA 4	0.5
6	S3 CIPRO 1/10 PIA 1	2
7	S3 CIPRO 1/10 EMB 2	1
8	S6 CEF -4 EMB 3	2
9	S6 CEF -4 EMB 4	1
10	S6 CEF -5 EMB 4	1
11	S6 CIPRO 1/10 LAM 2	N/A
12	S5 CIPRO 1/10 PIA 3	2
13	S6 CIPRO 1/10 LAM 3	N/A
14	G1 COL -5 EMB 1	8
15	G1 COL-5 EMB 4	8
16	G1 IMI N PIA 1	2
17	G1 CEF -5 PIA 1	2
18	G1 CEF-5 PIA 2	2
20	G1 CEF -5 PIA 4	2
21	G1 CEF-5 LAM 2	2
22	G1 CEF 10-5 LAM 3	2
23	G1 CEF 10-5 LAM 4	2
24	G2 CEF 10-5 SC 1	2
26	G2 CEF 10-5 SC 3	2
27	G2 CEF 10-4 SC 1	2
28	G2 CEF 10-4 SC 2	2
29	G2 CIPRO 1/10 PIA 2	1
31	G2 IMI PIA 1	N/A
32	G2 IMI PIA 2	4
33	G2 IMI 1/10 PIA 3	N/A
34	G2 IMI 1/10 LAM 1	1
35	G2IMI 1/10 LAM 3	1
36	G2 IMI 1/10 LAM 4	2

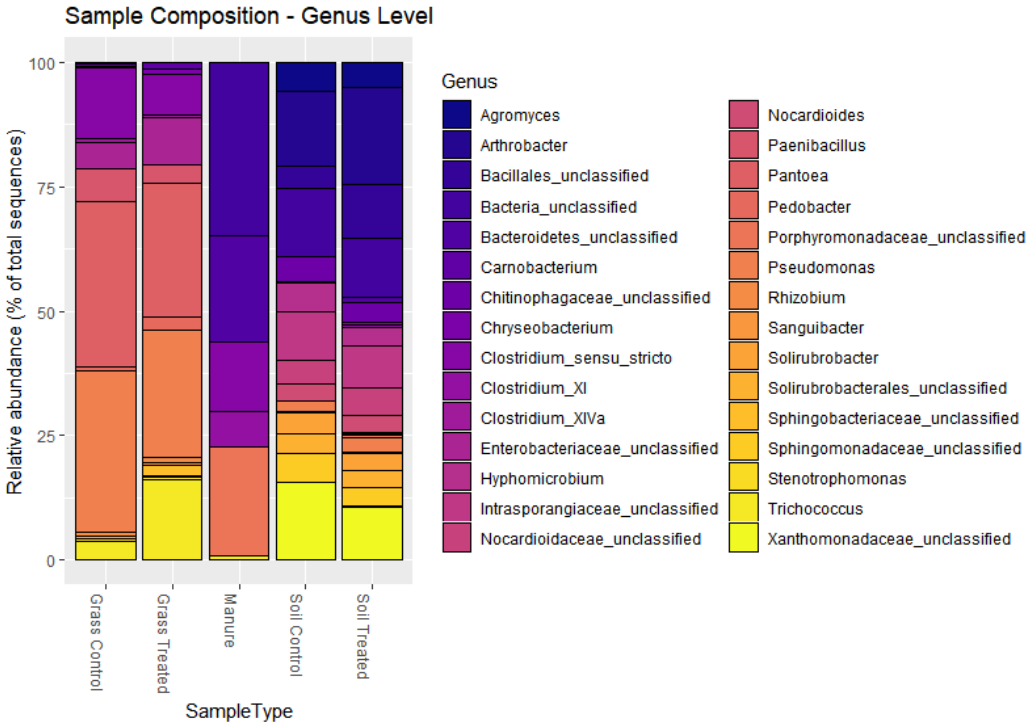
Isolate Code	Isolate Name	Colisitin MIC (mg/L)
37	G3 CIPRO 1/10 PIA 1	0.5
38	G3 CIPRO 1/10 PIA 2	1
39	G3 CIPRO 1/10 PIA 3	1
40	G3 IMI 1/10 LAM 1	2
41	G3 IMI 1/10 LAM 3	2
42	G3 CIPRO 1/10 LAMY 4	2
43	G3 IMI 1/10 EMB 1	2
44	G3 IMI 1/10 EMB 2	1
45	G3 IMI 1/10 EMB 4	2
46	G3 CEF 10-4 SC 1	1
47	G3 CEF 10-4 SC 2	2
48	G4 IMI N EMB 1	2
49	G4 IMI N EMB 4	2
50	G4 IMI N PIA 1	2
51	G4 CEF 10-4 PIA 2	2
52	G4 CEF 10-4 PIA 3	2
53	G4 CEF 10-4 PIA 4	2
54	G4 CEF 10-4 PIA 5	2
55	G4 CIPRO N PIA 1	2
56	G4 CEF 10-4 SC 1	1
57	G4 CEF 10-4 SC 2	2
58	G4 CEF 10-4 SC 3	1
59	G4 CEF 10-4 SC 4	2
60	G4 COL -5 LAM 2	>128
62	G5 CEF 10-4 SC 1	0.5
63	G5 CEF 10-4 SC 2	1
64	G5 CEF 10-4 SC 3	0.5
65	G5 CEF 10-4 SC 4	1
66	G5 CEF 10-5 SC 1	2
67	G5 CEF 10-5 SC 2	2
68	G5 CEF-4 EMB 1	2
69	G5 CEF-4 EMB 3	2
70	G5 IMI N EMB 1	2
71	G5 IMI N EMB 2	2
72	G5 IMI N EMB 3	2
73	G5 IMI N EMB 4	1
74	G5 CIPRO 1/10 EMB 1	
75	G5 CIPRO 1/10 EMB 4	N/A
76	G5 CEF-4 PIA 1	N/A
77	G5 CEF -4 PIA 2	N/A
78	G5 CEF -4 PIA 3	N/A

Isolate Code	Isolate Name	Colisitin MIC (mg/L)
79	G5 CEF -4 PIA 4	1
80	G5 IMP N LAM 3	2
81	G5 IMP N LAM 4	2
82	G5 CEF-4 LAM 2	1
83	G6 CEF 10-5 PIA 1	0.5
84	G6 CIPRO N PIA 1	1
85	G6 CIPRO N PIA 6	N/A
86	G6 CIPRO N PIA 5	N/A
87	G6 CEF 10-4 LAM 8	1
88	G6 CIPRO 1/10 EMB 4	0.5
89	G6 CEF LAM 2	1
90	G6 CEF -4 SC 2	N/A
92	G6 CEF 10-5 PIA 2	2
93	G6 CEF -5 EMB 2	2
94	G6 CEF 10-5 PIA 3	N/A
95	G6 CEF LAM 4	2
96	PIA COL 1/10,000 3	128
97	PIA CEF 1/10,00- 3	128
98	LAM 1/10,000 CEF 3	N/A

Appendix 12: Summary of sequencing depth for 16S rRNA sequencing for each sample in Chapter 3.



Appendix 13: Bar chart displaying the top 40 genera detected in grass, manure and soil samples from Chapter 3.



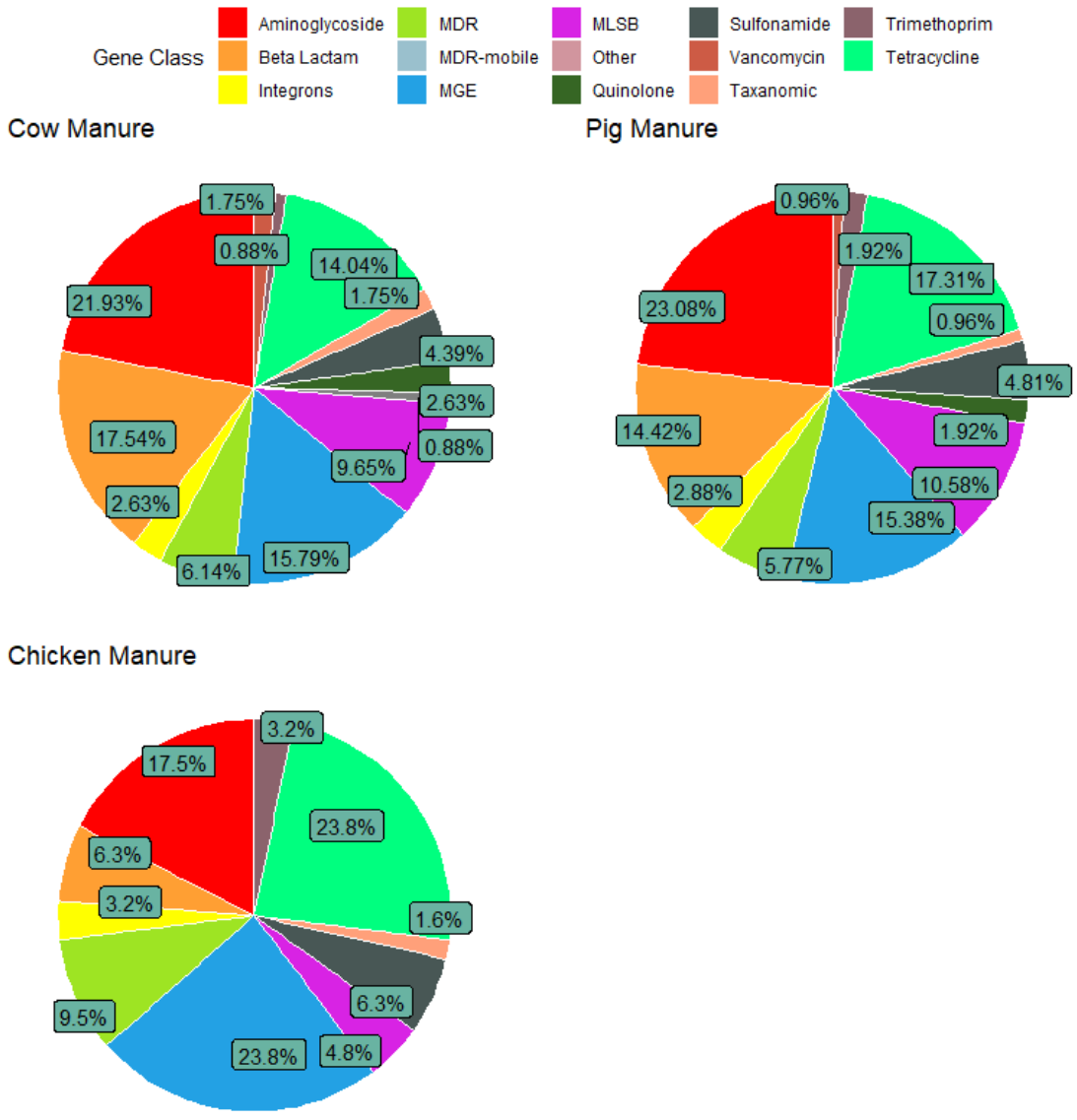
Appendix 14: Details of DESeq2 log fold changes of OTUs in treated soil in comparison to control soil.

OTU	log2FoldChange	pvalue	padj	Phylum	Family	Genus
Otu00006	4.011455313	0.000112871	0.006362343	Firmicutes	Carnobacteriaceae	Trichococcus
Otu00011	8.485727446	1.61E-11	2.02E-08	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00014	7.463462312	4.02E-09	1.27E-06	Bacteroidetes	Porphyromonadaceae	Porphyromonadaceae_unclassified
Otu00018	7.097322662	1.92E-08	3.81E-06	Firmicutes	Clostridiaceae_1	Clostridium_sensu_stricto
Otu00022	7.678611707	1.10E-09	4.35E-07	Bacteroidetes	Bacteroidetes_unclassified	Bacteroidetes_unclassified
Otu00024	7.271440149	6.76E-09	1.79E-06	Bacteroidetes	Bacteroidetes_unclassified	Bacteroidetes_unclassified
Otu00035	5.388002162	1.14E-05	0.000900906	Firmicutes	Peptostreptococcaceae	Clostridium_XI
Otu00058	6.653589336	2.01E-07	3.18E-05	Firmicutes	Ruminococcaceae	Ruminococcaceae_unclassified
Otu00075	8.010303154	2.55E-11	2.02E-08	Proteobacteria	Pseudomonadaceae	Pseudomonas
Otu00080	5.501302783	0.000102717	0.006026063	Firmicutes	Enterococcaceae	Enterococcus
Otu00083	5.663413568	7.38E-05	0.004675523	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00097	6.420677694	1.15E-06	0.000130009	Proteobacteria	Desulfobacteraceae	Desulfobacterium
Otu00098	5.065107183	0.000602274	0.025561574	Firmicutes	Bacillales_unclassified	Bacillales_unclassified

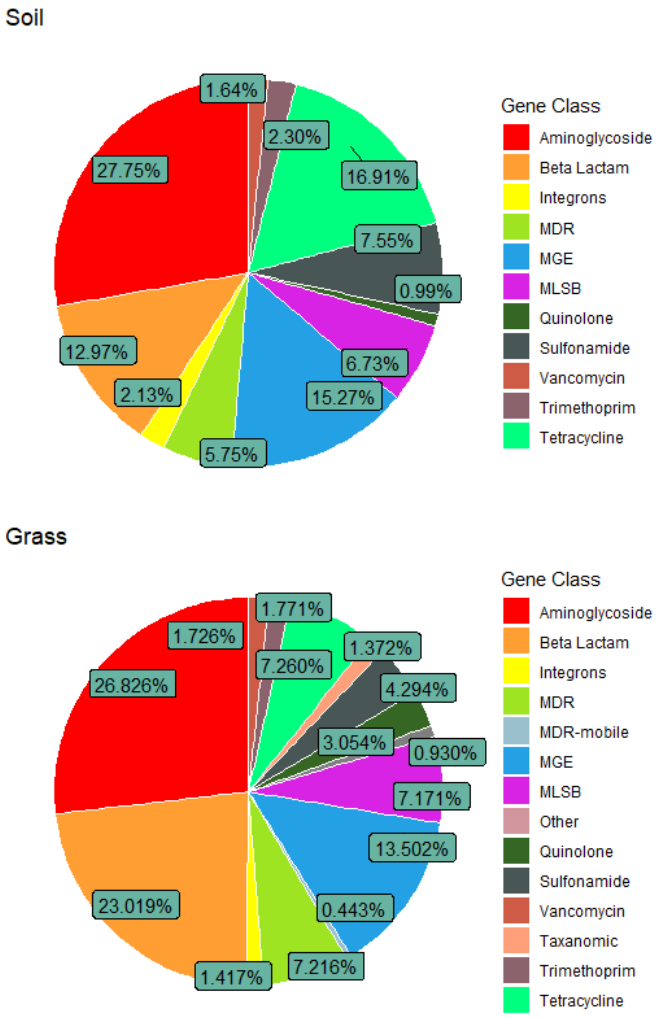
OTU	log2FoldChange	pvalue	padj	Phylum	Family	Genus
Otu00120	5.922011984	1.82E-05	0.001375902	Firmicutes	Ruminococcaceae	Saccharofermentans
Otu00131	6.641332762	2.78E-07	4.00E-05	Firmicutes	Ruminococcaceae	Saccharofermentans
Otu00133	8.003275125	1.92E-10	1.01E-07	Bacteroidetes	Bacteroidetes_unclassified	Bacteroidetes_unclassified
Otu00141	6.535403996	4.06E-07	4.94E-05	Firmicutes	Clostridiales_Incertae_Sedis_XIII	Anaerovorax
Otu00163	5.072317166	0.00061322	0.025561574	Firmicutes	Firmicutes_unclassified	Firmicutes_unclassified
Otu00166	7.016854649	2.54E-08	4.47E-06	Firmicutes	Ruminococcaceae	Ruminococcaceae_unclassified
Otu00189	4.963059458	0.001149417	0.043349442	Bacteroidetes	Porphyromonadaceae	Paludibacter
Otu00212	5.539624691	7.74E-05	0.004717087	Verrucomicrobia	Subdivision5_family_incertae_sedis	5_genus_incertae_sedis
Otu00226	5.722659167	2.32E-06	0.000245416	Firmicutes	Clostridiales_Incertae_Sedis_XI	Tissierella
Otu00235	5.416038103	0.000116482	0.006362343	Firmicutes	Ruminococcaceae	Ruminococcaceae_unclassified
Otu00246	5.275087423	0.000212441	0.010515843	Firmicutes	Clostridiales_Incertae_Sedis_XI	Tissierella
Otu00279	22.53014769	8.12E-09	1.84E-06	Proteobacteria	Gammaproteobacteria_unclassified	Gammaproteobacteria_unclassified
Otu00297	6.217997025	2.70E-06	0.000266942	Verrucomicrobia	Subdivision5_family_incertae_sedis	5_genus_incertae_sedis
Otu00307	5.992747492	1.08E-05	0.00089701	Firmicutes	Firmicutes_unclassified	Firmicutes_unclassified
Otu00323	5.267310518	0.000364863	0.016998316	Firmicutes	Ruminococcaceae	Saccharofermentans

OTU	log2FoldChange	pvalue	padj	Phylum	Family	Genus
Otu00335	5.039545542	0.00078156	0.031743354	Firmicutes	Ruminococcaceae	Ruminococcaceae_unclassified
Otu00336	5.94728573	8.91E-06	0.000820272	Firmicutes	Firmicutes_unclassified	Firmicutes_unclassified
Otu00337	5.763486224	2.92E-05	0.002102158	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00349	6.572210798	3.40E-07	4.49E-05	Firmicutes	Lachnospiraceae	Lachnospiraceae_unclassified
Otu00370	4.425042224	0.001016349	0.039265786	Firmicutes	Clostridiales_Incertae_Sedis_XI	Clostridiales_Incertae_Sedis_XI_unclassified
Otu00385	5.386074594	0.000271377	0.013026118	Tenericutes	Acholeplasmataceae	Acholeplasma
Otu00410	3.502299957	9.32E-06	0.000820272	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00423	5.404591359	0.00016229	0.008568906	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00502	5.671288078	4.75E-05	0.003274005	Proteobacteria	Syntrophaceae	Syntrophaceae_unclassified
Otu00592	4.423394439	0.001230768	0.045338053	Firmicutes	Peptococcaceae_1	Desulfonispora
Otu00684	5.312868832	0.000208546	0.010515843	Proteobacteria	Syntrophaceae	Smithella
Otu00687	4.967993902	0.00092623	0.036678724	Proteobacteria	Pseudomonadaceae	Pseudomonas
Otu00726	5.115644441	0.000416674	0.018857455	Actinobacteria	Actinomycetales_unclassified	Actinomycetales_unclassified
Otu00733	4.728524982	5.64E-05	0.003722222	Actinobacteria	Microbacteriaceae	Leucobacter
Otu00755	5.148458585	0.000525687	0.023130215	Proteobacteria	Alcaligenaceae	Alcaligenaceae_unclassified

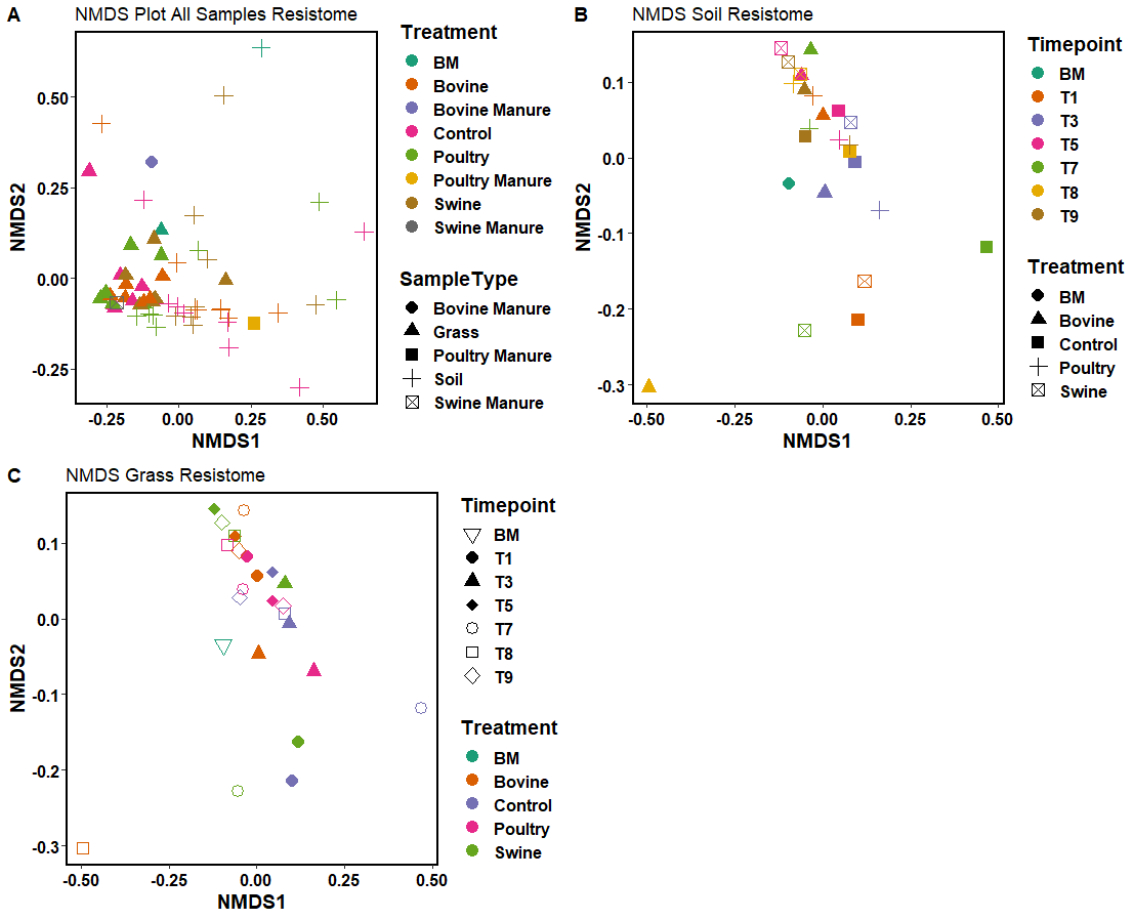
Appendix 15: Pi Chart of the percentage gene class in the manure samples.



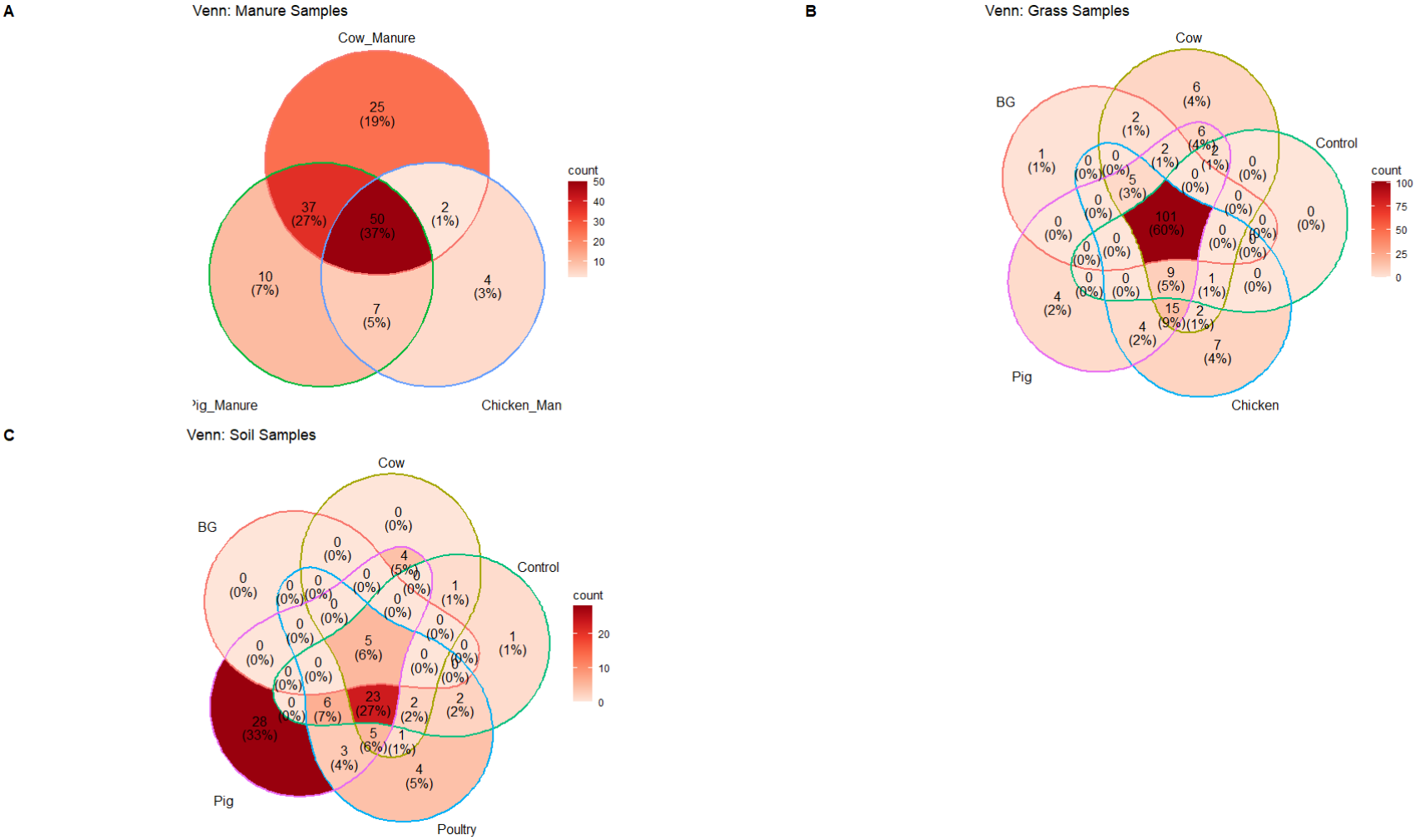
Appendix 16: Pi Chart of the percentage gene class in grass and soil samples



Appendix 17: (A) NMDS ordination plot of HT-qPCR relative abundance data for all samples: K =2, stress value = 0.1 using Bray-Curtis distances. (B) NMDS ordination plot of HT-qPCR relative abundance data for soil samples: K=2, stress value = 0.09 using Bray-Curtis distances. (C) NMDS ordination of HT-qPCR relative abundances for grass samples: K=2, stress value = 0.07 using Bray-Curtis distances.



Appendix 18: Venn Diagrams illustrating the number of target genes in common in different samples and treatments. Graph A displays the Venn diagram for manure samples Graph B shows the Venn diagram for grass samples. Graph C shows the Venn diagram for soil samples.



Appendix 19: Table displaying the genes in common and differing between manure samples

Names	total	elements
Chicken Manure Cow Manure Pig Manure	50	tolC_2
		intI2_2
		tetG
		tetD
		ISAba3
		aphA3
		acrF
		IS1133
		tetL_2
		acrB_1
		aadA2_1
		Tp614
		ermB_2
		tet39
		tetX
		tnpA_4
		sul1_1
		mdtE
		intI1_3
		tnpA_3
		tetH
		aadA16
		ermF
		trbC
		spcN
		sul2_1
		aph(3")-ia
		acrA_1
		tetQ
		tnpA_1
		tet32
		A. baumannii
		aadA2_3
		aadB
		dfra17
		tetO_2
		IS613
		tetT
		tet44

Names	total	elements
		tetW
		tetR
		sul2_2
		tnpA_2
		tnpA_5
		ampC_6
		blaCTX-M
		sul1_2
		aph3-iii
		aadA7
		aac(6)-iv_ih
Cow Manure Pig Manure	37	aph6-ia
		blaCTX-M_2
		tetPA
		blaSFO
		cphA_1
		IncI1_repI1
		ermA/ermTR
		ermA
		blaNDM
		ant4-ib
		ermC_2
		ampC_5
		ant6-ia
		tetS
		ampC_3
		aadA6
		blaCTX-M_4
		ermO
		oprD
		blaMIR
		sul3_1
		blaCTX-M_1
		ant6-ib
		IncN_rep
		vanA
		erm36
		blaCARB
		aadA10
		ermT_1
		tet36_1
		orf37-IS26
		aac(3)-xa_1
		intI3_1
		IncQ_oriT

Names	total	elements
		ampC_2
		IncP_oriT
		ermE
Chicken Manure Pig Manure	7	tetC_2
		blaTEM_1
		aadD
		Tn3
		ermX_1
		dfrA1_1
		tetA/B_2
Chicken Manure Cow Manure	2	mexA
		lncF_FIC
Pig Manure	10	ermY
		aadA5_2
		blaROB
		qnrD
		aacC2
		aac3-IVa
		apmA
		aac(6')-II
		aph4-ia
		qnrS_1
Cow Manure	25	aph3-ib
		aac(6)-ir
		trfA
		aac(6)-iic
		P. aeruginosa
		aac(3)-iid_iia
		qnrVC1_VC3_VC6
		aac(6)-ig
		ISPps
		aph4-ib
		mcr1
		blaFOX
		ermD/K
		ampC/blaDHA
		blaOXA48
		aac(3)-ib
		vanB_1
		blaMOX/blaCMY
		qnrB4
		aac(3)-id_ie
		blaPAO
		ereB_2
		qepA

Names	total	elements
		blaOXY
		ampC_4
Chicken Manure	4	traN
		ampC_1
		IS6/257
		ISEfm1

Appendix 20: Table displaying genes in common and differing between grass treatments and manure samples.

Names	total	elements
Background Grass, Cow Manured Grass, Chicken Manured Grass, Control Grass and Pig Manured Grass	101	aph6-ia
		tolC_2
		aph3-ib
		aac(6)-ir
		blaCTX-M_2
		tetG
		blaSFO
		tetD
		cphA_1
		bl1acc
		IncI1_repI1
		rmtB
		acrF
		IS1111
		blaCTX-M_6
		IS1133
		dfrA8
		pikR1
		tetJ
		aac(6)-iic
		qacF/H
		blaNDM
		ant4-ib
		tetL_2
		P. aeruginosa
		acrB_1

Names	total	elements
		vgaB_1
		ermB_2
		ampC_5
		ant6-ia
		vgaA_1
		aac(3)-iid_iia
		sul1_1
		aph_viii
		aac(6)-iz
		ampC_3
		aac(6)-ig
		mdtE
		ampC_1
		tnpA_3
		aadA6
		blaCTX-M_4
		aadA16
		aph4-ib
		spcN
		mcr1
		ermO
		oprD
		blaMIR
		sul3_1
		blaCTX-M_1
		sul2_1
		ant6-ib
		aph3-via

Names	total	elements
		IS6/257
		mexA
		acrA_1
		IncN_rep
		blaCTX-M_3
		vanA
		tnpA_1
		blaFOX
		erm36
		A. baumannii
		ermD/K
		aadA10
		blaCARB
		ampC/blaDHA
		aac3-IVa
		blaCTX-M_5
		blaOXA48
		orf37-IS26
		aadB
		aac(3)-ib
		blaKPC
		vanB_1
		Tn3
		ereA
		aac(3)-xa_1
		aac(6')-Ib_1
		intI3_1
		tetR

Names	total	elements
		qnrB4
		sul2_2
		tnpA_2
		blaPAO
		oqxA
		IncQ_oriT
		ampC_6
		qepA
		blaCTX-M
		ampC_2
		aph3-iii
		aadA7
		blaOXY
		aac(6)-iv_ih
		IncP_oriT
		blaPER
		ampC_4
		ermE
		qnrS2
Background Grass, Cow Manured Grass, Chicken Manured Grass and Pig Manured Grass	5	traN
		tetX
		tetH
		aph(3")-ia
		aadA2_3
Cow Manured Grass, Chicken Manured Grass, Control Grass and Pig Manured Grass	9	blaIMP_1
		blaROB
		qnrD
		dfrA12

Names	total	elements
		intI1_3
		ISPps
		trbC
		lncF_FIC
		aac(3)-id_ie
Background Grass, Cow Manured Grass, Pig Manured Grass	2	aac(6)-is_iu_ix
		blaIMI
Cow Manured Grass, Chicken Manured Grass and Control Grass	1	tetA_1
Cow Manured Grass, Chicken Manured Grass and Pig Manured Grass	15	intI2_2
		ISAba3
		ermA
		aac6-aph2
		aadA2_1
		tet39
		aadD
		tetS
		dfra17
		ermX_1
		tetW
		dfrA1_1
		blaACC
		ISEfm1
		sul1_2
Cow Manured Grass, Control Grass, Pig Manured Grass	2	aadA5_2
		Tp614
Background Grass, Cow Manured Grass	2	blaSHV11
		blaVIM

Names	total	elements
Cow Manured Grass, Chicken Manured Grass	2	qnrVC1_VC3_VC6
		armA
Cow Manured Grass, Pig Manured Grass	6	tetPA
		blaTEM_1
		tnpA_4
		aacC2
		aph4-ia
		tetA/B_2
Chicken Manured Grass, Pig Manured Grass	4	IncHI2-smr0018
		IS613
		tet44
		tetE
Background Grass	1	trfA
Cow Manured Grass	6	tetC_2
		K. pneumoniae
		ermD
		marR_3
		aacA/aphD
		tnpA_5
Chicken Manured Grass	7	msrA_1
		blaZ
		ermC_2
		tetK
		mecA
		msrC_1
		blaOKP
Pig Manured Grass	4	ermF

Names	total	elements
		ermT_1
		tetO_2
		qnrS_1

Appendix 21: Table displaying the genes in common and different between treated soil samples and manure samples.

Names	total	elements
Soil Background Soil Cow Soil Control Soil Pig Soil Chicken	5	oprD
		tetG
		tetD
		aadA7
		trbC
Soil Cow Soil Control Soil Pig Soil Chicken	23	spcN
		aph6-ia
		ermO
		blaMIR
		blaSFO
		cphA_1
		vanA
		IS1133
		aadB
		aac(3)-ib
		tetL_2
		aac(3)-xa_1
		tetR
		sul1_1
		qepA
		blaCTX-M
		ampC_2
		tnpA_3
		IncP_oriT
		blaCTX-M_4
		aadA16
		ermE

Names	total	elements
		aph4-ib
Soil Cow Soil Control Soil Chicken	2	aph3-ib
		mdtE
Soil Control Soil Pig Soil Chicken	6	sul3_1
		aadA10
		dfrA8
		ampC_5
		aac(3)-iid_iia
		IncQ_oriT
Soil Cow Soil Pig Soil Chicken	5	sul2_1
		IncI1_repI1
		aadA2_3
		blaIMP_1
		tnpA_2
Soil Cow Soil Control	1	erm36
Soil Control Soil Chicken	2	blaOXA48
		ant4-ib
Soil Cow Soil Pig	4	intI2_2
		tet44
		aadA2_1
		ermB_2
Soil Cow Soil Chicken	1	orf37-IS26
Soil Pig Soil Chicken	3	tetX
		intI1_3
		sul1_2
Soil Control	1	aac(6)-iic
Soil Pig	28	ant6-ib
		aph(3")-ia
		tetQ

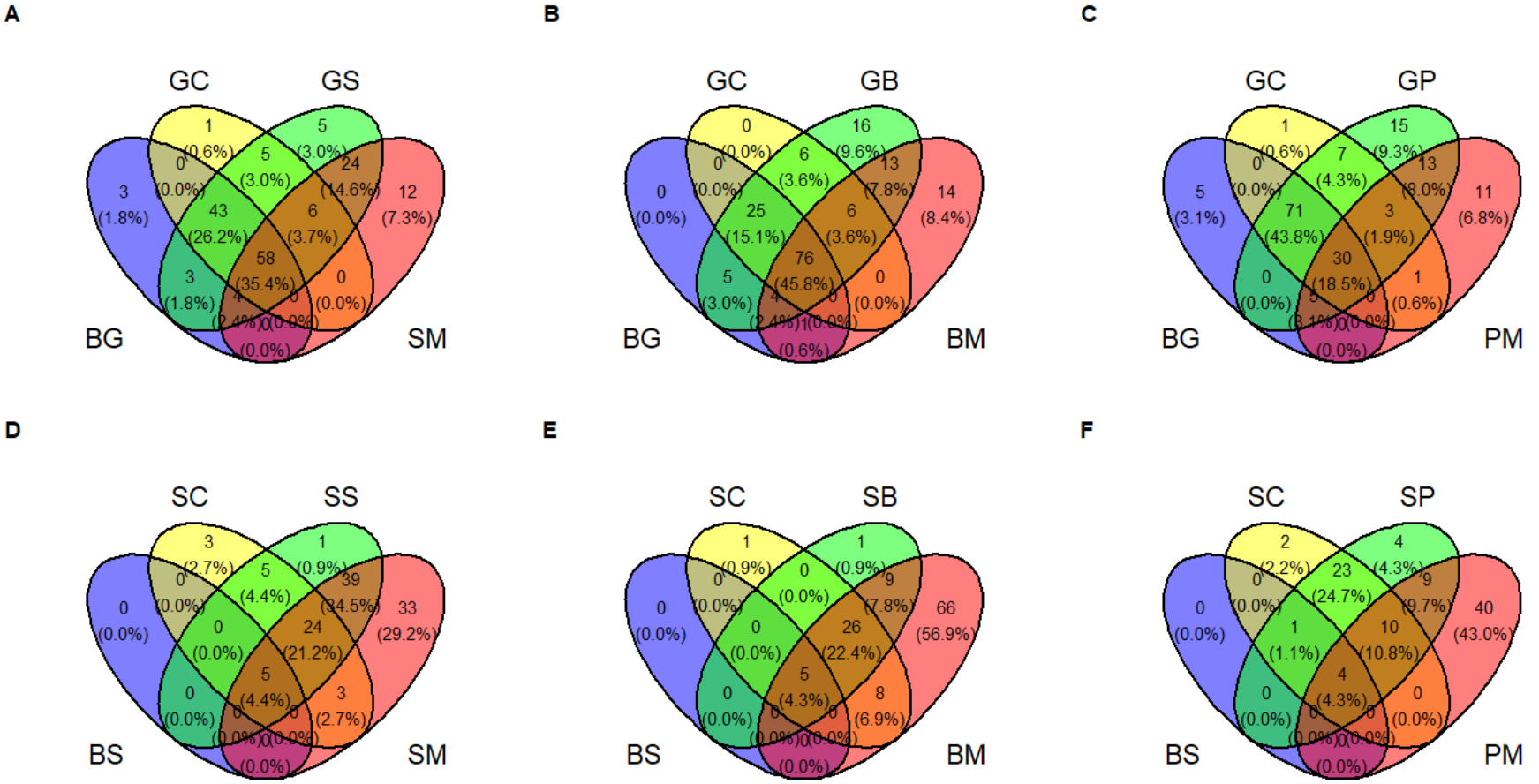
Names	total	elements
		tetPA
		blaTEM_1
		tnpA_1
		tet32
		ermT_1
		ermA
		tet36_1
		aadA5_2
		dfra17
		tetO_2
		IS613
		Tn3
		ermX_1
		Tp614
		tetW
		tet39
		dfrA1_1
		sul2_2
		tnpA_4
		aadD
		aph4-ia
		aacC2
		aph3-iii
		aadA6
		ermF
Soil Chicken	4	tolC_2
		IS6/257
		ermC_2
		ampC_6

Appendix 22: List of gene overlaps between grass samples and manure samples. BG= Background Grass, GB = cow manured grass, GC = control grass, GP = chicken manured grass, GS = pig manured grass, CM = cow manure, SM = pig manure and PM = chicken manure. TRUE indicates the gene was present in that sample, FALSE indicates it was not.

BG	GB	GC	GP	GS	CM	SM	PM	Genes
TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	c("A. baumannii", "aac(6)-iv_ih", "aadA16", "aadA7", "aadB", "acrA_1", "acrB_1", "acrF", "ampC_6", "aph3-iii", "blaCTX-M", "ermB_2", "IS1133", "mdtE", "spcN", "sul1_1", "sul2_1", "sul2_2", "tetD", "tetG", "tetL_2", "tetR", "tnpA_1", "tnpA_2", "tnpA_3", "tolC_2")
FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	c("intI1_3", "trbC")
TRUE	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	c("aadA2_3", "aph(3")-ia", "tetH", "tetX")
FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	c("aadA2_1", "dfra17", "intI2_2", "ISAba3", "sul1_2", "tet39", "tetW")
FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	c("IS613", "tet44")
FALSE	TRUE	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE	Tp614
FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	TRUE	TRUE	tnpA_4
FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	TRUE	c("ermF", "tetO_2")
FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	tnpA_5
FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	c("aphA3", "tet32", "tetQ", "tetT")
TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE	TRUE	Tn3
FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	TRUE	TRUE	c("aadD", "dfrA1_1", "ermX_1")
FALSE	TRUE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	c("blaTEM_1", "tetA/B_2")
FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	tetC_2
TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE	mexA
FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE	lncF_FIC
TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	TRUE	c("ampC_1", "IS6/257")
TRUE	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	TRUE	traN
FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	TRUE	ISEfm1
TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	c("aac(3)-xa_1", "aadA10", "aadA6", "ampC_2", "ampC_3", "ampC_5", "ant4-ib", "ant6-ia", "ant6-ib", "aph6-ia", "blaCARB", "blaCTX-M_1", "blaCTX-M_2", "blaCTX-M_4", "blaMIR", "blaNDM", "blaSFO", "cphA_1", "erm36", "ermE", "ermO", "IncI1_repI1", "IncN_rep", "IncP_oriT", "IncQ_oriT", "intI3_1", "oprD", "orf37-IS26", "sul3_1", "vanA")
FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE	FALSE	c("ermA", "tetS")
FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	tetPA

BG	GB	GC	GP	GS	CM	SM	PM	Genes
FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	ermT_1
FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	ermC_2
FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	FALSE	c("ermA/ermTR", "tet36_1")
TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE	FALSE	aac3-IVa
FALSE	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE	FALSE	c("blaROB", "qnrD")
FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	TRUE	FALSE	aadA5_2
FALSE	TRUE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	c("aacC2", "aph4-ia")
FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	qnrS_1
FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	c("aac(6')-II", "apmA", "ermY")
TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	c("aac(3)-ib", "aac(3)-iid_iia", "aac(6)-ig", "aac(6)-iic", "aac(6)-ir", "ampC/blaDHA", "ampC_4", "aph3-ib", "aph4-ib", "blaFOX", "blaOXA48", "blaOXY", "blaPAO", "ermD/K", "mcr1", "P. aeruginosa", "qepA", "qnrB4", "vanB_1")
FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	c("aac(3)-id_ie", "ISPps")
FALSE	TRUE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	qnrVC1_VC3_VC6
TRUE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	trfA
FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	c("blaMOX/blaCMY", "ereB_2")
TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	c("aac(6')-Ib_1", "aac(6)-iz", "aph_viii", "aph3-via", "bl1acc", "blaCTX-M_3", "blaCTX-M_5", "blaCTX-M_6", "blaKPC", "blaPER", "dfrA8", "ereA", "IS1111", "oqxA", "pikR1", "qacF/H", "qnrS2", "rmtB", "tetJ", "vgaA_1", "vgaB_1")
FALSE	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	c("blaIMP_1", "dfrA12")
FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	c("aac6-aph2", "blaACC")
FALSE	FALSE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	c("IncHI2-smr0018", "tetE")
TRUE	TRUE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	c("aac(6)-is_iu_ix", "blaIMI")
FALSE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	tetA_1
FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	armA
FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	c("blaOKP", "blaZ", "mecA", "msrA_1", "msrC_1", "tetK")
TRUE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	c("blaSHV11", "blaVIM")
FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	c("aacA/aphD", "ermD", "K. pneumoniae", "marR_3")

Appendix 23: Venn diagrams of grass and soil samples with manure samples HT-qPCR samples. Figure A-C are grass samples, Figure D-F are soil samples. Sample codes are: BG= Background, GC = Grass Control, GS = Pig Manured Grass, SM = Pig manure, GB = Cow manured grass, BM = Cow manure, GP = chicken manured grass, PM = Chicken manure, BS= Background Soil, SC = Control Soil, SS= pig manured soil, SB = Cow manured soil, SP = chicken manured soil

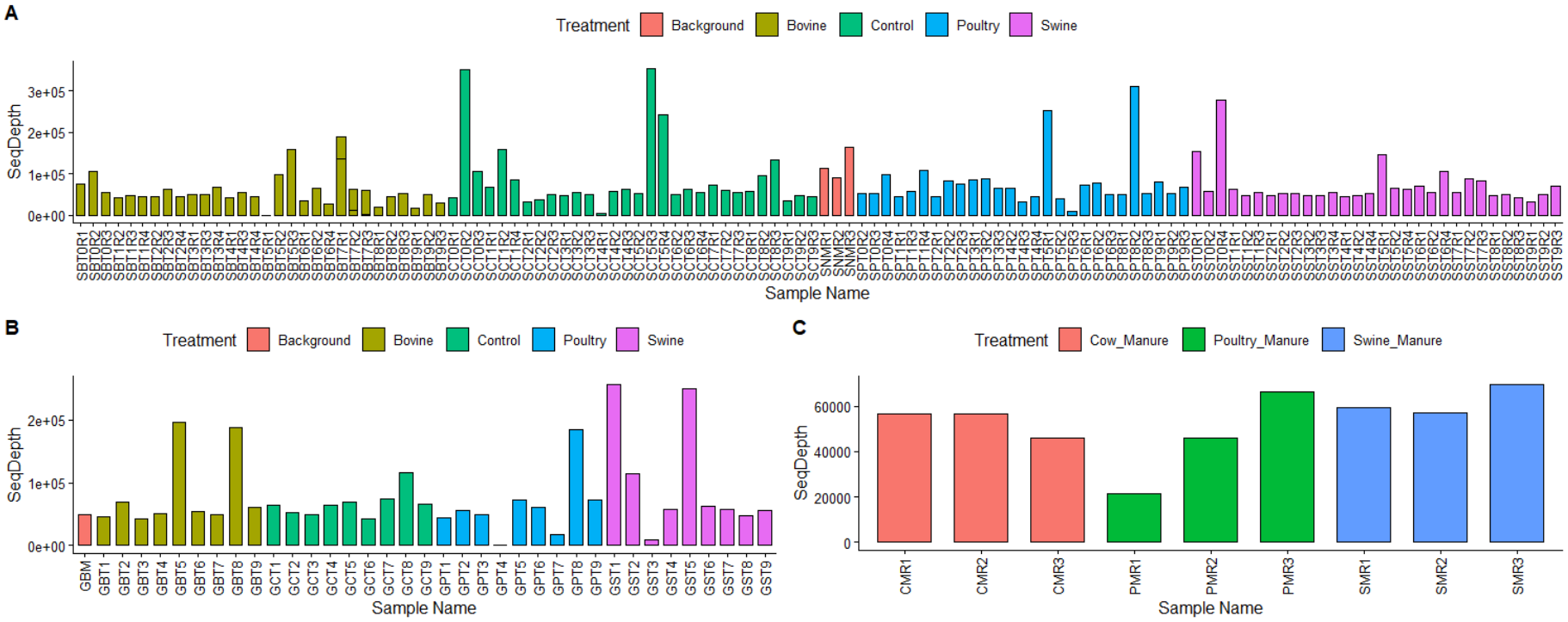


Appendix 24: List of gene overlaps between grass samples and manure samples. BS= Background Soil, SB = cow manured soil, SC = control soil, SP = chicken manured soil, SS = pig manured soil, CM = cow manure, SM = pig manure and PM = chicken manure. TRUE indicates the gene was present in that sample, FALSE indicates it was not.

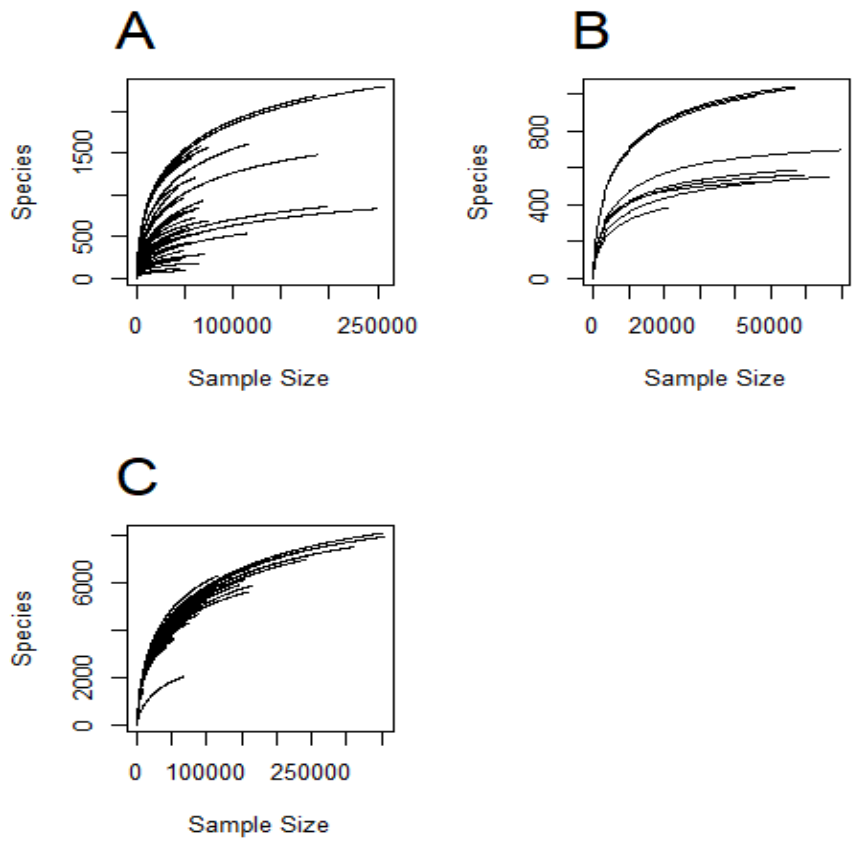
BG	SB	SC	SP	SS	CM	SM	PM	Genes
TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	c("aadA7", "tetD", "tetG", "trbC")
FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	c("aadA16", "aadB", "blaCTX-M", "IS1133", "spcN", "sul1_1", "tetL_2", "tetR", "tnpA_3")
FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	c("aadA2_3", "sul2_1", "tnpA_2")
FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	c("intI1_3", "sul1_2", "tetX")
FALSE	TRUE	FALSE	FALSE	TRUE	TRUE	TRUE	TRUE	c("aadA2_1", "ermB_2", "intI2_2", "tet44")
FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	TRUE	c("aph(3")-ia", "aph3-iii", "dfra17", "ermF", "IS613", "sul2_2", "tet32", "tet39", "tetO_2", "tetQ", "tetW", "tnpA_1", "tnpA_4", "Tp614")
FALSE	TRUE	TRUE	TRUE	FALSE	TRUE	TRUE	TRUE	mdtE
FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	c("ampC_6", "tolC_2")
FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	c("A. baumannii", "aac(6)-iv_ih", "acrA_1", "acrB_1", "acrF", "aphA3", "ISAba3", " tetH", "tetT", "tnpA_5")
FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	c("aadD", "blaTEM_1", "dfrA1_1", "ermX_1", "Tn3")
FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	c("tetA/B_2", "tetC_2")
FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	c("lncF_FIC", "mexA")
FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	IS6/257
FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	c("ampC_1", "ISEfm1", "traN")
TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	oprD
FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	c("aac(3)-xa_1", "ampC_2", "aph6-ia", "blaCTX-M_4", "blaMIR", "blaSFO", "cphA_1", "ermE", "ermO", "IncP_oriT", "vanA")
FALSE	FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	c("aadA10", "ampC_5", "IncQ_oriT", "sul3_1")
FALSE	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE	FALSE	IncI1_repI1
FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	c("aadA6", "ant6-ib", "ermA", "ermT_1", "tet36_1", "tetPA")
FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	TRUE	FALSE	ant4-ib

BG	SB	SC	SP	SS	CM	SM	PM	Genes
FALSE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	orf37-IS26
FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	ermC_2
FALSE	TRUE	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	erm36
FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	FALSE	c("ampC_3", "ant6-ia", "blaCARB", "blaCTX-M_1", "blaCTX-M_2", "blaNDM", "ermA/ermTR", "IncN_rep", "intI3_1", "tetS")
FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	FALSE	c("aacC2", "aadA5_2", "aph4-ia")
FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	c("aac(6')-II", "aac3-IVa", "apmA", "blaROB", "ermY", "qnrD", "qnrS_1")
FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	c("aac(3)-ib", "aph4-ib", "qepA")
FALSE	FALSE	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	aac(3)-iid_iia
FALSE	TRUE	TRUE	TRUE	FALSE	TRUE	FALSE	FALSE	aph3-ib
FALSE	FALSE	TRUE	TRUE	FALSE	TRUE	FALSE	FALSE	blaOXA48
FALSE	FALSE	TRUE	FALSE	FALSE	TRUE	FALSE	FALSE	aac(6)-iic
FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	c("aac(3)-id_ie", "aac(6)-ig", "aac(6)-ir", "ampC/blaDHA", "ampC_4", "blaFOX", "blaMOX/blaCMY", "blaOXY", "blaPAO", "ereB_2", "ermD/K", "ISPps", "mcr1", "P. aeruginosa", "qnrB4", "qnrVC1_VC3_VC6", "trfA", "vanB_1")
FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE	dfrA8
FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	blaIMP_1

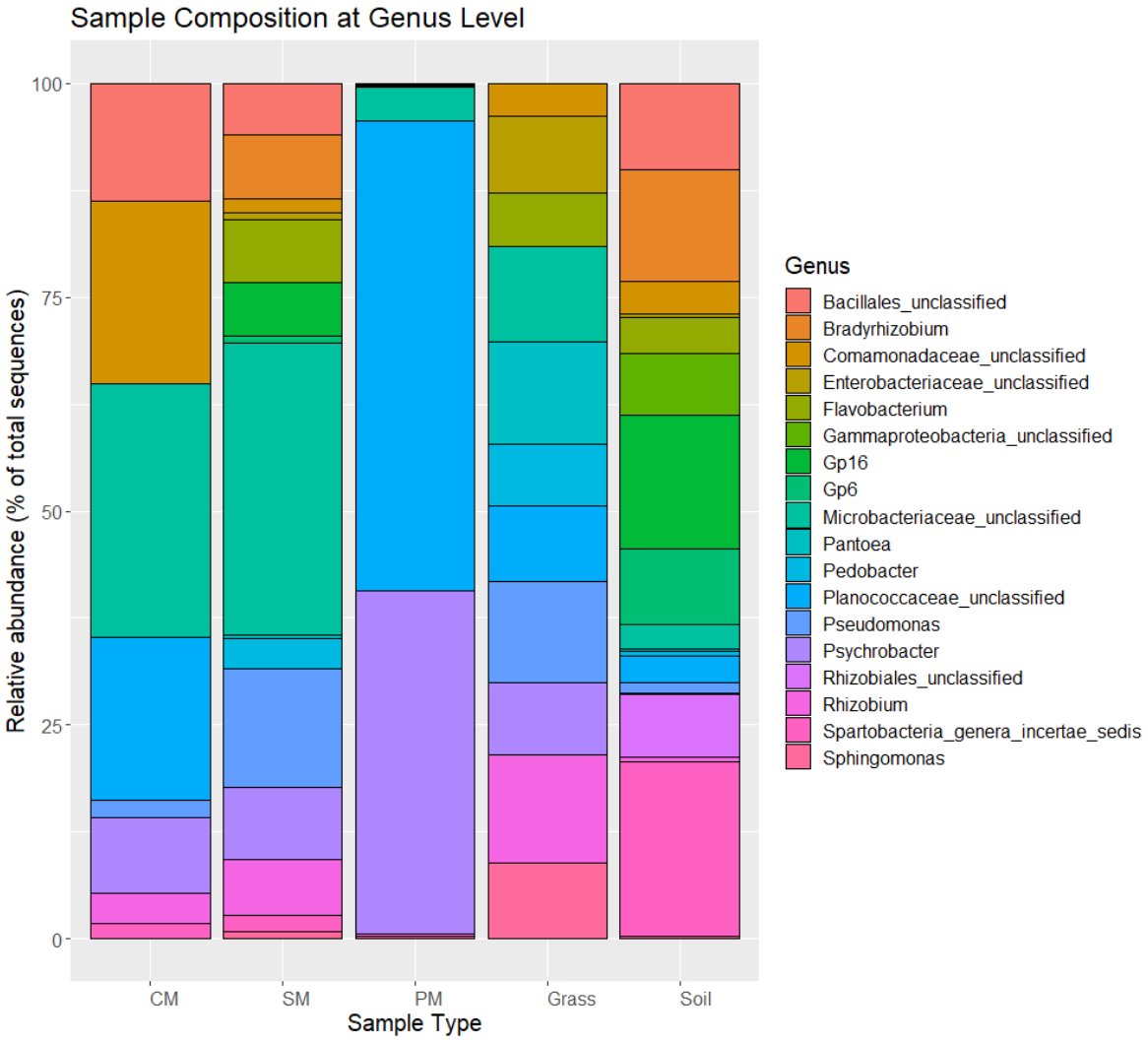
Appendix 25: Sequencing depth for each (A) Soil samples (B) Grass Samples and (C) Manure samples



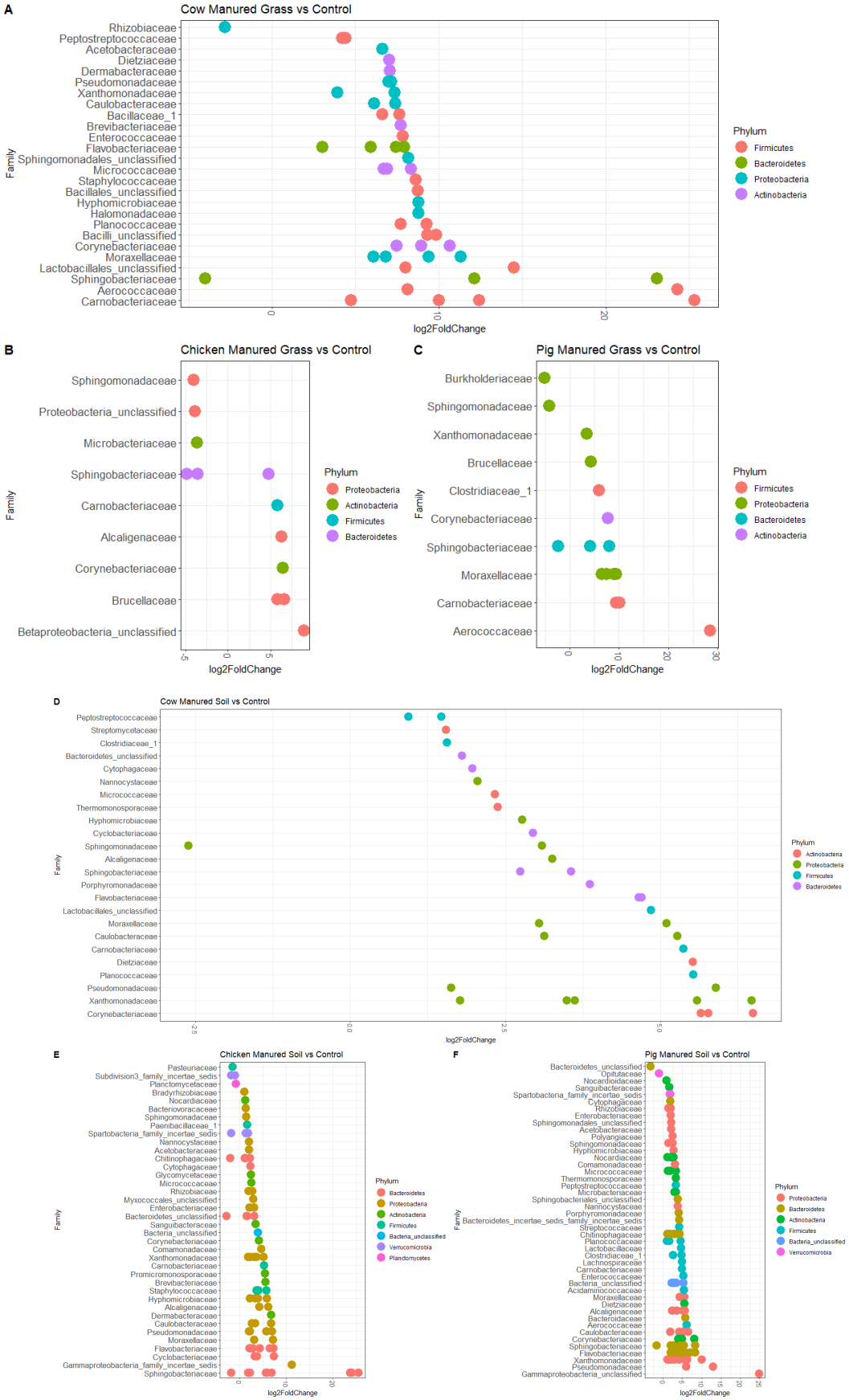
Appendix 26: Rarefaction curves for (A) Grass (B) Manure and (C) Soil.



Appendix 27: Sample composition at Genus Level.



Appendix 28: DESeq2 results for Grass (A-C) and Soil (D-F).



Appendix 29: Table displaying identification results for the isolated bacteria.

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
1	PM PIA COL 4	Chicken Manure	Pseudomonas Isolation Agar	*Enterococcus*	*faecalis*	2.21		
2	PM LAM CEF 3	Chicken Manure	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.26		
3	PM LAM CEF 4	Chicken Manure	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.08		
4	PM LAM COL 1	Chicken Manure	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.25		
5	PM LAM COL 2	Chicken Manure	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.11		
6	PM LAM COL 3	Chicken Manure	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.07		
7	PM LAM COL 6	Chicken Manure	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.26		
8	SM EMB CEF 5	Pig Manure	EMB	*Escherichia*	*coli*	2.55		
9	SM EMB CIPRO 1	Pig Manure	EMB	*Escherichia*	*coli*	2.49		
10	SM EMB CIPRO 2	Pig Manure	EMB	*Escherichia*	*coli*	2.48		
11	SM EMB CIPRO 3	Pig Manure	EMB	*Escherichia*	*coli*	2.54		
12	SM EMB CIPRO 4	Pig Manure	EMB	*Escherichia*	*coli*	2.54		
13	SM EMB CIPRO 5	Pig Manure	EMB	*Escherichia*	*coli*	2.51		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
14	SM EMB CIPRO 6	Pig Manure	*EMB*	*Escherichia*	coli	2.53		
15	SM PIA COL 2	Pig Manure	*Pseudomonas Isolation Agar*	*Providencia*	rettgeri	2.42		
16	SM PIA KAN 1	Pig Manure	*Pseudomonas Isolation Agar*	*Citrobacter*	freundii	2.45		
17	CM EMB CEF 1	Cow Manure	*EMB*	*Escherichia*	coli	2.42		
18	CM EMB CEF 2	Cow Manure	*EMB*	*Escherichia*	coli	2.37		
19	CM EMB CEF 3	Cow Manure	*EMB*	*Escherichia*	coli	2.57		
20	CM EMB CEF 4	Cow Manure	*EMB*	*Escherichia*	coli	2.54		
21	CM SC COL 1	Cow Manure	*Simmon Citrate Agar*	*Providencia*	rettgeri	2.4		
22	CM SC COL 3	Cow Manure	*Simmon Citrate Agar*	*Providencia*	rettgeri	2.38		
23	CM PIA COL 2	Cow Manure	*Pseudomonas Isolation Agar*	*Providencia*	rettgeri	2.2		
24	CM PIA COL 4	Cow Manure	*Pseudomonas Isolation Agar*	*Providencia*	rettgeri	2.33		
25	CM PIA COL 5	Cow Manure	*Pseudomonas Isolation Agar*	*Providencia*	rettgeri	2.37		
26	CM PIA COL 6	Cow Manure	*Pseudomonas Isolation Agar*	*Providencia*	rettgeri	2.32		
27	CM EMB KAN 1	Cow Manure	*EMB*	*Escherichia*	coli	2.45		
28	CM EMB KAN 2	Cow Manure	*EMB*	*Escherichia*	coli	2.46		
29	CM EMB KAN 3	Cow Manure	*EMB*	*Escherichia*	coli	2.28		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
30	CM EMB KAN 4	Cow Manure	EMB	*Escherichia*	*coli*	2.53		
31	CM LAM CEF 1	Cow Manure	Leeds Agar (Acinetobacter)	*Escherichia*	*coli*	2.43		
32	CM LAM CEF 2	Cow Manure	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.1		
33	CM LAM CEF 4	Cow Manure	Leeds Agar (Acinetobacter)	*Escherichia*	*coli*	2.39		
34	CM LAM KAN 1	Cow Manure	Leeds Agar (Acinetobacter)	*Escherichia*	*coli*	2.46		
35	CM EMB CIPRO 1	Cow Manure	EMB	*Escherichia*	*coli*	2.54		
36	CM EMB CIPRO 2	Cow Manure	EMB	*Escherichia*	*coli*	2.45		
37	CM EMB CIPRO 3	Cow Manure	EMB	*Escherichia*	*coli*	2.4		
38	G2 BM SC CEF 3	Grass	Simmon Citrate Agar	*Acinetobacter*	*baumannii*	2.38		
39	G2 BM SC CEF 5	Grass	Simmon Citrate Agar	*Acinetobacter*	*baumannii*	2.3		
40	G2 BM SC CEF 6	Grass	Simmon Citrate Agar	*Acinetobacter*	*baumannii*	2.37		
41	G2 BM SC KAN 1	Grass	Simmon Citrate Agar	*Citrobacter*	*gillenii*	2.5		
42	G2 BM PIA COL 1	Grass	Pseudomonas Isolation Agar	*Serratia*	*fonticola*	2.38		
43	G2 BM PIA COL 2	Grass	Pseudomonas Isolation Agar	*Serratia*	*fonticola*	2.43		
44	G2 BM PIA COL 3	Grass	Pseudomonas Isolation Agar	*Serratia*	*fonticola*	2.46		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
45	G2 BM PIA COL 5	Grass	Pseudomonas Isolation Agar	*Serratia*	*fonticola*	2.38		
46	G2 BM PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.09		
47	G2 BM SC KAN 1	Grass	SImmon Citrate Agar	*Citrobacter*	*gillenii*	2.44		
48	G2 BM EMB COL 5	Grass	EMB	*Proteus*	*vulgaris*	2.18		
49	G2 BM EMB COL 7	Grass	EMB	*Hafnia*	*paralevi*	N/A	EFP113	99.67%
50	G2 EMB CEF 6	Grass	EMB	*Acinetobacter*	*baumannii*	2.36		
51	G2 EMB CEF 7	Grass	EMB	*Acinetobacter*	*baumannii*	2.3		
52	SC NM SC COL 1	Soil	Simmon Citrate Agar	*Providencia*	*rettgeri*	2.07		
53	SC NM SC COL 2	Soil	Simmon Citrate Agar	*Providencia*	*stuartii*	2.48		
54	SC NM PIA CEF 1	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.01		
55	SC NM PIA COL 1	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.03		
56	SC NM PIA COL 3	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.11		
57	SC NM PIA COL 5	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.02		
58	SC NM LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.01		
59	SC NM LAM CEF 3	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.44		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
60	SC NM LAM CEF 6	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.39		
61	SC NM LAM COL 2	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.19		
62	SC NM LAM COL 3	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.35		
63	SC NM LAM COL 5	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.23		
64	SC NM LAM COL 6	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.35		
65	SC NM EMB KAN 1	Soil	EMB	*Escherichia*	*coli*	2.35		
66	SC NM EMB KAN 2	Soil	EMB	*Escherichia*	*coli*	2.58		
67	SC NM EMB KAN 3	Soil	EMB	*Escherichia*	*coli*	2.51		
68	SC NM EMB KAN 4	Soil	EMB	*Escherichia*	*coli*	2.37		
69	SC NM EMB KAN 5	Soil	EMB	*Escherichia*	*coli*	2.15		
70	SC NM EMB KAN 6	Soil	EMB	*Escherichia*	*coli*	2.37		
71	G2 BM LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.35		
72	G2 BM LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.38		
73	G2 BM LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.38		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
74	G2 BM LAM CEF 4	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.48		
75	G2 BM LAM CEF 5	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.32		
76	SB T3 EMB COL 1	Soil	EMB	*Serratia*	*marcescens*	2.17		
77	SB T3 EMB COL 2	Soil	EMB	*Serratia*	*marcescens*	2.17		
78	SB T3 EMB CIPRO 1	Soil	EMB	*Stenotrophomonas*	*maltophilia*	2.1		
79	SB T3 EMB KAN 1	Soil	EMB	*Ochrobactrum*	*intermedium*	2.21		
80	SB T2 SC COL 6	Soil	Simmon Citrate Agar	*Serratia*	*marcescens*	2.31		
81	SB T3 SC CEF 6	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.03		
82	SB T4 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.37		
83	SB T4 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.26		
84	SB T4 LAM CEF 3	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.39		
85	SB T4 LAM CEF 4	Soil	Leeds Agar (Acinetobacter)	*Myroides*	*odoratus*	2.18		
86	SB T4 LAM CEF 5	Soil	Leeds Agar (Acinetobacter)	*Citrobacter*	*gillenii*	2.43		
87	SB T4 LAM CEF 6	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.05		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
88	SB T3 PIA COL 1	Soil	Pseudomonas Isolation Agar	*Serratia*	*marcescens*	2.17		
89	SB T3 PIA COL 2	Soil	Pseudomonas Isolation Agar	*Serratia*	*marcescens*	2.34		
90	SB T3 PIA COL 3	Soil	Pseudomonas Isolation Agar	*Serratia*	*marcescens*	2.31		
91	SB T3 PIA KAN 4	Soil	Pseudomonas Isolation Agar	*Stenotrophomonas*	*maltophilia*	2.31		
92	SB T3 PIA CIPRO 2	Soil	Pseudomonas Isolation Agar	*Serratia*	*marcescens*	2.37		
93	SP T3 EMB CEF 9	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.19		
94	SP T3 EMB CEF 10	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.22		
95	SP T3 EMB KAN 3	Soil	EMB	*Citrobacter*	*gillenii*	2.47		
96	SP T3 EMB KAN 4	Soil	EMB	*Proteus*	*vulgaris*	2.31		
97	SP T3 EMB KAN 5	Soil	EMB	*Citrobacter*	*gillenii*	2.4		
98	SP T3 EMB KAN 6	Soil	EMB	*Bacillus*	*cereus*	2.31		
99	SP T3 SC CIPRO 5	Soil	Simmon Citrate Agar	*Alcaligenes*	*faecalis*	2.33		
100	SP T3 PIA KAN 1	Soil	Pseudomonas Isolation Agar	*Proteus*	*vulgaris*	2.61		
101	SP T3 PIA KAN 3	Soil	Pseudomonas Isolation Agar	*Proteus*	*vulgaris*	2.22		
102	SP T3 PIA KAN 6	Soil	Pseudomonas Isolation Agar	*Proteus*	*vulgaris*	2.56		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
103	SP T3 SC CEF 3	Soil	Simmon Citrate Agar	*Serratia*	*marcescens*	2.26		
104	SP T3 EMB KAN 1	Soil	EMB	Citrobacter	*gillenii*	2.45		
105	SC T3 SC CIPRO 1	Soil	Simmon Citrate Agar	*Serratia*	*marcescens*	2.19		
				Providencia	*rettgeri*	2.16		
106	SC T3 SC CEF 1	Soil	Simmon Citrate Agar	*Proteus*	*hauseri*	2.28		
107	SC T3 EMB COL 10	Soil	EMB	*Proteus*	*hauseri! (vulgaris?)*	2.27		
108	SC T3 EMB CEF 4	Soil	EMB	*Proteus*	*vulgaris*	2.35		
109	SC T3 PIA COL 3	Soil	EMB	*Proteus*	*vulgaris*	2.26		
110	SC T3 EMB COL 9	Soil	EMB	*Proteus*	*vulgaris*	2.1		
111	SS T3 PIA COL 3	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.24		
112	SS T3 PIA COL 6	Soil	Pseudomonas Isolation Agar	*Serratia*	*marcescens*	2.29		
113	SS T3 PIA CEF 6	Soil	Pseudomonas Isolation Agar	*Escherichia*	*coli*	2.45		
114	SS T3 PIA CIPRO 3	Soil	Pseudomonas Isolation Agar	*Bacillus*	spp	1.58	EFP139	99.82%
115	SS T3 PIA KAN 1	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.25		
116	GB T3 SC COL 4	Grass	Simmon Citrate Agar	*Providencia*	*rettgeri*	2.23		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
117	GB T3 SC COL 1	Grass	Simmon Citrate Agar	*Acinetobacter*	*baumannii*	2.19		
118	GB T3 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.48		
119	GB T3 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.42		
120	GB T3 LAM CEF 5	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.36		
121	GB T3 LAM CEF 6	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.4		
122	GB T3 LAM CIPRO 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*indicus*	2.01		
123	GB T3 LAM CIPRO 4	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.16		
124	GB T3 LAM COL 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.39		
125	GB T3 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.2		
126	GB T3 PIA KAN 2	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.17		
127	GB T3 PIA KAN 3	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.16		
128	GB T3 PIA KAN 4	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.21		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
129	GB T3 PIA KAN 5	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.18		
130	GB T3 PIA KAN 6	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.14		
131	GB T3 PIA CIPRO 1	Grass	Pseudomonas Isolation Agar	*Bacillus*	*paralicheniformis*	1.51	EFP147	99.90%
132	GB T3 PIA CIPRO 3	Grass	Pseudomonas Isolation Agar	*Bacillus*	*subtilis*	2.07		
133	GB T3 PIA CEF 5	Grass	Pseudomonas Isolation Agar	*Pseudomonas*	*fulva*	2.24		
134	GB T3 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.08		
135	GB T3 PIA KAN 2	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.15		
136	GB T3 PIA CEF 6 (white colony)	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.22		
137	GB T3 PIA CEF 2 (white colony)	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.06		
138	GB T3 PIA CEF 2 (yellow colony)	Grass	Pseudomonas Isolation Agar	*Pseudomonas*	*fulva*	2.1		
139	GB T3 PIA CEF 1 (yellow)	Grass	Pseudomonas Isolation Agar	*Pseudomonas*	*fulva*	2.06		
140	GB T3 PIA CEF 1 (white colony)	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.11		
141	GB T3 EMB COL 2	Grass	EMB	*Serratia*	*marcescens*	2.11		
142	GB T3 EMB COL 7	Grass	EMB	*Serratia*	*marcescens*	2.14		
143	GB T3 EMB CEF 1	Grass	EMB	*Acinetobacter*	*baumannii*	2.1		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
144	GB T3 EMB CEF 2	Grass	EMB	*Acinetobacter*	*baumannii*	2.22		
145	GP T3 SC CEF 1	Grass	Simmon Citrate Agar	*Acinetobacter*	*baumannii*	2.06		
146	GP T3 LAM COL 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.34		
147	GB T3 EMB COL 4	Grass	EMB	*Moellerella*	*wisconsensis*	2.07		
148	GP T3 LAM COL 3	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.45		
149	GP T3 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.15		
150	GP T3 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.27		
151	GP T3 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.12		
152	GP T3 LAM CEF 4	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.02		
153	GP T3 EMB COL 1	Grass	EMB	*Acinetobacter*	*baumannii*	2.21		
154	GP T3 EMB COL 2	Grass	EMB	*Escherichia*	*Coli*	2.4		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
155	GS T3 SC CEF 2	Grass	Simmon Citrate Agar	*Acinetobacter*	*baumannii*	2.21		
156	GS T3 PIA KAN 3	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.06		
157	GS T3 PIA KAN 4	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.25		
158	GS T3 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.36		
159	GS T3 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.18		
160	GC T3 SC CEF 1	Grass	Simmon Citrate Agar	*Acinetobacter*	*baumanii*	2.24		
161	GC T3 PIA CEF 2	Grass	Pseudomonas Isolation Agar	*Stenotrophomonas*	*maltophilia*	2.11		
162	GC T3 PIA CEF 3	Grass	Pseudomonas Isolation Agar	*Stenotrophomonas*	*maltophilia*	2.04		
163	GC T3 PIA CIPRO 5	Grass	Pseudomonas Isolation Agar	*Escherichia*	*Coli*	2.02		
164	GC T3 PIA CIPRO 4	Grass	Pseudomonas Isolation Agar	*Stenotrophomonas*	*maltophilia*	2.08		
165	GC T3 PIA CEF 1	Grass	Pseudomonas Isolation Agar	*Stenotrophomonas*	*maltophilia*	2.12		
166	SP T3 EMB CEF 1	Soil	EMB	*Acinetobacter*	*baumannii*	2.12		
167	SP T3 EMB CEF 2	Soil	EMB	*Proteus*	*vulgaris*	N/A	EFP126	100%

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
168	SP T3 EMB CEF 3	Soil	EMB	*Acinetobacter*	*baumannii*	2.16		
169	SP T3 EMB CEF 4	Soil	EMB	*Acinetobacter*	*baumannii*	2.18		
170	SP T5 LAM CIPRO 1	Soil	LAM	*Glutamicibacter*	*creatinolyticus*	2.21		
171	SP T3 SC CEF 1	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.23		
172	SP T3 SC CEF 2	Soil	Simmon Citrate Agar	*Acinetobacter*	*baumannii*	2.14		
175	SP T5 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.38		
176	SP T5 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.28		
177	SP T5 LAM CEF 3	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.22		
178	SP T5 LAM CEF 4	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.27		
179	SP T5 LAM CEF 5	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.28		
180	SP T5 LAM CEF 6	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.32		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
181	SB T5 EMB COL 3	Soil	EMB	*Serratia*	*marcescens*	2.28		
182	SB T5 PIA KAN 2	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.11		
183	SB T5 PIA COL 1	Soil	Pseudomonas Isolation Agar	*Serratia*	*marcescens*	2.16		
184	SB T5 PIA COL 2	Soil	Pseudomonas Isolation Agar	*Serratia*	*marcescens*	2.35		
185	SB T5 PIA COL 3	Soil	Pseudomonas Isolation Agar	*Serratia*	*marcescens*	2.02		
186	SB T5 PIA COL 4	Soil	Pseudomonas Isolation Agar	*Serratia*	*marcescens*	2.36		
187	SB T5 SC COL 6	Soil	Simmon Citrate Agar	*Serratia*	*marcescens*	2.19		
188	SB T5 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.24		
189	SC T5 SC CEF 1	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.04		
190	SC T5 SC CEF 2	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.34		
191	SC T5 EMB CEF 1	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.17		
192	SC T5 EMB CEF 2	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.02		
193	SC T5 EMB CEF 3	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.3		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
194	SC T5 EMB CEF 4	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.08		
195	SC T5 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.32		
196	SC T5 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.33		
197	SC T5 LAM CEF 3	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.22		
198	SC T5 LAM CEF 4	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.31		
199	SC T5 LAM CEF 5	Soil	Leeds Agar (Acinetobacter)	*Kluyvera*	*intermedia*	2.16		
200	SC T5 LAM CEF 6	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.25		
201	SC T5 PIA KAN 1	Soil	Pseudomonas Isolation Agar	*Achromobacter*	*spanius*	2		
202	SC T5 PIA KAN 2	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.18		
203	SC T5 PIA KAN 3	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.08		
204	SC T5 PIA KAN 4	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.05		
205	SC T5 PIA KAN 5	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.2		
206	SC T5 PIA KAN 6	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.13		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
207	SC T5 PIA COL 2	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.15		
208	SC T5 PIA COL 3	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.06		
209	SC T5 PIA COL 4	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.08		
210	SS T5 EMB CIPRO 1	Soil	EMB	*Bordetella*	*trematum*	2.22		
211	SS T5 LAM COL 1	Soil	Leeds Agar (Acinetobacter)	*Serratia*	*marcescens*	2.27		
212	SS T5 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.26		
213	SS T5 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.29		
214	SS T5 LAM CEF 3	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.06		
215	SS T5 LAM CEF 4	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.07		
216	GB T5 PIA KAN 5	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.24		
217	GB T5 PIA KAN 6	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.22		
218	GB T5 PIA KAN 7	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.11		
219	GB T5 EMB COL 1	Grass	EMB	*Moellerella*	*wisconsensis*	2.09		
220	GB T5 EMB COL 2	Grass	EMB	*Moellerella*	*wisconsensis*	2.27		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
221	GB T5 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Morganella*	*morganii*	2.6		
222	GB T5 LAM KAN 1	Grass	Leeds Agar (Acinetobacter)	*Escherichia*	*Coli*	2.42		
223	GB T5 PIA KAN 4	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.05		
224	GB T5 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.23		
225	GB T5 LAM KAN 2	Grass	Leeds Agar (Acinetobacter)	*Escherichia*	*Coli*	2.08		
226	GB T5 LAM KAN 3	Grass	Leeds Agar (Acinetobacter)	*Escherichia*	*Coli*	2.4		
227	GB T5 EMB COL 3	Grass	EMB	*Providencia*	*rettgeri*	2.18		
228	GC T5 SC CEF 1	Grass	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.29		
229	GB T1 LAM COL 5	Grass	Leeds Agar (Acinetobacter)	*Enterobacter*	*cloacae*	2.28		
230	GB T5 EMB KAN 1	Grass	EMB	*Escherichia*	*coli*	2.35		
231	GB T5 EMB KAN 2	Grass	EMB	*Escherichia*	*coli*	2.56		
232	GB T5 EMB KAN 3	Grass	EMB	*Escherichia*	*coli*	2.42		
233	GB T5 EMB KAN 4	Grass	EMB	*Escherichia*	*coli*	2.48		
234	GB T5 EMB KAN 5	Grass	EMB	*Escherichia*	*Coli*	2.45		
235	GB T5 EMB KAN 6	Grass	EMB	*Escherichia*	*Coli*	2.37		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
236	GC T5 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.18		
237	GC T5 PIA KAN 2	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.1		
238	GC T5 PIA KAN 3	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.17		
239	GC T5 PIA CIPRO 1	Grass	Pseudomonas Isolation Agar	*Stenotrophomonas*	*maltophilia*	2.18		
240	GC T4 SC COL 2	Grass	Simmon Citrate Agar	*Klebsiella*	*pneumoniae*	2.42		
241	GC T4 SC COL 3	Grass	Simmon Citrate Agar	*Klebsiella*	*pneumoniae*	2.36		
242	GC T1 EMB CIPRO 2	Grass	EMB	*Hafnia*	*alvei*	2.43		
243	GC T1 EMB CIPRO 3	Grass	EMB	*Hafnia*	*alvei*	2.27		
244	GC T1 EMB CIPRO 4	Grass	EMB	*Hafnia*	*alvei*	2.28		
245	GC T1 EMB CIPRO 5	Grass	EMB	*Hafnia*	*alvei*	2.48		
246	GC T1 EMB CEF 1	Grass	EMB	*Acinetobacter*	*baumannii*	2.38		
247	GC T1 EMB CEF 2	Grass	EMB	*Acinetobacter*	*baumannii*	2.43		
248	GC T1 EMB CEF 3	Grass	EMB	*Acinetobacter*	*baumannii*	2.41		
249	GC T1 EMB CEF 4	Grass	EMB	*Acinetobacter*	*baumannii*	2.47		
250	GS T1 EMB CEF 1	Grass	EMB	*Acinetobacter*	*baumannii*	2.45		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
251	GS T1 EMB CEF 2	Grass	EMB	*Acinetobacter*	*baumannii*	2.54		
252	GS T1 EMB CEF 4	Grass	EMB	*Acinetobacter*	*baumannii*	2.51		
253	GS T1 EMB CEF 5	Grass	EMB	*Acinetobacter*	*baumannii*	2.37		
254	GS T1 EMB CEF 6	Grass	EMB	*Acinetobacter*	*baumannii*	2.38		
255	GS T1PIA CEF 1	Grass	Pseudomonas Isolation Agar	*Citerobacter*	*braakii*	2.35		
256	GS T1 PIA CIPRO 1	Grass	Pseudomonas Isolation Agar	*Citerobacter*	*braakii*	2.33		
257	GS T1 PIA CIPRO 2	Grass	Pseudomonas Isolation Agar	*Citerobacter*	*braakii*	2.15		
258	GS T1 PIA CIPRO 3	Grass	Pseudomonas Isolation Agar	*Citerobacter*	*braakii*	2.33		
259	GS T1 PIA CIPRO 4	Grass	Pseudomonas Isolation Agar	*Citerobacter*	*braakii*	2.31		
260	GS T1 PIA CIPRO 5	Grass	Pseudomonas Isolation Agar	*Citerobacter*	*braakii*	2.33		
261	GS T1 PIA CIPRO 6	Grass	Pseudomonas Isolation Agar	*Citerobacter*	*braakii*	2.41		
262	GS T1 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.14		
263	GS T1 PIA KAN 2	Grass	Pseudomonas Isolation Agar	*Citerobacter*	*braakii*	2.37		
264	GS T1 PIA KAN 3	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.05		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
265	GS T1 PIA KAN 4	Grass	Pseudomonas Isolation Agar	*Citero bacter*	*braakii*	2.32		
266	GS T1 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Provid encia*	*rustigianii*	2.15		
267	GS T1 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Provid encia*	*rustigianii*	2.26		
268	GS T1 PIA COL 3	Grass	Pseudomonas Isolation Agar	*Proteu s*	*vulgaris*	2.27		
269	GC T3 EMB 3	Grass	EMB	*Hafnia*	*alvei*	2.17		
270	GC T3 EMB 4	Grass	EMB	*Provid encia*	*rettgeri*	2.17		
271	GC T3 EMB 5	Grass	EMB	*Provid encia*	*rettgeri*	2.06		
272	GC T1 SC CEF 1	Grass	Simmon Citrate Agar	*Acinet obacte r*	*baumannii*	2.15		
273	GC T1 SC CEF 2	Grass	Simmon Citrate Agar	*Acinet obacte r*	*baumannii*	2.34		
274	GC T1 SC CEF 3	Grass	Simmon Citrate Agar	*Acinet obacte r*	*baumannii*	2.35		
275	GC T1 SC CEF 4	Grass	Simmon Citrate Agar	*Acinet obacte r*	*baumannii*	2.42		
276	GC T1 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Provid encia*	*rettgeri*	2.07		
277	GC T1 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Provid encia*	*rettgeri*	2.16		
278	GC T1 PIA COL 3	Grass	Pseudomonas Isolation Agar	*Provid encia*	*rettgeri*	2.46		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
279	GC T1 PIA COL 4	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.03		
280	GC T1 PIA COL 5	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.01		
281	GC T1 PIA COL 6	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.08		
282	GC T1 PIA CEF 2	Grass	Pseudomonas Isolation Agar	*Pseudomonas*	*fulva*	2.27		
283	GC T1 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.21		
284	GC T1 PIA KAN 2	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.2		
285	GC T1 PIA KAN 3	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.14		
286	GC T1 PIA KAN 4	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.01		
287	GC T1 PIA KAN 5	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.08		
288	GC T1 PIA KAN 6	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.12		
289	GP T1 SC KAN 1	Grass	Simmon Citrate Agar	*Citerobacter*	*gillenii*	2.47		
290	GP T1 SC KAN 2	Grass	Simmon Citrate Agar	*Citerobacter*	*gillenii*	2.43		
291	GP T1 SC KAN 3	Grass	Simmon Citrate Agar	*Citerobacter*	*gillenii*	2.1		
292	GP T1 SC KAN 4	Grass	Simmon Citrate Agar	*Citerobacter*	*gillenii*	2.36		
293	GP T1 EMB CEF 1	Grass	EMB	*Acinetobacter*	*baumannii*	2.24		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
294	GP T1 EMB CEF 2	Grass	EMB	*Acinetobacter*	*baumannii*	2.43		
295	GP T1 EMB CEF 3	Grass	EMB	*Acinetobacter*	*baumannii*	2.32		
296	GP T1 EMB CEF 4	Grass	EMB	*Acinetobacter*	*baumannii*	2.4		
297	GP T1 EMB CEF 5	Grass	EMB	*Acinetobacter*	*baumannii*	2.36		
298	GP T1 EMB CEF 6	Grass	EMB	*Acinetobacter*	*baumannii*	2.37		
299	GP T1 SC CEF 3	Grass	Simmon Citrate Agar	*Acinetobacter*	*baumannii*	2.37		
300	GP T1 SC CEF 4	Grass	Simmon Citrate Agar	*Acinetobacter*	*baumannii*	2.34		
301	GP T1 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.31		
302	GP T1 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.19		
303	GP T1 PIA COL 3	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.28		
304	GP T1 PIA COL 4	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.35		
305	GP T1 PIA COL 5	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.18		
306	GP T1 PIA COL 6	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.05		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
307	GP T1 PIA CEF 5	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.36		
308	GP T1 PIA CEF 6	Grass	Pseudomonas Isolation Agar	*Citerobacter*	*braakii*	2.28		
309	SC T1 EMB CEF 1	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.2		
310	SC T1 EMB CEF 2	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.26		
311	SC T1 EMB CEF 3	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.28		
312	SC T1 EMB CEF 4	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.41		
313	SC T1 EMB CEF 5	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.42		
314	SC T1 EMB CEF 6	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.38		
315	SC T1 EMB CEF 7	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.26		
316	SC T1 EMB CEF 8	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.26		
317	SC T1 PIA KAN 1	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.22		
318	SC T1 PIA KAN 2	Soil	Pseudomonas Isolation Agar	*Citerobacter*	*gillenii*	2.4		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
319	SC T1 PIA KAN 3	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.26		
320	SC T1 PIA KAN 4	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.19		
321	SC T1 PIA KAN 5	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	N/A	EFP138	99.49%
322	SC T1 PIA KAN 6	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.13		
323	SC T1 LAM COL 5	Soil	Leeds Agar (Acinetobacter)	*Hafnia*	*alvei*	2.34		
324	SB T1 EMB CEF 3	Soil	EMB	*Serratia*	*marcescens*	2.39		
325	SB T1 EMB CEF 4	Soil	EMB	*Serratia*	*marcescens*	2.32		
326	SB T1 LAM KAN 5	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.21		
327	SB T1 SC CEF 1	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.41		
328	SB T1 SC CEF 2	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.06		
329	SB T1 SC CEF 3	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.32		
330	SB T1 PIA KAN 1	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.12		
331	SB T1 PIA KAN 2	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2		
332	SB T1 PIA KAN 3	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.23		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
333	SB T1 PIA KAN 4	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.21		
334	SB T1 PIA KAN 5	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.12		
335	SB T1 PIA KAN 6	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.16		
336	SC NM LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.23		
337	SC NM LAM CEF 3	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.28		
338	SC T3 SC CEF 2	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.16		
339	SC T3 SC CEF 3	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.06		
340	SC T3 SC CEF 4	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.19		
341	GB T1 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.19		
342	SC T1 SC CEF 1	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.3		
343	SC T1 SC CEF 2	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.18		
344	SC T1 SC CEF 3	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.17		
345	SC T1 SC CEF 4	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.31		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
346	SC T1 EMB COL 2	Soil	EMB	*Hafnia*	*alvei*	2.47		
347	SC T1 EMB COL 3	Soil	EMB	*Hafnia*	*alvei*	2.49		
348	SP T1 EMB CEF 1	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.31		
349	SP T1 EMB CEF 2	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.3		
350	SP T1 EMB CEF 3	Soil	EMB	*no ID*	*no ID*	no ID		
351	SP T1 EMB CEF 4	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.14		
352	SP T1 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.41		
353	SP T1 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.05		
355	SP T1 LAM CEF 5	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.11		
356	SP T1 LAM COL 1	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.33		
357	SP T1 LAM COL 2 (Pink colony)	Soil	Leeds Agar (Acinetobacter)	*Lysinibacillus*	*pakistanensis*	1.7	EFP142	99.83
358	SP T1 LAM COL 2 (Yellow colony)	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*megaterium*	2.22		
359	GB T1 LAM COL 3	Grass	Leeds Agar (Acinetobacter)	*Enterobacter*	*cloacae*	2.36		
360	SP T1 LAM COL 4	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*megaterium*	2.16		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
361	SP T1 LAM COL 5 (Pink colony)	Soil	Leeds Agar (Acinetobacter)	*Lysinibacillus*	*fusiformis*	1.75	EFP137	100%
362	GB T1 LAM COL 2	Grass	Leeds Agar (Acinetobacter)	*Enterobacter*	*cloacae*	2.42		
363	SP T1 PIA CIPRO 1	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.22		
364	SP T1 PIA KAN 1	Soil	Pseudomonas Isolation Agar	*Citerobacter*	*gillenii*	2.38		
365	SP T1 PIA KAN 2	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.13		
366	SP T1 PIA KAN 3	Soil	Pseudomonas Isolation Agar	*Citerobacter*	*gillenii*	2.4		
367	SP T1 PIA KAN 4	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.52		
368	SP T1 PIA KAN 5	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.05		
369	GB T1 LAM COL 1	Grass	Leeds Agar (Acinetobacter)	*Enterobacter*	*cloacae*	2.27		
370	SB T1 EMB COL 1	Soil	EMB	*Serratia*	*marcescens*	2.32		
371	SB T1 EMB COL 2	Soil	EMB	*Serratia*	*marcescens*	2.33		
372	SB T1 EMB COL 3	Soil	EMB	*Serratia*	*marcescens*	2.35		
373	SB T1 EMB COL 4	Soil	EMB	*Serratia*	*marcescens*	2.3		
374	SB T1 EMB COL 5	Soil	EMB	*Serratia*	*marcescens*	2.36		
375	SB T1 EMB COL 6	Soil	EMB	*Serratia*	*marcescens*	2.3		
376	SB T1 PIA CIPRO 1	Soil	Pseudomonas Isolation Agar	*Providencia*	*rustigianii*	2.29		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
377	GB T1 PIA COL 3	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.21		
378	GB T1 PIA COL 4	Grass	Pseudomonas Isolation Agar	*Klebsiella*	*pneumoniae*	2.44		
379	GB T1 PIA KAN 4	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.1		
380	SB T1 PIA CIPRO 5	Soil	Pseudomonas Isolation Agar	*Providencia*	*rustigianii*	2.18		
381	GB T1 SC CEF 1	Grass	Simmon Citrate Agar	*Acinetobacter*	*baumannii*	2.34		
382	SS T1 SC CEF 1	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.26		
383	SS T1 SC CEF 2	Soil	Simmon Citrate Agar	*Myriodes*	*odoratus*	2.07		
384	SS T1 SC CEF 3	Soil	Simmon Citrate Agar	*Myriodes*	*odoratus*	2.42		
385	SS T1 EMB CEF 1	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.35		
386	SS T1 EMB CEF 2	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.38		
387	SS T1 EMB CEF 3	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.13		
388	GB T1 PIA KAN 3	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.19		
389	SS T1 LAM CEF 4 (Pink colony)	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.34		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
390	GS T5 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.17		
391	GS T5 PIA KAN 4	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.16		
392	GC T5 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Morganella*	*morganii*	2.31		
393	GC T5 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Morganella*	*morganii*	2.12		
394	GC T5 PIA COL 3	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.27		
395	GC T5 PIA COL 4	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.41		
396	GC T5 LAM COL 1	Grass	Leeds Agar (Acinetobacter)	*Morganella*	*morganii*	2.17		
397	GP T5 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.39		
398	GP T5 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.26		
399	GP T5 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Pseudomonas*	*putida*	2.21		
400	GP T5 PIA CEF 3	Grass	Pseudomonas Isolation Agar	*Pseudomonas*	*putida*	2.17		
401	GP T5 PIA CEF 1	Grass	Pseudomonas Isolation Agar	*Pseudomonas*	*putida*	2.22		
403	GS T5 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.48		
404	GS T5 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Enterococcus*	*faecalis*	N/A	EFP108	99.36%

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
405	GS T5 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Stenotrophomonas*	*maltophilia*	N/A	EFP107	98.57%
406	GS T5 LAM CEF 4	Grass	Leeds Agar (Acinetobacter)	*Enterococcus*	*faecalis*	N/A	EFP112	98.89%
408	GB T1 LAM CIPRO 2	Grass	Leeds Agar (Acinetobacter)	*Serratia*	*marcescens*	2.15		
409	GB T1 LAM CIPRO 5	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*indicus*	2.02		
410	GB T1 LAM CIPRO 6	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*indicus*	2.07		
411	GP T1 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.44		
412	GP T1 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*ppbaumannii*	2.36		
413	GP T1 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.21		
414	GP T1 LAM CEF 4	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.41		
415	GP T1 LAM CEF 5	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.16		
416	GP T1 LAM CEF 6	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.42		
417	GS T1 LAM CIPRO 1	Grass	Leeds Agar (Acinetobacter)	*Escherichia*	*coli*	2.38		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
418	GS T1 LAM CIPRO 3	Grass	Leeds Agar (Acinetobacter)	*Escherichia*	*coli*	2.43		
419	GS T1 LAM CIPRO 4	Grass	Leeds Agar (Acinetobacter)	*Escherichia*	*coli*	2.47		
420	GS T1 LAM CIPRO 5	Grass	Leeds Agar (Acinetobacter)	*Escherichia*	*coli*	2.42		
421	GC T1 LAM COL 1	Grass	Leeds Agar (Acinetobacter)	*Klebsiella*	*pneumoniae*	2.48		
422	GC T1 LAM COL 2	Grass	Leeds Agar (Acinetobacter)	*Klebsiella*	*pneumoniae*	2.41		
423	GC T1 LAM COL 3	Grass	Leeds Agar (Acinetobacter)	*KLebsiella*	*pneumoniae*	2.43		
424	GC T1 LAM COL 4	Grass	Leeds Agar (Acinetobacter)	*Klebsiella*	*pneumoniae*	2.45		
425	GC T1 LAM CEF 6	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.46		
426	GC T1 LAM COL 6	Grass	Leeds Agar (Acinetobacter)	*Klebsiella*	*pneumoniae*	2.42		
427	GC T1 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.3		
428	GC T1 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.43		
429	GC T1 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.4		
430	GC T1 LAM CEF 4	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.39		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
431	GC T1 LAM CEF 5	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*baumannii*	2.43		
432	SB T7 EMB CEF 4	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.12		
433	SB T7 PIA KAN 1	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.2		
434	SB T7 PIA KAN 2	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.28		
435	SB T7 PIA COL 1	Soil	Pseudomonas Isolation Agar	*Providencia*	*alcalifaciens*	2.4		
436	SB T7 PIA COL 2	Soil	Pseudomonas Isolation Agar	*Providencia*	*alcalifaciens*	2.4		
437	SN T7 SC CEF 1	Soil	Simmon Citrate Agar	*Proteus*	*hauseri*	2.38		
438	SN T7 SC CEF 2	Soil	Simmon Citrate Agar	*Proteus*	*hauseri*	2.22		
439	SN T7 SC CEF 4	Soil	Simmon Citrate Agar	*Proteus*	*hauseri*	2.37		
440	SB T7 SC CIPRO 3	Soil	Simmon Citrate Agar	*Proteus*	*hauseri*	2.36		
441	SB T7 PIA CEF 1	Soil	Pseudomonas Isolation Agar	*Achromobacter*	*spanicus*	2.09		
442	SB T7 EMB CIPRO 1	Soil	EMB	*Serratia*	*fonticola*	2.51		
443	SB T7 EMB CIPRO 2	Soil	EMB	*Achromobacter*	*piechaudii*	2.21		
444	SB T7 EMB CIPRO 3 (Purple colony)	Soil	EMB	*Achromobacter*	*spanius*	2.04		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
445	SB T7 EMB CIPRO 3 (Pink colony)	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.11		
446	SB T7 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.35		
447	SB T7 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.22		
448	SB T7 LAM CIPRO 2	Soil	Leeds Agar (Acinetobacter)	*Achromobacter*	*mucicolens*	2.02		
449	SC T7 SC CEF 1	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.41		
450	SC T7 PIA COL 4	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.26		
452	SC T7 PIA KAN 2	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.28		
453	SC T7 PIA KAN 3	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.03		
454	SC T7 PIA KAN 4	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.24		
455	SC T7 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.34		
456	SC T7 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.03		
457	SC T7 LAM CEF 3	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.25		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
458	SC T7 LAM CEF 4	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.25		
459	SC T7 LAM CEF 5	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.25		
460	SC T7 LAM CEF 6	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.22		
461	SC T7 EMB COL 1	Soil	EMB	*Hafnia*	*alvei*	2.53		
462	SC T7 EMB COL 2	Soil	EMB	*Escherichia*	*coli*	2.36		
463	SC T7 EMB COL 3	Soil	EMB	*Escherichia*	*coli*	2.46		
464	SC T7 EMB COL 4	Soil	EMB	*Escherichia*	*coli*	2.33		
465	SS T7 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.4		
466	SS T7 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Bacillus*	*cereus*	2.32		
467	SS T7 LAM CEF 3	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.03		
468	SS T7 LAM CEF 4	Soil	Leeds Agar (Acinetobacter)	*Serratia*	*marcescens*	2.33		
469	SS T7 EMB 1	Soil	EMB	*Hafnia*	*alvei*	2.5		
470	SS T7 EMB 4	Soil	EMB	*Hafnia*	*alvei*	2.46		
471	SS T7 EMB CEF 3	Soil	EMB	*Escherichia*	*coli*	2.46		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
472	SS T7 EMB CEF 4	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.03		
473	SS T7 SC COL 1	Soil	Simmon Citrate Agar	*Serratia*	*marcescens*	2.36		
474	SS T7 SC COL 2	Soil	Simmon Citrate Agar	*Serratia*	*marcescens*	N/A	EFP134	99.73
475	SS T7 SC COL 3	Soil	Simmon Citrate Agar	*Serratia*	*marcescens*	2.25		
476	SS T7 SC COL 4	Soil	Simmon Citrate Agar	*Serratia*	*marcescens*	2.35		
477	SS T7 SC CEF 1	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.56		
478	SS T7 SC CEF 2	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.24		
479	SS T7 SC CEF 3	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.35		
480	SS T7 SC CEF 4	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.35		
481	SS T7 SC CEF 5	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.43		
482	SS T7 SC CEF 6	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.35		
483	SS T7 PIA COL 1	Soil	Pseudomonas Isolation Agar	*Providencia*	*alcalifaciens*	2.34		
484	SS T7 PIA COL 2	Soil	Pseudomonas Isolation Agar	*Providencia*	*alcalifaciens*	2.38		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
485	SS T7 PIA KAN 1	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.29		
486	SS T7 PIA KAN 2	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.37		
487	SS T7 PIA KAN 3	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.2		
488	SS T7 PIA KAN 4	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.02		
489	SS T7 PIA KAN 5	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.16		
490	SS T7 PIA KAN 6	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.12		
491	SP T7 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.01		
492	SP T7 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.14		
493	SP T7 LAM CEF 3	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.18		
494	SP T7 LAM CEF 4	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.02		
495	SP T7 LAM CIPRO 1	Soil	Leeds Agar (Acinetobacter)	*Achromobacter*	*piechaudii*	2.03		
496	SP T7 LAM CIPRO 2	Soil	Leeds Agar (Acinetobacter)	*Achromobacter*	*piechaudii*	2.07		
497	SP T7 LAM CIPRO 5	Soil	Leeds Agar (Acinetobacter)	*Achromobacter*	*mucicolens*	2.12		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
498	SP T7 EMB COL 1	Soil	EMB	*Hafnia*	*alvei*	2.48		
499	SP T7 EMB COL 2	Soil	EMB	*Providencia*	*alcalifaciens*	2.50		
500	SP T7 EMB CEF 1	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.43		
501	SP T7 EMB CEF 2	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.16		
502	SP T7 EMB CEF 3	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.33		
503	SP T7 EMB CEF 4	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.43		
504	SP T7 EMB CEF 5	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.21		
505	SP T7 EMB CEF 6	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.38		
506	SP T7 PIA CIPRO 1	Soil	Pseudomonas Isolation Agar	*Stenotrophomonas*	*maltophilia*	2.19		
507	SP T7 PIA CIPRO 2	Soil	Pseudomonas Isolation Agar	*Achromobacter*	*spanius*	2.05		
508	SP T7 PIA CIPRO 3	Soil	Pseudomonas Isolation Agar	*Stenotrophomonas*	*maltophilia*	2.13		
509	SP T7 PIA KAN 1	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.21		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
510	SP T7 PIA KAN 2	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.14		
511	SP T7 PIA KAN 3	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.12		
512	SP T7 PIA KAN 4	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.24		
513	SP T7 PIA KAN 6	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.13		
514	SP T7 PIA COL 1	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.07		
515	SP T7 PIA COL 2	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.18		
516	SP T7 PIA COL 3	Soil	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.38		
517	GB T7 EMB COL 1	Grass	EMB	*Hafnia*	*alvei*	2.53		
518	GB T7 EMB CEF 3	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.42		
519	GB T7 EMB CEF 4	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.35		
520	GB T7 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Providencia*	*alcalifaciens*	2.45		
521	GB T7 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.37		
522	GB T7 PIA COL 3	Grass	Pseudomonas Isolation Agar	*Providencia*	*alcalifaciens*	2.42		
523	GB T7 PIA COL 4	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.39		
524	GB T7 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.22		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
525	GB T7 PIA KAN 2	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.30		
526	GB T7 PIA KAN 3	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.27		
527	GB T7 PIA KAN 4	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.18		
528	GB T7 PIA KAN 5	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.23		
529	GB T7 PIA KAN 6	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.17		
530	GB T7 PIA CEF 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.17		
531	GS T7 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.21		
532	GS T7 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.17		
533	GS T7 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.48		
534	GS T7 LAM CEF 4	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.28		
535	GS T7 SC KAN 1	Grass	Simmon Citrate Agar	*Citerobacter*	*gillenii*	2.39		
536	GS T7 SC CEF 1	Grass	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.39		
537	GS T7 SC CEF 2	Grass	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.4		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
538	GS T7 SC CEF 3	Grass	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.35		
539	GS T7 SC CEF 4	Grass	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.24		
540	GS T7 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Providencia*	*alcalifaciens*	2.44		
541	GS T7 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Providencia*	*alcalifaciens*	2.47		
542	GS T7 PIA COL 3	Grass	Pseudomonas Isolation Agar	*Providencia*	*alcalifaciens*	2.44		
543	GS T7 PIA COL 4	Grass	Pseudomonas Isolation Agar	*Providencia*	*alcalifaciens*	2.36		
544	GS T7 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.17		
545	GS T7 PIA KAN 2	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.25		
546	GS T7 PIA KAN 3	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.05		
547	GS T7 PIA KAN 4	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.13		
548	GS T7 PIA KAN 5	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.13		
549	GS T7 PIA KAN 6	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.31		
550	GS T7 EMB CEF 2	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.32		
551	GS T7 EMB CEF 3	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.45		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
552	GS T7 EMB CEF 4	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.43		
553	GS T7 EMB CIPRO 2	Grass	EMB	no ID	no ID	1.31	EFP149	Psuedomonas/Stenotrophomonas
554	GC T7 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.15		
555	n/a	n/a	n/a	*Kluyvera*	*intermedia*	2.06		
556	GC T7 PIA KAN 2	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.19		
557	GC T7 PIA KAN 3	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.02		
558	GC T7 PIA KAN 4	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.14		
559	GC T7 PIA KAN 5	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.2		
560	GC T7 PIA KAN 6	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.22		
561	GC T7 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.06		
562	GC T7 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Providencia*	*retteri*	2.43		
563	GC T7 PIA COL 3	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.03		
564	GC T7 PIA COL 4	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgerii*	2		
565	GC T7 PIA CEF 3	Grass	Pseudomonas Isolation Agar	*Pseudomonas*	*fulva*	2.22		
566	GC T7 PIA CEF 4	Grass	Pseudomonas Isolation Agar	*Pseudomonas*	*fulva*	2.22		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
567	GC T7 EMB COL 2	Grass	EMB	*Providencia*	*stuartii*	2.44		
568	GC T7 EMB COL 3	Grass	EMB	*Providencia*	*stuartii*	2.32		
569	GC T7 EMB COL 4	Grass	EMB	*Serratia*	*liquefaciens*	2.53		
570	GC T7 SC COL 1	Grass	Simmon Citrate Agar	*Raoultella*	*planticola*	2.53		
571	GC T7 SC COL 2	Grass	Simmon Citrate Agar	*Raoultella*	*planticola*	2.46		
572	GC T7 SC CEF 2	Grass	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.19		
573	GC T7 SC CEF 3	Grass	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.27		
574	GC T7 SC CEF 4	Grass	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.26		
575	GC T7 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.17		
576	GC T7 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2		
577	GC T7 EMB CIPRO 2	Grass	EMB	*Stenotrophomonas*	*maltophilia*	2.17		
578	GS T7 LAM KAN 1	Grass	Leeds Agar (Acinetobacter)	*Citerobacter*	*gillenii*	2.41		
579	GS T7 SC COL 1	Grass	Simmon Citrate Agar	*Serratia*	*liquefaciens*	2.42		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
580	GC T7 PIA CIPRO 4	Grass	Pseudomonas Isolation Agar	*Achromobacter*	*spanius*	2.05		
581	GP T7 LAM COL 1	Grass	Leeds Agar (Acinetobacter)	*Kurthia*	*gibsonii*	2.24		
582	GP T7 LAM COL 2	Grass	Leeds Agar (Acinetobacter)	*Kurthia*	*gibsonii*	2.23		
583	GP T7 LAM COL 3	Grass	Leeds Agar (Acinetobacter)	*Kurthia*	*gibsonii*	2.21		
584	GP T7 LAM COL 4	Grass	Leeds Agar (Acinetobacter)	*Kurthia*	*gibsonii*	2.21		
585	GP T7 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.26		
586	GP T7 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.29		
587	GP T7 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.29		
588	GP T7 LAM CEF 4	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.2		
589	GP T7 LAM CEF 5	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.36		
590	GP T7 LAM CEF 6	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.3		
591	GP T7 EMB CEF 3	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.2		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
592	GP T7 EMB CEF 4	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.17		
593	GP T7 SC CEF 1	Grass	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.22		
594	GP T7 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.18		
595	GP T7 PIA KAN 2	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2		
596	GP T7 PIA KAN 3	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.22		
597	GP T7 PIA KAN 4	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.26		
598	GP T7 PIA KAN 5	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.24		
599	GP T7 PIA KAN 6	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.2		
600	GP T7 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.32		
601	GP T7 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.49		
602	GP T7 PIA COL 3	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.41		
603	GP T7 PIA COL 4	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.41		
604	SP T8 PIA KAN 1	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.13		
605	SP T8 PIA KAN 3	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.14		
606	SP T8 PIA COL 1	Soil	Pseudomonas Isolation Agar	*Providencia*	*rustigianii*	2.4		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
607	SP T8 PIA COL 4	Soil	Pseudomonas Isolation Agar	*Providencia*	*rustigianii*	2.49		
608	SP T8 SC COL 1	Soil	Simmon Citrate Agar	*Raoultella*	*ornithinolytica*	2.57		
609	SP T8 SC CEF 1	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	N/A	EFP136	99.81%
610	SP T8 SC CEF 2	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.31		
611	SP T8 EMB CEF 1	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.5		
612	SP T8 EMB CEF 2	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.03		
613	SP T8 EMB CEF 3	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.28		
614	SP T8 EMB CEF 4	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.38		
615	SP T8 EMB KAN 1	Soil	EMB	*Citrobacter*	*gillenii*	2.45		
616	SP T8 EMB KAN 2	Soil	EMB	*Citrobacter*	*gillenii*	2.35		
617	SP T8 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.32		
618	SP T8 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.22		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
620	SS T8 EMB KAN 1	Soil	EMB	*Citrobacter*	*gillenii*	2.41		
621	SS T8 EMB KAN 2	Soil	EMB	*Citrobacter*	*gillenii*	2.28		
622	SS T8 EMB KAN 3	Soil	EMB	*Citrobacter*	*gillenii*	2.44		
623	SS T8 EMB CEF 2	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.03		
624	SS T8 EMB CEF 3	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.33		
625	SS T8 EMB CEF 4	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.1		
626	SS T8 EMB CIPRO 2	Soil	EMB	*Stenotrophomonas*	*maltophilia*	N/A	EFP135	99.28%
628	SB T8 EMB CEF 1	Soil	EMB	*Acinetobacter*	*calcoaceticus*	N/A	EFP125	99.02%
629	SB T8 EMB CEF 2	Soil	EMB	*Kluyvera*	*intermedia*	2.1		
630	SB T8 EMB CEF 3	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.39		
631	SB T8 EMB CEF 4	Soil	EMB	*Acinetobacter*	*calcoaceticus*	N/A	EFP132	99.64%
632	SB T8 EMB CEF 5	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.15		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
633	SB T8 SC CEF 2	Soil	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.03		
634	SB T8 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.17		
635	SB T8 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.12		
636	SB T8 LAM CEF 3	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.16		
637	SB T8 LAM CEF 4	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.37		
638	SC T8 EMB KAN 1	Soil	EMB	*Citrobacter*	*gillenii*	2.43		
639	SC T8 EMB KAN 2	Soil	EMB	*Citrobacter*	*gillenii*	2.4		
640	SC T8 EMB COL 1	Soil	EMB	*Serratia*	*fonticola*	2.55		
641	SC T8 EMB COL 2	Soil	EMB	*Serratia*	*fonticola*	2.49		
642	SC T8 EMB COL 4	Soil	EMB	*Serratia*	*liquefaciens*	2.29		
643	SC T8 EMB CEF 1	Soil	EMB	*Acinetobacter*	*calcoaceticus*	N/A	EFP130	99.81
644	SC T8 EMB CEF 2	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.48		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
645	SC T8 EMB CEF 3	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.12		
646	SC T8 EMB CIPRO 1	Soil	EMB	*Raoultella*	*ornithinolytica*	2.53		
647	SC T8 PIA KAN 1	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.15		
648	SC T8 PIA KAN 2	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.16		
650	SC T8 PIA KAN 4	Soil	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.22		
651	SC T8 SC COL 1	Soil	Simmon Citrate Agar	*Raoultella*	*ornithinolytica*	2.43		
652	SC T8 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	N/A	EFP129	100%
653	SC T8 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.15		
654	SC T8 LAM CEF 3	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	N/A	EFP131	99.91%
655	SC T8 LAM CEF 4	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	N/A	EFP128	99.18%
656	GP T8 EMB COL 1	Grass	EMB	*Hafnia*	*alvei*	2.45		
657	GP T8 EMB CEF 1	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.45		
658	GP T8 EMB CEF 2	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.24		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
659	GP T8 EMB CEF 3	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.34		
660	GP T8 EMB KAN 1	Grass	EMB	*Citrobacter*	*gillenii*	2.4		
662	GP T8 EMB KAN 3	Grass	EMB	*Citrobacter*	*gillenii*	2.3		
663	GP T8 EMB KAN 4	Grass	EMB	*Citrobacter*	*gillenii*	2.41		
664	GP T8 PIA CEF 2	Grass	Pseudomonas Isolation Agar	*Pseudomonas*	*punonesis*	N/A	EFP150	99.8-%
665	GP T8 PIA CEF 3	Grass	Pseudomonas Isolation Agar	*Pseudomonas*	*flavescens*	2.08		
666	GP T8 SC COL 3	Grass	Simmon Citrate Agar	*Raoultella*	*planticola*	2.29		
667	GP SC CEF 1	Grass	Simmon Citrate Agar	*Acinetobacter*	*calcoaceticus*	2.32		
668	GP T8 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	N//A	EFP146	99.67%
669	GP T8 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	N//A	EFP151	100%
670	GP T8 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Pseudomonas*	*flavescens*	2.07		
671	GP T8 LAM CEF 4	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.38		
672	GS T8 EMB CEF 1	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.25		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
673	GS T8 EMB CEF 2	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.09		
674	GS T8 EMB CEF 3	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.38		
675	GS T8 EMB CIPRO 3	Grass	EMB	*Stenotrophomonas*	*maltophilia*	2.04		
676	GS T8 EMB CIPRO 4	Grass	EMB	*No ID*	*No ID*	No ID		
677	GS T8 EMB KAN 1	Grass	EMB	*Citrobacter*	*gillenii*	2.14		
678	GS T8 EMB KAN 2	Grass	EMB	*Citrobacter*	*freundii*	2.03		
679	GS T8 SC COL 1	Grass	Simmon Citrate Agar	*Raoultella*	*ornithinolytica*	2.5		
680	GS T8 SC CEF 1	Grass	Simmon Citrate Agar	*No ID*	*No ID*	N/A		
681	GS T8 EMB COL 1	Grass	EMB	*Serratia*	*liquefaciens*	2.43		
682	GS T8 PIA CEF 1	Grass	Pseudomonas Isolation Agar	*Enterococcus*	*gallinarum*	2.11		
683	GS T8 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.24		
684	GS T8 PIA KAN 2	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.11		
686	GS T8 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Kluyvera*	*intermedia*	2.13		
687	GS T8 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.31		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
688	GB T8 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.41		
689	GB T8 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Serratia*	*liquefaciens*	2.37		
690	GB T8 PIA CEF 1	Grass	Pseudomonas Isolation Agar	*Stenotrophomonas*	*maltophilia*	2.2		
691	GB T8 PIA CIPRO 2	Grass	Pseudomonas Isolation Agar	*Stenotrophomonas*	*maltophilia*	N/A	EFP148	99.75
692	GB T8 PIA KAN 2	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.1		
694	GB T8 EMB COL 4	Grass	EMB	*Hafnia*	*alvei*	2.48		
695	GB T8 EMB CEF 2	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.19		
696	GB T8 EMB CEF 4	Grass	EMB	*Acinetobacter*	*calcoaceticus*	N/A	EFP145	99.82
697	GB T8 EMB KAN 1	Grass	EMB	*Citrobacter*	*gillenii*	2.32		
698	GB T8 EMB KAN 2	Grass	EMB	*Providencia*	*rettgeri*	2.39		
699	GB T8 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	N/A	EFP146	99.67
700	GB T8 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.26		
701	GB T8 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.17		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
702	GB T8 LAM CEF 4	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.21		
703	GC T8 EMB COL 1	Grass	EMB	*Providencia*	*rettgeri*	2.39		
704	GC T8 EMB CEF 1	Grass	EMB	*Acinetobacter*	*calcoaceticus*	N/A	EFP141	99.41
705	GC T8 EMB CEF 2	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.3		
706	GC T8 EMB CEF 3	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.48		
707	GC T8 EMB CEF 4	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.41		
708	GC T8 EMB KAN 1	Grass	EMB	*Citrobacter*	*gillenii*	2.47		
709	GC T8 EMB KAN 2	Grass	EMB	*Citrobacter*	*gillenii*	2.36		
710	GC T8 EMB KAN 3	Grass	EMB	*Citrobacter*	*gillenii*	2.46		
711	GC T8 EMB CIPRO 1	Grass	EMB	*Stenotrophomonas*	*maltophilia*	2.03		
712	GC T8 EMB CIPRO 2	Grass	EMB	*Stenotrophomonas*	*maltophilia*	2.05		
713	GC T8 EMB CIPRO 3	Grass	EMB	*Stenotrophomonas*	*maltophilia*	2.08		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
714	GC T8 EMB CIPRO 4	Grass	EMB	*Stenotrophomonas*	*maltophilia*	2.11		
715	GC T8 PIA CEF 1	Grass	Pseudomonas Isolation Agar	*Stenotrophomonas*	*maltophilia*	2.04		
716	GC T8 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.28		
717	GC T8 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Providencia*	*rettgeri*	2.32		
718	GC T8 PIA CIPRO 1	Grass	Pseudomonas Isolation Agar	*Stenotrophomonas*	*maltophilia*	2.07		
719	GC T8 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.24		
720	GC T8 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.04		
721	GC T8 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	N/A	EFP140	100
722	GC T8 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.01		
723	GC T8 LAM CEF 4	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.12		
724	GS T8 LAM CIPRO 1	Grass	Leeds Agar (Acinetobacter)	*Stenotrophomonas*	*maltophilia*	N/A	EFP154	99.82
725	GC T9 SC COL 1	Grass	Simmon Citrate Agar	*Serratia*	*liquefaciens*	2.29		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
726	GC T9 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Pseudomonas*	*flavescens*	2.1		
727	GC T9 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.43		
728	GC T9 LAM CEF 4	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.32		
729	GC T9 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Serratia*	*liquefaciens*	2.23		
730	GC T9 EMB CEF 1	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.2		
731	GC T9 EMB COL 1	Grass	EMB	*Hafnia*	*alvei*	2.5		
732	GP T9 EMB CEF 1	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.36		
733	GP T9 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Providencia*	*rustigianii*	2.21		
734	GP T9 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.24		
735	GP T9 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Pseudomonas*	*flavescens*	2.13		
736	GP T9 LAM CEF 3	Grass	Leeds Agar (Acinetobacter)	*Pseudomonas*	*flavescens*	2.14		
737	GS T9 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Serratia*	*fonticola*	2.44		
738	GS T9 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Providencia*	*rustigianii*	2.33		
739	GS T9 PIA COL 3	Grass	Pseudomonas Isolation Agar	*Enterobacter*	*cloacae*	2.04		

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
740	GB T9 PIA KAN 1	Grass	Pseudomonas Isolation Agar	*Kluyvera*	*intermedia*	2.19		
741	GB T9 PIA COL 1	Grass	Pseudomonas Isolation Agar	*Serratia*	*liquefaciens*	2.20		
742	GB T9 PIA COL 2	Grass	Pseudomonas Isolation Agar	*Serratia*	*fonticola*	2.45		
743	SP T9 EMB CEF 1	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.03		
744	SP T9 EMB CEF 5	Soil	EMB	*Acinetobacter*	*calcoaceticus*	2.08		
745	SP T9 SC CEF 1	Soil	SC	*Acinetobacter*	*calcoaceticus*	2.08		
746	SP T9 SC KAN 1	Soil	SC	*Alcaligenes*	*faecalis*	2.2		
747	SP T9 PIA KAN 1	Soil	PIA	*Achromobacter*	*spanius*	2.27		
748	SP T9 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.21		
749	SP T9 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.29		
750	SP T9 LAM CEF 3	Soil	Leeds Agar (Acinetobacter)	*Citerobacter*	*gillenii*	2.5		
751	SP T9 LAM CEF 4	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	N/A	EFP133	99.74

Sample Number	Sample Name	Origin of sample	Agar Isolate was Selected On	Genus	Species	Score Value	Identified with PCR: BARCODE	Identified with PCR: % ID
752	SS T9 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.3		
753	SS T9 LAM CEF 2	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.53		
754	SS T9 EMB KAN 4	Soil	EMB	*Citrobacter*	*gillenii*	2.54		
755	SS T9 EMB COL 1	Soil	EMB	*Hafnia*	*alvei*	2.31		
756	SB T9 SC COL 1	Soil	Simmon Citrate Agar	*Serratia*	*marcescens*	2.24		
757	SB T9 SC COL 2	Soil	Simmon Citrate Agar	*Serratia*	*marcescens*	2.29		
758	SB T9 LAM CEF 1	Soil	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.04		
759	GP T9 EMB CEF 1	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.11		
760	GP T9 EMB CEF 2	Grass	EMB	*Acinetobacter*	*calcoaceticus*	2.23		
761	GS T9 LAM CEF 1	Grass	Leeds Agar (Acinetobacter)	*Serratia*	*fonticola*	2.48		
762	GS T9 LAM CEF 2	Grass	Leeds Agar (Acinetobacter)	*Acinetobacter*	*calcoaceticus*	2.07		
763	GS T9 SC COL 1	Grass	Simmon Citrate Agar	*Serratia*	*fonticola*	2.53		
764	GS T9 SC COL 2	Grass	Simmon Citrate Agar	*Serratia*	*fonticola*	2.25		

Appendix 30: Disk test results for E.coli, K.pneumonaie and Acinetobacter spp. *Green indicates the bacteria was susceptible to an antibiotic where as orange indicates intermediate susceptibility and red indicates resistance to an antibiotic.*

Isolate Name	Sample Origin	Tet (30 µg)	Cefotaxime (5 µg)	Kanamycin (30 µg)	Amikacin (30 µg)	Ciprofloxacin (5 µg)	Imipenem (10 µg)
Pig Manure		Zone Diameter in millimetres (mm)					
SM EMB CEF 5	Pig Manure	0	28	0	23	33	27
SM EMB CIPRO 1	Pig Manure	0	26	13	22.5	0	28.5
SM EMB CIPRO 2	Pig Manure	0	26	12.5	23	0	26
SM EMB CIPRO 3	Pig Manure	13.5	22	13	18.5	0	23.5
SM EMB CIPRO 4	Pig Manure	0	25	12	22.5	0	28
SM EMB CIPRO 5	Pig Manure	0	25	22.5	20	0	27
SM EMB CIPRO 6	Pig Manure	0	26.5	21	21.5	0	28
Cow Manure							
CM EMB CEF 1	Cow Manure	0	0	20	21	0	27
CM EMB CEF 2	Cow Manure	0	0	17.5	18.5	0	27.5
CM EMB CEF 3	Cow Manure	0	0	19	19.5	0	27.5
CM EMB CEF 4	Cow Manure	0	0	20	20.5	0	28
CM EMB KAN 1	Cow Manure	16	27.5	0	20	31	27
CM EMB KAN 2	Cow Manure	17	21.5	0	21.5	32	26
CM EMB KAN 3	Cow Manure	15	27	0	21	30	27
CM EMB KAN 4	Cow Manure	0	28.5	0	20	35	27.5

Isolate Name	Sample Origin	Tet (30 µg)	Cefotaxime (5 µg)	Kanamycin (30 µg)	Amikacin (30 µg)	Ciprofloxacin (5 µg)	Imipenem (10 µg)
CM EMB CIPRO 1	Cow Manure	0	0	20.5	20.5	0	27.5
CM EMB CIPRO 2	Cow Manure	8	0	18	18	0	28
CM EMB CIPRO 3	Cow Manure	0	0	20	20	0	27
CM LAM CEF 3	Cow Manure	0	0	18.5	21.5	0	28.5
CM LAM CEF 4	Cow Manure	0	0	19	20	0	26
CM LAM KAN 1	Cow Manure	16.5	27	0	23	32	25
Soil							
SC NM EMB KAN 1	Soil	0	24	0	20.5	33	26
SC NM EMB KAN 2	Soil	0	27.5	0	21.5	32	27.5
SC NM EMB KAN 3	Soil	0	24.5	0	22	34	25.5
SC NM EMB KAN 4	Soil	0	27	0	23	33	26
SC NM EMB KAN 5	Soil	0	25.5	0	21	33	25
SC NM EMB KAN 6	Soil	0	25.5	0	21.5	33	28
SS T3 PIA CEF 6	Soil	15.5	29	21.5	19.5	32	27
SC T7 EMB COL 2	Soil	16.5	25	21	18	29	27
SC T7 EMB COL 3	Soil	16.5	28	20	22	29	27.5
SC T7 EMB COL 4	Soil	17.5	28	20	22	31.5	27
SS T7 EMB CEF 3	Soil	20	38	22	20	31	27
Grass							

Isolate Name	Sample Origin	Tet (30 µg)	Cefotaxime (5 µg)	Kanamycin (30 µg)	Amikacin (30 µg)	Ciprofloxacin (5 µg)	Imipenem (10 µg)
GS T1 LAM CIPRO 1	Grass	0	27	19	20.5	24.5	27
GS T1 LAM CIPRO 3	Grass	0	28	21	21.5	26	27
GS T1 LAM CIPRO 4	Grass	0	26.5	19.5	21	25	29
GS T1 LAM CIPRO 5	Grass	0	26.5	20.5	21	24.5	31
GP T3 EMB COL 2	Grass	0	24.5	18	18	12	30
GB T5 LAM KAN 1	Grass	0	28	0	21	35	29
GB T5 LAM KAN 2	Grass	19.5	22	19	20	30	21
GB T5 LAM KAN 3	Grass	0	26	0	22	35	27.5
GB T5 EMB KAN 1	Grass	0	29	0	21.5	34	28
GB T5 EMB KAN 2	Grass	0	27	0	21	34	26.5
GB T5 EMB KAN 3	Grass	0	27	0	22	31	26
GB T5 EMB KAN 4	Grass	0	28	0	21.5	31	25.5
GB T5 EMB KAN 5	Grass	0	27.5	0	22	35	27
GB T5 EMB KAN 6	Grass	0	28	0	22	35	28
K.pneumonaie							
Isolate Name	Sample Origin	Tet (30 µg)	Cefotaxime (5 µg)	Kanamycin (30 µg)	Amikacin (30 µg)	Ciprofloxacin (5 µg)	Imipenem (10 µg)
GC T5 SC COL 2	Grass	19	25	20.5	20	28	24.5

Isolate Name	Sample Origin	Tet (30 µg)	Cefotaxime (5 µg)	Kanamycin (30 µg)	Amikacin (30 µg)	Ciprofloxacin (5 µg)	Imipenem (10 µg)
GC T5 SC COL 3	Grass	20.5	19.5	16.5	20.5	29	23.5
GB T1 PIA COL 4	Grass	0	28	23	21	27	18.5
GC T1 LAM COL 1	Grass	20	22	19.5	20	22.5	24.5
GC T1 LAM COL 2	Grass	22.5	23.5	19.5	19.5	24	25
GC T1 LAM COL 3	Grass	20	22.5	20	19.5	23.5	24.5
GC T1 LAM COL 4	Grass	20.5	23	20	19	25	24.5
GC T1 LAM COL 6	Grass	20	22.5	20	19	22.5	25.5

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
Grass Background								
G2 BM SC CEF 3	baumannii	Grass	19.5	27	25.5	18.5	21.5	21
G2 BM SC CEF 5	baumannii	Grass	19	28	25.5	18	21	21.5
G2 BM SC CEF 6	baumannii	Grass	18.5	26	25	18	21	21
G2 EMB CEF 6	baumannii	Grass	18.5	25	23	19.5	21	20
G2 EMB CEF 7	baumannii	Grass	20	25	25	19	20.5	20.5
G2 BM LAM CEF 1	baumannii	Grass	20	28	25.5	20	21.5	20.5
G2 BM LAM CEF 2	baumannii	Grass	19.5	26.5	22.5	19	21.5	21

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
G2 BM LAM CEF 3	baumannii	Grass	21.5	29	24	15.5	21.5	21.5
G2 BM LAM CEF 4	baumannii	Grass	18.5	28	25	19	21.5	21.5
G2 BM LAM CEF 5	baumannii	Grass	20	27	25	18.5	20	20
Grass Control								
GC T1 EMB CEF 1	baumannii	Grass	19.5	31	25	20	22.5	21
GC T1 EMB CEF 2	baumannii	Grass	18.4	27	23.5	20	21.5	21.5
GC T1 EMB CEF 3	baumannii	Grass	18	26	24.5	18.5	20.5	21.5
GC T1 EMB CEF 4	baumannii	Grass	19	29	23.5	19	21.5	21
GC T1 SC CEF 1	baumannii	Grass	18.5	29.5	23.5	20.5	21.5	21.5
GC T1 SC CEF 2	baumannii	Grass	19	26	23	20	22	22.5
GC T1 SC CEF 3	baumannii	Grass	18	25.5	25	18	21	20
GC T1 SC CEF 4	baumannii	Grass	15.5	23.5	23.5	18	20	20.5
GC T1 LAM CEF 6	baumannii	Grass	19	25	24	18	20.5	20
GC T1 LAM CEF 1	baumannii	Grass	19	27	22.5	20	21	21
GC T1 LAM CEF 2	baumannii	Grass	19	25	23	18.5	20	20
GC T1 LAM CEF 3	baumannii	Grass	19	26.5	25	20	21.5	21

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
GC T1 LAM CEF 4	baumannii	Grass	19.5	28	22.5	20	20.5	20.5
GC T1 LAM CEF 5	baumannii	Grass	18.5	25	25	19	20.5	19.5
GC T3 SC CEF 1	baumanii	Grass	18	26.5	23.5	17.5	20.5	20.5
GC T5 SC CEF 1	calcoaceticus	Grass	17	25	22	19.5	22.5	22.5
GC T7 SC CEF 2	calcoaceticus	Grass	18.5	24	22.5	18.5	21.5	21.5
GC T7 SC CEF 3	calcoaceticus	Grass	16.5	27	23	20	23.5	23.5
GC T7 SC CEF 4	calcoaceticus	Grass	19	26	24.5	20	24	21
GC T7 LAM CEF 1	calcoaceticus	Grass	15.5	24.5	21	19.5	20	21.5
GC T7 LAM CEF 3	calcoaceticus	Grass	19.5	25	21.5	20	20	23
GC T8 EMB CEF 1	calcoaceticus	Grass	20	30	24	22	24.5	23.5
GC T8 EMB CEF 2	calcoaceticus	Grass	20	30	25	22.5	25	24
GC T8 EMB CEF 3	calcoaceticus	Grass	17	30.5	22	21.5	24	24
GC T8 EMB CEF 4	calcoaceticus	Grass	19.5	27.5	21.5	21.5	24.5	24.5
GC T8 LAM CEF 1	calcoaceticus	Grass	18.5	27.5	23.5	24	25.5	24
GC T8 LAM CEF 2	calcoaceticus	Grass	20	30	26	22	25	24.5
GC T8 LAM CEF 3	calcoaceticus	Grass	18	27	23	21.5	23.5	22.5

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
GC T8 LAM CEF 4	calcoaceticus	Grass	21	26.5	26	22.5	25	25
GC T9 LAM CEF 3	calcoaceticus	Grass	17.5	25	21.5	18	22.5	21
GC T9 LAM CEF 4	calcoaceticus	Grass	18.5	25	23	19	23	21.5
GC T9 EMB CEF 1	calcoaceticus	Grass	19.5	25.5	22	20.5	23	22
Grass Pig								
GS T1 EMB CEF 1	baumannii	Grass	19	26	24	18.5	20	20
GS T1 EMB CEF 2	baumannii	Grass	20	29	24.5	20	23.5	21
GS T1 EMB CEF 4	baumannii	Grass	20	26	23.5	20	22	21
GS T1 EMB CEF 5	baumannii	Grass	20	27.5	25	20	22	21.5
GS T1 EMB CEF 6	baumannii	Grass	18.5	27	25	20.5	21.5	21.5
GS T3 SC CEF 2	baumannii	Grass	18	28	24	20	25	22.5
GS T5 LAM CEF 1	baumannii	Grass	17.5	29	23.5	22	23.5	24
GP T1 LAM CEF 5	baumannii	Grass	17.5	25	24.5	21	20	20
GS T7 LAM CEF 1	calcoaceticus	Grass	20	25	22.5	17.5	20.5	21
GS T7 LAM CEF 2	calcoaceticus	Grass	20	26	23.5	20	23.5	21.5
GS T7 LAM CEF 3	calcoaceticus	Grass	18.5	25.5	22.5	22	23.5	23.5

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
GS T7 LAM CEF 4	calcoaceticus	Grass	20	23	23	20	21.5	22.5
GS T7 SC CEF 1	calcoaceticus	Grass	20	24	23.5	20.5	22.5	21
GS T7 SC CEF 2	calcoaceticus	Grass	21	30	23	21	24	23.5
GS T7 SC CEF 3	calcoaceticus	Grass	21	25	23	21	22.5	22.5
GS T7 SC CEF 4	calcoaceticus	Grass	20.5	25	24.5	17.5	21	19
GS T7 EMB CEF 2	calcoaceticus	Grass	19	24	23.5	23.5	20.5	21
GS T7 EMB CEF 3	calcoaceticus	Grass	18	27	22.5	21.5	21	20
GS T7 EMB CEF 4	calcoaceticus	Grass	19	25.5	23	21.5	20.5	21.5
GS T8 EMB CEF 1	calcoaceticus	Grass	23.5	27	27	20	24.5	23.5
GS T8 EMB CEF 2	calcoaceticus	Grass	20	30	23	20	24.5	23
GS T8 EMB CEF 3	calcoaceticus	Grass	19	26.5	23.5	20.5	24.5	23.5
GS T8 LAM CEF 3	calcoaceticus	Grass	19.5	27.5	24	20	24.5	23.5
GS T9 LAM CEF 2	calcoaceticus	Grass	19	24.5	23.5	20	22	22.5
Grass Chicken								
GP T1 EMB CEF 1	baumannii	Grass	25	29.5	25	20	21.5	21
GP T1 EMB CEF 2	baumannii	Grass	20	28	25	20	21	21

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
GP T1 EMB CEF 3	baumannii	Grass	19.5	26	23.5	21	21	21.5
GP T1 EMB CEF 4	baumannii	Grass	19.5	28	24	19.5	21.5	20
GP T1 EMB CEF 5	baumannii	Grass	19.5	25	24.5	19.5	21	21.5
GP T1 EMB CEF 6	baumannii	Grass	19	27	24	20	21	21
GP T1 SC CEF 3	baumannii	Grass	16.5	30	25	19	22.5	23
GP T1 SC CEF 4	baumannii	Grass	17.5	22.5	26	18.5	22.5	21
GP T1 LAM CEF 1	baumannii	Grass	18.5	26.5	25	18.5	21	20
GP T1 LAM CEF 2	baumannii	Grass	17	30	25	19	23	22
GP T1 LAM CEF 3	baumannii	Grass	17	25	26	18.5	23	22
GP T1 LAM CEF 4	baumannii	Grass	18.5	26.5	24.5	19	21	20
GP T1 LAM CEF 6	baumannii	Grass	17.5	30	25.5	19	23	22
GP T3 SC CEF 1	baumannii	Grass	19.5	25.5	23.5	19	21	21
GP T3 LAM COL 2	baumannii	Grass	20.5	27.5	25	21	21.5	20.5
GP T3 LAM COL 3	baumannii	Grass	17	26.5	23.5	15.5	23.5	22
GP T3 LAM CEF 1	baumannii	Grass	20	27	25	20	21	21
GP T3 LAM CEF 2	baumannii	Grass	17	24.5	25	17.5	22	22

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
GP T3 LAM CEF 3	baumannii	Grass	20.5	27	24	19.5	20.5	20.5
GP T3 LAM CEF 4	baumannii	Grass	17.5	25	21	19.5	20	21.5
GP T3 EMB COL 1	baumannii	Grass	19.5	25	25	23.5	20	21
GP T5 LAM CEF 1	calcoaceticus	Grass	18.5	26.5	23	21.5	23	23
GP T5 LAM CEF 2	calcoaceticus	Grass	19	25	23	20	23	22.5
GP T7 LAM CEF 1	calcoaceticus	Grass	18.5	22	23.5	17.5	20	19
GP T7 LAM CEF 2	calcoaceticus	Grass	19.5	29.5	24.5	20	25.5	24
GP T7 LAM CEF 3	calcoaceticus	Grass	20.5	25	21	19.5	21	20
GP T7 LAM CEF 4	calcoaceticus	Grass	17	25.5	21.5	20	21.5	20
GP T7 LAM CEF 5	calcoaceticus	Grass	20	29	21.5	18.5	22.5	19.5
GP T7 LAM CEF 6	calcoaceticus	Grass	18.5	25	24	19	23	22
GP T7 EMB CEF 3	calcoaceticus	Grass	18	25.5	22	20	22.5	23
GP T7 EMB CEF 4	calcoaceticus	Grass	18.5	24.5	22.5	20.5	22.5	22
GP T7 SC CEF 1	calcoaceticus	Grass	19	25.5	22.5	19	22	22.5
GP T8 EMB CEF 1	calcoaceticus	Grass	21.5	28	24	20.5	22.5	24
GP T8 EMB CEF 2	calcoaceticus	Grass	22	25.5	23	20.5	25	20

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
GP T8 EMB CEF 3	calcoaceticus	Grass	19.5	29	23	21.5	24.5	24
GP T8 SC CEF 1	calcoaceticus	Grass	18.5	28	22.5	19.5	23.50	23
GP T9 LAM CEF 1	calcoaceticus	Grass	20	27.5	21	20.5	23	21.5
GP T8 LAM CEF 1	calcoaceticus	Grass	18.5	27.5	24.5	22.5	25	24
GP T8 LAM CEF 2	calcoaceticus	Grass	20	31	25.5	21	24	23.5
GP T8 LAM CEF 4	calcoaceticus	Grass	18.5	28	25	20	22.5	23
GP T9 EMB CEF 1	calcoaceticus	Grass	17.5	25.5	22.5	20.5	23.5	23.5
GP T9 EMB CEF 1	calcoaceticus	Grass	20	25	23	20.5	22.5	22.5
GP T9 EMB CEF 2	calcoaceticus	Grass	17.5	25	22.5	18	20.5	22.5
Grass Cow								
GB T1 LAM CIPRO 5	indicus	Grass	20.5	29	21	20.5	14.5	23
GB T1 LAM CIPRO 6	indicus	Grass	20	29.5	23	19.5	14	23
GB T1 SC CEF 1	baumannii	Grass	19	29	23	18.5	21	20.5
GB T3 LAM CEF 1	baumannii	Grass	19.5	29	24.5	20	24	20
GB T3 LAM CEF 2	baumannii	Grass	19	29	25.5	20	21.5	20
GB T3 LAM CEF 5	baumannii	Grass	19	26	23	19.5	23	21

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
GB T3 LAM CEF 6	baumannii	Grass	20	30	25.5	20	23	21.5
GB T3 LAM CIPRO 2	indicus	Grass	20	28	21	19.5	0	24.5
GB T3 LAM CIPRO 4	baumannii	Grass	19	22.5	25	20	21	21
GB T3 LAM COL 1	baumannii	Grass	18.5	29.5	26	19.5	21	20.5
GB T3 EMB CEF 1	baumannii	Grass	19	26.5	24	18.5	21	21
GB T3 EMB CEF 2	baumannii	Grass	18.5	26.5	23.5	19	20	20
GB T7 EMB CEF 3	calcoaceticus	Grass	18	30	23.5	22	23	22.5
GB T7 EMB CEF 4	calcoaceticus	Grass	18	28	25.5	21	23	23.5
GB T8 EMB CEF 2	calcoaceticus	Grass	19.5	28	23.5	21.5	23	23.5
GB T8 EMB CEF 4	calcoaceticus	Grass	19.5	27	25	22	23.5	23.5
GB T8 LAM CEF 1	calcoaceticus	Grass	17	29	24	21.5	23.5	23
GB T8 LAM CEF 2	calcoaceticus	Grass	18	25	21	20	23.5	23
GB T8 LAM CEF 3	calcoaceticus	Grass	21	26	23.5	22	23.5	23.5
GB T8 LAM CEF 4	calcoaceticus	Grass	18	27.5	23	22	24.5	23.5
Soil Background								
SC NM LAM CEF 1	calcoaceticus	Soil	20	26	27	21.5	23.5	22
Soil Control								

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
SC T1 EMB CEF 1	calcoaceticus	Soil	23	33	24	22	26	27
SC T1 EMB CEF 2	calcoaceticus	Soil	19	26	23	20	23	23.5
SC T1 EMB CEF 3	calcoaceticus	Soil	20	32	22.5	20	23.5	22
SC T1 EMB CEF 4	calcoaceticus	Soil	19	30	23.5	20	24	22
SC T1 EMB CEF 5	calcoaceticus	Soil	20	29	22	22.5	23	24
SC T1 EMB CEF 6	calcoaceticus	Soil	19.5	25.5	21.5	21.5	24	24.5
SC T1 EMB CEF 7	calcoaceticus	Soil	17	28	21	20	23.5	22.5
SC T1 EMB CEF 8	calcoaceticus	Soil	18.5	26	23.5	21.5	23	25
SC T1 SC CEF 1	calcoaceticus	Soil	18.5	24	21	21.5	23.5	22
SC T1 SC CEF 2	calcoaceticus	Soil	17.5	24.5	22	20	21.5	23
SC T1 SC CEF 3	calcoaceticus	Soil	20	24.5	23	21	23.5	22.5
SC T1 SC CEF 4	calcoaceticus	Soil	19	29	23	19	22	22
SC T3 SC CEF 2	calcoaceticus	Soil	20	30	24	21	18.5	20
SC T3 SC CEF 3	calcoaceticus	Soil	19	28.5	23.5	18	19.5	18.5
SC T3 SC CEF 4	calcoaceticus	Soil	20	29	23	17.5	20	21.5
SC T5 SC CEF 1	calcoaceticus	Soil	18.5	25.5	22.5	20.5	23.5	23.5

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
SC T5 SC CEF 2	calcoaceticus	Soil	19	30	22.5	20	25	25
SC T5 EMB CEF 1	calcoaceticus	Soil	20	25.5	23.5	20	25	24
SC T5 EMB CEF 2	calcoaceticus	Soil	17.5	28	22	20	24.5	22
SC T5 EMB CEF 3	calcoaceticus	Soil	20.5	30	23	20	23	23
SC T5 EMB CEF 4	calcoaceticus	Soil	17.5	25.5	21.5	20.5	22.5	22
SC T5 LAM CEF 1	calcoaceticus	Soil	17.5	25	22	19	22.5	24
SC T5 LAM CEF 2	calcoaceticus	Soil	18	25	21	21	25	23.5
SC T5 LAM CEF 3	calcoaceticus	Soil	19	25.5	23	20.5	25	24.5
SC T5 LAM CEF 4	calcoaceticus	Soil	20	28	25	20.5	24	23
SC T5 LAM CEF 6	calcoaceticus	Soil	17.5	27	24.5	26	25	24.5
SC T7 SC CEF 1	calcoaceticus	Soil	20	23	24	18	23	31
SC T7 LAM CEF 1	calcoaceticus	Soil	19.5	29	23	22.5	25	23.5
SC T7 LAM CEF 2	calcoaceticus	Soil	20	29	23	21	18.5	20
SC T7 LAM CEF 3	calcoaceticus	Soil	19.5	30	24	18	25	20
SC T7 LAM CEF 4	calcoaceticus	Soil	18	24.5	23.5	20.5	23	23.5
SC T7 LAM CEF 5	calcoaceticus	Soil	19	23	21.5	20	23	21

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
SC T7 LAM CEF 6	calcoaceticus	Soil	19	25.5	23.5	19.5	23.5	20.5
SC T8 EMB CEF 1	calcoaceticus	Soil	20	26.5	24,5	21	24.5	22.5
SC T8 EMB CEF 2	calcoaceticus	Soil	20.5	29	24.5	21,5	25.5	24
SC T8 EMB CEF 3	calcoaceticus	Soil	20	25.5	22	21	24	24
SC T8 LAM CEF 1	calcoaceticus	Soil	19.5	30	24.5	22	25	23
SC T8 LAM CEF 2	calcoaceticus	Soil	20	28.5	24	21.5	25	23
SC T8 LAM CEF 3	calcoaceticus	Soil	20.5	35	25	22	25.5	24
SC T8 LAM CEF 4	calcoaceticus	Soil	21	27	24.5	21	25	24
Soil Pig								
SS T1 SC CEF 1	calcoaceticus	Soil	18	25	22.5	20	22.5	22
SS T1 EMB CEF 1	calcoaceticus	Soil	18.5	28	25	21.5	23	22.5
SS T1 EMB CEF 2	calcoaceticus	Soil	17.5	28	21.5	20.5	22.5	22
SS T1 EMB CEF 3	calcoaceticus	Soil	20	27	26	23	21.5	20.5
SS T1 LAM CEF 4	calcoaceticus	Soil	16	25	22	20.5	23	22.5
SS T5 LAM CEF 1	calcoaceticus	Soil	19.5	29	21.5	21	24	23.5
SS T5 LAM CEF 2	calcoaceticus	Soil	17	26	21	20	24	23

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
SS T5 LAM CEF 3	calcoaceticus	Soil	17	27.5	22	20	23.5	23.5
SS T5 LAM CEF 4	calcoaceticus	Soil	17.5	25.5	21.5	20.5	23	21.5
SS T7 LAM CEF 1	calcoaceticus	Soil	21	29	23.5	21	21.5	22
SS T7 LAM CEF 3	calcoaceticus	Soil	17	26	21.5	20.5	23.5	22
SS T7 EMB CEF 4	calcoaceticus	Soil	18	27	22	22	23	24
SS T7 SC CEF 1	calcoaceticus	Soil	20	27	21	22	22.5	22.5
SS T7 SC CEF 2	calcoaceticus	Soil	17.5	25	23	20	21	23
SS T7 SC CEF 3	calcoaceticus	Soil	22	28	25	22	23	22
SS T7 SC CEF 4	calcoaceticus	Soil	20	26	23.5	22	22	22
SS T7 SC CEF 5	calcoaceticus	Soil	20	25	23	21	21	21.5
SS T7 SC CEF 6	calcoaceticus	Soil	20	27	22.5	20.5	21	22
SS T8 EMB CEF 2	calcoaceticus	Soil	19	28	24	22	25	24.5
SS T8 EMB CEF 3	calcoaceticus	Soil	19.5	29	25	23.5	26	24.5
SS T8 EMB CEF 4	calcoaceticus	Soil	19	27	23.5	20.5	24	22.5
SS T9 LAM CEF 1	calcoaceticus	Soil	17	23.5	22.5	20	23	22
SS T9 LAM CEF 2	calcoaceticus	Soil	18.5	34	22.5	20.5	22.5	22.5

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
Soil Chicken								
SP T1 EMB CEF 1	calcoaceticus	Soil	17	27	25	21	21.5	22
SP T1 EMB CEF 2	calcoaceticus	Soil	18	25.5	25	20	23.5	23.5
SP T1 EMB CEF 4	calcoaceticus	Soil	17.5	28	25	19	22.5	23
SP T1 LAM CEF 1	calcoaceticus	Soil	17.5	27	27.5	20	22.5	23
SP T1 LAM CEF 2	calcoaceticus	Soil	18,5	23	25.5	19	22.5	21.5
SP T1 LAM CEF 5	calcoaceticus	Soil	18.5	25	28	20.5	23.5	23.5
SP T3 EMB CEF 9	calcoaceticus	Soil	20	26.5	23.5	21	24	23
SP T3 EMB CEF 10	calcoaceticus	Soil	21	28	25.5	22	24.5	24.5
SP T3 EMB CEF 1	baumannii	Soil	20	26	22	19.5	23.5	23.5
SP T3 EMB CEF 3	baumannii	Soil	20	27.5	22	21.5	23.5	23.5
SP T3 EMB CEF 4	baumannii	Soil	18.5	30	24	20.5	24	23
SP T3 SC CEF 1	calcoaceticus	Soil	19.5	28	22.5	19	22.5	24
SP T3 SC CEF 2	baumannii	Soil	19	27	23	20	23	23.5
SP T5 LAM CEF 1	calcoaceticus	Soil	19	25.5	23	21	23.5	23.5
SP T5 LAM CEF 2	calcoaceticus	Soil	17.5	26	21.5	20.5	22.5	24.5

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
SP T5 LAM CEF 3	calcoaceticus	Soil	17.5	25	24	20.5	21.5	22
SP T5 LAM CEF 4	calcoaceticus	Soil	18	27	22.5	20	23	22
SP T5 LAM CEF 5	calcoaceticus	Soil	15.5	26	23	21	23	23
SP T5 LAM CEF 6	calcoaceticus	Soil	17.5	24	23.5	20	23	22
SP T7 EMB CEF 1	calcoaceticus	Soil	18	26.5	25	21.5	24	24
SP T7 EMB CEF 2	calcoaceticus	Soil	19	25.5	23	20	23	21
SP T7 EMB CEF 3	calcoaceticus	Soil	18	25	23	20.5	22	22
SP T7 EMB CEF 4	calcoaceticus	Soil	17.5	25	21.5	21	22.5	22
SP T7 EMB CEF 5	calcoaceticus	Soil	17.5	26	25	23	24.5	23.5
SP T7 EMB CEF 6	calcoaceticus	Soil	18	26	22.5	21.5	23.5	22
SP T7 LAM CEF 1	calcoaceticus	Soil	18.5	26.5	23.5	21.5	24	22.5
SP T7 LAM CEF 2	calcoaceticus	Soil	18.5	28	23.5	20.5	22.5	22.5
SP T7 LAM CEF 3	calcoaceticus	Soil	18	27.5	23	20.5	23	22.5
SP T7 LAM CEF 4	calcoaceticus	Soil	19	27.5	24	21	24	22
SP T8 SC CEF 1	calcoaceticus	Soil	19	30	23.5	20.5	24.5	23.5
SP T8 SC CEF 2	calcoaceticus	Soil	19.5	27	24.5	22.5	25	24

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
SP T8 EMB CEF 1	calcoaceticus	Soil	18	29	24	20	22	22
SP T8 EMB CEF 2	calcoaceticus	Soil	17.5	28	24	23	24	23.5
SP T8 EMB CEF 3	calcoaceticus	Soil	17.5	27.5	22.5	22	22.5	22
SP T8 EMB CEF 4	calcoaceticus	Soil	17.5	25	22.5	21	23	22.5
SP T8 LAM CEF 1	calcoaceticus	Soil	20	32	27	24	25	23.5
SP T8 LAM CEF 2	calcoaceticus	Soil	19	29	23	21	23.5	23.5
SP T9 SC CEF 1	calcoaceticus	Soil	16.5	26	23	20	23	22.5
SP T9 LAM CEF 1	calcoaceticus	Soil	17	27	26.5	21	23	23
SP T9 LAM CEF 2	calcoaceticus	Soil	19	28	23.5	21	24	24
SP T9 LAM CEF 4	calcoaceticus	Soil	19.5	32	25	21	24.5	23.5
SP T9 EMB CEF 1	calcoaceticus	Soil	19	26.5	22	20.5	23	23.5
SP T9 EMB CEF 5	calcoaceticus	Soil	19.5	28	23.5	21	25	25
Soil Cow								
SB T1 LAM KAN 5	calcoaceticus	Soil	18	25	23	20	24	22.5
SB T1 SC CEF 2	calcoaceticus	Soil	17	25	23	20	21.5	22
SB T1 SC CEF 3	calcoaceticus	Soil	17.5	26.5	23.5	20	24	23

Isolate Name	Species	Origin	Ceftazidime (30 µg)	Imipenem (10 µg)	Ciprofloxacin (5 µg)	Tigecycline (15 µg)	Kanamycin (30 µg)	Amikacin (30 µg)
SB T3 SC CEF 6	calcoaceticus	Soil	18	24.5	23.5	22	24	22.5
SB T7 EMB CEF 4	calcoaceticus	Soil	20	26	21	20.5	22.5	22
SB T7 EMB CIPRO 3	calcoaceticus	Soil	28	25	21	24	21	22.5
SB T7 LAM CEF 2	calcoaceticus	Soil	16.5	24.5	22	20.5	23.5	22.5
SB T1 SC CEF 1	calcoaceticus	Soil	18	25.5	22	20	22.5	23
SB T7 LAM CEF 1	calcoaceticus	Soil	20.5	26	23	22	23	24
SB T8 EMB CEF 1	calcoaceticus	Soil	21	32	25.5	21	25	24.5
SB T8 EMB CEF 3	calcoaceticus	Soil	17	28	23	19.5	23	22.5
SB T8 EMB CEF 4	calcoaceticus	Soil	18.5	30	23	22.5	24	23.5
SB T8 EMB CEF 5	calcoaceticus	Soil	20.5	30	26	26	27	24
SB T8 SC CEF 2	calcoaceticus	Soil	19.5	27	21	20.5	22.5	22
SB T8 LAM CEF 1	calcoaceticus	Soil	18	28.5	24	21.5	23.5	24.5
SB T8 LAM CEF 2	calcoaceticus	Soil	18	28	23	21	24	23
SB T8 LAM CEF 3	calcoaceticus	Soil	20	26	23.5	20.5	23	22
SB T8 LAM CEF 4	calcoaceticus	Soil	20	28	25.4	20	25.5	22.5
SB T9 LAM CEF 1	calcoaceticus	Soil	18.5	27	22	21.5	23	23

Appendix 31: AmpC and ESBL double disk test results for cefotaxime resistant E.coli. Cells in green indicate the isolate was negative for the tested enzyme while red indicates the isolate was positive for enzyme production.

Isolate Name	**AmpC Disk Tests**			**ESBL Disk Test**		
Cow Manure	**Zone measurement with boronic acid (mm)**	**Zone measurement without boronic acid (mm)**	Zone difference	**Zone measurement with clauvalanic acid (mm)**	**Zone measurement without clauvalanic acid (mm)**	Zone Difference
CM EMB CEF 1	24	25.5	1.5	12	21	9
CM EMB CEF 2	21.5	24	2.5	13	22	9
CM EMB CEF 3	21.5	24	2.5	12	23	11
CM EMB CEF 4	20	22	2	12	23	11
CM EMB CIPRO 1	25	26.5	1.5	12	24.5	12.5
CM EMB CIPRO 2	21	24.5	3.5	11	22.5	11.5
CM EMB CIPRO 3	21.5	24	2.5	12	22.5	10.5
CM LAM CEF 3	23.5	24	0.5	11.5	21	9.5
CM LAM CEF 4	20	23	3	11.5	20.5	9

Appendix 32: Table displaying WGS data for E. coli,

Sample_Name	Taxon identified	ST	Plasmid Replicons Detected	Antibiotic Resistance Genes	Point Mutations Detected
CM EMB KAN 1	*E. coli*	106	ColRNAI_1, IncFIB(AP001918)_1, IncFII_1	aph(3'')-Ib_5, aph(6)-Id_1,aph(3')-Ia_1, blaTEM-1B_1, sul1_5, sul2_3, dfrA5_1,mdf(A)_1, sul1_5, sul2_3	parC(p.S57T)
CM EMB KAN 2	*E. coli*	1126	Col440II_1, Col44)11_1, IncFIB(AP001918)_1	aac(3)-IVa_1,aph(3'')-Ib_2, aph(3')-Ia_10, aph(4)-Ia_1, aph(6)-Id_1, floR_2, mdf(A)_1	None detected
CM EMB KAN 3	*E. coli*	1126	Col440II_1, Col44)11_1, IncFIB(AP001918)_1	aac(3)-IVa_1,aph(3'')-Ib_2, aph(3')-Ia_10, aph(4)-Ia_1, aph(6)-Id_1, floR_2, mdf(A)_1	None detected
CM EMB KAN 4	*E. coli*	58	Col(BS512)_1,Col156_1.,Col8282_1,Col440II_1,ColpVC_1, IncFIB(AP001918)_1 IncFII_1	aph(3'')-Ib_5,aph(3')-Ia_1, aph(6)-Id_1, blaTEM-104_1, dfrA5_1, floR_2, mdf(A)_1, tet(A)_6	None detected
CM EMB CIPRO 1	*E. coli*	1431	Col440II_1, IncY_1	aph(3'')-Ib_5,aph(6)-Id_1,blaCTX-M-	gyrA(p.D87N, p.S83L), parC

Sample_Name	Taxon identified	ST	Plasmid Replicons Detected	Antibiotic Resistance Genes	Point Mutations Detected
				15_1, blaTEM-1B_1, mdf(A)_1, qnrS1_1, sul2_2, tet(A)_6	(p.S80I), parE (p.S458A)
CM EMB CIPRO 2	*E. coli*	1431	Col440II_1	mdf(A)_1	gyrA(p.D87N, p.S83L), parC (p.S80I), parE (p.S458A)
CM EMB CIPRO 3	*E. coli*	1431	Col440II_1, IncY_1	aph(3")-Ib_5,aph(6)-Id_1,blaCTX-M-15_1, blaTEM-1B_1, mdf(A)_1, qnrS1_1, sul2_2, tet(A)_6	gyrA(p.D87N, p.S83L), parC (p.S80I), parE (p.S458A)
CM LAM CEF 3	*E. coli*	1431	Col440II_1, IncY_1	aph(3")-Ib_5,aph(6)-Id_1, blaCTX-M-15_1, blaTEM-1B_1, mdf(A)_1, qnrS1_1, sul2_2, tet(A)_6	gyrA(p.D87N, p.S83L), parC (p.S80I), parE (p.S458A)
CM LAM CEF 4	*E. coli*	1431	Col440II_1, IncY_1	aph(3")-Ib_5,aph(6)-Id_1, blaCTX-M-15_1, blaTEM-1B_1, mdf(A)_1, qnrS1_1, sul2_2, tet(A)_6	gyrA(p.D87N, p.S83L), parC (p.S80I), parE (p.S458A)
CM LAM KAN 1	*E. coli*	1126	Col440II_1, Col440II_1, IncFIB(AP001918)_1	aac(3)-IVa_1,aph(3")-Ib_2, aph(3')-Ia_10, aph(4)-Ia_1, aph(6)-	None detected

Sample_Name	Taxon identified	ST	Plasmid Replicons Detected	Antibiotic Resistance Genes	Point Mutations Detected
				Id_1, floR_2, mdf(A)_1	
CM EMB CEF 1	*E. coli*	1431	Col440II_1, IncY_1	aph(3'')-Ib_5, aph(6)-Id_1,blaCTX-M-15_1,blaTEM-1B_1, mdf(A)_1, qnrS1_1, sul2_2, tet(A)_6	gyrA(p.D87N)
CM EMB CEF 2	*E. coli*	1431	Col440II_1, IncY_1	aph(3'')-Ib_5, aph(6)-Id_1,blaCTX-M-15_1,blaTEM-1B_1, mdf(A)_1, qnrS1_1, sul2_2, tet(A)_6	gyrA(p.D87N,p.S83L)
CM EMB CEF 3	*E. coli*	1431	Col440II_1, IncY_1	aph(3'')-Ib_5, aph(6)-Id_1,blaCTX-M-15_1,blaTEM-1B_1, mdf(A)_1, qnrS1_1, sul2_2, tet(A)_6	gyrA(p.D87N,p.S83L), parC(p.S80I)
CM EMB CEF 4	*E. coli*	1431	Col440II_1, IncY_1	aph(3'')-Ib_5, aph(6)-Id_1,blaCTX-M-15_1,blaTEM-1B_1, mdf(A)_1, qnrS1_1, sul2_2, tet(A)_6	gyrA(p.D87N,p.S83L),parC(p.S80I), parE(p.S458A)
SM EMB CEF 5	*E. coli*	58	Col(BS512)_1, Col156_1,	aph(3'')-Ib_5,aph(3')-Ia_1,	None detected

Sample_Name	Taxon identified	ST	Plasmid Replicons Detected	Antibiotic Resistance Genes	Point Mutations Detected
			Col8282_1, ColRNAI_1, ColpVC_1, IncFIB, IncFII_1	aph(6)-Id_1, blaTEM-190_1, dfrA5_1, floR_2, mdf(A)_1, tet(A)_6	
SM EMB CIPRO 1	*E. coli*	156	Col440I_1, ColRNAI_1, IncFII(pECLA)_1_pECLA, IncFII_1, IncI1_1_Alpha ,p0111_1	blaTEM-1B_1,catA1_1, catB3_2, dfrA1_10, erm(B)_18, mdf(A)_1, mph(A)_2, sul1_5,tet(B)_2	gyrA(p.D87N,p.S83L), parC(p.S80I)
SM EMB CIPRO 2	*E. coli*	156	Col440I_1, ColRNAI_1, IncFII(pECLA)_1_pECLA, IncFII_1, IncI1_1_Alpha ,p0111_1	blaTEM-1B_1,catA1_1, catB3_2, dfrA1_10, erm(B)_18, mdf(A)_1, mph(A)_2, sul1_5,tet(B)_2	gyrA(p.D87N,p.S83L), parC(p.S80I)
SM EMB CIPRO 3	*E. coli*	156	Col440I_1, ColRNAI_1, IncFII(pECLA)_1_pECLA, IncFII_1, IncI1_1_Alpha ,p0111_!	blaTEM-1B_1,catA1_1, catB3_2, dfrA1_10, erm(B)_18, mdf(A)_1, mph(A)_2, sul1_5,tet(B)_2	gyrA(p.D87N,p.S83L), parC(p.S80I)
SM EMB CIPRO 4	*E. coli*	156	Col440I_1, ColRNAI_1, IncFII(pECLA)_1_pECLA, IncFII_1, IncI1_1_Alpha ,p0111_1	blaTEM-1B_1,catA1_1, catB3_2, dfrA1_10, erm(B)_18, mdf(A)_1, mph(A)_2, sul1_5,tet(B)_2	gyrA(p.D87N,p.S83L), parC(p.S80I)

Sample_Name	Taxon identified	ST	Plasmid Replicons Detected	Antibiotic Resistance Genes	Point Mutations Detected
SM EMB CIPRO 5	*E. coli*	617	ColRNAI_1, IncFIB, IncFIC(FII)_1, IncFII_1	ant(3")-Ia_1,aph(3")-Ib_2,aph(6)-Id_1,blaTEM-1A_1,catA1_1, catB3_2, dfrA1_10, erm(B)_18, mdf(A)_1, mph(A)_2, sul1_5,tet(B)_1	gyrA(p.D87N,p.S83L), parC(p.S80I),parE(p.S458A)
SM EMB CIPRO 6	*E. coli*	617	IncFIB(AP001918)_1, IncFII_	ant(3")-Ia_1,aph(3")-Ib_2,aph(6)-Id_1,blaTEM-1A_1,catA1_1, catB3_2, dfrA1_10, erm(B)_18, mdf(A)_1, mph(A)_2, sul1_5,tet(B)_1	gyrA(p.D87N,p.S83L), parC(p.S80I),parE(p.S458A)
GS T1 LAM CIPRO 1	*E. coli*	189	IncFII_1, IncX2_1, IncX5_1	aadA5_1,ant(3")-Ia_1,blaTEM-1A_1,dfrA17_1,erm(B)_18,mdf(A)_1,mph(A)_2,qnrS1_1,sul1_5,tet(A)_6	None detected
GS T1 LAM CIPRO 3	*E. coli*	10	Col440II_1, ColRNAI_1, IncFIB(pKPHS1)_1_pKPHS1, IncFII_1, IncN_1 IncX1_1, IncX3_1	aph(6)-Id_1,blaTEM-1A_1,dfrA14_5,mdf(A)_1,mph(A)_2,qnrS1_1,sul2_2,tet(A)_6	None detected

Sample_Name	Taxon identified	ST	Plasmid Replicons Detected	Antibiotic Resistance Genes	Point Mutations Detected
GS T1 LAM CIPRO 4	*E. coli*	10	Col440II_1, ColRNAI_1, IncFIB(pKPHS1)_1_pKPHS1, IncFII_1, IncN_1 IncX1_1, IncX3_1	aph(6)-Id_1,blaTEM-1A_1,dfrA14_5,mdf(A)_1,mph(A)_2,qnrS1_1,sul2_2,tet(A)_6	None detected
GS T1 LAM CIPRO 5	*E. coli*	189	IncFII_1, IncX2_1, IncX5_1	aadA5_1,ant(3")-Ia_1,blaTEM-1A_1,dfrA17_1,erm(B)_18,mdf(A)_1,mph(A)_2,qnrS1_1,sul1_5,tet(A)_6	None detected
GP T3 EMB COL 2	*E. coli*	453	IncFIB(AP001918)_1	ant(3")-Ia_1,blaTEM-1A_1,dfrA1_10,mdf(A)_1,sul1_5,tet(A)_6	gyrA(p.D87N, p.S83L), parC (p.S80I)
GB T5 LAM KAN 1	*E. coli*	58	Col156_1, Col440II_1, ColRNAI_1, IncFIB(AP001918)_1, IncF11_1	aph(3")-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-1B_1,dfrA5_1,mdf(A)_1,sul2_3,tet(B)_2	None detected
GB T5 LAM KAN 2	*E. coli*	58	Col156_1, Col440II_1, ColRNAI_1, IncFIB(AP001918)_1, IncF11_1	aph(3")-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-1B_1,dfrA5_1,mdf(A)_1,sul2_3,tet(B)_2	None detected

Sample_Name	Taxon identified	ST	Plasmid Replicons Detected	Antibiotic Resistance Genes	Point Mutations Detected
GB T5 LAM KAN 3	*E. coli*	58	Col156_1, Col440II_1, ColRNAI_1, IncFIB(AP001918)_1, IncF11_1	aph(3'')-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-1B_1,dfrA5_1,mdf(A)_1,sul2_3,tet(B)_2	None detected
GB T5 EMB KAN 1	*E. coli*	58	Col156_1, Col440II_1, ColRNAI_1, IncFIB(AP001918)_1, IncF11_1	aph(3'')-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-1B_1,dfrA5_1,mdf(A)_1,sul2_3,tet(B)_2	None detected
GB T5 EMB KAN 2	*E. coli*	58	Col156_1, Col440II_1, ColRNAI_1, IncFIB(AP001918)_1, IncF11_1	aph(3'')-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-1B_1,dfrA5_1,mdf(A)_1,sul2_3,tet(B)_2	None detected
GB T5 EMB KAN 3	*E. coli*	58	Col156_1, Col440II_1, ColRNAI_1, IncFIB(AP001918)_1, IncF11_1	aph(3'')-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-1B_1,dfrA5_1,mdf(A)_1,sul2_3,tet(B)_2	None detected
GB T5 EMB KAN 4	*E. coli*	58	Col156_1, Col440II_1, ColRNAI_1,	aph(3'')-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-	None detected

Sample_Name	Taxon identified	ST	Plasmid Replicons Detected	Antibiotic Resistance Genes	Point Mutations Detected
			IncFIB(AP001918)_1, IncF11_1	1B_1,dfrA5_1,mdf(A)_1,sul2_3,tet(B)_2	
GB T5 EMB KAN 5	*E. coli*	58	Col156_1, Col440II_1, ColRNAI_1, IncFIB(AP001918)_1, IncF11_1	aph(3'')-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-1B_1,dfrA5_1,mdf(A)_1,sul2_3,tet(B)_2	None detected
GB T5 EMB KAN 6	*E. coli*	58	Col156_1, Col440II_1, ColRNAI_1, IncFIB(AP001918)_1, IncF11_1	aph(3'')-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-1B_1,dfrA5_1,mdf(A)_1,sul2_3,tet(B)_2	None detected
SC NM EMB KAN 1	*E. coli*	58	Col(BS512)_1, Col156_1, Col8282_1, ColRNAI_1, ColpVC_1, IncFIB, IncFII_1	aph(3'')-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-190_1,dfrA5_1,floR_2,mdf(A)_1,tet(A)_6	None detected
SC NM EMB KAN 2	*E. coli*	58	Col(BS512)1, Col156_1, Col 8282_1, ColRNAI_1,ColpVC_1, IncFI_1	aph(3'')-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-104_1,dfrA5_1,floR_2,mdf(A)_1,tet(A)_6	None detected

Sample_Name	Taxon identified	ST	Plasmid Replicons Detected	Antibiotic Resistance Genes	Point Mutations Detected
SC NM EMB KAN 3	*E. coli*	58	Col(BS512)1, Col156_1, Col 8282_1, ColRNAI_1,ColpV C_1, IncFI_1	aph(3")-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-104_1,dfrA5_1,floR_2,mdf(A)_1,tet(A)_6	None detected
SC NM EMB KAN 4	*E. coli*	58	Col(BS512)1, Col156_1, Col 8282_1, ColRNAI_1,ColpV C_1, IncFI_1	aph(3")-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-190_1,dfrA5_1,floR_2,mdf(A)_1,tet(A)_6	None detected
SC NM EMB KAN 5	*E. coli*	58	Col(BS512)1, Col156_1, Col 8282_1, ColRNAI_1,ColpV C_1, IncFI_1	aph(3")-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-190_1,dfrA5_1,floR_2,mdf(A)_1,tet(A)_6	None detected
SC NM EMB KAN 6	*E. coli*	58	Col(BS512)1, Col156_1, Col 8282_!, ColRNAI_1,ColpV C_1, IncFI_1	aph(3")-Ib_5,aph(3')-Ia_1,aph(6)-Id_1,blaTEM-190_1,dfrA5_1,floR_2,mdf(A)_1,tet(A)_6	None detected
SC T7 EMB COL 2	*E. coli*	446	IncFIA(HI1)_1_HI1, IncFIB(pB171)_1_pB17	mdf(A)_1	None detected

Sample_Name	Taxon identified	ST	Plasmid Replicons Detected	Antibiotic Resistance Genes	Point Mutations Detected
SC T7 EMB COL 3	*E. coli*	446	IncFIA(HI1)_1_HI1, IncFIB(pB171)_1_pB17	mdf(A)_1	None detected
SC T7 EMB COL 4	*E. coli*	446	IncFIA(HI1)_1_HI1, IncFIB(pB171)_1_pB17	mdf(A)_1	None detected
SS T3 PIA CEF 6	*E. coli*	1248	Col440II_1, ColRNAI_1, IncFIA_1, IncFIB(AP001918)	mdf(A)_1	None detected
SS T7 EMB CEF 3	*E. coli*	446	IncFIA(HI1)_1_HI1, IncFIB(pB171)_1_pB17	mdf(A)_1	Non detected

Appendix 33: Table displaying WGS data for A. baumanii isolates.

Sample Name	Schema	ST	Antibiotic Resistance Genes	Plasmids Detected	Point Mutations Detected
G2 BM LAM CEF 1	abaumannii_2	44	blaADC-25_1, blaOXA-106_1	None detected	None detected
G2 BM LAM CEF 2	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
G2 BM LAM CEF 3	abaumannii_2	44	blaADC-25_1, blaOXA-106_1	None detected	None detected
G2 BM LAM CEF 4	abaumannii_2	44	blaADC-25_1, blaOXA-106_1	None detected	None detected
G2 BM LAM CEF 5	abaumannii_2	-	blaADC-25_1,blaOXA-70_1	None detected	None detected
G2 BM SC CEF 3	abaumannii_2	462	blaADC-25_1,blaOXA-262_1	None detected	None detected
G2 BM SC CEF 5	abaumannii_2	462	blaADC-25_1,blaOXA-262_1	None detected	None detected
G2 BM SC CEF 6	abaumannii_2	462	blaADC-25_1,blaOXA-262_1	None detected	None detected
G2 EMB CEF 6	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
G2 EMB CEF 7	abaumannii_2	-	blaADC-25_1,blaOXA-70_1	None detected	None detected
GB T1 SC CEF 1	abaumannii_2	1190	blaADC-25_1,blaOXA-430_1	None detected	None detected
GB T3 EMB CEF 1	abaumannii_2	-	blaADC-25_1,blaOXA-70_1	None detected	None detected
GB T3 EMB CEF 2	abaumannii_2	-	blaADC-25_1,blaOXA-70_1	None detected	None detected
GB T3 LAM CEF 1	abaumannii_2	44	blaADC-25_1,blaOXA-106_1	None detected	None detected
GB T3 LAM CEF 2	abaumannii_2	44	blaADC-25_1,blaOXA-106_1	None detected	None detected
GB T3 LAM CEF 5	abaumannii_2	44	blaADC-25_1,blaOXA-106_1	None detected	None detected
GB T3 LAM CEF 6	abaumannii_2	44	blaADC-25_1,blaOXA-106_1	None detected	None detected

Sample Name	Schema	ST	Antibiotic Resistance Genes	Plasmids Detected	Point Mutations Detected
GB T3 LAM CIPRO 4	abaumannii_2	584	blaADC-25_1,blaOXA-125_1	None detected	None detected
GB T3 LAM COL 1	abaumannii_2	44	blaADC-25_1,blaOXA-106_1	None detected	None detected
GB T3 SC COL 1	Proteus?	-	blaADC-25_1,	None detected	None detected
GC T1 EMB CEF 1	abaumannii_2	44	blaADC-25_1,blaOXA-106_1	None detected	None detected
GC T1 EMB CEF 2	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
GC T1 EMB CEF 3	abaumannii_2	462	blaADC-25_1,blaOXA-262_1	None detected	None detected
GC T1 EMB CEF 4	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
GC T1 LAM CEF 1	abaumannii_2	-	blaADC-25_1,blaOXA-106_1	None detected	None detected
GC T1 LAM CEF 2	abaumannii_2	462	blaADC-25_1,blaOXA-262_1	None detected	None detected
GC T1 LAM CEF 3	abaumannii_2	44	blaADC-25_1,blaOXA-106_1	None detected	None detected
GC T1 LAM CEF 4	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
GC T1 LAM CEF 5	abaumannii_2	462	blaADC-25_1,blaOXA-262_1	None detected	None detected
GC T1 LAM CEF 6	abaumannii_2	462	blaADC-25_1,blaOXA-262_1	None detected	None detected
GC T1 SC CEF 1	abaumannii_2	-	blaADC-25_1,blaOXA-106_1	None detected	None detected
GC T1 SC CEF 2	abaumannii_2	-	blaADC-25_1,blaOXA-106_1	None detected	None detected
GC T1 SC CEF 3	abaumannii_2	1027	blaADC-25_1,blaOXA-203_1	None detected	None detected

Sample Name	Schema	ST	Antibiotic Resistance Genes	Plasmids Detected	Point Mutations Detected
GC T1 SC CEF 4	abaumannii_2	1027	blaADC-25_1,blaOXA-203_1	None detected	None detected
GC T3 SC CEF 1	abaumannii_2	44	blaADC-25_1,blaOXA-106_1	None detected	None detected
GP T1 EMB CEF 1	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
GP T1 EMB CEF 2	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
GP T1 EMB CEF 3	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
GP T1 EMB CEF 4	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
GP T1 EMB CEF 5	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
GP T1 EMB CEF 6	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
GP T1 LAM CEF 1	abaumannii_2	462	blaADC-25_1,blaOXA-262_1	None detected	None detected
GP T1 LAM CEF 2	abaumannii_2	1027	blaADC-25_1,blaOXA-203_1	None detected	None detected
GP T1 LAM CEF 3	abaumannii_2	1027	blaADC-25_1,blaOXA-203_1	None detected	None detected
GP T1 LAM CEF 4	abaumannii_2	N/A	blaADC-25_1,blaOXA-262_1	None detected	None detected
GP T1 LAM CEF 5	abaumannii_2	462	blaADC-25_1,blaOXA-262_1	None detected	None detected
GP T1 LAM CEF 6	abaumannii_2	1027	blaADC-25_1,blaOXA-203_1	None detected	None detected
GP T1 SC CEF 3	abaumannii_2	1027	blaADC-25_1,blaOXA-203_1	None detected	None detected
GP T1 SC CEF 4	abaumannii_2	1027	blaADC-25_1,blaOXA-203_1	None detected	None detected

Sample Name	Schema	ST	Antibiotic Resistance Genes	Plasmids Detected	Point Mutations Detected
GP T3 EMB COL 1	abaumannii_2	584	blaADC-25_1,blaOXA-125_1	None detected	None detected
GP T3 LAM CEF 1	abaumannii_2	-	blaADC-25_1,blaOXA-70_1	None detected	None detected
GP T3 LAM CEF 2	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
GP T3 LAM CEF 3	abaumannii_2	-	blaADC-25_1,blaOXA-70_1	None detected	None detected
GP T3 LAM CEF 4	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
GP T3 LAM COL 2	abaumannii_2	44	blaADC-25_1,blaOXA-106_1	None detected	None detected
GP T3 LAM COL 3	abaumannii_2	44	blaADC-25_1,blaOXA-106_1	None detected	None detected
GP T3 SC CEF 1	abaumannii_2	462	blaADC-25_1,blaOXA-262_1	None detected	None detected
GS T1 EMB CEF 1	abaumannii_2	-	blaADC-25_1,blaOXA-70_1	None detected	None detected
GS T1 EMB CEF 2	abaumannii_2	44	blaADC-25_1,blaOXA-106_1	None detected	None detected
GS T1 EMB CEF 4	abaumannii_2	-	blaADC-25_1,blaOXA-180_1	None detected	None detected
GS T1 EMB CEF 5	abaumannii_2	-	blaADC-25_1,blaOXA-70_1	None detected	None detected
GS T1 EMB CEF 6	abaumannii_2	44	blaADC-25_1,blaOXA-106_1	None detected	None detected
GS T3 SC CEF 2	abaumannii_2	-	blaADC-25_1,blaOXA-332_1	None detected	None detected
GS T5 LAM CEF 1	abaumannii_2	-	blaADC-25_1,blaOXA-322_1	None detected	None detected
SP T3 EMB CEF 1	abaumannii_2	-	blaADC-25_1,blaOXA-326_1	None detected	None detected
SP T3 EMB CEF 2	Proteus?	-	blaADC-25_1,blaOXA-354_1	None detected	None detected
SP T3 EMB CEF 3	abaumannii_2	-	blaADC-25_1,blaOXA-326_1	None detected	None detected

Sample Name	Schema	ST	Antibiotic Resistance Genes	Plasmids Detected	Point Mutations Detected
SP T3 EMB CEF 4	abaumannii_2	-	blaADC-25_1,blaOXA-332_1	None detected	None detected
SP T3 SC CEF 2	abaumannii_2	-	blaADC-25_1,blaOXA-354_1	None detected	None detected