Molecular Epidemiology of Antibiotic Resistant *Escherichia coli* from Intensively-Produced Poultry in a Farm-to-Fork Continuum in KwaZulu-Natal, South Africa

Katherine McIver

217080743

A dissertation submitted in fulfilment of the requirements for the degree of Master of Medical Science (Medical Microbiology) in the School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal.

Supervisor

Professor Sabiha Yusuf Essack

Co-Supervisors

Dr. Linda Antionette Bester

Dr. Luther King Abia Akebe

September 2020

Molecular Epidemiology of Antibiotic Resistant *Escherichia coli* from Intensively-Produced Poultry in a Farm-to-Fork Continuum in KwaZulu-Natal, South Africa

Katherine McIver

217080743

A dissertation submitted to the School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Westville Campus, for the degree of Master of medical Science (Medical Microbiology)

This is a dissertation by manuscript with an overall introduction and final summary.

This is to certify that the content of the dissertation is the original research work of Dr Katherine McIver, supervised by;

Supervisor: Signed:_____ **Prof Sabiha Y. Essack** Date:_____

Co-Supervisor: Signed:_____ **Dr Linda A. Bester** Date:_____

Co-Supervisor: Signed:_____ **Dr Luther King Abia Akebe** Date:_____

DECLARATION

I, Dr Katherine McIver, declare as follows:

1) That the work described in the dissertation has not been submitted to the University of KwaZulu-Natal or any other tertiary institution for the purposes of obtaining an academic qualification, by myself or any other party.
2) That my contribution to the project was as follows:
    a) The research reported in this dissertation is my original work except where otherwise stated.
3) This dissertation does not contain any data, pictures, graphs or other information from any other persons work unless specifically acknowledged.
4) This dissertation does not contain any writing from another person unless specifically acknowledged. Where written sources have been quoted:
    a) Information is rewritten in my own words and the source referenced
    b) If exact words are used, they are placed inside quotation marks and referenced

Signed:

_____

K. McIver

217080743

Date:_____

ACKNOWLEDGEMENTS

- I would like to express my sincere thanks to the following people for their role in the research
- Professor Sabiha Essack for the opportunity to take part in this study and continued guidance throughout.
- Dr Linda Bester for her help with technical queries and laboratory procedures.
- Dr Daniel Gyamfi Amoaka for his help with laboratory processes.
- Dr Luther King Abia Akebe for his help with laboratory processes, especially in helping with Polymerase Chain Reactions.
- Chantel Molechan, Stephanie Pillay and Melissa Ramtahal for their help through-out the project.
- I would like to acknowledge the financial contribution of the National Research Foundation.

Any omissions or shortcomings of this research is the sole responsibility of the researcher.

K McIver

Durban

May 2020

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 2

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

AAC	Aminoglycoside Acetyl-Transferase
ABR	Antibiotic Resistance
AMC	Amoxycillin-clavulanic acid
AME	Aminoglycoside-Modifying Enzymes
AMK	Amikacin
AMP	Ampicillin
AMR	Antimicrobial Resistance
ANT	Aminoglycoside Nucleotideyl- Transferase
APEC	Avian Pathogenic *Escherichia coli*
APH	Aminoglycoside Phosphoryl-Transferase
AST	Antibiotic Susceptibility Testing
ARG	Antibiotic Resistance Gene
AZM	Azithromycin
CAZ	Ceftazidime
CFU	Colony Forming Unit
CHL	Chloramphenicol
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CRO	Ceftriaxone
CTX	Cefotaxime
DDD	Daily Defined Dose

DEC	Diarrhoeagenic *Escherichia coli*
DHFR	Dihydrofolate Reductase
DHPS	Dihydropteroate Synthetase
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
EMB	Eosin Methylene Blue
ERIC-PCR	Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction
ESBL	Extended Spectrum β-lactamase
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ExPEC	Extra Intestinal Pathogenic *Escherichia coli*
FAO	Food and Agricultural Organisation of the United Nations
FEP	Cefepime
FOX	Cefoxitin
GEN	Gentamicin
HGT	Horizontal Gene Transfer
HIC	High Income Country
ICE	Integrative Conjugative Elements
IMP	Imipenem
Int	Integron
IS	Insertion Sequence
LEX	Cefalexin

LGT	Lateral Gene Transfer
LMIC	Low and Middle Income Country
LPS	Lipopolysaccharide
MDR	Multidrug Resistance
MEM	Meropenem
MGE	Mobile Genetic Element
NAL	Nalidixic acid
NEMEC	Neonatal Meningitis *Escherichia coli*
OIE	World Organisation for Animal Health
PABA	Para-aminobenzoic acid
PBP	Penicillin Binding Protein
PCR	Polymerase Chain Reaction
QPCR	Quantitative Polymerase Chain Reaction
RNA	Ribonucleic Acid
rpm	Revolutions per minute
RPP	Ribosomal Protective Proteins
SCI	Sedentary Chromosomal Integrons
SEPEC	Sepsis-Associated *Escherichia coli*
SXT	Trimethroprim-sulphamethoxazole
TAE	Tris-Acetate EDTA
TET	Tetracycline
TGC	Tigecycline

TSB	Trypticase Soya Broth
Tn	Transposon
TSI	Triple Sugar Iron
UK	United Kingdom
UPEC	Uropathogenic *Escherichia coli*
USA	United States of America
WHO	World Health Organisation

LIST OF APPENDICES

SUPPLEMENTARY MATERIAL

Electronic copy of this dissertation in portable digital format on Compact Disc (CD)

ABSTRACT

The increased use of antibiotics in intensively produced food animals has resulted in the selection of drug-resistant bacteria across the farm-to-fork continuum. There is a risk of transfer of this resistance to humans and as such a public health risk. The aim of this study was to investigate the molecular epidemiology of antibiotic resistant *Escherichia coli* from intensively produced poultry in the uMgungundlovu district of Kwa-Zulu Natal, South Africa.

This was a longitudinal descriptive study with the aim to determine the epidemiology of antibiotic resistance of *E.coli* from hatching through to the final retail product from an intensive poultry farm house. The farm reported the use of zinc bacitracin and Salinomycin included in the feed, but no therapeutic antibiotics used in this batch of chickens. However, the following antibiotics were used on the farm in the previous 12 months: Doxycycline, Sulfadiazine and Trimethoprim, Enrofloxacin, Ceva olaquindox 10%, Avilamycin, Tylosin 10% and Kitasamycin tartate. During the first five weeks, ten samples from litter and faeces were collected. During transfer from the house to abattoir ten swabs from transport trucks and transport crates were taken. At the abattoir ten samples from carcass wash were collected. After slaughter and dressing ten caecums, whole chickens, thighs and necks were collected. Again, during house washing, ten samples were collected. *E.coli* was putatively identified using Eosin Methylene Blue agar followed by Sorbitol MacConkey agar and confirmed by identification of the *uidA* gene by polymerase chain reaction. Susceptibility to a panel of antibiotics recommended by the World Health Organization Advisory Group on the Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR) was ascertained by the Kirby-Bauer disk diffusion method for 20 antibiotics according to CLSI guidelines. Realtime PCR was used to test for resistance genes *tetA, tetB, qnrB, qnrS, aac(6)-lb-cr, sul1, sul2, sul3,* bla_{SHV}, bla_{CTX-M}, bla_{TEM} conferring resistance to tetracyclines, quinolones, sulphonamides and cephalosporin antibiotics. Clonal similarities were investigated using ERIC-PCR.

A total of 266 *E.coli* isolates constituted the sample size with a non-susceptibility profile of ampicillin 48.1%, tetracycline 27.4%, nalidixic acid 20.3%, trimethoprim-sulphamethoxazole 13.9%, chloramphenicol 11.7%, cefalexin 4.5%, ciprofloxacin 4.1%, amoxycillin-clavulanic acid 3.4%, gentamicin 1.9%, cefoxitin 1.1%, cefepime 1.1%, cefotaxime 1.1%, amikacin 1.1%, ceftriaxone 0.8% and azithromycin 0.8%. Isolates were fully susceptible to ceftazidime, imipenem, meropenem and tigecycline.

Of the 266 isolates 6.4% were multidrug resistant (resistant to one or more antibiotics in three or more distinct antibiotic classes). The most frequently observed resistance genes were bla_{CTX-M} (100%), *sul1*(80%), *tetA*(77%), *tetB*(71%). Using ERIC-PCR the isolates were grouped into 27 clusters with a 75% similarity. Eight clusters comprised of isolates from only one sample.

There was an increase in MDR and resistance genes over the farm to fork continuum with lowest and highest levels seen in transport and waste-water samples respectively. ERIC-PCR did not indicate the transmission of clones across the farm-to-fork continuum. There instead appeared to be *de novo* or evolution of resistance genes or the introduction of plasmids over the time period. As the only antimicrobials used in this flock were salinomycin and zinc bacitracin it is postulated that the resistance observed could be attributed to the co-selection of resistance genes and/or horizontal gene transfer from the environment, insects, chicken food and workers. Overall resistance levels were low over the six weeks of the study, MDR and the prevalence of resistance genes increased over time. The diverse clonality shown by the ERIC PCR results did not support the transmission of clones across the farm-to-fork continuum but indicated a *de novo* evolution of resistance genes and/or the loss or gain of plasmids over the time period.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Antibiotic resistance is a threat to the way we all live, with drug-resistant pathogens increasingly affecting humans, animals and the environment (OIE, 2016; WHO, 2017a). The cost of resistance is found both directly and indirectly in humans and animals. Directly, treatment costs increase due to more expensive drugs being needed and longer durations of treatment. Indirectly, morbidity and mortality rates increase, resulting in loss of productivity both in humans and in animals (FAO, 2016).

To combat the threat of antibiotic resistance the WHO, OIE and FAO have formed a tripartite alliance. As antibiotic resistance crosses between humans, animals, and the environment, a One Health approach is needed to address the problem. The tripartite alliance was needed for input from the human health, animal health and the food animal health sectors represented by each organisation (WHO, 2015; FAO, 2016; OIE, 2016).

Animals and specifically production animals are thought to be a reservoir for antibiotic resistant genes (ARGs). With the use of antibiotics for growth promotion, disease prevention and treatment in animal production, selection of resistant organisms has occurred (WHO, 2019). This resistance can transfer to humans either directly from contact or indirectly via the food chain, resulting in a public health threat (Van Boeckel et al., 2017). Drug- resistant organisms also threaten animal welfare and our food supply if animals become ill or die (OIE, 2016).

With an increasing demand for animal protein, especially in developing countries, poultry farming has become one of the fastest growing industries (FAO, 2016). In South Africa, the poultry industry is the largest animal product contributor making up 43% of gross value in 2018. Poultry meat is the most consumed meat in South Africa (SAPA, 2018). A large proportion of poultry farming is intensive therefore, reliance on antibiotics for animal health, growth and for public safety with regard zoonotic pathogens is often deemed necessary. This has highlighted poultry as an important reservoir of antibiotic resistance for humans (Singer and Hofacre, 2006).

In order to stop the threat of antibiotic resistance more information is needed on the epidemiology of antibiotic resistance (WHO, 2015; FAO, 2016; OIE, 2016). Research is being done on resistance in food-borne pathogens and indicator bacteria to provide this information. Indicator bacteria are used as they are generally found in humans, animals, and the environment and, as such, provide a good overview of the resistance present in each population. *Escherichia coli* is an indicator bacterium that is used in surveillance of antibiotic resistance (Aarestrup, 2004). *E.coli* is easily cultured and is found in all animals making it a good choice as an indicator bacteria. It is often a contaminant of food and is

found in the environment. *E.coli* is able to move between humans, animals and the environment as both a commensal or as a pathogen (Kunert Filho et al., 2015; Manges, 2016).

1.2 Antibiotic Resistance (ABR)

Antibiotic resistance (ABR) is the ability of bacteria to adapt and survive in the presence of antibiotics (WHO, 2015). Without effective antibiotics, commonly treatable infections become untreatable and possibly fatal, resulting in a threat to human health, animal health, and food security (WHO, 2015; FAO, 2016; OIE, 2016).

1.2.1 Development and Dissemination of Antibiotic Resistance

Selective pressure in bacteria follow the same laws of natural selection as eukaryotes. Selective pressure is any phenomenon that allows a certain phenotype to have a competitive advantage. Selective pressure drives natural selection and evolution. In the case of antibiotic resistance, their use, imparts a selective pressure on bacteria selecting for resistant isolates. Fitness to survive however, is not only reliant on genetic mutations and random selection of genes down cell lines as with eukaryotes. Bacteria can acquire genetic material to their advantage from lateral gene transfer (LGT) also known as horizontal gene transfer (HGT). This allows for genes in a microbial biome to become a shared resource available to all bacteria in a population. The ability of bacteria to use LGT has meant that the time frame for evolution of resistant phenotypes is much shorter in comparison to eukaryotes (Stokes and Gillings, 2011).

In production animals, selective pressures are imposed by inappropriate prescribing, treating and poor quality/sub-standard or falsified antibiotics being used (Chang et al., 2015). The pool of resistant genes in animals is thought to be a threat to human health either directly or indirectly. Direct transfer of antibiotic resistance between animals and humans can occur through contact in animal husbandry situations. Drug-resistant bacteria and ARGs can also transfer to meat and meat products, becoming a potential source to humans indirectly through consumption of animal products (Bester and Essack, 2010).

At the moment there is inconsistent data on the use of antimicrobials in agriculture both in the high income countries (HICs) and especially in low and middle income countries (LMICs) (FAO, 2016). A study on antimicrobial use in food animals in South Africa from 2002 to 2004 using information from eight out of the twenty five pharmaceutical companies in the country, showed that South Africa is more lenient than the European Union (EU) with regard to antimicrobial use in food production animals. In 2015 6.2 tons of antimicrobials were imported into South Africa of which 26% were estimated for animal use. Majority of antimicrobials sold for animal use were classified as growth promoters which included a number of antimicrobials not used in human medicine such as ionophores, flavomycin, olaquindox, zinc bacitracin and tylosin. Tetracycline is also registered as a growth promoter and made up 27% of total antimicrobial sales (South African Department of Health, 2018) Most main classes

and types of antimicrobials which are permitted to be used in feed premixes in South Africa were banned for the same use in the EU. Only chloramphenicol and nitrofurans were not permitted to be used in food producing animals in South Africa at the time of the survey (Eagar, Swan and Van Vuuren, 2012).

Complicating the above factors, is the globalization present today (WHO, 2015; Robinson et al., 2016). Travel internationally is easy, and, with movement of people there is increased spread of certain resistance genes from country to country (Founou, Founou and Essack, 2016; Robinson et al., 2016). This has been seen with the *mcr-1* and *NDM* genes spreading from China and India respectively to Africa, Europe and the Americas (Robinson et al., 2016). Food products and animals are also being shipped globally, and with them resistant bacteria (Founou, Founou and Essack, 2016; Robinson et al., 2016).

Antibiotic resistance can be intrinsic or acquired. Intrinsic resistance is where the whole species is resistant to a certain antibiotic. Intrinsic resistance is often when the bacterium does not have the drugs target molecule or is impermeable to the drug. With acquired resistance only certain isolates or clones are resistant due to genetic changes allowing them to become resistant (Greenwood et al., 2007). Acquired resistance is either via a mutation or transmission.

Mutation of genes in the bacterium's genome can allow that isolate to survive in the presence of an antibiotic and replicate. These changes are either to antibiotic target sites, impermeability of a bacterium's cell membrane or wall to the antibiotic, inactivating enzymes or bypass pathways allowing the bacterium to survive in the presence of the antibiotics. They can be either single large step mutations, or smaller multistep mutations (Greenwood et al., 2007).

Transmission of genes between bacteria horizontally or vertically can spread resistance in a population of bacteria (Greenwood et al., 2007; Holmes et al., 2016).

Genetic information can be transferred between bacterial cells through transformation, transduction and conjugation and is known as lateral gene transfer (LGT) or horizontal gene transfer (HGT):

a) Transformation is where free deoxyribonucleic acid (DNA) is taken up by a bacterium and incorporated into its genome.
b) Transduction is where bacterial DNA is incorporated into a bacteriophage during lytic phase and then transferred to new bacteria during infection.
c) Conjugation is where DNA is transferred as a plasmid from one bacterium to another, this can allow the transfer of numerous resistance genes at the same time (Greenwood et al., 2007; Bester and Essack, 2010; Holmes et al., 2016) .

Mobile genetic elements (MGEs) enable bacteria to transfer genes by horizontal or vertical transfer. MGEs include insertion sequences (IS), transposons (Tn), Gene cassettes, and Integrons (Int) which can be found in combinations in plasmids and genomic islands (Partridge et al., 2018).

Insertion sequences (IS) are short mobile elements containing a transposase enzyme, flacked by inverted repeats, and some have passenger genes (including resistance genes). They can, either be cut from a section of DNA and inserted into another section or replicate and inserted in a recipient DNA. ISs often contain promoter genes which drive expression of the passenger or resistance genes (Partridge et al., 2018).

IS's are often found associated with transposons or can be contained within them. Transposons typically have two inverted repeats with a transposase gene (larger than found in ISs), a resolvase and a resolution site containing passenger genes. They are able to replicate with the copy being able to insert into site specific regions of a recipient DNA (Partridge et al., 2018).

Gene cassettes contain 1 or 2 genes which can include resistance genes. They do not contain any promoter genes and cannot replicate on their own. They are, for this reason, associated with ISs, Tns and integrons. They contain an attC recombinant site to allow replication when associated with integrons. Integrons contain the relevant attl recombination site, promoter and tyrosine recombinase gene to allow replication of the gene cassette. Multiple gene cassettes can be found together and are known as a cassette array (Partridge et al., 2018; Akrami, Rajabnia and Pournajaf, 2019).

Integrons facilitate the replication and ordering of gene cassettes on the chromosome and on mobile genetic units. On the chromosome they are known as sedentary chromosomal integrons (SCI), otherwise outside the chromosome as mobile integrons. Mobile integrons are thought to allow bacteria to adapt quickly to a changing environment by increasing expression of necessary genes for survival. Mobile integrons also copy and insert gene cassettes onto plasmids for HGT to another bacterium. SCI allow the cell to maintain a wide genetic variability, but these genes are slower to be expressed (Loot et al., 2017).

Plasmids are DNA molecules found outside the bacterium's chromosome which can be replicated and transferred to other bacterium carrying with them several genes. The structure of plasmids are comprised of a backbone of genes needed for replication and maintenance, combined with accessory genes necessary for non essential functions. Genes found on plasmids are distinct from those found on the bacterium's chromosome. Some of the accessory genes found in plasmids can carry several resistance genes. Plasmids have the ability to replicate and move between bacterium via conjugation, allowing the horizontal spread of resistance (Frost et al., 2005).

Genomic islands are found on chromosomes and are acquired via horizontal gene transfer. They will often contain resistance genes and are known as resistance islands. If they carry virulence genes, they are known as pathogenicity islands. Genomic islands that contain elements that allow them to be self-transmissible are known as integrative conjugative elements (ICE). ICE can replicate, excise, and transfer genetic material via conjugation to another bacterium where they are able to integrate into the recipient's chromosome (Partridge et al., 2018).

Bacteria use several mechanisms of drug resistance which are mentioned below and are coded for by resistance genes:

a) Alteration of cell permeability or increased efflux of drugs from the cell:
    i) Porin loss from the outer membranes of bacterial cells results in reduced drug influx, allowing lower intercellular drug concentrations and cell survival (Santajit and Indrawattana, 2016).
    ii) Efflux pumps remove antimicrobials from within the cell, not allowing a sufficient concentration of drug to kill the bacterium (Santajit and Indrawattana, 2016). A bacterium can possess more than one efflux system, targeting different drugs. Efflux systems are one of the main mechanisms found in multidrug resistant organisms (Lin et al., 2015).
b) Inactivating enzymes which deactivate the drug (Santajit and Indrawattana, 2016) such as β-lactamases and aminoglycoside-modifying enzymes (AMEs) which target β-lactams and aminoglycosides respectively (Li et al., 2014).
c) Modification of drug binding sites such as changes to PBP's (penicillin binding proteins) in the cytoplasmic membrane. Changes to the PBP's prevent penicillin binding and inhibit cell wall synthesis resulting in penicillin resistance (Santajit and Indrawattana, 2016)
d) Biofilm formation where the bacteria as a group attach to a substrate and secrete a matrix which provides protection from antibiotics (Santajit and Indrawattana, 2016).

Antibiotic resistance transfer is complex and although selective pressure from antibiotics might drive the development and acquisition of ARGs, there are cases where ARGs persist and disseminate without continued antibiotic pressure. There also does not seem to be a loss of fitness of resistant bacteria when compared to their susceptible counterparts and therefore removal of antibiotic selective pressure does not necessary revert a bacterial community back to being susceptible. Cross-selection and co-selection of several ARGs at one time in response to a single antibiotic promotes the development of multidrug resistance organisms (Holmes et al., 2016).

1.2.2 Implications of ABR

Antibiotics, since their discovery in the 1930's, have been important in the improvement of human health and animal welfare (WHO, 2015). Before the introduction of antibiotics, diseases which are now treatable were life threatening. Antibiotics have improved survival rates and welfare of humans and animals (FAO, 2016; OIE, 2016), however ABR places these advances at risk (Robinson et al., 2016).

a) Public health

With the development of ABR doctors are having to resort to last line medications or critically important medications which for common diseases. These antibiotics are generally reserved for serious infections in humans and infections that originate in non -humans but can transfer to humans and where organisms can acquire resistance from non-human sources (WHO, 2017b). Examples of these are tuberculosis

and gonorrhoea which are now starting to show resistance to the last resort antibiotics. Antibiotics are used to reduce the risk of secondary infections following surgery. They are also used in immune compromised patients to prevent infections. Resistant organisms threaten the safety of these patients, making procedures riskier (WHO, 2015).

One of the main areas of concern is the use of antibiotics in agriculture and the potential impact on human health. There are several proposed mechanisms for the threat to humans from the use of antibiotics in agriculture, but the magnitude of each is unknown (Chang et al., 2015). Three possible mechanisms are:

i) Infection by resistant bacteria from animals to humans which then do not circulate in the human population (Chang et al., 2015).
ii) Transfer between humans in a population of an animal-derived resistant pathogen (Chang et al., 2015).
iii) Transfer of resistant genes from animal organism to a human pathogen (Chang et al., 2015).

Of the above mechanisms the last is the hardest to prove but possibly the greatest threat for human health (Bester and Essack, 2010; Chang et al., 2015) .

Humans can be directly or indirectly exposed to bacteria of animal origin carrying ABR. Transfer occurs from animals, or animal excretions, to humans via direct contact, while indirect contact is through the food chain (Hammerum and Heuer, 2009; Founou, Founou and Essack, 2016). The direct contact is more common as an occupational hazard for veterinarians and farmers but there has been evidence of spread to their immediate family and the community. Indirect contact via the food chain also includes bacteria acquired during transport, slaughter, packaging and food preparation (Founou, Founou and Essack, 2016).

b) Animal Health

With the use of antibiotics in agriculture, food animals are healthier ensuring a better food supply and reducing the risk of zoonotic diseases (FAO, 2016; OIE, 2016). However, the use of antibiotics in agriculture also drives the development of ABR. There needs to be a balance between safety and welfare of animals and humans and the public health risk of development of ABR. As antibiotics try to prevent disease in animals and therefore suffering, it is hard to stop using antibiotics in food animals completely (Founou, Founou and Essack, 2016).

More information is still needed on antibiotic use and resistance in agriculture in order to make an informed decision on the use of antibiotics in agriculture going forward (Chang et al., 2015).

This is especially the case where there is an increasing demand for lower cost protein such as in LMICs which has led to rapid intensification of farming without the necessary improvement in farming techniques (Founou, Founou and Essack, 2016). In upper middle income countries such as South Africa

there is a switch from extensive farming to more intensive farming practices as incomes rise and demand for animal protein increases (Van Boeckel et al., 2015). This has resulted in crowding of animals, more stress amongst animals, faster disease spread and as a result, increased reliance on antibiotics (Founou, Founou and Essack, 2016).

Antibiotic consumption is estimated to rise 67% between 2010 and 2030 if efforts to reduce the use in agriculture are not made. The majority of this increase will be in China, India and other Asian countries (Van Boeckel et al., 2015). In the period 2000 to 2015 antibiotic consumption increased 65% when calculated using daily defined dosage (DDD) of antibiotics. Most of the increase was seen in LMICs where DDD increased 114%, while the DDD only increased by 6% in HICs (Klein et al., 2018).

With a predicted 67% increase in global antimicrobial use for animal production, solutions are needed which can reduce this use without influencing food security or negatively affecting LMICs. Proposed solutions have been to improve regulation of antimicrobial use, reduction in meat consumption and user fees on all veterinary antimicrobials. All of these have their advantages and disadvantages, but models predict that they could reduce consumption up to 60% if used in isolation and up to 80% if used together. This would need co-operation globally and especially from China and other large consumer countries to make a difference (Van Boeckel et al., 2017).

The different antibiotic use in HICs compared to LMICs is due to the use of better farming practices, biosecurity and vaccination programs. This has allowed intensive farming to be less reliant on antibiotics such as has been seen in Scandinavian countries (Founou, Founou and Essack, 2016). In Europe, regulations have limited the use of antibiotics and there has not been a reduction in production even though they use less than half the global average antibiotics per kilogram animal produced. The range of antimicrobial use globally is lowest in Norway, at 8mg/Kg animal product, compared to the highest recorded amount of 318mg/Kg animal product in China (Van Boeckel et al., 2017).

c) Economic implications

The economic implications of antibiotic resistance are increased treatment costs and loss of production. This is due to doctors being forced to use more expensive medications, patients having longer hospital stays, and decreased productivity of both animals and humans (WHO, 2015; FAO, 2016). In 2016 estimated deaths from drug resistant pathogens were 700 000 and this is predicted to increase to 10 million by 2050 if no steps are taken to slow the progression of antimicrobial resistance. The economic estimate of loss in global production is $100 trillion if this occurs. LMICs will be the worst affected due to poorer health care systems, poorer sanitation and reduced access to effective medications (O'Neill, 2016; Robinson et al., 2016). This has been made worse by increasing populations of immune suppressed individuals such as those with AIDS (Bester and Essack, 2010).

The effect on livestock production has been modelled by the World Bank and shows that global production could decrease by 7.9% by 2050 if no changes are made to slow the development of

antimicrobial resistance. LMICs will be the worst affected with livestock playing a larger role in their economies. Added to the above decreased access to protein in these countries will inhibit development especially in children and young women (World Bank, 2017).

In poultry, one of the largest economic losses is due to bacterial infections by *E.coli* which causes colibacillosis. Economic losses due to colibacillosis are from cost of treatment, mortality, condemnation at slaughter and increased production time in broilers. It is a major cause of death in the first week of production and condemnation of layers at slaughter (Mellata, 2013). Contaminated hatching eggs can also be a source of infection in young chicks resulting in omphalitis (Kunert Filho et al., 2015).

1.3 AMR and One Health

The "one health" approach emphasizes the connectiveness between the environment, humans and animals (FAO, 2016). Antimicrobial resistance is the ability of organisms to resist the effects of antimicrobials, a subgroup of which is the ability of bacteria to survive the effects of antibiotics. AMR affects the health of humans, animals and the environment and for this reason needs a "one health" approach to solving this global problem (WHO, 2015; FAO, 2016; OIE, 2016) . Antimicrobial resistance is a problem affecting both HICs and LMICs.

In 2015 the World Health Organization (WHO), the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) formed a tripartite alliance to tackle the problem of AMR (WHO, 2015; FAO, 2016; OIE, 2016) . Each agency has slightly different focusses to combatting the threat of AMR within their area of expertise.

The resulting strategy from the WHO to combat AMR is summarized below (WHO, 2015).

a) Improve awareness and understanding of AMR through effective communication, education, and training.
b) Strengthen the knowledge and evidence base through surveillance and research.
c) Reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures.
d) Optimize the use of antimicrobial medicines in human and animal health.
e) Develop the economic case for sustainable investment that takes account of the needs of all countries and increase investment in new medicines, diagnostic tools, vaccines and other interventions (WHO, 2015).

The OIE developed a similar strategy with more emphasis on animal health and welfare within the One Health approach. Elements in their strategy and similar to the WHO strategy are:

a) Improving awareness.

b) Understanding and strengthening of the knowledge and evidence base through surveillance and research within the animal and veterinary sector.
c) Promoting good governance and capacity building in member countries through helping to implement National Action Plans. These plans aim to improve the use and regulation of antimicrobials in the animal sector. Included in this, is the improvement of animal husbandry to reduce the need for antimicrobials (OIE, 2016).
d) Promoting the use of OIE standards to improve biosecurity, animal welfare and public heath while standardizing acquired information globally (OIE, 2016).

FAO's emphasis is on food and agricultural safety. The organization has expertise in aquatic and terrestrial animal health and welfare, as well as crop production and, as such, feed and food security. Their aim is to reduce the misuse and use of antimicrobials without negatively affecting the agricultural industry (FAO, 2016). The FAO has proposed the following strategy to combat AMR:

a) Increase in awareness and information to all parties in the agricultural sector.
b) Improve surveillance specifically in the agriculture and food industries.
c) Surveillance includes the use of antimicrobials, the presence of AMR as well as detection of antimicrobial residues.
d) Using available data, strengthen the use of policies to reduce the use of antimicrobials and reduce residues in food producing systems.
e) Promote measures to improve farming and food producing practices to reduce the use of antimicrobials through information, research and good governance. This includes implementation of biosecurity measures and knowledge on appropriate antimicrobial uses in the agriculture and food production sectors (FAO, 2016).

The data collected by all three organisations will be combined to provide an overview of resistance across human, animal, and environmental health.

The types of antibiotics used in animals are frequently the same as used in human medicine. As use of antibiotics in non-humans changes the occurrence of resistant bacteria in animals and food, there is an increased risk of the exposure of humans to resistant bacteria. The more pathogens, in this case from food animals, that are resistant to critically important antibiotics, the greater the consequences for human health (WHO, 2019). Based on the need to preserve the efficacy of antibiotics for human health, it has been proposed that critical and highly important antibiotics not be used as growth promoters in food producing animals, even though the risk of transfer from animals to humans has not been quantified yet (Hammerum and Heuer, 2009).

Antibiotics are classified into three groups; critically important, highly important and important in human medicine. Two criteria are used to determine the classification. The first criteria is if an 'antibiotic class is a sole or one of limited available therapies to treat serious bacterial infections in

people. The second criteria is that if an 'antibiotic class is used to treat infections in people caused by either: (1) bacteria that may be transmitted to humans from non-human sources, or (2) bacteria that may acquire resistance genes from non-human sources' (WHO, 2019). Critically important antibiotics meet both criteria above, highly important meet one of the criteria and important meet neither of the criteria (WHO, 2019).

Examples of critically important antimicrobial groups relevant to *E.coli* and poultry are the aminoglycosides, 3rd, 4th and 5th generation cephalosporins, carbapenems, glycylcyclines, macrolides, penicillin's, polymyxins and quinolones. Highly important antimicrobial groups include amphenicols, 1st and 2nd generation cephalosporins, sulphonamides and tetracyclines. Important groups include aminocyclitols, cyclic polypeptides and nitrofurantoin which often include products used as growth promoters (WHO, 2019).

This needs to be compared to a list of critical important antibiotics for veterinary medicine as described by the OIE (OIE, 2007). A balance needs to be achieved to between the health of humans and animals (WHO, 2019).

Aminoglycosides, cephalosporins, macrolides and quinolones are on both WHO and OIE lists as critically important antimicrobial groups for humans and animals respectively (OIE, 2007; WHO, 2019).

The third aspect of the one health approach to ABR is the environment. The environment can also be a source of resistance genes. Before the introduction of antibiotics, resistance genes existed in the environment. Resistance genes allowed certain bacteria to inhabit niches where resistance provided a competitive advantage against naturally occurring antibiotics (Stokes and Gillings, 2011). With the introduction of antibiotic selective pressure by humans there has been a concentration of resistance genes in the microbial biome of the environment. Selective pressure has been driven by both antibiotic residues and resistance bacteria being introduced into the environment from agriculture and human use (Founou, Founou and Essack, 2016).

Antibiotic residues can be introduced into the environment in animal and human waste where the antibiotics are not or only partially metabolized in the animal's system. Residues can also be released directly from manufacturing plants into the environment in countries where there are no regulations preventing this pollution from occurring (Founou, Founou and Essack, 2016).

Animal and human waste containing resistant bacteria released into the environment is another source of resistance genes for the environmental microbial biome. Lateral gene transfer between bacteria in the environment has allowed concentration of genes and of multidrug resistance mobile units to persist (Founou, Founou and Essack, 2016).

An example of the interaction between production animals and the environment was a study in Canada which found antibiotic residues in litter from broilers in both experimental feeding farms and commercial farms. The same study found multidrug resistance in *E.coli* isolates in the broiler litter. It was concluded that untreated litter was a source of both antibiotic residues and antibiotic resistant organisms for the environment (Furtula et al., 2010).

Extended Spectrum β-lactamase (ESBL) *E.coli* are becoming increasingly important in human medicine with resistant infection causing morbidity and mortality. A study done in the Netherlands involving five layer and three broiler farms compared faecal and rinse water (from cleaning the poultry houses) ESBL *E.coli* with environmental ESBL E.coli samples. It was found 60% of environmental samples matched faecal and rinse water with respect to strain type (ST), ESBL-genotype and ABR profile. It was concluded that poultry farms were a major source of ESBL *E.coli* environmental contamination (Blaak et al., 2015).

Gram-negative bacteria from intensive single crop production farm soil showed a high degree of multidrug resistance, including ESBLs when compared to soils from extensive multi crop farms and organic multi crop farms. All farms used varying amounts of organic fertilisers with the intensive farms using highest levels as well as pesticides and chemical fertilisers. None of the farms used antibiotics. Organic farm soil where only small amounts of organic fertiliser was used on previously pristine land was associated with a reduced risk of multidrug resistance. Similarities were found between the genes coding for ESBL in the soil and clinical isolates from humans and animals showing resistance, highlighting the risk of the environment as a reservoir of ABR (Jones-Dias, Manageiro and Canica, 2016).

1.4 Antibiotic Classes, Mechanisms of Action and Mechanisms of Resistance

a) β-lactam antibiotics

β-lactam antibiotics consist of the penicillin's, cephalosporins, cephamycins, carbapenems and monobactams. They all contain a four membered β-lactam ring. They prevent the maintenance of the bacteria's cell wall by binding to penicillin binding proteins (PBPs). PBPs are transpeptidase enzymes necessary for maintenance of the peptidoglycan cross linking in the bacterial cell wall (Bush and Bradford, 2016). Damage to the cell wall results in cell stress and lysis. They have a broad spectrum of activity against Gram-negative and Gram-positive bacteria as well as aerobic and anaerobic species (Fair and Tor, 2014).

The addition of β-lactam inhibitors such as clavulanic acid, sulbactam and tazobactam to a β-lactam antibiotic act by binding to certain β-lactamases, deactivating the enzyme (so protecting the β-lactam antibiotic) and allowing the β-lactamase to continue working (Drawz and Bonomo, 2010).

Resistance mechanisms include enzymatic degradation by β-lactamases, mutations to PBPs and removal of antibiotic via increased efflux pumps.

The main mechanism of resistance to this class is due to enzyme breakdown of the β-lactam ring (Fair and Tor, 2014). β-Lactamases hydrolyse the β-lactam ring of penicillin, cephalosporins and carbapenems (Hammerum and Heuer, 2009; Fair and Tor, 2014). These enzymes are coded for by chromosomal genes or genes found on plasmids (Hammerum and Heuer, 2009). β-lactamases are divided into 4 groups according to the Ambler classification (Fair and Tor, 2014).

Class A contain β-lactamases that inactivate penicillin's and some cephalosporins. Some enzymes can also inactivate carbapenems. Most extended spectrum β-lactamases (ESBLs) fall into this group and can hydrolyse a wide variety of penicillin's and cephalosporins (Greenwood et al., 2007; Hammerum and Heuer, 2009; Fair and Tor, 2014). ESβLs in this group are usually found on plasmids allowing for spread within and between species. Class B consist of the metallo-β-lactamases (MBLs) and are often found on class 1 integrons associated with gene cassettes containing aminoglycoside modifying enzymes (AMEs) conferring co-resistance to quinolones. Class C consists of the AmpC β-lactamases which are usually found on chromosomes. They inactivate a wide range of β-lactams including most of the cephalosporins, aztreonam but not carbapenems. Class D consists of the OXA β-lactamases which hydrolyse cephalosporins, aztreonam and some carbapenems, they are often found on integrons making them transferable (Fair and Tor, 2014). β-lactamases are a major threat to the efficacy of β-lactam antibiotics and therefore human health (Li et al., 2014).

There are a number of penicillin-binding proteins (PBPs) found in each species of bacteria. Mutation of the PBPs results in reduced binding of the β-lactam thus resulting in resistance (Greenwood et al., 2007; Fair and Tor, 2014)).

Increase in efflux by RND and ABC efflux pumps result in increased efflux of antibiotic. β-lactam antibiotics usually enter the bacterial cell via porins. Changes to porin structure such as size and function, block the antibiotic from entering, result in resistance to β-lactams (Greenwood et al., 2007; Fair and Tor, 2014).

Ceftiofur a third generation cephalosporin, is sometimes injected *in ovo* or into 1 day old chicks in the hatchery to prevent *E.coli* infections (Baron, 2014).

b) Quinolones

Quinolones have a broad spectrum of activity against aerobic Gram-negative and Gram-positive bacteria as well as some anaerobic Gram-negatives (Fair and Tor, 2014). They work by inhibiting DNA coiling and protein synthesis. They do this by binding to either DNA gyrase in the case of Gram-negative bacteria or topoisomerase IV in the case of Gram-positive bacteria (Redgrave et al., 2014; Hooper and Jacoby, 2015).

Resistance to quinolones can be due to several mechanisms working alone or in conjunction with each other. They can be chromosomal changes or plasmid mediated. The first is resistance via mutations to DNA gyrase or topoisomerase IV resulting in reduced drug binding. Second is an increase in transport of the drug out of the bacteria via efflux pumps and reduced influx of drug through porin loss. This results in reducing intracellular drug and as a result causes resistance. Third is mutation of an aminoglycoside acetylating enzyme which develops the ability to acetylate certain fluoroquinolones such as ciprofloxacin and norfloxacin. Fourthly plasmids can carry qnr genes which code for proteins that protect the binding sites on DNA gyrase and topoisomerase IV from drug binding (Redgrave et al., 2014; Hooper and Jacoby, 2015).

Enrofloxacin, a fluoroquinolone, is used to treat respiratory disease in poultry by adding it to the drinking water, resistance to enrofloxacin results in cross resistance to ciprofloxacin which is often used in humans (Nelson, 2007).

c) Aminoglycosides

Aminoglycosides are used mostly against Gram-negative bacteria but are active against some Gram-positive ones and are used in the treatment of drug resistant tuberculosis. Aminoglycosides bind to the A site of the 16S rRNA unit on the 30S sub-unit of the bacterial ribosome. This binding causes a mRNA and tRNA mismatch resulting in a mistranslation of proteins being produced affecting bacterial functioning. Abnormal proteins are thought to be incorporated into the cell wall and eventually result in cell death. Some aminoglycosides are capable of also binding to the 50S sub-unit and affecting protein translation in two sites (Garneau-Tsodikova and Labby, 2016).

Resistance in aminoglycosides is most commonly caused by aminoglycoside-modifying enzymes (AMEs) found near the inner cytoplasmic membrane (Li et al., 2014). These enzymes are divided into three groups determined by how they modify the aminoglycoside molecule. The three groups are the aminoglycoside-acetyltransferases (AAC), aminoglycoside nucleotidyltransferases (ANT) and aminoglycoside -phosphotransferases (APH). They are found on their own or in combinations. One of the most common and clinically significant is coded for by the aac(6)-lb gene (Garneau-Tsodikova and Labby, 2016). The different aminoglycosides have different susceptibilities to AMEs depending on their amino and hydroxyl groupings. The AACs work by attaching an acetate to the amino group on the aminoglycoside. For this reason, they only work on aminoglycosides containing deoxystreptamine and do not affect streptomycin and spectinomycin. The ANTs attach nucleotides to exposed hydroxyl groups while APHs attach a phosphate molecule to the exposed hydroxyl group. For this reason, the combination of AMEs present in a bacteria will determine which of the aminoglycosides the bacterium will be resistant to as they do not all have the same amino and hydroxyl groups (Greenwood et al., 2007).

Aminoglycoside modifying enzymes are often encoded on mobile genetic elements allowing for transfer between and within species (Fair and Tor, 2014).

Resistance can also develop by changes to the ribosome target site, preventing aminoglycoside binding. This occurs by either mutation of the binding site or enzymatic changes to the binding site by RNA methyltrasnferases. Mutations of the binding site are often lethal to the bacterium. RNA Methytransferases methylate the 16S rRNA so preventing the aminoglycoside from binding and resulting in resistance. These enzymes are coded for by genes which can be transferred on mobile genetic elements. They however do not provide resistance to aminoglycosides that bind to the 50S unit, such as neomycin and apramycin (Garneau-Tsodikova and Labby, 2016).

Cell wall changes can also result in resistance due to reduced intracellular amounts of drug. These can be due to down regulation of porin channels reducing inflow. Incorporation of positively charged arabinose molecules into the lipopolysaccharide outer layer causes a decreased affinity to the cationic aminoglycoside molecule reducing influx of drug. Increase in efflux pumps so increasing movement of drug out of the bacterium causing resistance (Garneau-Tsodikova and Labby, 2016).

Neomycin is commonly used for bacterial gastrointestinal infections in poultry (EMEA, 2002) while gentamicin is injected into day old chicks for treatment of *E.coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* (Agunos, 2012).

d) Amphenicols

Amphenicols are phenylpropanoid antibiotics of which chloramphenicol was used in humans and florfenicol is registered for use in veterinary medicine. Chloramphenicol has a broad spectrum of activity against Gram-positive and Gram -negative bacteria and can diffuse into cerebrospinal fluid and cells making it good for meningitis and intracellular infections. It does have however serious side effects such as fatal aplastic anaemia, and for this reason, is no longer routinely used. It is still used in some LMICs however as it is inexpensive (Greenwood et al., 2007). Florfenicol does not have the nitro ring that chloramphenicol has making it less toxic and safe for use in food producing animals (Al-Shahrani and Naidoo, 2015). It has a wide spectrum of activity and is used in the treatment of *E.coli* as well as other enteric bacterial infections (Pokrant, 2018).

Chloramphenicol binds to the peptidyl transferase centre on the 23S rRNA of the 50s Ribosome unit preventing elongation during protein synthesis (Fair and Tor, 2014).

There are several mechanisms of resistance to chloramphenicol antibiotics. The most common cause is due to chloramphenicol acetyltransferases, which inactivate chloramphenicol, and are transferred on MGEs via cat genes. The gene cfr confers resistance via methylation of the target site on the 23s rRNA, so preventing chloramphenicol binding. Similarly, mutations on the 23s rRNA can prevent drug

binding and result in resistance. Chloramphenicol can also be inactivated by 3-O-phosphotransferases (Roberts and Swartz, 2009).

e) Macrolides

Macrolides work by binding to the 50s ribosomal subunit preventing elongation of the translation during protein synthesis. They have a moderately broad spectrum of activity and are mostly bacteriostatic (Fair and Tor, 2014).

The main mechanism of resistance is efflux pumps but modification of number of target molecules and macrolide modifying enzymes also play a role (Fair and Tor, 2014).

Erythromycin, the first discovered antibiotic in the group, induces the production of a methylation enzyme which acts on the 23 s ribosomal RNA and is stored on a plasmid. If this enzyme is induced by erythromycin, the bacteria will also be resistant to other macrolides, lincosamides and streptogramins. If enzyme is not induced the rest of the macrolide group retain their effectiveness (Greenwood et al., 2007).

Tylosin is a macrolide often used in poultry as a growth promotant as well as treatment of necrotic enteritis and respiratory diseases caused by *mycoplasma* sp. It however is classified as a critically important antibiotic according to the WHO. This is due to the development of cross resistance to erythromycin an important antibiotic in human medicine (Paranhos et al, 2020)

Tiamulin fumarate, a pleuromutilin antibiotic and related to the macrolides is used for treating airsacculitis caused by *Mycoplasma* sp and spirochaetes in poultry. It can be added to either water or food (Islam et al, 2009).

f) Tetracyclines

Tetracyclines are broad spectrum antibiotics with activity against Gram-positive, Gram-negative, spirochetes, chlamydia and some protozoan parasites. They are generally bactericidal but can be bactericidal in certain instances such as tigecycline. Tetracyclines primarily bind to the 16S rRNA of the 30S sub-unit of the ribosome and inhibit elongation of protein synthesis (Grossman, 2016).

Resistance to tetracycline develops due to four acquired mechanisms. The primary mechanism of action is due to excretion of drug through efflux pumps. The majority of pumps are part of the major facilitator superfamily (MFS) where tetracycline is exchanged for protons and is energy dependent. The genes tetA and tetB encode for these efflux pumps and are transferable on mobile genetic elements. Resistance is also caused by binding site mutations reducing tetracycline affinity and ribosomal protection proteins (RPP) which can release tetracycline from its binding to the 16S rRNA and subsequently cause resistance. RPP are mediated by Tet(O) and Tet(M) and are transmitted by MGEs. The last major mechanism of resistance is the presence of enzymatic inactivation. Mono-oxygenases

add a hydroxyl group to tetracyclines. They are transferable by MGEs and mediated by Tet(X) and Tet(37) as well as others (Nguyen et al., 2014; Grossman, 2016).

Added to the above mechanisms tetracycline can induce multidrug resistance through intrinsic systems. AraC family of transcriptional activators respond to environmental stress including antibiotics. Tetracycline is known to activate MarA found in *E.coli* which leads to overexpression of a multidrug efflux pump AcrAB and down regulating the porin OmpF leading to MDR (Grossman, 2016).

Chlortetracycline and Oxytetracycline, both tetracyclines, are used in broilers to treat airsacculitis as they can be added to drinking water (Singer and Hofacre, 2006)

g) Sulphonamides

Sulphonamides have a broad spectrum of activity but due to side effects such as allergic reactions and blood dyscrasias are now used less frequently (Fair and Tor, 2014). Sulphonamides are analogues of para-aminobenzoic acid (PABA), a precursor in the production of folate. Bacteria produce folate to produce purine nucleotides and thiamine necessary for cell division. Sulphonamides competitively inhibit the enzyme dihydropteroate synthetase (DHPS), which converts PABA to dihydropteic acid (Huovinen et al., 1995; Greenwood et al., 2007).

Resistance is caused by either mutation of the dhps gene on the chromosome leading to DHPS enzymes with a lower affinity to sulphonamides or via mobile genes sul1, sul2, sul 3 transferred on plasmids. These genes code for drug resistant variants of DHPS resulting in resistance (Huovinen et al., 1995; Kim et al., 2019). Recently a monooxygenase enzyme has been discovered in soil bacteria that can degrade sulphonamides named sulX (Kim et al., 2019).

h) Trimethoprim

Trimethoprim are diaminopyrimidines which inhibit the production of folate at a later stage in the production compared to sulphonamides. Diaminopyrimidines inhibit the enzyme dihydrofolate reductase (DHFR), preventing the conversion of dihydrofolate to tetrahydrofolate, the active form of folate (Huovinen et al., 1995; Greenwood et al., 2007). Combined with sulphonamides there has been found to be a 100-fold synergistic effect compared to either drug used individually (Fair and Tor, 2014).

Resistance to trimethoprim is by the production of DHFR enzymes which are not susceptible to trimethoprim either via mutations on the chromosome or transferable genes found on mobile genetic elements. There are a number of dhfr genes that code for DHFRs which are not susceptible to trimethoprim (Huovinen et al., 1995).

Trimethoprim sulfamethoxazole is broad spectrum antibiotic used in broilers to treat Gram negative organisms causing pneumonias as well as treating coccidiosis (Poultry DVM, 2020).

i) Polymixins

Polymixins have a bactericidal effect against Gram-negative bacteria. They act on the disrupting the lipopolysaccharide (LPS) outer layer of the gram negative cell membrane resulting in cell lysis (Fair and Tor, 2014). They have historically not been used due to neurotoxicity and nephrotoxicity, but colistin has recently become a last line antibiotic in human medicine (Fair and Tor, 2014; Quesada et al., 2015). Colistin is however still used in poultry production in some countries (Quesada et al., 2015).

Resistance is due to changes in LPS molecules either by mutation of chromosomal genes or as a result of the plasmid-mediated mcr-1 gene (Perreten et al., 2016; Robinson et al., 2016). The mcr-1 gene codes for a phosphoethanolamine transferase enzyme, which attaches a phosphoethanolamine to lipid A in the outer layer of the cell membrane, preventing any polymixin binding. Prevention of binding results in antibiotic resistance, as the polymixin cannot cause cell lysis (Liu et al., 2016). Resistance can also be due to increase in protein H1 which changes the divalent cations of the LPS protecting it or increasing the polysaccharide capsule so protecting the LPS layer. There have also been cases of enzyme breakdown by a colistinases (Fair and Tor, 2014).

1.5 Poultry Production

1.5.1 Antibiotic use

After World War 2, demand for poultry as a source of protein increased, and with it the expansion of the poultry industry into intensive commercial farms. Initially in the 1960s broilers took 112 days until market size of 1.1kg with a feed conversion of 4.7. Through genetic selection, improved biosecurity, vaccination programs, disease prevention and correct nutrition as well as the use of growth promoters' broilers are now ready for market in 35-42 days and have a feed conversion of 1.8 at a weight of 2.7 kg (Hanning, 2015).

Antibiotics are used extensively in poultry production for several reasons. First introduced in the 1940s, it was discovered that adding antibiotics at sub-therapeutic dosages to chicken feed improved feed conversion and decreased mortality (Graham, Boland and Silbergeld, 2007). This is thought to be due to changes in intestinal microbiota, resulting in more nutrient availability and absorption (Hanning, 2015; Mehdi, Letourneau-Montminy, et al., 2018). Antibiotics are also used to prevent diseases in chickens such as necrotic enteritis, caused by *Clostridium perfringens* (Mehdi, Letourneau-Montminy, et al., 2018), and colibacillosis, caused by Avian Pathogenic *Escherichia coli* (APEC) (Kunert Filho et al., 2015). Colibacillosis can cause severe morbidity with carcass condemnation and mortalities resulting in economic loss (Kunert Filho et al., 2015). Antibiotics are used to reduce potential zoonotic pathogens from entering the food chain from poultry production. Diseases such as campylobacteriosis,

salmonellosis and infections caused by *E.coli* can cause disease in humans from ingestion of contaminated meat. Antibiotics have helped control these diseases (Mehdi, Letourneau-Montminy, et al., 2018).

Antibiotics have been used as growth promoters and been implicated in the development of antibiotic resistance (Graham, Boland and Silbergeld, 2007; Fair and Tor, 2014; FAO, 2016). Antibiotics added as growth promoters to animal feed are often at sub-therapeutic dosages therefore increasing selection for resistant organisms (Van Boeckel et al., 2015). This was demonstrated in a study in India that showed that on farms using antibiotics for growth promotion, there was an increased rate of antibiotic resistance amongst *E.coli* isolates in comparison to farms not using growth promoters (Brower et al., 2017).

With the increased importance and focus on the development of antibiotic resistance, the use of antibiotics as growth promoters has been debated. A study by one of Americas largest poultry producers compared houses given antibiotics for growth promotion and houses given no antibiotics (Graham, Boland, Silbergeld, 2007). The results showed that growth promoting antibiotics cost the producer more than the resultant gain from reduced loss in mortality and condemnation at slaughter. An argument was made that growth promoting antibiotics added no advantage to production and due to the promotion of antibiotic resistance should be discontinued (Graham, Boland and Silbergeld, 2007).

The European Union banned the use of antibiotics as growth promoters in 1999 (Graham, Boland and Silbergeld, 2007). Since then some European countries have been able to maintain production with significantly less use of antibiotics (Van Boeckel et al., 2017). To maintain production without the use of antibiotics requires good biosecurity, vaccination programs, lower stocking densities and animal husbandry. In some developing countries the increased demand for animal protein has led to agriculture moving from extensive systems to intensive systems. In these situations antibiotics are unfortunately used to compensate for inadequate husbandry and biosecurity systems (Van Boeckel et al., 2017).

In an intensive production animal system, there are large numbers of animals being grouped together (Van Boeckel et al., 2015). With close contact of animals, disease spreads quickly between individual animals making maintaining a healthy herd or flock difficult (Van Boeckel et al., 2015). In poultry for instance there can be thousands of birds in a poultry house. It is therefore difficult to treat individual birds, so instead the entire group will be medicated. For this reason, antibiotics are often being added to feed or water. Due to these administration methods, each individual bird in not given an optimal dose as it is dependent on how much they eat or drink (Founou, Founou and Essack, 2016). When dosing is done for preventing disease it is known as prophylaxis. In comparison when an entire flock is dosed to treat a few individuals it is known as metaphylaxis (FAO, 2016; Founou, Founou and Essack, 2016).

In extensive and small holder livestock farming, the situation is different. Individual animals are more likely to receive therapeutic treatment. The choice and route of administration of antibiotics would also be different when compared to intensive farming (FAO, 2016). This could result in different antibiotic resistance profiles when comparing different farming systems (Bester and Essack, 2010). Subsistence farming also increases the risk of zoonotic diseases due to more direct contact between humans and animals (Gummow, 2003; Bester and Essack, 2010).

In South Africa, the majority of antimicrobials used in animals are administered in feed (68,5%) of which Tylosin, a pleuromutilin, make up 61%, tetracyclines 14% and polipeptides 9%. Sulphonamides make up 95,4% of antimicrobials sold for in-water use. In feed and in water medication make up the majority of applications in the poultry industry. The majority, 72% of antimicrobials, fall under the Stock Remedies Act and can therefore be bought without a veterinary prescription (Eager, Swan, van Vuuren, 2012).

1.5.2 Farming methods, processing, contamination

There are many types and intensities of poultry farming. These range from a few chickens in the back yard for either eggs or meat, to large poultry houses with floor areas up to 1860m^2 and a stocking density of two birds/m^2 in commercial units. This equates to houses of about 30000 birds. In between are a continuum of sizes and intensifications (Karcher and Mench, 2018).

In HICs majority of poultry production is done by large commercial farms. These are generally intensive farming set ups with strict biosecurity measures, climate control of houses, vaccination programs and controlled feeding. Antimicrobials are often used for growth promotion and disease control (Hanning, 2015).

LMICs often have small scale back yard set ups or small production houses with between 50 and 500 birds. The farmers will provide shelter and feed (Ahuja and Sen, 2007). They may be using vaccinations and treatments, if available, but are usually reactive rather than preventative, so antibiotics are not used as a standard used (FAO, 2013). Biosecurity is minimal if present. The main disadvantages of small-scale production units are that they can be outcompeted by large companies who control genetic stock and economies of scale. Small scale production units have increased prevalence and risk of spreading disease to the public as there are less controls in place down the food chain (Ahuja and Sen, 2007).

Backyard and subsistence poultry have been advocated by the FAO as a means of poverty alleviation, improved nutrition and improvement of welfare of people in LMICs. Poultry farming has fewer barriers to starting than other forms of farming making it an attractive option. Chickens provide a source of protein while eggs provide protein and essential nutrients (FAO, 2013). In LMICs backyard chickens

can make up most of the overall chicken production of a country far exceeding commercial production (Ahuja and Sen, 2007).

Measures to reduce the consumption of antibiotics in the poultry industry focus around disease prevention. Disease prevention is done through vaccinations programs, improved quarantine methods and biosecurity of houses and farms. Good husbandry is necessary to reduce stress of birds and so reducing disease in the flock (Cox and Pavic, 2010).

In the slaughter and processing plant there are areas where contamination with bacteria can occur. Correct handling of birds is essential to prevent contamination during defeathering and evisceration. Cleanliness and disinfection of facilities, equipment and staff is necessary to prevent bacteria transferring to the end retail products. Correct chilling and storage of meat products are necessary to stop bacterial replication. All these factors help to reduce the risk of contamination of meat with zoonotic bacteria or commensal bacteria with the potential to harbor antimicrobial resistant genes (Cox and Pavic, 2010).

1.5.3 ABR in Poultry Production

Antibiotic resistance in production animals, including poultry, has been studied in recent years with the emergence of multidrug resistant bacteria and the threat they pose for human, animal and environmental health (Singer and Hofacre, 2006). Studies of antibiotic resistance have been done in bacteria from faecal or cloacal samples, the poultry farm environment, clinical samples from diseased birds, farm workers and meat products from both abattoirs and retail outlets (Oguttu, Veary and Picard, 2008; Saidi, Mafirakureva and Mbanga, 2013; Adelowo, Fagade and Agersø, 2014; Brower et al., 2017; Johnson et al., 2017).

Resistance levels vary between countries and continents. This is related to what antibiotics are being used and in what way. Denmark stopped the use of Avoparcin in 1995 which was followed by the rest of the European Union in 1997. Avoparcin causes cross resistance with vancomycin, an important human antibiotic. Studies since the ban have had variable decreases in vancomycin resistance thought to be due to the perseverance of genes in the environment (Singer and Hofacre, 2006). Scandinavian countries have relatively low levels of antibiotic resistance compared to other regions due to low use of antibiotics in poultry farming. Sweden stopped using antibiotics for growth promotion in 1986, while Denmark stopped in 1999, neither have had significant decreases in production (WHO, 2017b).

It is difficult to compare studies due to different study design, but some general significant trends are seen in the resistance profiles. MDR is commonly found. A study in India found MDR in 94% of layer and 60% of broiler cloacal samples (Brower et al., 2017) while cloacal samples from chickens in China showed 72,8% MDR in *E.coli* isolates (Li et al., 2014). Studies of resistance in *E.coli* from chicken

meat have also had high levels. Samples from Kenya had 42,9% showed MDR (Odwar et al., 2014) while 100% of samples from Iran where multi-drug resistant (Talebiyan et al., 2014).

Another concerning finding is the increase in ESBL found in bacteria of poultry origin. This a public health concern if resistance transfers across to humans, as ESBL threaten the usefulness of many commonly used antibiotics for human infections. This leads to difficult to treat infections or necessitates the use of last resort antibiotics. ESBLs have been found in studies of poultry farms, the environment, and meat. A study in the Netherlands found 65% of layers and 81% of broilers had ESBL positive *E.coli* in faecal samples (Blaak et al., 2015). In India 42% of layers and 87% of boilers cloacal samples had ESBL positive *E.coli* (Brower et al., 2017). In China 49,5% of chicken faecal samples contained ESBL positive *E.coli* (Li et al., 2014). Meat samples have also been found to have high levels of ESBLs. For example, a study in Ghana found 44% of local poultry meat and 31% of imported poultry meat contained ESBL positive *E.coli* (Eibach et al., 2018). Much lower levels were seen in a Finnish study with only 8,1% of poultry meat containing ESBL positive *E.coli* (Päivärinta et al., 2016).

Studies comparing conventional farming practices to free range or organic farming have been done. A study in Brazil in 2015, comparing antibiotic resistance in meat from conventional poultry farming versus free range farming, found bacteria from conventional farming practices had higher levels of antibiotic resistance, virulence genes and genes for ESBLs than those from free range poultry meat (Koga et al., 2015). A similar study done in Arizona, United States of America in 2012, compared conventional farmed turkey and chicken meat with poultry raised without antibiotics and organically farmed poultry. They found a similar difference with meat from conventionally raised poultry having higher levels of antibiotic resistance compared to organic or raised without antibiotic categories. This difference was greater in turkey meat compared to chicken meat. They also found that in chicken meat differences between brands was greater than between production types. This highlighted the possible influence of production facilities on the resistances found in poultry meat (Davis et al., 2018).

Higher levels of resistance have also been found in enteric *E.coli* from poultry workers compared to community members. In a study done in poultry workers from Maryland and Virginia in the United States of America in 2007, poultry workers had higher levels of resistant bacteria compared to non-poultry workers. Poultry workers also had increased levels of multi-drug resistant isolates in comparison to non-poultry workers from the community (Price et al., 2007). A similar study in South Africa in 2008 and found that resistance in poultry workers was higher than in the non-worker community members, however the difference was not statistically significant (Oguttu, Veary and Picard, 2008).

1.5.4 Transfer of ABR from food animals and meat products to humans

There are two proposed mechanisms of transfer of antibiotic resistance from the food production chain to humans and could impact human health adversely. The first is directly from animals to humans via

contact with live animals, body fluids, faeces, urine, and semen. The direct route affects occupations such as farmers, farm workers, veterinarians, and abattoir workers more than the general public. It is more prevalent in rural settings where humans and animals interact more closely. The indirect route is via food products to the end consumers. The end product can be contaminated with resistant bacteria at several stages along the food chain from the farm, through transport, to the processing plant and as a retail product at market. It is harder to determine the extent that the indirect pathway is impacting human health, but it could potentially expose a very large number of people to resistant isolates of animal origin (Bester and Essack, 2010; Founou, Founou and Essack, 2016).

In either route it is proposed that health of humans is affected by either infection with resistant pathogenic bacteria, or from resistant commensal bacteria of animal origin, which then have the potential to transfer genes to pathogenic or commensal bacteria in humans. The extent to which resistance genes move from animal bacteria to human bacteria is difficult to quantify and track. This makes determining the contribution of animal source resistance to the cases of human resistance difficult to quantify. There are some authors that think that the transfer is not significant while others think the that resistance through the food chain is a threat to human health and measures need to be taken to slow the progress (Bester and Essack, 2010).

E.coli and *Enterococcus* are indicator organisms in poultry. They represent commensal bacteria but are potentially zoonotic. They carry resistance genes and are ubiquitous in poultry (Aarestrup, 2004). *E.coli* was chosen for this study for the reasons explained in the next section.

1.6 Escherichia coli (E.coli)

1.6.1 Bacterial characteristics

E.coli is a species of bacteria that belongs to the family Enterobacteriaceae . *E. coli* are Gram-negative, facultative, anaerobic bacteria that are normally motile due to the presence of flagella. They are rod shaped and approximately 1.1-1.5 µm to 6 µm in size (Kunert Filho et al., 2015).

E. coli are found as commensals in the digestive tract of animals and birds (Mahon, Lehman and Manuselis, 2011; Kunert Filho et al., 2015). Fimbriae allow adhesion within the intestinal tract. *E. coli* have sex pili which allow transfer of genetic information between organisms, including genes for antibiotic resistance. *E.coli* can also be pathogenic to both humans and animals (Mahon, Lehman and Manuselis, 2011).

1.6.2 Pathotypes and virulence factors

Pathogenic forms of *E. coli* occur in both the intestinal tract and in extra-intestinal tissues of humans, animals and birds (Kunert Filho et al., 2015). Pathogenicity is determined by the presence of virulence factors. Virulence factors include flagella providing motility, fimbria allowing adhesion and toxins.

Toxins allow the *E. coli* to survive in the host by causing lysis of immune cells, inhibiting phagocytosis and preventing chemotaxis of white blood cells (Mahon, Lehman and Manuselis, 2011).

a) Diarrhoeagenic *E.coli* (DEC)

In humans intestinal pathogenic *E. coli* or diarrheagenic *E. coli* are divided into five categories, determined by their effects on the host and virulence factors. These categories are enterotoxigenic, enteroinvasive, enteropathogenic, enterohaemorrhagic and enteroadherent which in turn is divided into sub-categories diffusely adherent and enteroaggregative. The diffusely adherent category is also implicated in urinary tract infections (Kunert Filho et al., 2015). Enterohaemorrhagic *E coli* are associated with the release of verotoxins and shiga toxins resulting in hemorrhagic diarrhea (Mahon, Lehman and Manuselis, 2011). Food borne infection routes are often the cause of DEC (Manges, 2016).

b) Extraintestinal *E.coli*

Extra-intestinal *E coli* (ExPEC) are commonly found as the cause of urinary tract infections as uropathogenic *E. coli* (UPEC), septicemia-associated *E. coli* (SEPEC) and neonatal meningitis *E. coli* (NEMEC) (Mellata, 2013). ExPEC bacteria can be found as commensals in the gastrointestinal tract, causing no harm until they opportunistically infect extra-intestinal tissues. There is a time lag between infection from original source into the intestine and final infection in extra intestinal tissue. This makes identification of the original source difficult (Manges, 2016).

In humans, the number of extraintestinal *E.coli* infections (ExPEC) has increased in recent years and with it, an increase in multidrug resistance (Mellata, 2013).

c) Avian Extraintestinal *E.coli*

Avian Extraintestinal *E.coli* (APEC) is a subgroup of ExPEC. APEC are a heterogenous group of virulent *E.coli* which infect poultry. APEC can belong to several different serogroups and contain a range of virulence genes and mobile genetic elements (Kunert Filho et al., 2015).

Intestines and the environment serve as a reservoir for APEC in poultry. APEC can infect several different organ systems in poultry. Infection can be localised or generalised and is collectively known as colibacillosis (Mellata, 2013).

Omphalitis and yolk sac infections are seen in the first week after hatching and is the most common cause of mortality during this time. Cellulitis of the lower abdomen and legs following injury is often found at slaughter resulting in condemnations. Salpingitis is found in layers, often with only a loss in egg production and an increased embryonic mortality. If the infection spreads to peritoneum it can cause septicemia and increased mortality (Kunert Filho et al., 2015).

Damage to the respiratory tract either mechanically, via dust and ammonia, or due to viral disease can allow secondary APEC infections in the respiratory tract. Infection of the air sacs can spread systemically resulting in colisepticemia (Kunert Filho et al., 2015).

1.6.3 APEC as a source of ExPEC.

DEC has often been linked to contaminated food as a source of infection. In comparison, food sources are suspected as being a reservoir for ExPEC, but due to the time lag between colonisation of the gut and extra intestinal infection, this is difficult to prove (Manges, 2016). Of the potential food sources, poultry products seem to be the largest potential sources of ExPEC pathogens. Johnston et al. (2005) investigated a range of retail food sources for *E.coli* and ExPEC and found that poultry had the highest levels of *E.coli* contamination and potential ExPEC compared to other food sources (Johnson et al., 2005).

The theory that APEC from-poultry products can be a source of ExPEC infections in humans, is based on several factors such as genetic similarities in virulence factors and mobile genetic elements. The pathogenic potential of APEC in human-mice models and ExPEC from human clinical samples in chicken models has been shown. Molecular epidemiological data of animal and human clinical isolates have been shown to have close links. This combined with cryptic outbreaks of ExPEC infections in communities with specific strains have led to the conclusion that APEC could be a source of ExPEC in humans (Manges, 2016).

1.6.4 Use as an Indicator Bacteria

E.coli are used in surveillance programs as an indicator bacteria. This is because they are found in both animals and human's intestinal tracts as commensal bacteria. *E.coli* are relatively easily cultured, making laboratory work less difficult. Due to *E.coli* being able to accept and transfer plasmids easily, they accumulate ARGs and are therefore representative of the antibiotic resistance present in a population. Bacteria selected for monitoring, generally fall into one or more of the following categories: animal pathogens, zoonotic organisms and indicator bacteria (Aarestrup, 2004).

Animal pathogen specimens might give an early warning system for emerging resistance. But due to inconsistencies in submission by veterinarians, the possibility of previous antimicrobial treatments, or treatment failure prior to submission, animal pathogen samples will not give an accurate overview of antimicrobial resistance. Zoonotic bacteria are included for their public health implications and include *Salmonella* and *Campylobacter spp* (Aarestrup, 2004). Indicator bacteria should be included as they can be isolated from healthy humans and animals and give a better overview of resistance, e.g. *Escherichia coli* and *Enterococcus* sp.

1.7 South African Perspective

Antibiotic usage in food producing animals in South Africa is governed by two Acts; the Fertilizers, Farm Feeds and Agricultural Remedies and Stock Remedies Act (Act No. 36 of 1947) and the Medicines and Related Substances Control Act (Act No. 101 of 1965). These Acts are regulated by different government departments: the national Department of Agriculture, Land Reform and Rural Development (DALRRD) and the national Department of Health respectively (Eagar, Swan and Van

Vuuren, 2012). Information on antimicrobial usage in animals is still scarce (Schellack et al., 2017), but it has been reported that 72% of the registered antibiotics used in food animals fall into the Fertilisers, Farm Feeds and Agricultural Remedies and Stock Remedies Act. Of the infeed registered antimicrobials, 64 were stock remedies and 5 registered as veterinary medicines. Stock remedies are sold over the counter and do not require a prescription while veterinary medicines require a prescription from a veterinarian for use in animals. Tylosin, the largest selling antimicrobial in the South African study, spiramycin, bacitracin and virginiamycin are authorised for use in South Africa, but have been banned for use in the European Union as Growth Promoting Antibiotics as they are structurally related to important human medications. It seems that in South Africa, antimicrobial use in food animals is poorly controlled when compared to Europe where farmers require prescriptions from veterinarians in order to use antimicrobials in food animals (Eagar, Swan and Van Vuuren, 2012).

Only a few studies of antibiotic resistance in *E.coli* in poultry in South Africa have been published.

One study published in 2008 looked at antibiotic resistance in *E.coli* and *Salmonella* from poultry, poultry abattoir workers and volunteer controls. Poultry isolates showed a high degree of resistance to doxycycline (98.2%), sulphamethoxazole (78.7%), ampicillin (75%), enrofloxacin (75.6%), fosfomycin (98.2%) and nalidixic acid (90.5%). These antibiotics were all either given to the chickens or included in feed in the period before sampling except for nalidixic acid which has a cross resistance with enrofloxacin (Oguttu, Veary and Picard, 2008).

A study of ABR in colibacillosis samples from 2009 to 2015, from South African poultry farms, showed high levels of MDR. However, MDR levels were dropping towards the end of the study. MDR was still however 77% in 2015. The study found that resistance to antibiotics tended to match the antibiotics being used on farms at the time of sampling (Theobald et al., 2019).

Close to South Africa, a study done using clinical avian pathogenic *E.coli* in Zimbabwe had similar resistance patterns for ampicillin (high 94.1%) and tetracycline (100%). However, all isolates were susceptible to ciprofloxacin, which is the same group as enrofloxacin, which was high, when tested in the South African study. It seems that enrofloxacin is not yet widely used to treat colibacillosis in chickens in Zimbabwe and may account for the differences. Chloramphenicol is often used in Zimbabwe to treat colibacillosis with resistance to chloramphenicol being 36.9%, and isolates falling into the intermediate group accounting for 45.6% (Saidi, Mafirakureva and Mbanga, 2013).

The South African Veterinary Council has recently made it unprofessional by veterinarians to use colistin, a last line human antibiotic, in food animals unless there is no alternative based on culture and sensitivity (Schellack et al., 2017). This followed the detection of colistin resistance isolates in clinical colibacillosis from broilers in 2015 and the detection of the *mcr-1* gene in 19 of these cases (Perreten et al., 2016). The *mcr-1* gene is transferable within a bacterial species and between species of bacteria, increasing the risk of colistin resistance spread. In 2016, nine clinical human cases of colistin resistant

organisms with the *mcr-1* gene were found in South Africa, raising concerns (Coetzee et al., 2016). The *mcr-1* gene has also been found in bacteria in Tunisia and Algeria (Alonso et al., 2017).

Overall resistance in food animals in Africa has been found to be high to tetracyclines, penicillin's and sulphonamides, all of which are commonly used in animal husbandry. There have been increasing reports of quinolone resistance and the presence of ESBL resistance emerging from Africa in food animals and animal products (Alonso et al., 2017).

1.8 Conclusion

There is still very little information on the levels of antibiotic resistance in poultry in South Africa. Surveillance is necessary to monitor resistance trends to allow interventions and to identify new resistance profiles, especially to last resort antibiotics.

This study is unique as no other studies to our knowledge in South Africa have looked at resistance in *E.coli* over a farm to fork continuum from hatching to final retail product in chicken.

Study design

2.1 Aim

The aim of this study was to investigate the molecular epidemiology of antibiotic resistant *E coli* from farm-to-fork in intensively produced chicken in the uMgungundlovu district, KwaZulu Natal, South Africa.

2.2 Objective

1) To isolate *E.coli* from chicken samples collected across the farm-to-fork continuum,
2) To ascertain the antibiotic susceptibility of each isolate to relevant antibiotics
3) To identify selected resistance genes responsible for the susceptibility profiles observed
4) To ascertain the clonal relatedness between the isolates collected over the time period.

2.3 Methodology

Samples were taken from the poultry house from week one to week five (litter and faecal), from transport of chickens from the houses to the abattoir (crates and truck samples), from the abattoir (carcass rinse and caecal samples) and from retail products (whole chickens, thigh and necks). Lastly samples of the wastewater from cleaning the chicken house were collected. The same flock of chickens was followed throughout the study.

E.coli was identified putatively using selective media and biochemical tests and definitively identified using qPCR testing for the *uidA* gene. Antibiotic susceptibility was performed using the Kirby-Bauer disc diffusion method and results were evaluated according to Clinical and Laboratory Standards

Institute guidelines. Antibiotics tested were based on the WHO-AGISAR recommended panel for *E.coli*.

ERIC PCR was performed on a selection of isolates based on antibiograms. This was to determine clonality between isolates. The same isolates were tested using qPCR for the following resistance genes, where relevant, according to their antibiograms: *sul1, sul2, sul3, tetA, tetB, qnrB, qnrS, aac(6)-lb-cr*, bla_{CTX-M}, bla_{SHV}, bla_{TEM}. (Ebomah, Adefisoye and Okoh, 2018).

References

Aarestrup, F. M. (2004) Monitoring of antimicrobial resistance in animals: Principles and Limitations, *Journal of Veterinary Medicine*, 51, pp. 380–388.

Adelowo, O. O., Fagade, O. E. and Agersø, Y. (2014) Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, southwest Nigeria, *Journal of Infection in Developing Countries*, 8(9), pp. 1103–1112

Agunos, A., Léger, D., Carson, C. (2012) Review of antimicrobial therapy of selected bacterial diseases in broiler chickens in Canada, *Canadian Veterinary Journal*, 53 pp 1289-1300.

Ahuja, V. and Sen, A. (2007) Poultry in the 21st Century: avian influenza and beyond. International Poultry Conference Bangkok, November 2007, in *Scope and Space for Small-scale Poultry Production in Developing Countries*. Bangkok: Food and Agriculture Organization of the United Nations, pp. 549–568. Available at: http://www.fao.org/3/i0323e/i0323e00.htm.

Al-Shahrami, S., Naidoo, V. (2015) Florfenicol induces early embryonic death in eggs collected from treated hens, *BMC Veterinary Research*, 11:213

Akrami, F., Rajabnia, M. and Pournajaf, A. (2019) Resistance integrons; A mini review, *Caspian Journal of Internal Medicine*, 10(4), pp. 370–376.

Alonso, C. A., Zagara, M., Ben Sallem, R., Jouini, A., Ben Slama, K., Torres, C. (2017) Editor ' s Choice Antibiotic resistance in *Escherichia coli* in husbandry animals : the African perspective, *Society for Applied Microbiology*, 64, pp. 318–334.

Baron, S. Jouy,, E. Larvor, E., Eono, F., Bougeard, S., Kempf, I., (2014) Impact of third-generation-cephalosporin and administration in hatcheries on fecal *Escherichia coli* Antimicrobial resistance in broilers and layers. *Antimicrobial Agents and Chemotherapy*, 58(9) p 5428-5434

Bester, L. A. and Essack, S. Y. (2010) Antibiotic resistance via the food chain: Fact or fiction? *South African Journal of Science*, 106(9–10), pp. 1–5.

Blaak, H., van Hoek, A.H.AM., Hamidjaja, R.A., van der Plaats, R.Q.J., Kerkhof-de Heer, L., de Roda Husman, A., Schets, F.M. (2015) Distribution, numbers, and diversity of ESBL-producing *E. coli* in the poultry farm environment, *PLoS ONE*, 10(8), pp. 1–23.

Brower, C. H., Mandal, S., Hayer, S., Sran, M., Zehra, A., Patel, S.J., Kaur, R., Chatterjee, L., Mishra, S., Das, B.R., Singh, P., Singh, R., Gill, J.P.S., Laxminarayan, R. (2017) The prevalence of extended-spectrum beta-lactamase-producing multidrug-resistant *Escherichia coli* in poultry chickens and variation according to farming practices in Punjab, India, *Environmental Health Perspectives*, 125(7), pp. 1–10.

Bush, K. and Bradford, P. A. (2016) β -Lactams and β -Lactamase Inhibitors: An Overview, *Cold Spring Harbor Perspectives in Medicine*, 6(8): a025247

Chang, Q. Wang, W., Regev-Yochay, G., Lipsitch, M., Hanage, W.P. (2015) Antibiotics in agriculture and the risk to human health: How worried should we be?, *Evolutionary Applications*, 8(3), pp. 240–247.

Coetzee, J. Corcoran, C., prentice, E., Moodley, M., Mendelson, M., Poirel, L., Nordmann, P. Brink, A.J. (2016) Emergence of plasmid-mediated colistin resistance (MCR-1) among *Escherichia coli* isolated from South African patients, *South African Medical Journal*, 106(5), pp. 449–450.

Cox, J. M. and Pavic, A. (2010) Advances in enteropathogen control in poultry production, *Journal of Applied Microbiology*, 108(3), pp. 745–755.

Davis, G. S. Waits, K., Nordstroom, L., Grande, H., Weaver, B., Papp, K., Horwinski, J., Koch, B., Hungate, B.R., Liu, C.M., Price, L.B. (2018) Antibiotic-resistant *Escherichia coli* from retail poultry meat with different antibiotic use claims, *BMC Microbiology*. 18(1), pp. 1–7.

Drawz, S. M. and Bonomo, R. A. (2010) Three Decades of B -Lactamase Inhibitors, *Clinical Microbiology Reviews*, 23(1), pp. 160–201.

Eagar, H., Swan, G. and Van Vuuren, M. (2012) A survey of antimicrobial usage in animals in South Africa with specific reference to food animals, *Journal of the South African Veterinary Association*, 83(1), pp. 1–9.

European Agency for the Evaluation of Medicinal Products Veterinary medicines and Inspections. (2002) Neomycin: Summary Report, Canary Warf, United Kingdom. Available at: https://www.ema.europa.eu/en/documents/mrl-report/neomycin-summary-report-2-committee-veterinary-medicinal-products_en.pdf .[Accessed: 30th November 2020].

Eibach, D. Dekker, D. Boahen, K.B., Akenten, C.W., Sarpong, N., Campos, C.B., Berneking, L., Aepfelbacher, M., Krumkamp, R., Owusu-Dabo, E., May, J. (2018) Extended-spectrum beta-lactamase-

producing *Escherichia coli* and *Klebsiella pneumoniae* in local and imported poultry meat in Ghana, *Veterinary Microbiology*, 217, pp. 7–12.

Fair, R. J. and Tor, Y. (2014) Perspectives in Medicinal Chemistry Antibiotics and Bacterial Resistance in the 21st Century, *Perspectives in Medicinal Chemistry*, 6, pp. 25–64.

Food and Agriculture Organisation (FAO) (2016) 'The FAO action plan on antimicrobial resistance 2016-2020'. Rome, Italy. http://www.fao.org/3/a-i5996e.pdf.

Food and Agriculture Organization of the United Nations (FAO)(2013) *POULTRY DEVELOPMENT REVIEW*. Rome, Italy. Available at: http://www.fao.org/3/i3531e/i3531e00.htm.

Founou, L. L., Founou, R. C. and Essack, S. Y. (2016) Antibiotic resistance in the food chain: A developing country-perspective, *Frontiers in Microbiology*, 7, pp. 1–19.

Frost, L. S. Leplae, R., Summers, A.O., Toussaint, A. (2005) Mobile genetic elements: The agents of open source evolution, *Nature Reviews Microbiology*, 3(9), pp. 722–732.

Furtula, V. Farrell, E.G., Diarrassouba, F., Rempel, H., Pritchard, J., Diarra, M.S. (2010) Veterinary pharmaceuticals and antibiotic resistance of *Escherichia coli* isolates in poultry litter from commercial farms and controlled feeding trials, *Poultry Science*, 89(1), pp. 180–188.

Garneau-Tsodikova, S. and Labby, K. J. (2016) Mechanisms of Resistance to Aminoglycoside Antibiotics: Overview and Perspectives, *Medchemcomm*, 7(1), pp. 11–27.

Graham, J. P., Boland, J. J. and Silbergeld, E. (2007) Growth Promoting Antibiotics in Food Animal Production: An Economic Analysis, *Public Health Reports*, 122, pp. 79–87.

Greenwood, D. Finch, R., Davey, P., Wilcox, M. (2007) *Antimicrobial Chemotherapy*. 5th Edition. Oxford University Press. London, United Kingdom

Grossman, T. H. (2016) Tetracycline antibiotics and resistance, *Cold Spring Harbor Perspectives in Medicine*, 6(4):a025387.

Gummow, B. (2003) A survey of zoonotic diseases contracted by South African veterinarians., *Journal of the South African Veterinary Association*, 74(3), pp. 72–76.

Hammerum, A. M. and Heuer, O. E. (2009) Human Health Hazards from Antimicrobial-Resistant *Escherichia coli* of Animal Origin, *Clinical Infectious Diseases*, 48(7), pp. 916–921.

Hanning, I. (2015) Antibiotic Use in Poultry : A Driving Force for Organic Poultry Production, *Food Protection Trends*, 35(6), pp. 440–447.

Holmes, A. H., Moore, L.S.P, Sundsford, A., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P.J., Hooper, D. C. and Jacoby, G. A. (2015) Mechanisms of drug resistance: quinolone resistance. *Annuals of the New York Acadamy of Sciences.*, 1354(1), pp. 12–31.

Huovinen, P. Sundstrom, L., Swedberg, G., Skold, O. (1995) Trimethoprim and sulfonamide resistance, *Antimicrobial Agents and Chemotherapy*, 39(2), pp. 279–289.

Islam, K.M.S., Klein, U., Burch, D.G.S. (2009) The activity and compatibility of the antibiotic tiamulin with other drugs in poultry medicine-A Review, *Poultry Science*, 88, pp 2353-2359

Johnson, J. R. Kuskowski, M.A., Smith, K., O'Bryan, T.T., Tatini, S. (2005) Antimicrobial-Resistant and Extraintestinal Pathogenic *Escherichia coli* in Retail Foods, *The Journal of Infectious Diseases*, 191(7), pp. 1040–1049.

Johnson, J. R. Porter, S.B. Johnston, B. Thuras, P. Clock, S. Crupain, M. Ragan, U. (2017) Extraintestinal Pathogenic and Antimicrobial-Resistant *Escherichia coli*, Including Sequence Type 131 (ST131), from Retail Chicken Breasts in the United States in 2013, *Applied and Environmental Microbiology* 83 (6) pp. 1-9

Jones-Dias, D., Manageiro, V. and Canica, M. (2016) Influence of agricultural practice on mobile bla genes: IncI1-bearing CTX-M, SHV, CMY and TEM in *Escherichia coli* from intensive farming soils, *Environmental Microbiology*, 18(1), pp. 260–272.

Karcher, D. M. and Mench, J. A. (2018) Overview of commercial poultry production systems and their main welfare challenges. In Mench, J.A. (eds). *Advances in Poultry Welfare*. Elsevier Ltd. Woodhead Publishing, Sawston, United Kingdom

Kim, D. W. Thawng, C.N., Lee, K., Wellington, E.M.H. (2019) A novel sulfonamide resistance mechanism by two-component flavin-dependent monooxygenase system in sulfonamide-degrading actinobacteria, *Environment International*. Elsevier, 127, pp. 206–215.

Klein, E. Y. van Boekel, T.P., Martinez, E.M., Pant, S., Gandra, S., Levin, S.A., Goossens, H., Laxminarayan, R. (2018) Global increase and geographic convergence in antibiotic consumption between 2000 and 2015, *Proceedings of the National Academy of Sciences*, 115 (15) E3463-E3470.

Koga, V. L. Scandoriero, S., Vespero, E.C., Oba, A., de Brito, B.G., de Brito, K.C.T., Nakazato, G., Kobayashi, R.K.T. (2015) Comparison of Antibiotic Resistance and Virulence Factors among *Escherichia coli* Isolated from Conventional and Free-Range Poultry, *BioMed Research International*. 2015: 618752.

Kunert Filho, H. C. Brito, K.C.T., Cavalli, L.S., Brito, B.G. (2015) Avian Pathogenic *Escherichia coli* (APEC) - an update on the control. In: *The Battle Against Microbial Pathogens: Basic science,*

Technological Advances and Educational Programs, Medez-Vilas, E. (Ed), Formatex Research Centre, Badajoz, Spain.

Li, L. Wang, B., Feng, S., Li, J., Wu, C., Wang, Y., Ruan, X., Zeng, M. (2014) Prevalence and Characteristics of Extended-Spectrum b - Lactamase and Plasmid-Mediated Fluoroquinolone Resistance Genes in *Escherichia coli* Isolated from Chickens in Anhui Province , China, *PLoS ONE*, 9(8), pp. 1–6.

Lin, J. Nishino, K., Roberts, M.C., Tolmasky, M., Aminov, R.I., Zhang, L. (2015) Mechanisms of antibiotic resistance, *Frontiers in Microbiology*, 6, pp. 1–3.

Liu, Y.-Y. Wang, Y., Walsh, T.R., Yi, L., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L., Gu, H., ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J., Shen, J. (2016) Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study, *The Lancet: Infectious Diseases*, 16(2), pp. 161–168.

Loot, C. Nivina, A., Cury, J., Escudero, J.E., Ducos-Galand, M., Bikard, D., Rocha, E.P.C., Mazel, D. (2017) Differences in integron cassette excision dynamics shape a trade-off between evolvability and genetic capacitance, *American Society for Microbiology*, 8(2):e02296-16.

Mahon, C. R., Lehman, D. C. and Manuselis, G. (Eds) (2011) *Textbook of Diagnostic Microbiology*, *4th Edn.* Saunders Elsevier, Missouri, USA.

Manges, A. R. (2016) *Escherichia coli* and urinary tract infections: The role of poultry-meat, *Clinical Microbiology and Infection*. 22(2), pp. 122–129.

Mehdi, Y., Létourneau-montminy, M. P., Gaucher, M., Chorfi, Y., Suresh, G., Rouissi, T., Brar, S.K., Cote, C., Ramirez, A.A., Godbout, S. (2018) Use of antibiotics in broiler production: Global impacts and alternatives, *Animal Nutrition*. 4(2), pp. 170–178.

Mellata, M. (2013) Human and Avian Extraintestinal Pathogenic *Escherichia coli* : Infections, zoonotic risks, and antibiotic resistance trends, *Foodborne Pathogens and Disease*, 10 (11), pp. 916–932.

Nelson, J.M., Chiller, T.M., Powers, J.H., Angulo, F.J. (2007) Fluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones from use in poultry: A public health success story, *Clinical Infectious Diseases,* 44 pp. 977-980

Nguyen, F. Starosta, A.L., Arenz, S., Sohmen, D., Dönhöfer, A., Wilson, D.N. (2014) Tetracycline antibiotics and resistance mechanisms, *Biological Chemistry*, 395(5), pp. 559–575.

O'Neill, J. (2016) Tackling Drug-Restistant Infections Globally: Final Report and Recommendations, The Review on Antimicrobial Resistance. HM Government and Wellcome Trust; London, United Kingdom

Odwar, J. A. Kikuvi, G., Kariuki, J.N., Kariuki, S. (2014) A cross-sectional study on the microbiological quality and safety of raw chicken meats sold in Nairobi, Kenya, *BMC Research Notes*, 7(1), pp. 1–8.

Oguttu, J. W., Veary, C. M. and Picard, J. A. (2008) Antimicrobial drug resistance of *Escherichia coli* isolated from poultry abattoir workers at risk and broilers on antimicrobials, *Journal of the South African Veterinary Association*, 79(4), pp. 161–166.

Päivärinta, M. Pohjola, L., Fredriksson-Ahomaa, M., Heikinheimo, A. (2016) Low Occurrence of Extended-Spectrum b -lactamase-Producing *Escherichia coli* in Finnish Food-Producing Animals, *Zoonosis and Public Health*, 63, pp. 624–631.

Paranhos, A.G.O., Pereira, A.R., da Fonseca, I.C., Sanson, A.L., Afonso, R.J.C.F., Aquino, S.F. (2020) Analysis of Tylosin in poultry litter by HPLC-UV and HPLC-MS/MS after LTPE, *International Journal of Environmental Analytical Chemistry*, published online at:

https://www.tandfonline.com/doi/full/10.1080/03067319.2019.1694921?casa_token=MXaMHljgqHUAAAAA%3A0aWyHhNlcEgkHXsyNzmpSQNu6hR-xAPvsEcEBA3jR-_ggptNVSFNdpFJBOjG0ButsVYY3oImnq9a73c

Partridge, S. R. Kwong, S., Firth, N., Jensen, S.O. (2018) Mobile genetic elements associated with antimicrobial resistance, *Clinical Microbiology Reviews*, 31(4), pp. 1–61.

Perreten, V. Strauss, C., Collard A., Gerber, D. (2016) Colistin resistance gene mcr-1 in avian pathogenic *Escherichia coli* in South Africa. *Antimicrobial Agents and Chemotherapy*, 60(7), pp. 4414-4415.

Pokrant, E., Riquelme, R., Maddaleno, A., San Martin, B., Cornejo, J. (2018) Residue Depletion of Florfenicol and Florfenicol Amine in Broiler Chicken Claws and a Comparison of Their Concentrations in Edible Tissues Using LC-MS/MS, *Molecules*, 23(9):2211

Poultry DVM, (2020) Trimethoprim sulfamethoxazole (Bactrim) [Online] Available from: http://www.poultrydvm.com/drugs/trimethoprim-sulfamethoxazole. [Accessed: 18th November 2020].

Price, L. B. Graham, J.T., Lackey, L.G., Roess, A., Vailes, R., Silbergeld, E. (2007) Elevated risk of carrying gentamicin-resistant *Escherichia coli* among U.S. poultry workers, *Environmental Health Perspectives*, 115(12), pp. 1738–1742.

Redgrave, L. S. Sutton, S.B., Webber, M.A., Piddock, L.J.V. (2014) Fluoroquinolone resistance: Mechanisms, impact on bacteria, and role in evolutionary success, *Trends in Microbiology*. 22(8), pp. 438–445.

Roberts, M. C. and Swartz, S. (2009) Tetracycline and Chloramphenicol Resistance Mechanisms. In Mayers D.L. (eds) Antimicrobial Drug Resistance. Infectious Disease. Humana Press. New Jersey, USA.

Robinson, T. P., Bru, D.P., Carrique-Mas, J., Fèvre, E.M., Gilbert, M., Grace, D., Hay, S.I., Jiwakanon, J., Kakkar, M., Kariuki, S., Laxminarayan, R., Lubroth, J., Magnusson, U., Thi Ngoc, P., van Boekel, T.P., Woolhouse, M.E.J. (2016) Antibiotic resistance is the quintessential One Health issue, *Transactions of The Royal Society of Tropical Medicine and Hygiene*, 110(7), pp. 377–380.

Saidi, B., Mafirakureva, A. P. and Mbanga, J. (2013) Antimicrobial Resistance of *Escherichia coli* Isolated from Chickens with Colibacillosis in and Around Harare , Zimbabwe, *Avian Diseases*, 57(1), pp. 152–154.

Santajit, S. and Indrawattana, N. (2016) Mechanisms of antimicrobial resistance in bacteria in ESKAPE pathogens, *BioMed Research International*, 2016:2475067.

Schellack, N. benjamin, D., Brink, A., Duse, A., Faure, K., Goff, D., Mendelson, M., Meyer, J., Miot, J., Perovic, O., Pople, T., Suleman, F., van Vuuren, M., Essack, S. (2017) A situational analysis of current antimicrobial governance, regulation, and utilization in South Africa, *International Journal of Infectious Diseases*. 64, pp. 100–106.

Singer, R. S. and Hofacre, C. L. (2006) Invited Minireview : Potential Impacts of Antibiotic Use in Poultry Production, *Avian Diseases*, 50(2), pp. 161–172.

South African Department of Health (2018) *Surveillance for Antimicrobial Resistance and Consumption of Antimicrobials in South Africa*.

http://www.health.gov.za/index.php/component/phocadownload/category/199-antimicobial-resistance

South African Poultry Association (SAPA) (2018) *Industry Report 2018*. Honeydew, South Africa

http://sapoultry.co.za/pdf-docs/sapa-industry-profile.pdf

Stokes, H. W. and Gillings, M. R. (2011) Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens, *Federation of European Microbiological Societies Microbiology Reviews*, 35(5), pp. 790–819.

Talebiyan, R., Kheradmand, M., Khamesipour, F., Rubiee-Faradonbeh, M. (2014) Multiple Antimicrobial Resistance of *Escherichia coli* Isolated from Chickens in Iran, *Veterinary Medical International*, 2014: 491418.

Theobald, S. Etter, E., Gerber, D., Abonlik, C. (2019) Antibiotic Resistance Trends in *Escehrichia coli* in South African Poultry, *Foodborne Pathogens and Disease*, 16(9), pp. 1–9.

van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A., Laxminarayan, R. (2015) Global trends in antimicrobial use in food animals, *Proceedings of the National Academy of Sciences*, 112(18), pp. 5649–5654.

van Boeckel, T. P. Glennon, E., Chen, D., gilbert, M., Robinson, T.p., Grenfell, B.T., Levin, S.A., Bonhoeffer, S., Laxaminarayan, R. (2017) Reducing antimicrobial use in food animals, *Science*, 357(6358), pp. 1350–1352.

World Bank (2017) *Drug Resistant Infections: A Threat to Our Economic Future*, Washington, DC, USA.

World Health Organisation (WHO) (2015) *Global action plan on antimicrobial resistance*. Geneva, Switzerland.

World Health Organisation (WHO) (2017a) *Integrated Surveillance of Antimicrobial Resistance in Foodborne Bacteria*. Geneva, Switzerland.

World Health Organisation (WHO) (2017b) *WHO guidelines on use of medically important antimicrobials in food-producing animals*, Geneva, Switzerland.

World health Organisation (WHO) (2018) *Antimicrobial resistance; Key Facts.* http://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance (Accessed: 13 September 2018).

World Health Organisation (WHO) (2019) *Critically Important Antimicrobials for Human Medicine, 6th Revision*. Geneva, Switzerland.

World Organisation for Animal Health (OIE) (2016) *The OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials*, Paris, France

World Organisation for Animal Health (OIE) (2007) OIE list of Antimicrobials of Veterinary Importance, Paris, France.

CHAPTER 2

This dissertation is in manuscript format as follows:

K.S. McIver, D.G. Amoako, A.L.K. Abia, L.A. Bester, H.Y. Chenia, S.Y. Essack; Molecular epidemiology of antibiotic-resistant *Escherichia coli* from farm-to-fork in an intensive poultry production in KwaZulu-Natal, South Africa. To be submitted to Avian Pathology.

Contributions

- Katherine McIver, as the principle investigator, worked on the design of the study and protocols for laboratory work with the assistance of supervisors, executed the laboratory work and wrote the manuscript.
- Dr Daniel Gyamfi Amoako and Dr Akebe Luther King assisted with laboratory protocols.
- Dr Linda A. Bester, co-supervisor, designed the study and facilitated laboratory work
- Dr Hafiza Y. Chenia, helped with data analysis of ERIC-PCR results and production and review of the dendrogram.
- Prof Sabiha Y. Essack, principle supervisor, co-conceptualized the study, guided the literature review and ethical clearance application, facilitated data collection and analysis and undertook critical revision of the manuscript.

Molecular epidemiology of antibiotic-resistant *Escherichia coli* from farm-to-fork in an intensive poultry production in KwaZulu-Natal, South Africa

K.S. McIver1, D.G. Amoako1,2, A.L.K. Abia1, L.A. Bester2, H.Y. Chenia3, S.Y. Essack1*

[1]Antimicrobial Research Unit, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa;

[2]Biomedical Resource Unit, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal; Durban, South Africa;

[3]Discipline of Microbiology, School of Life Sciences, College of Agriculture, Engineering and Sciences, University of KwaZulu-Natal; Durban, South Africa;

Corresponding Author*

Sabiha Y. Essack

Antimicrobial Research Unit

College of Health Sciences

University of KwaZulu-Natal

Private Bag X54001

Durban

4000

Email: essacks@ukzn.ac.za

Abstract

The increased use of antibiotics in intensively produced food animals has resulted in the selection of drug-resistant bacteria across the farm-to-fork continuum, including in food products. There is a risk of transfer of this resistance to humans and as such a public health risk. The aim of this study was to investigate the molecular epidemiology of antibiotic resistant *Escherichia coli* from intensively produced poultry in the uMgungundlovu district of Kwa-Zulu Natal, South Africa.

An intensive poultry house was followed from hatching through to final retail product in order to investigate the epidemiology of antibiotic resistance of *E.coli* from Ross chickens. Each week for five weeks. ten samples from litter and faeces were collected. During transfer from the house to abattoir ten swabs from transport trucks and transport crates were taken. At the abattoir ten samples from carcass wash were collected. After slaughter and dressing ten of each caecum's, whole chickens, thighs and necks were collected. During house washing, another ten samples were collected. *E.coli* was putatively identified using eosin methylene blue agar followed by Sorbitol MacConkey agar and confirmed by identification of the *uidA* gene by polymerase chain reaction. Susceptibility to a panel of antibiotics recommended by the World Health Organization Advisory Group on the Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR) was ascertained by the Kirby-Bauer disk diffusion method for 20 antibiotics according to CLSI guidelines. Realtime PCR was used to test for resistance genes *tetA, tetB, qnrB, qnrS, aac(6)-lb-cr, sul1, sul2, sul3,* bla_{SHV}, $bla_{CTX\text{-}M}$, bla_{TEM} genes conferring resistance to tetracyclines, quinolones, sulphonamides and cephalosporin antibiotics. Clonal similarities were investigated using ERIC-PCR.

A total of 266 *E.coli* isolates were tested, with a non-susceptibility profile of ampicillin 48.1%, tetracycline 27.4%, nalidixic acid 20.3%, trimethoprim-sulphamethoxazole 13.9%, chloramphenicol 11.7%, cefalexin 4.5%, ciprofloxacin 4.1%, amoxycillin-clavulanic acid 3.4%, gentamicin 1.9%, cefoxitin 1.1%, cefepime 1.1%, cefotaxime 1.1%, amikacin 1.1%, ceftriaxone 0.8% and azithromycin 0.8%. Isolates were fully susceptible to ceftazidime, imipenem, meropenem and tigecycline.

Of the 266 isolates 6.4% were multidrug resistant (resistant to one or more antibiotics in three or more distinct antibiotic classes). The most frequently observed resistance genes were $bla_{CTX\text{-}M}$ (100%), *sul1*(80%), *tetA*(77%), *tetB*(71%). Using ERIC-PCR the isolates were grouped into 27 clusters with a 75% similarity, eight clusters comprised of isolates from only one sample.

There was an increase in MDR and resistance genes over the farm to fork continuum with lowest and highest levels seen in transport and waste water samples respectively. ERIC-PCR did not indicate the transmission of clones across the farm-to-fork continuum. There instead appeared to be de novo or evolution of resistance genes or the introduction of plasmids over the time period. As the only antimicorbials used in this flock were salinomycin and zinc bacitracin it is postulated that the resistance

observed could be attributed to the co-selection of resistance genes and/or horizontal gene transfer from the environment, insects, chicken food and workers.

Overall resistance levels were low over the six weeks of the study, MDR and the prevalence of resistance genes increased over time. The diverse clonality shown by ERIC PCR results did not support the transmission of clones across the farm-to-fork continuum. However it did provide a scenario of *de novo* evolution of resistance genes and the loss or gain of plasmids over the time period.

Keywords: Antibiotic-resistance; antibiotic resistance genes; *Escherichia coli*; intensive poultry production; farm to fork continuum; South Africa.

Introduction

Poultry farmers have been using antibiotics since the 1940s, when it was discovered that using antibiotics at sub-therapeutic levels resulted in better feed conversion and reduced mortality (Graham, Boland and Silbergeld, 2007). Antibiotics reduced diseases such as necrotic enteritis and colibacillosis (Kunert Filho et al., 2015; Mehdi, Létourneau-montminy, et al., 2018) as well as the incidence of human illness from chicken products such as enteritis caused by *Salmonella, Campylobacter* or *Escherichia coli* (Singer, 2015; Mehdi, Létourneau-montminy, et al., 2018).

Increased demand for animal protein has led to an intensification of poultry farming to provide for the demand (Blaak et al., 2015). Intensive farming involves much higher stocking densities than extensive or subsistence farming, resulting in close contact of animals and increases in stress levels. These factors predispose the animals to disease development from poor immunity and the spread of disease due to close contact. Factors such as good husbandry practices, good biosecurity and immunisation programs help to prevent disease outbreaks in flocks. Where these factors are not in place, antibiotics are used to compensate and maintain production (Founou, Founou and Essack, 2016), resulting in reservoirs of antibiotic resistant bacteria and antibiotic resistance genes that have the capacity to move to humans through the food chain (Chang et al., 2015).

In poultry, *E.coli* causes colibacillosis which is a major cause of economic losses in broiler and layer production (Mellata, 2013). *E.coli* are ubiquitous and can be transferred between humans, animals and the environment. They are also able to transfer plasmids and other mobile genetic units easily (Aarestrup, 2004). *E.coli* thus makes a good indicator bacteria in antibiotic resistance surveillance and was the choice for this study to delineate the molecular epidemiology of antibiotic resistant in *E.coli* from farm-to-form in an intensive poultry production system in the uMgungundlovu District in KwaZulu Natal, South Africa.

Methodology

Ethical considerations: This study forms part of a larger project for which ethical clearance had been obtained from the Animal Research Ethics Committee (Reference: AREC 073/016PD) and the Biomedical Research Ethics Committee (Reference BCA444/16) of the University of KwaZulu-Natal. The study was further placed on record with the South African National Department of Agriculture, Forestry and Fisheries (Reference: 12/11/1/5 (879)).

Study Population: Samples were taken from one batch of Cobb chickens from an intensive poultry farm in the uMgungundlovu District in KwaZulu Natal. Poultry were housed in houses with closed ventilation. Stocking densities were approximately 25 000 to 30 000 birds per house. The poultry were given no therapeutic antibiotics during study but Salinomycin and Zinc Bacitracin were included in the starter, grower and finisher feeds, post finisher feeds had no medication. Other antimicrobials used on

the farm in the previous 12 months but not given to the sample population included Doxycycline, Sulfadiazine and trimethoprim, Enrofloxacin, Ceva Olaquindox 10%, Avilamycin, Tylosin 10% and Kitasamycin Tartate. The same poultry house was followed from hatching to slaughter and final retail product using a farm-to-fork methodology. The sampling took place in August and September of 2017.

Only one poultry was sampled due to avian influenza outbreak at the time, although this was a typical intensive poultry house it would have been preferrable to sample more than one house and more than one farm for comparative purposes.

Sampling protocol: The sampling protocol recommended by the World Health Organization Advisory Group on the Integrated Surveillance of Antimicrobial Resistance (AGISAR) was used. In the growth phase, weekly samples were taken over five weeks, using a block-sampling method where 10 samples were taken from both litter and faeces each week. Week 1 litter samples were taken from the house prior to the hatchlings being introduced. The house was cleaned prior to new litter being placed in the house. Faecal samples were taken from the trays the hatchlings were transported on. From Week 2 to 5, samples were taken from litter and faeces in the house. Ten samples were pooled and transported, on ice within four hrs, for isolation and further analysis. Ten swabs from transport trucks and ten samples from transport crates were taken and pooled when the chickens were transported to the abattoir. At the abattoir, ten samples of carcass rinsate were taken and pooled, ten caeca were collected for contents to be extracted in the laboratory and 10 of each of whole chickens, thighs and necks were collected for testing. Samples of the rinsate from cleaning the house were collected in the last week of sampling.

Sample Processing: One gram (1 g) of pooled litter or faeces were mixed with 40ml of Tryptone Soya Broth (TSB) (Oxoid, Hamshire, United Kingdom). Swabs from transport trucks and crates were pooled and added to 40ml of TSB while 4 ml of pooled carcass rinsate was combined with 36ml of TSB. One gram of caecal contents was added to 5ml of TSB. Each was homogenised and then pooled to make a final volume of 50ml. 1ml of the pooled sample was combined with 9ml of TSB. Each of the whole chickens, thighs and necks was rinsed in 10ml of saline inside a sterile plastic bag. All whole chicken rinses were pooled, thigh rinses pooled, and neck rinses pooled to give 100ml from each retail meat type. Four millilitre from each was added to 36ml of TSB. All samples were then incubated for 2 hrs at 37°C.

After incubation serial dilutions were done and 100µl of each dilution was pour-plated onto Eosin Methylene Blue (EMB) Agar (Sigma-Aldrich, Missouri, USA) to calculate colony forming units (CFU's). Plates were incubated for 18 to 24 hours at 37°C.

Colonies were counted after incubation to quantify the *E. coli* in each sample.

Isolation: Twenty typical *E.coli* black green shiny colonies were selected from EMB plates and plated onto Sorbitol MacConkey Agar with 5-bromo-4-chloro-3-indolyl- b-D-glucuronide (BCIG) (Oxoid, Hampshire, United Kingdom). Plates were incubated at 37°C for 18 to 24 hours after which one typical colony for *E.coli* (blue purple) was selected from each plate and re-plated onto Nutrient Agar (Neogen, Langing, USA) and incubated for a further 18 to 24 hours at 37°C.

Isolates were subjected to biochemical tests. Isolates that were Gram-negative by the Gram string test, oxidase negative on oxidase strip test and catalase positive where then inoculated into Triple Sugar Iron (TSI) Agar slants. Isolates with typical TSI slant results (acid in base and on slant with gas production) were presumed to be *E.coli* and stored in 10% glycol TSB solution at -80°C until needed for further testing.

DNA Extraction: Colonies were suspended in 300µl of distilled water and boiled for 20 minutes. Samples were then placed on ice for 5 minutes before being centrifuged at 13000rpm for 3 minutes. Supernatant was extracted and stored at -20°C for further use.

Molecular confirmation as *Escherichia Coli*: Realtime polymerase chain reaction (PCR) was done to confirm isolates as *E.coli* by detecting the *uidA* gene. The following protocol was used with primers described in Supplementary Table 1:

PCR was conducted on a QuantStudio 5 (Thermo Fischer Scientific). Conditions included an Uracil-DNA glycosylase (UDG) activation at 50 °C for 2 min and dual-LockTM polymerase activation at 95 °C for 2 min. This was followed by 35 cycles of denaturation (95 °C for 15 s), annealing (60 °C for 15 s) and extension (72 °C for 10 s). A final extension was achieved at 72 °C for 5 min (Ebomah, Adefisoye and Okoh, 2018). *E.coli* ATCC 25922 was used as a positive control and nuclease-free water was used as negative control.

Antibiotics Susceptibility Testing (AST): Antibiotic sensitivity testing was done using the Kirby-Bauer Disc Diffusion Assay according to Clinic and Laboratory Standards Institutes (CLSI) guidelines and isolates were categorized as sensitive, intermediate or resistant using CLSI /EUCAST breakpoints as appropriate (CLSI, 2017; EUCAST, 2013). The following antibiotics were tested ampicillin 10µg, cefepime 30µg, cefotaxime 30µg, ceftazidime 30µg, cefoxitin 30µg, cefalexin 30µg (EUCAST), gentamicin 10µg, imipenem 10µg, meropenem 10µg, nalidixic acid 30µg, ciprofloxacin 5µg, tigecycline 15µg (EUCAST), amoxycillin-clavulanic acid 20µg +10µg, amikacin 30µg, chloramphenicol 30µg, azithromycin 15µg, tetracycline 30µg, trimethoprim-sulphamethoxazole 1,25 µg +23,75µg, ceftriaxone 30µg based on WHO AGISAR recommended list (WHO, 2017a).

Multidrug resistance was determined as resistance to at least one antibiotic in three or more classes.

Antibiotic resistance gene detection: Isolates that were selected for further molecular testing consisted of one randomly selected isolate from each sample for each antibiogram. Real-time PCR was

used to detect the following genes informed by the AST results: *tetA, tetB, qnrB, qnrS, aac(6)-lb-cr, sul1, sul2, sul3, bla$_{SHV}$, bla$_{CTX-M}$, bla$_{TEM}$* genes conferring resistance to tetracyclines, quinolones, sulphonamides and cephalosporin antibiotics (Adelowo et al., 2014; Awad et al., 2016; Dessie et al., 2013; Li et al., 2014; Sengeløv et al., 2003; Wang et al., 2008; Byrne-Bailey et al., 2009). (please see Supplementary Table 2 for primer sequences).

PCR conditions included a UDG activation at 98°C or 50 seconds. This was followed by 30 cycles of denaturation (95 °C for 10 s), annealing (62 °C for 30 s) and extension (72 °C for 20 s). A final extension was achieved at 72 °C for 5 minutes.

Conventional PCR using a BIO-RAD T100 Thermal Cycler was used to test for *bla$_{TEM}$* resistance genes. Conditions were an initial activation of 94°C for 3 minutes followed by 34 cycles of denaturation (94 °C for 1minute), annealing (55°C for 1 minute) and extension (72 °C for 1minute and 30 seconds). A final extension was achieved at 72 °C for 7 minutes (Ebomah, Adefisoye and Okoh, 2018).

PCR products from conventional PCR were subject to electrophoresis in a 1% agarose gel containing 5µl Ethidium Bromide at 100V for 45 min in a 1X Tris-acetate-EDTA (TAE) buffer. A 1 Kb ladder from Quick-load (New England Biolabs) was used as the standard. Gels were visualised using the Gel Doc™ XR+ imaging system (Bio-Rad, South Africa) and photographed.

Clonality: Representative isolates were selected for clonality experiments based on antibiograms, where isolates from different sources belonging to the same antibiogram were subjected to Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR). DNA was extracted using GeneJET Genomic DNA purification kit (ThermoFisher Scientific) following the manufacturer's guidelines. An amount of 12.5µl of DreamTaq Green PCR Master mix (2X) (ThermoFisher Scientific), 0,1µl of each forward and reverse primers, 3,3µl of nuclease free water and 4 µl of DNA to combined to make up a final PCR reaction of 20µl (please see Supplementary Table 3 for primer sequences). PCR was run on BIO-RAD T100 Thermal Cycler with the following conditions: initial denaturing of 95°C for 2 minutes followed by 34 cycles of denaturation (90°C for 30s), annealing (52°C for 1min) and extension (65°C for 8min) and a final extension of 65°C for 16 minutes. PCR products were subject to electrophoresis in a 1% agarose gel at 75V for 150 min in a 1X Tris-acetate-EDTA (TAE) buffer. A 1 Kb ladder from Quick-load (New England Biolabs) was used as the standard. Gels were stained in ethidium bromide solution for 15 minutes before distaining for 10 to 30 minutes in distilled water. Gels were visualised using Gel Doc™ XR+ imaging system (Bio-Rad, South Africa). Gels were analysed using Bionumerics software version 6.6 (Applied Maths NV, Belgium). A band tolerance of 10% was used for inputting gel images. Cluster generation used Pearson correlation with a 1% optimisation and an unweighted pair group with arithmetic averages (UPGMA) to create a dendrogram. Clusters were determined using a 75% similarity cut-off.

Results

Prevalence: Three hundred and forty five putative *E.coli* isolates were cultured from all sample sources over the 5-week period and included samples from the farm, transport vehicles and crates, the abattoir, retail meat and waste water recovered from house cleaning. Of these, 266 (77%) were confirmed as *E. coli* by biochemical tests and PCR (Supplementary Table 4). No isolates were cultured from truck samples.

Colony forming units were calculated for each house sample and are displayed in the Figure 1. The highest results were for week 2 with $9.1x10^8$ cfu followed by week 3 with $5.7x10^8$ cfu .

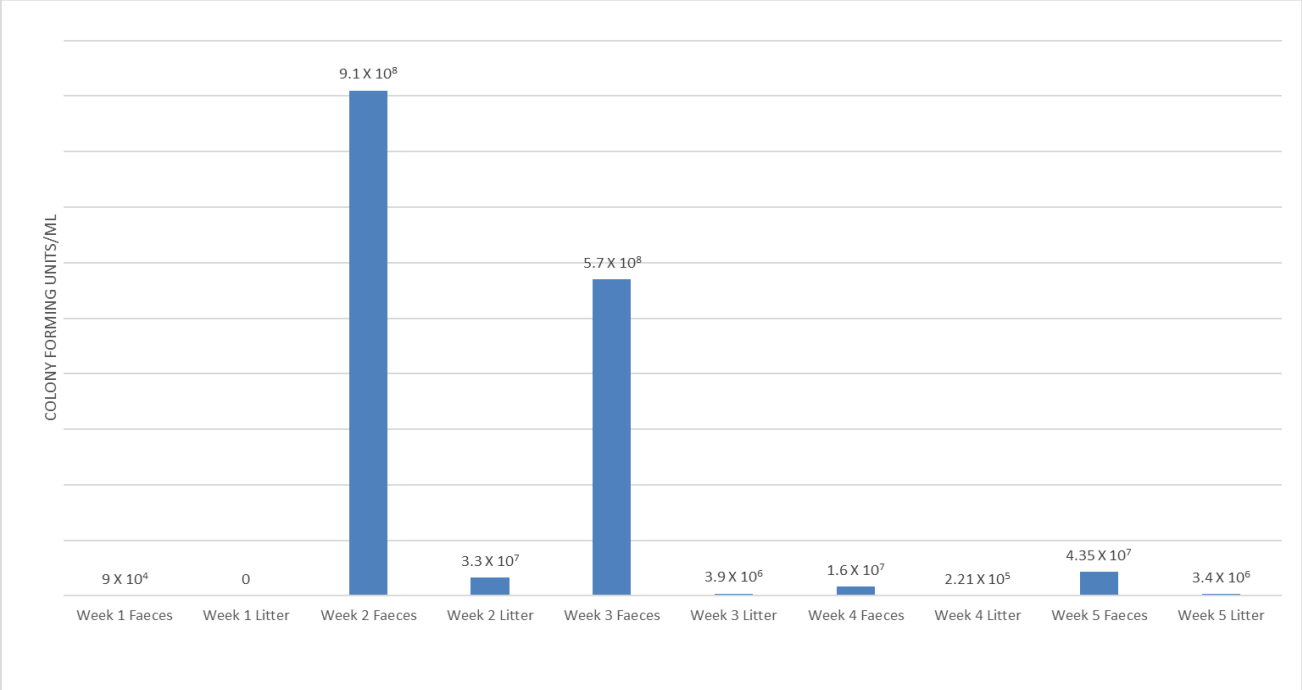


Figure 1: Quantification of *Escherichia coli* per sample.

Antibiotic susceptibility: Figure 2 shows the percentage non-susceptibility (as per EUCAST guidelines) of all isolates (n=266) tested against 20 antibiotics: ampicillin 48.1%, tetracycline 27.4%, nalidixic acid 20.3%, trimethoprim-sulphamethoxazole 13.9%, chloramphenicol 11.7%, cefalexin 4.5%, ciprofloxacin 4.1%, amoxycillin-clavulanic acid 3.4%, gentamicin 1.9%, cefoxitin 1.1%, cefepime 1.1%, cefotaxime 1.1%, amikacin 1.1%, ceftriaxone 0.8% and azithromycin 0.8%. Isolates were fully susceptible to ceftazidime, imipenem, meropenem and tigecycline.

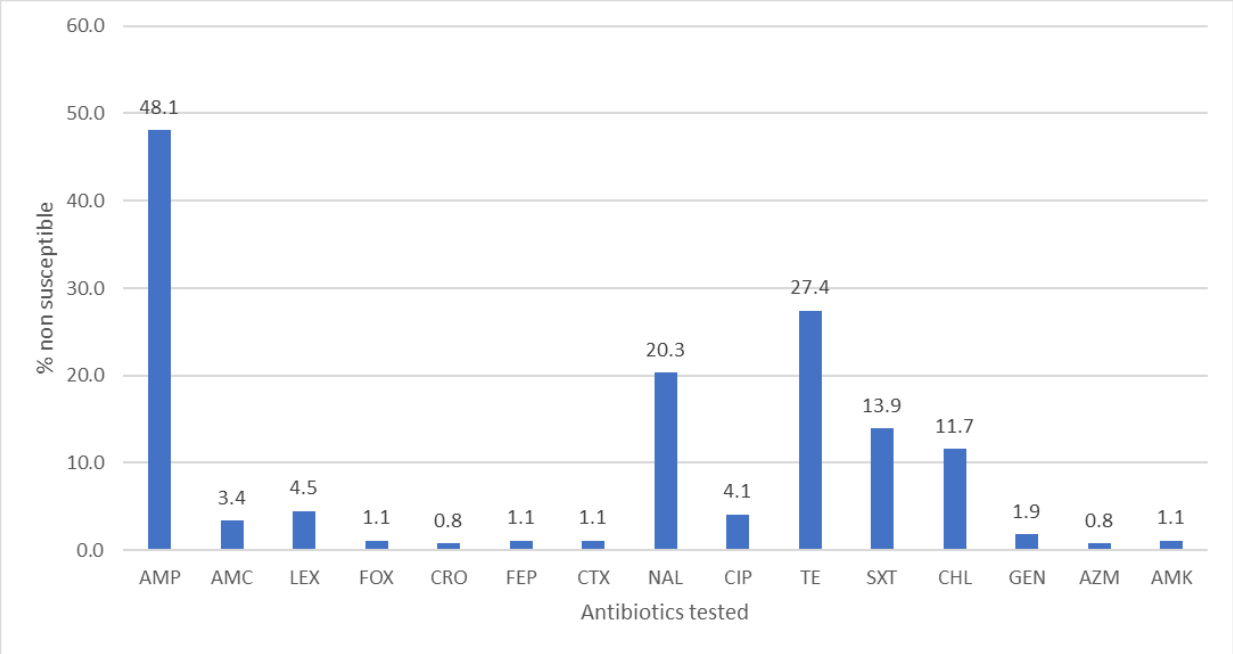


Figure 2: Percentage non susceptibility of all isolates to the antibiotics tested

Ampicillin(AMP), Amoxycillin-clavulanic acid (AMC), Cefalexin(LEX), Cefoxitin(FOX), Ceftriaxone(CRO), , Cefepime(FEP), Cefotaxime(CTX), Nalidixic Acid(NAL), Ciprofloxacin(CIP), , Tetracycline(TET), Trimethoprim-sulphamethoxazole (SXT), Chloramphenicol(CHL), Gentamicin(GEN), Azithromycin(AZM), Amikacin(AMK).

Figure 3 below shows the % non-susceptibility for each sample across the sample collection period of the five antibiotics with the highest resistance rates, i.e., ampicillin, nalidixic acid, tetracycline, trimethoprim-sulphamethoxazole and chloramphenicol. Ampicillin showed increased resistant levels in week 2 and week 5 as well as in the abattoir and waste water samples. Nalidixic acid also showed a peak in week 2 and again increased in abattoir and waste water samples. Tetracycline had high levels of resistance in weeks 3 to 5 and was highest in caecal samples. Trimethoprim-sulphamethoxazole had highest levels in week 2, caecal and waste water samples. Chloramphenicol had highest levels of resistance in week 2 and in waste water. Resistance levels to chloramphenicol were lower for the remaining samples. Resistance levels in retail meat were lower for all antibiotics compared with abattoir, caecal and waste water samples.

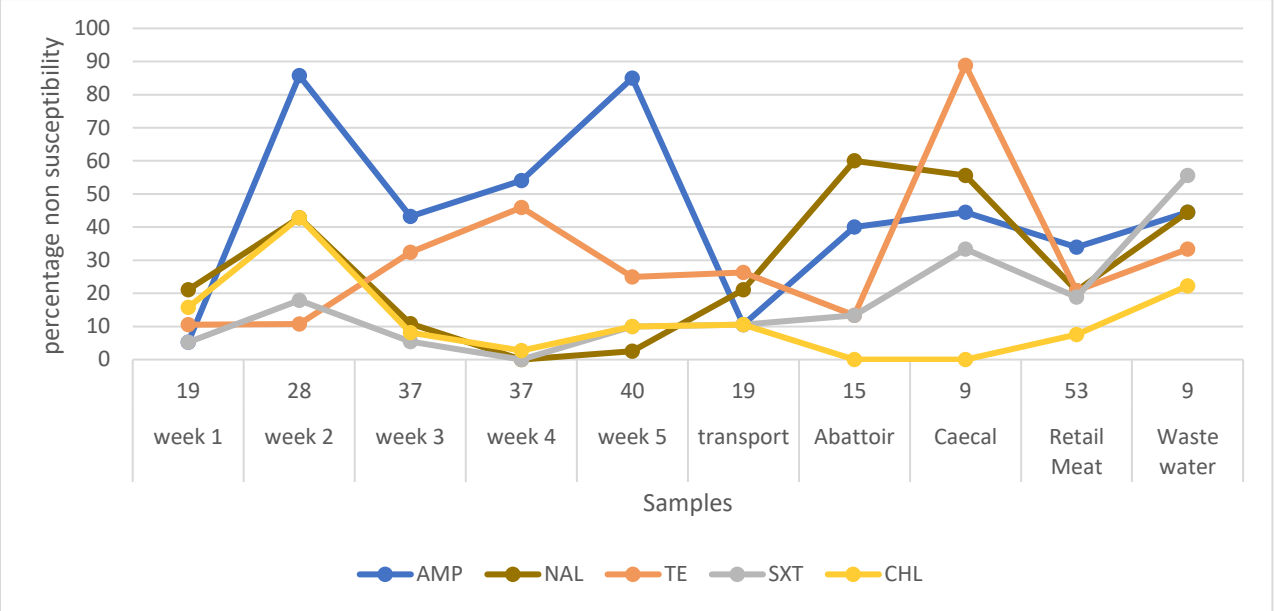


Figure 3: Percentage non-susceptibility of isolates to selected antibiotics stratified by sample source. ampicillin (AMP), nalidixic acid (NAL), tetracycline (TE), trimethoprim-sulphamethoxazole (SXT), chloramphenicol (CHL) stratified by sample source. (Numbers above samples represent sample size.)

Antibiograms: Antibiograms were created using zone diameters and CLSI guidelines for resistant organisms (except for cefalexin and tigecycline where EUCAST guidelines were used), yielding 33 antibiograms (Table 1). The largest groups were isolates with no resistance (86), ampicillin resistance (56) and tetracycline resistance (38) only. The majority of the remaining antibiograms had 5 or fewer isolates in each except for NAL-TET with 10 isolates and 2 profiles with 9 isolates in each which were NAL and SXT-CHL.

Table 1: Number of Isolates per Antibiogram Stratified by Source.

Antibiogram	Farm n=161	Transport n=19	Abattoir n=15 Carcass rinsate samples from abattoir	Caecal n=9	Retail n=53 (Whole chicken, neck and thighs)	Waste Water n=9	Total n=266
No resistance	48	9	3	0	25	2	87
AMP	46	1		1	7	1	56
AZM	1				1		2
CHL	1	1					2
CIP					1		1
GEN			2				2
LEX	2				2		4
NAL	2		4		3		9
SXT	1	1	1		1	1	5
TET	33	4	1				38
AMP-AMK	1						1
AMP-CHL	3						3
AMP-LEX	2		1				3
AMP-NAL	2	1					3
AMP-SXT					1		1
AMP-TET	1						1
LEX-TET	1						1
NAL-TET		1	1	4	4		10
SXT-CHL	5	1			2	1	9

TET-CHL	1						1
TET-GEN	1						1
TET-SXT	1			2	2		5
AMP-LEX-CHL	1						1
AMP-NAL-GEN			1				1
AMP-NAL-TET				1	1		2
AMP-TET-SXT	1			1	1		3
NAL-TET-SXT					2	3	5
AMP-TET-SXT-CHL	2						2
NAL-CIP-TET-SXT					1		1
AMP-CTX-TET-SXT-CHL	1						1
AMP-LEX-CRO-CTX-CHL	1						1
AMP-AMC-FOX-SXT-CHL-AMK	1						1
AMP-AMC-LEX-FOX-NAL-CIP						1	1
AMP-LEX-CRO-CTX-NAL-TET-CHL	1						1

Ampicillin(AMP), Amoxycillin-clavulanic acid(AMC), Cefalexin(LEX), Cefoxitin(FOX), Ceftriaxone(CRO, Cefepime(FEP), Cefotaxime(CTX), Nalidixic Acid(NAL), Ciprofloxacin(CIP), Tetracycline(TET), Trimethoprim-sulphamethoxazole (SXT), Chloramphenicol(CHL), Gentamicin(GEN), Tigecycline(TGC), Azithromycin(AZM), Amikacin(AMK).

Multidrug Resistance: Seventeen isolates showed resistance to antibiotics in three or more different antibiotic classes and were classified as multidrug resistant (MDR) (Table 1). Overall 6.4% of isolates showed multidrug resistance, with waste water having the highest percentage of multidrug resistance with 33%, followed by caecal samples with 22%. The lowest levels of multidrug resistance were found in farm, transport and abattoir samples with 3.7%, 0% and 6.7% respectively. Retail meat had 9.4% MDR isolates.

Resistance genes: Table 2 below shows the number and percentage of isolates positive for each resistance gene stratified by source. The predominant resistance genes were $bla_{CTX\text{-}M}$ (100%), *sul1* (80%), *tetA* (77%) and *tetB* (71%).

Table 2: Frequency of isolation of antibiotic resistance genes stratified by source

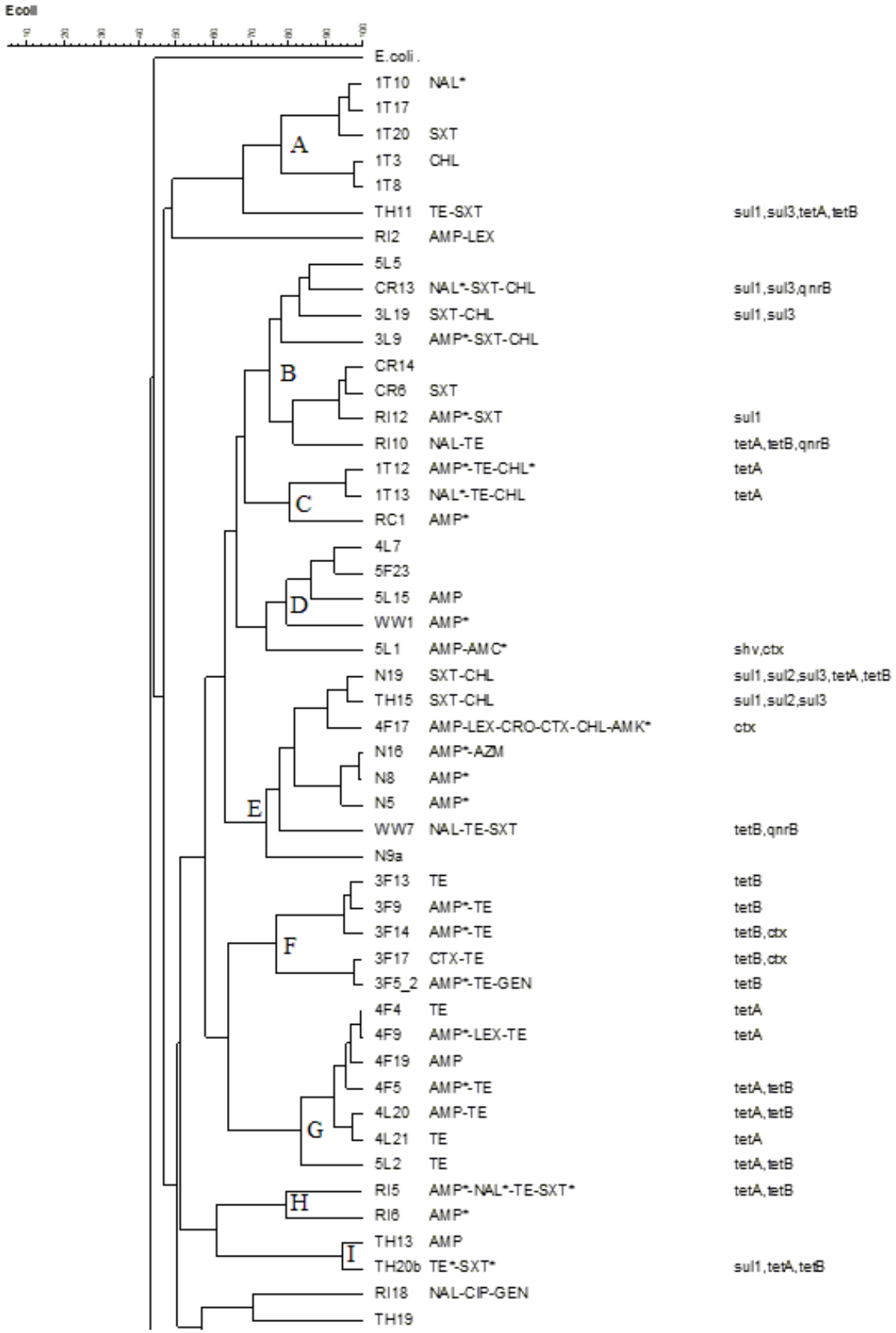
	Farm	Transport	Abattoir	Caecal	Retail Meat	Waste water	Total
tetA	14(58%)	3(100%)	2(67%)	5(100%)	11(100%)	4(80%)	39(77%)
tetB	13(56%)	2(67%)	2(67%)	5(100%)	11(100%)	3(60%)	36(71%)
qnrB	1(5.6%)	1(33%)	1(17%)	2(67%)	7(78%)	4(100%)	16(37%)
qnrS	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
aac(6)-lb-cr	0(0%)	0(0%)	0(0%)	1(33%)	0(0%)	0(0%)	1(2%)
sul1	7(64%)	1(50%)	1(50%)	2(100%)	9(100%)	4(80%)	24(80%)
sul2	2(20%)	0(0%)	0(0%)	1(50%)	4(44%)	4(80%)	11(37%)
sul3	5(50%)	1(50%)	0(0%)	1(50%)	6(67%)	3(60%)	16(53%)
SHV	8(62%)	None Tested	1(100%)	None Tested	2(50%)	0(0%)	11(58%)
CTX-M	13(100%)	None Tested	1(100%)	None Tested	4(100%)	1(100%)	19(100%)
TEM	0(0%)	None Tested	0(0%)	None Tested	1(25%)	0(0%)	1(5%)
Per Sample	61(36%)	8(38%)	8(25%)	17(68%)	56(64%)	23(58%)	174(46%)

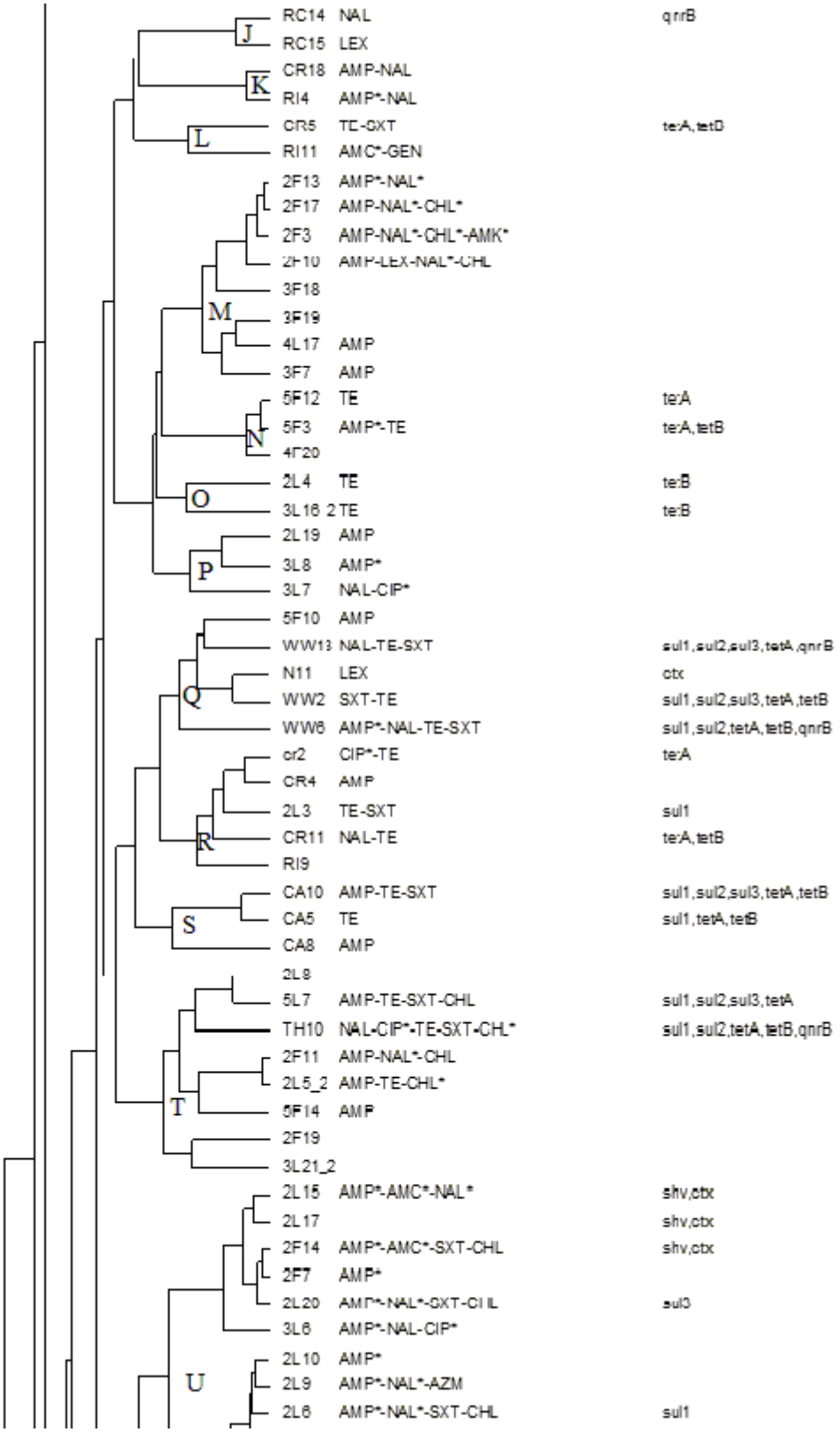
Dendrogram: Isolates could be divided into 27 clusters based on a 75% fingerprint similarity, with several isolates sharing greater than 90% similarity (Figure 4). Of the 27 clusters eight contained isolates from the same sample and two of these clusters contained five isolates in each. Cluster A contained isolates from week 1 faeces from the hatchling's trays. None of the antibiograms were the same. Cluster F were all from week 3 faecal samples but compared to cluster A there were some similarities in susceptibilities, with all isolates being non-susceptible to tetracycline and three to ampicillin.

The largest cluster had thirteen isolates from three samples (i.e., week 2 litter with ten isolates, week 2 faeces with two isolates and week 3 litter with one isolate). Three isolates from week 2 litter and one form week 2 faeces had the same antibiogram AMP-(Intermediate NAL)-SXT-CHL. All the isolates were non-susceptible to ampicillin. The rest of the antibiograms varied.

There were only a few instances demonstrating the same antibiograms but from different samples in a cluster. One cluster had a crate sample CR6 which showed greater than 90% similarity to a rinsate sample from the abattoir RI12 and both had the same antibiogram AMP-SXT. In another cluster both

a litter sample from week 2 and litter sample from week 3 showed resistance to tetracycline. Cluster P also had a week 2 litter and a week 3 litter sample with ampicillin non-susceptibility and >85% similarity. The dendogram indicated a relationship between isolates from different sources, their antibiograms and resistance genes.


Ecoli
E.coli .
1T10 NAL*
1T17
1T20 SXT
1T3 CHL
1T8
TH11 TE-SXT sul1,sul3,tetA,tetB
RI2 AMP-LEX
5L5
CR13 NAL*-SXT-CHL sul1,sul3,qnrB
3L19 SXT-CHL sul1,sul3
3L9 AMP*-SXT-CHL
CR14
CR6 SXT
RI12 AMP*-SXT sul1
RI10 NAL-TE tetA,tetB,qnrB
1T12 AMP*-TE-CHL* tetA
1T13 NAL*-TE-CHL tetA
RC1 AMP*
4L7
5F23
5L15 AMP
WW1 AMP*
5L1 AMP-AMC* shv,ctx
N19 SXT-CHL sul1,sul2,sul3,tetA,tetB
TH15 SXT-CHL sul1,sul2,sul3
4F17 AMP-LEX-CRO-CTX-CHL-AMK* ctx
N16 AMP*-AZM
N8 AMP*
N5 AMP*
WW7 NAL-TE-SXT tetB,qnrB
N9a
3F13 TE tetB
3F9 AMP*-TE tetB
3F14 AMP*-TE tetB,ctx
3F17 CTX-TE tetB,ctx
3F5_2 AMP*-TE-GEN tetB
4F4 TE tetA
4F9 AMP*-LEX-TE tetA
4F19 AMP
4F5 AMP*-TE tetA,tetB
4L20 AMP-TE tetA,tetB
4L21 TE tetA
5L2 TE tetA,tetB
RI5 AMP*-NAL*-TE-SXT* tetA,tetB
RI6 AMP*
TH13 AMP
TH20b TE*-SXT* sul1,tetA,tetB
RI18 NAL-CIP-GEN
TH19
A
B
C
D
E
F
G
H
I


J
RC14 NAL qnrB
RC15 LEX
K
CR18 AMP-NAL
RI4 AMP*-NAL
L
CR5 TE-SXT tetA,tetB
RI11 AMC*-GEN
M
2F13 AMP*-NAL*
2F17 AMP-NAL*-CHL*
2F3 AMP-NAL*-CHL*-AMK*
2F10 AMP-LEX-NAL*-CHL
3F18
3F19
4L17 AMP
3F7 AMP
N
5F12 TE tetA
5F3 AMP*-TE tetA,tetB
4F20
O
2L4 TE tetB
3L16_2 TE tetB
P
2L19 AMP
3L8 AMP*
3L7 NAL-CIP*
Q
5F10 AMP
WW13 NAL-TE-SXT sul1,sul2,sul3,tetA,qnrB
N11 LEX ctx
WW2 SXT-TE sul1,sul2,sul3,tetA,tetB
WW6 AMP*-NAL-TE-SXT sul1,sul2,tetA,tetB,qnrB
R
cr2 CIP*-TE tetA
CR4 AMP
2L3 TE-SXT sul1
CR11 NAL-TE tetA,tetB
RI9
S
CA10 AMP-TE-SXT sul1,sul2,sul3,tetA,tetB
CA5 TE sul1,tetA,tetB
CA8 AMP
T
2L8
5L7 AMP-TE-SXT-CHL sul1,sul2,sul3,tetA
TH10 NAL-CIP*-TE-SXT-CHL* sul1,sul2,tetA,tetB,qnrB
2F11 AMP-NAL*-CHL
2L5_2 AMP-TE-CHL*
5F14 AMP
2F19
3L21_2
U
2L15 AMP*-AMC*-NAL* shv,ctx
2L17 shv,ctx
2F14 AMP*-AMC*-SXT-CHL shv,ctx
2F7 AMP*
2L20 AMP*-NAL*-SXT-CHL sul3
3L6 AMP*-NAL-CIP*
2L10 AMP*
2L9 AMP*-NAL*-AZM
2L6 AMP*-NAL*-SXT-CHL sul1

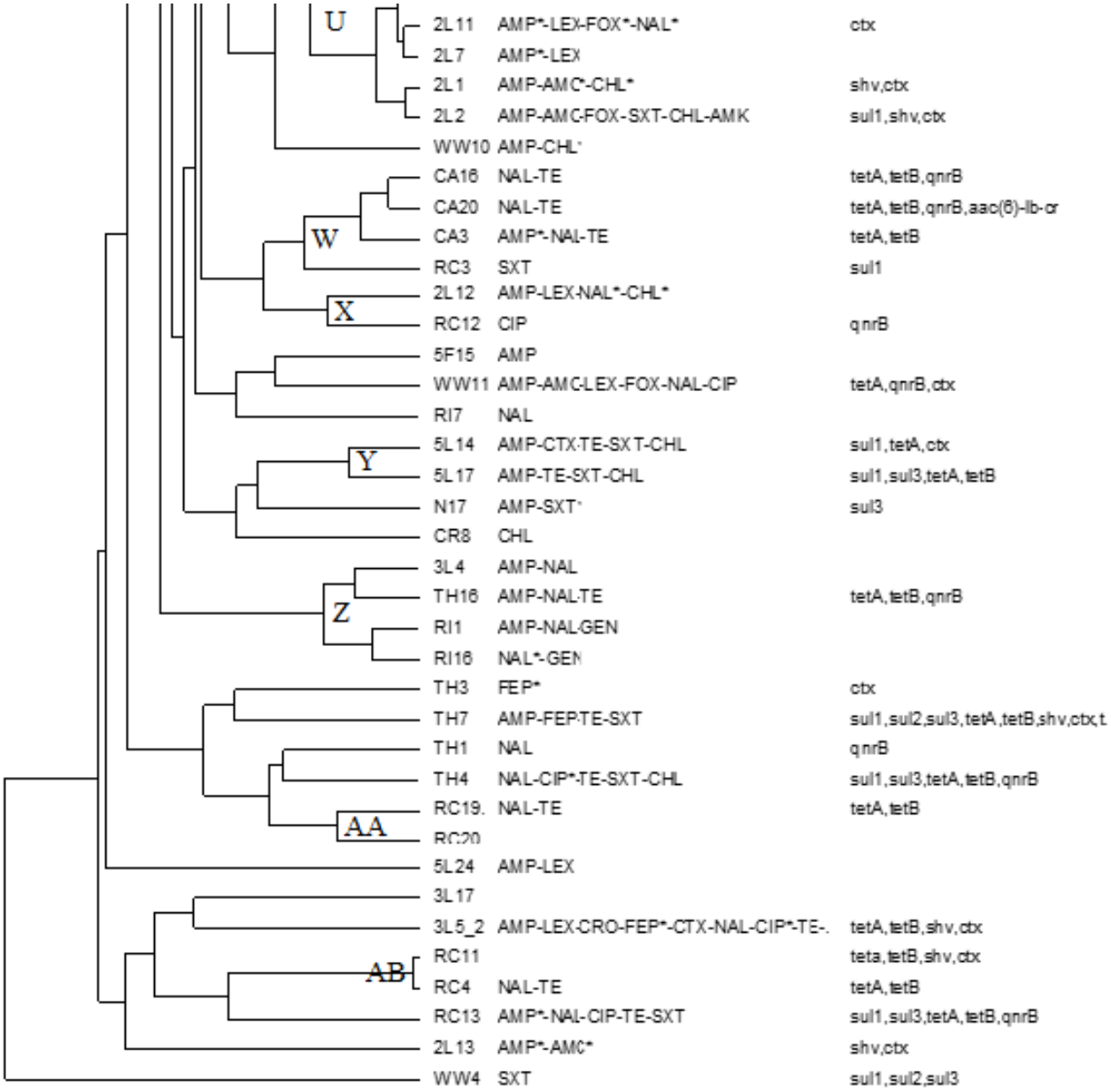


Figure 4: Dendrogram showing relationship between isolates (first column depicts isolate number, showing source) with antibiograms (second column) and resistance genes (third column).

Keys: Week 1 is denoted as 1T and are all hatching tray samples; Week 2 is denoted as 2L (Litter samples) or 2F (faecal samples); Week 3 is denoted as 3L (Litter samples) or 3F (faecal samples)

Week 4 is denoted as 4L (Litter samples) or 4F (faecal samples); Week 5 is denoted as 5L (Litter samples) or 5F (faecal samples); CR are transport crate samples; RI are abattoir rinsate samples; RC are whole chicken samples; N are neck sample; TH are thigh samples; WW are waste water samples; and these codes are followed by the isolate number.

Discussion

This study investigated antibiotic resistance in *Escherichia coli*, from hatching to final retail product, over six weeks in a flock of intensively produced chickens in the uMgungundlovu District in KwaZulu Natal. The flock received anticoccidial agent salinomycin and growth promoter zinc bacitracin in the feed. Both compounds were incorporated into starter, grower and finisher feeds while no ionophores nor antibiotics were present in the post finisher feed. According to farm management, no therapeutic antibiotics were used in this flock. Although overall resistance levels were low over the 6 weeks of the study compared to other studies done in South Africa (Oguttu et al, 2008), MDR and the prevalence of resistance genes increased over time, this is discussed in more detail below. The diverse clonality shown by the ERIC PCR results did not support the transmission of clones across the farm-to-fork continuum but indicated a de novo evolution of resistance genes and/or the loss or gain of plasmids over the time period. Resistance profiles could be corelated with historically used antibiotics that included doxycycline, sulfadiazine and trimethoprim, enrofloxacin, olaquindox, avilamycin, tylosin and kitasamycin tartrate. Resistance to tetracycline, trimethoprim-sulfamethoxazole and broad-spectrum β-lactam antibiotics could be attributed to transferrable resistance genes.

Average antibiotic resistance levels for all samples from this study were lower than in two other studies on *E.coli* from poultry in South Africa. Neither of these however was a farm to fork testing protocol but rather looking at caecal samples at processing (Oguttu, Veary and Picard, 2008) and colibacillosis samples (Theobald et al., 2019). The study done by Oguttu et al showed resistance of 98,2% for Doxycycline (compared to 89% for tetracycline in this study caecal sample), 78% sulphamethoxazole (33% this study), 75,6% for enrofloxacin and 90,5% for nalidixic acid (0% for ciprofloxacin and 56% for Nalidixic acid in this study). Colibacillosis samples would be expected to have higher resistance levels due increased incidence of therapeutic antibiotic use and an association between virulence and resistance genes in Avian Pathogenic Extraintestinal *E.coli* (APEC) (Johnson et al., 2012). Reasons for the low antibiotic resistance in this study could be due to low use of therapeutic antibiotics, good biosecurity and good processing practice at the abattoir with minimal contamination. The biosecurity on the farm included only allowing essential workers on the farm and showering in and out procedures. Samples were collected by staff and delivered to main office buildings for us to collect due to these protocols.

Although overall resistance levels were low in comparison to other studies except for ampicillin and tetracycline, there was an increase of both percentage of multidrug resistant isolates and resistance genes detected over the farm to fork continuum. The farm, transport and abattoir samples had the lowest levels of MDR. There was an increase in MDR towards the end of the study, followed by a decrease in retail meats samples corelating with antimicrobial withdrawal periods prior to slaughter. MDR was

expectedly high in the caecal sample and waste water samples and was matched by the presence of resistance genes with percentages of most of the genes being higher in caecal and waste water samples.

Seventy-four percent of isolates tested positive for the resistance genes investigated on this study. However, resistance could not be attributed to these genes in all isolates and may have been caused by other mechanisms that were not investigated. The majority of tetracycline non-susceptible isolates contained either *tetA* or *tetB* genes (Adelowo, Fagade and Agersø, 2014; Li et al., 2014; Awad, Arafat and Elhadidy, 2016). Resistance in the remaining isolates could be attributed to either ribosomal protective proteins (RPP) or mono-oxygenase enzymes which inactivate tetracycline (Grossman, 2016). *Sul1, sul2* and *sul3* encode resistance to sulphamethoxazole. In this study the numbers of *sul2* were low and numbers of *sul3* were high, which is different to other studies which found the reverse (Dessie, Bae and Lee, 2012; Adelowo, Fagade and Agersø, 2014; Awad, Arafat and Elhadidy, 2016).

Only a few mobile quinolone resistance genes were detected in this study with sixteen *qnrB* and one *aac(6)-lb-cr* gene detected. The other mechanism of resistance for quinolones is mutations to DNA gyrase in Gram -negative bacteria and increase in efflux or porin loss (Redgrave et al., 2014; Hooper and Jacoby, 2015).

All isolates with third and fourth generation cephalosporin non-susceptibility were screened for bla_{TEM}, $bla_{CTX\text{-}M}$ and bla_{SHV} genes. All isolates tested contained $bla_{CTX\text{-}M}$ which is higher than other reported rates. Of these, four of the nineteen were found in retail meats and could potentially be a public health risk of transfer to humans via the food chain if chicken is undercooked or contaminates surfaces (Eibach et al., 2018; Niero et al., 2018). Niero et al found ESBL resistance genes in 9% of broilers isolates (Niero et al., 2018) while Eibach et al found higher percentages with 67% CTX-M-15, 11% CTX-M-1 and 9% CTX-M-2 (Eibach etal., 2018).

It is generally accepted that use of antibiotics promote the selection for antibiotic resistant isolates (Stokes and Gillings, 2011; Fair and Tor, 2014; Chang et al., 2015; Founou, Founou and Essack, 2016). It has been shown that salinomycin, an ionophore used as a coccidiostat in poultry, is associated with development of MDR in *E.coli*. There seems to be an association between its use and the co-selection of a number of resistance genes for tetracycline, sulphonamides, chloramphenicol and aminoglycosides, all of which are often found on class 1 integrons. Zinc bacitracin has also been associated with increase in the *sul1* and *sul2* resistance genes which were found in this study (Diarra et al., 2007). Excreted antimicrobials in faeces contaminating litter and promoting antibiotic resistance in the litter which is then used as fertilizer. This process then increases antibiotic resistance in the environment (Furtula et al., 2010).

Resistance may also be introduced from the environment, with hatchlings guts being colonised within a few hours of hatching (Salenha et al., 2009; Baron et al., 2014). It is possible for horizontal gene

transfer to have occurred from bacteria in the hatchery or house to bacteria in the chickens guts (Baron et al., 2014).

This farm also has an all-in-all out system. This means after a flock is sent to slaughter the whole house is cleaned, if some bacteria remain this could be a source of resistance to the next flock entering. In this study the highest levels of MDR and resistant genes were from waste water after cleaning of the house. If any bacteria survive the house cleaning these could be a source of resistant organisms to the next batch of introduced hatchlings (Oguttu, Veary and Picard, 2008). Biosecurity is another source of resistance, flies and rodents have been known to carry resistant organisms and if houses are not sealed these can be transferred to poultry. The same applies to workers in the house. Chicken feed has also been found to be a source of multidrug resistant *E.coli* (Salenha et al., 2009). In future studies investigating these aspects could help to determine the extent of their involvement.

There was no evidence of the dissemination of bacterial clones across the farm-to-fork continuum as evident from the diversity in clusters, antibiograms and resistance genes. There, instead appeared to be a de novo emergence of resistance genes at different time points, or the loss of gain of MGEs across the period from hatching to final processing. Similar findings were seen in a study of *E.coli* from different stages of processing of chicken carcasses conducted by Geornaras and Hastings (2001), in which clonality (amplified fragment length polymorphism) did not correspond to plasmid profiles or antibiograms (Geornaras and Hastings, 2001).

The low levels of antibiotic resistance in retail samples is would a good indicator of food safety for South Africa if this consistent across all retail samples, however more studies from a variety of poultry meat sources would need to be done to investigate this.

Conclusion

In summary, although overall resistance levels were low over the six weeks of the study, MDR and the prevalence of resistance genes increased over time. The diverse clonality shown by the ERIC PCR results did not support the transmission of clones across the farm-to-fork continuum but indicated a *de novo* evolution of resistance genes and/or the loss or gain of plasmids over the time period. Resistance profiles could be corelated with historically used antibiotics and resistance to tetracycline, trimethoprim-sulfamethoxazole and broad-spectrum β-lactam antibiotics could be attributed to transferrable resistance genes. As the only antibiotics used in this flock were growth promoters and anticoccidials, it is postulated that co-selection of resistance genes and horizontal transfer may have contributed to this increase in resistance. Other contributions such as from the environment, insects, feed and workers would have to be investigated further in future studies.

Acknowledgements: The research reported in this publication was funded by the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) Research Project:

"Triangulation of Antibiotic Resistance from Humans, the Food Chain and Associated Environments – A One Health Project" (Reference ID: 204517) and the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation of South Africa (Grant No: 98342). We would like to thank Professor Olarniran Ademola of the School of Life Sciences, University of KwaZulu-Natal (UKZN), Durban, South Africa, for allowing us access to Bionumerics software version 6.6 (Applied maths NV, Belgium) to construct the dendrogram.

Disclaimers: The opinions, findings and conclusions expressed in this research are that of the authors and do not necessarily reflect the opinions or views of the organisations and agencies that funded the project.

Disclosure Statement: Professor Sabiha Essack is the chairperson of the Global Respiratory Infection partnership sponsored by an unconditional educational grant from Reckitt and Benckiser, UK.

References

Aarestrup, F. M. (2004) Monitoring of antimicrobial resistance in animals: Principles and Limitations, *Journal of Veterinary Medicine*, 51, pp. 380–388.

Adelowo, O. O., Fagade, O. E. and Agersø, Y. (2014) Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, southwest Nigeria, *Journal of Infection in Developing Countries*, 8(9), pp. 1103–1112

Awad, A., Arafat, N. and Elhadidy, M. (2016) Genetic elements associated with antimicrobial resistance among avian pathogenic *Escherichia coli*, *Annals of Clinical Microbiology and Antimicrobials*. 15(1), pp.59.

Baron, S., Jouy, E., Larvor, E., Eono, F., Bougeard, S., Kempf, I. (2014) Impact of third-generation-cephalosporin administration in hatcheries on fecal *Escherichia coli* antimicrobial resistance in broilers and layers, *Antimicrobial Agents and Chemotherapy*, 58(9), pp. 5428–5434.

Blaak, H., van Hoek, A.H.AM., Hamidjaja, R.A., van der Plaats, R.Q.J., Kerkhof-de Heer, L., de Roda Husman, A., Schets, F.M. (2015) Distribution, numbers, and diversity of ESBL-producing *E. coli* in the poultry farm environment, *PLoS ONE*, 10(8), pp. 1–23.

Byrne-Bailey, K. Gaze, W. Kay, P. Boxall, A.B.A. Hawkey, P.M. Wellington, E.M.H. (2009) Prevalence of Sulfonamide Resistance Genes in Bacterial Isolates from Manured Agricultural Soils and Pig Slurry in the United Kingdom, *Antimicrobial Agents and Chemotherapy*, 53(2), pp 696-702

Chang, Q. Wang, W., Regev-Yochay, G., Lipsitch, M., Hanage, W.P. (2015) Antibiotics in agriculture and the risk to human health: How worried should we be? *Evolutionary Applications*, 8(3), pp. 240–247.

Chirindze, L.M. Zimba, T.F. Sekyere, J.O. Govinden, U. Chenia, H.Y. Sundsfjord, A. Essack, S.Y. Simonsen, G.S. (2018) Faecal colonization of *E.coli* and *Klebsiella* spp. producing extended-spectrum beta-lactamases and plasmid-mediated AmpC in Mozambican university students, *BMC Infectious Diseases,* 18:244

Clinical and Laboratory Standards Institute (CLSI) (2017) *Performance standards for antimicrobial susceptibility testing. CLSI supplement M100.* 27th Ed. Wayne, Pennsylvania, USA.

Dessie, H. K., Bae, D. H. and Lee, Y. J. (2012) Characterization of integrons and their cassettes in *Escherichia coli* and Salmonella isolates from poultry in Korea, *Poultry Science*, 92 (11) , pp. 3036–3043.

Diarra, M. S. Silevrsides, F.G., Diarrassouba, F., Pritchard, J., Masson, L., Brousseau, R., Bonnet, C., Delaquis, P., Bach, S., Skura, B.J., Topp, E. (2007) Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, *Clostridium perfringens* and Enterococcus counts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in *Escherichia coli* Isolates, *Applied and Environmental Microbiology*, 73(20), pp. 6566–6576.

Dungeni, M. van der Merve, R.R. Momba, M.N.B. (2010) Abundance of pathogebic bacteria and viral indicators in chlorinated effluents produced by four wastewater treatment plants in Gauteng Province, South Africa, *Water South Africa*, 36(5), pp 607-614

Ebomah, K., Adefisoye, M. and Okoh, A. (2018) Pathogenic *Escherichia coli* Strains Recovered from AquaticResources in the Eastern Cape, South Africa, and its Significance to Public Health, *International Journal of Environmental Research and Public Health.*, 15 (7), pp. 1506.

Eibach, D. Dekker, D. Boahen, K.B., Akenten, C.W., Sarpong, N., Campos, C.B., Berneking, L., Aepfelbacher, M., Krumkamp, R., Owusu-Dabo, E., May, J. (2018) Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in local and imported poultry meat in Ghana, *Veterinary Microbiology*, 217, pp. 7–12.

European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2017). *Breakpoint tables for interpretation of MICs and zone diameters. Version 7.0, 2017.* http:/www.eucast.org.

Fair, R. J. and Tor, Y. (2014) Perspectives in Medicinal Chemistry Antibiotics and Bacterial Resistance in the 21st Century, *Perspectives in Medicinal Chemistry*, 6, pp. 25–64.

Founou, L. L., Founou, R. C. and Essack, S. Y. (2016) Antibiotic resistance in the food chain: A developing country-perspective, *Frontiers in Microbiology*, 7(NOV), pp. 1–19.

Georgnaras, I., Hasting, J.W., van Holy, A. (2001) Genotypic Analysis of *Escherichia Coli* from poultry Carcasses and Their Susceptibilities to Antimicrobial Agents. *Applied and Environmental Microbiology*, 67(4), pp 1940-1944.

Graham, J. P., Boland, J. J. and Silbergeld, E. (2007) Growth Promoting Antibiotics in Food Animal Production: An Economic Analysis, *Public Health Reports*, 122, pp. 79–87.

Grossman, T. H. (2016) Tetracycline antibiotics and resistance, *Cold Spring Harbor Perspectives in Medicine*, , 6(4):a025387.

Hooper, D. C. and Jacoby, G. A. (2015) Mechanisms of drug resistance: quinolone resistance, *Annuals of the New York Acadamy of Sciences.*, 1354(1), pp. 12–31.

Johnson, T. J. Logue, C.M., Johnson, J.R., Kuskowski, M.A., Sherwood, J.S., Barnes, H.J., DebRoy, C., Wannemuehler, Y.M., Obata-Yasouka, M., Spanjaard, L., Nolan, L.K. (2012) Associations Between Multidrug Resistance, Plasmid Content, and Virulence Potential Among Extraintestinal Pathogenic and Commensal *Escherichia coli* from Humans and Poultry, *Foodborne Pathogens and Disease*, 9(1), pp. 37–46.

Kunert Filho, H. C. Brito, K.C.T., Cavalli, L.S., Brito, B.G. (2015) Avian Pathogenic *Escherichia coli* (APEC) - an update on the control. In: *The Battle Against Microbial Pathogens: Basic science, Technological Advances and Educational Programs*, Medez-Vilas, E. (Ed), Formatex Research Centre, Badajoz, Spain.

Li, L. Wang, B., Feng, S., Li, J., Wu, C., Wang, Y., Ruan, X., Zeng, M. (2014) Prevalence and Characteristics of Extended-Spectrum b - Lactamase and Plasmid-Mediated Fluoroquinolone Resistance Genes in *Escherichia coli* Isolated from Chickens in Anhui Province , China, *PLoS ONE*, 9(8), pp. 1–6.

Mehdi, Y., Létourneau-Montminy, M. P., Gaucher, M., Chorfi, Y., Suresh, G., Rouissi, T., Brar, S.K., Cote, C., Ramirez, A.A., Godbout, S. (2018) Use of antibiotics in broiler production: Global impacts and alternatives, *Animal Nutrition*. 4(2), pp. 170–178.

Mellata, M. (2013) Human and Avian Extraintestinal Pathogenic *Escherichia coli* : Infections, Zoonotic Risks, and Antibiotic Resistance Trends, *Foodborne Pathogens and Disease*, 10(11), pp. 916–932.

Niero, G., Bortolaia, V., Vanni, M., Intorre, L., Guardabassi, L., Piccirillo, A. (2018) High diversity of genes and plasmids encoding resistance to third-generation cephalosporins and quinolones in clinical *Escherichia coli* from commercial poultry flocks in Italy, *Veterinary Microbiology*, 216, pp. 93–98.

Oguttu, J. W., Veary, C. M. and Picard, J. A. (2008) Antimicrobial drug resistance of *Escherichia coli* isolated from poultry abattoir workers at risk and broilers on antimicrobials, *Journal of the South African Veterinary Association*, 79(4), pp. 161–166.

Redgrave, L. S. Sutton, S.B., Webber, M.A., Piddock, L.J.V. (2014) Fluoroquinolone resistance: Mechanisms, impact on bacteria, and role in evolutionary success, *Trends in Microbiology*. 22(8), pp. 438–445.

Salenha, A. Myaing, T.T., Ganapathy, K.K., Zulkifli, I., Raha, R., Arifah, K. (2009) Possible Effect of Antibiotic-Supplemented Feed and Environment on the Occurrence of Multiple Antibiotic Resistant *Escherichia coli* in Chickens, *International Journal of Poultry Science*, 8(1), pp. 28–31.

Sengelov, G. Halling-Sorensen, B. Aarestrup, F.M. (2003) Susceptibility of *Escherichia coli* and *Enterococcus faecium* isolated from pigs and broiler chickens to tetracycline resistance determinants in *E.coli* from food animals, *Veterinary Microbiology*, 95 pp 91-101

Singer, R. (2015) Therapeutics and antibiotic resistance, in *19th World Veterinary Poultry Association Congress in South Africa*, pp. 1–7. Available at: https://poultryhealthtoday.com/randall_singer_therapeutics-and-antibiotic-resistance/.

Stokes, H. W. and Gillings, M. R. (2011) Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens, *Federation of European Microbiological Societies Microbiology Reviews*, 35(5), pp. 790–819.

Theobald, S. Etter, E., Gerber, D., Abonlik, C. (2019) Antibiotic Resistance Trends in *Escehrichia coli* in South African Poultry, *Foodborne Pathogens and Disease*, 16(9), pp. 1–9.

Versalovic, J. Koeuth, T. Lupski, J.R. (1991) Distribution of repetitive DNA sequence in eubacteria and application to fingerprinting of bacterial genomes, *Nucleic Acids Research*, 19(24), pp 6823-6831

World Health Organisation (WHO) (2017a) *Integrated Surveillance of Antimicrobial Resistance in Foodborne Bacteria*. Geneva, Switzerland.

Supplementary Information

Table 1: Primers used for the detection of the *uidA* gene to confirm *Escherichia coli* identity

Gene	Primer sequence 5'-3'	Control Strain	Reference
uidA-F	AAAACGGCAAGAAAAAGCAG	*E.coli* ATCC 25922	(Dungeni, van Der Merwe and Momba, 2010)
uidA-R	ACGCGTGGTTAACAGTCTTGCG		

Table 2: Primers and control strains for each of the resistance genes tested

Gene	Primers sequence 5'-3'	Control strain	Reference
tetA-F	GTAATTCTGAGCACTGTCGC	In house *Klebsiella pneumonia* strain GCKP12 verified by WGS	(Sengeløv, Halling-sørensen and Aarestrup, 2003)
tetA-R	CTGCCTGGACAACATTGCTT		
tetB-F	CTCAGTATTCCAAGCCTTTG	In house *Escherichia coli* strain PN091E1Il verified by WGS	(Sengeløv, Halling-sørensen and Aarestrup, 2003)
tetB-R	ACTCCCCTGAGCTTGAGGGG		
qnrB-F	GGAATCGAAATTGGCCACTG	In house *Klebsiella pneumonia* strain KP224 verified by WGS	(Li et al., 2014)
qnrB-R	TTTGCCGTTCGCCAGTCGAA		
qnrS-F	CACTTTGATGTCGCAGAT	In house *Klebsiella pneumonia* strain KP230 verified by WGS	(Li et al., 2014)
qnrS-R	CAACATACCCAGTGCTT		
aac(6)-lb-cr-F	GATGCTCTATGGGTGGCTAA	In house *Klebsiella pneumonia* strain GCKP12 verified by WGS	(Li et al., 2014)
aac(6)-lb-cr-R	GGTCCGTTTGGATCTTGGTGA		
sul1-F	CTTCGATGAGAGCCGGCGGC	In house strain 48 verified by WGS	(Byrne-Bailey et al., 2009)
sul1-R	GCAAGGCGGAAACCGCGCC		
sul2-F	TCGTCAACATAACCTCGGACAC	In house strain 48 verified by WGS	(Byrne-Bailey et al., 2009)

sul2-R	GTTGCGTTTGATACCGGCAC		
sul3-F	GAGCAAGATTTTTGGAATCG	In house strain 48 verified by WGS	(Byrne-Bailey et al., 2009)
sul3-R	CATCTGCAGCTAACCTAGGGCTTTGGA		
SHV-F	TTAACTCCCTGTTAGCCA	In house strain 21_S12(950117510)	(Chirindze et al., 2018)
SHV-R	GATTTGCTGATTTCGCCC		
CTXM-F	GGTTAAAAAATCACTGCGTC	In house strain 18_S10(945169659)	(Chirindze et al., 2018)
CTXM-R	TTGGTGACGATTTTAGCCGC		
TEM-F	AAAATTCTTGAAGACG	In house strain 15_S8(945165838)	(Chirindze et al., 2018)
TEM-R	TTACCAATGCTTAATCA		

Table 3: Primers and controls used for ERIC-PCR

Primer	Primer sequence 5'-3'	Control	Reference
ERIC 1	ATGTAAGCTCCTGGGGATTCAC	*E.coli* ATCC 25922	(Versalovic, Koeuth and Lupski, 1991)
ERIC 2	AAGTAAGTGACTGGGGTGAGCG		

Table 4: Putative and confirmed *Escherichia coli* across the farm-to-fork continuum

Sample	Putative *E. coli*	Confirmed *E. coli*
Week 1	41	19
Week 2	40	28
Week 3	40	37
Week 4	39	37
Week 5	50	40
Truck	0	0
Crate	20	19
Abattoir	20	15
Caecal	20	9
Retail Meat	62	53
Waste Water	13	9
Total	345	266

CHAPTER 3

This study describes the antibiotic profiles, antibiotic resistance genes and genetic relatedness of *E. coli* isolates from an intensive poultry-production farm in the uMgungundlovu area of KwaZulu-Natal, South Africa.

3.1 Conclusions

The following conclusions were made with reference to the objectives of this study:

- *Escherichia coli* were successfully isolated from week-one tray samples, week two to five faecal and litter samples, as well as transport crates, abattoir, caecal samples, retail products and waste water samples.
- Two Hundred and sixty-six isolates were confirmed as *Escherichia coli* by PCR
- The Kirby-Bauer disk diffusion method was used to obtain antibiotic susceptibility profiles for 20 antibiotics: ampicillin 48.1%, tetracycline 27.4%, nalidixic acid 20.3%, trimethoprim-sulphamethoxazole 13.9%, chloramphenicol 11.7%, cefalexin 4.5%, ciprofloxacin 4.1%, amoxycillin-clavulanic acid 3.4%, gentamicin 1.9%, cefoxitin 1.1%, cefepime 1.1%, cefotaxime 1.1%, amikacin 1.1%, ceftriaxone 0.8% and azithromycin 0.8%. Isolates were fully susceptible to ceftazidime, imipenem, meropenem and tigecycline.
- Thirty-six different antibiograms were observed with 6.4% of isolates being multidrug resistant (resistant to one antibiotic in three or more classes)
- Overall resistance genes prevalence was *CTX-M* (100%), *sul1* (80%), *tetA* (77%), *tetB* (71%), *SHV* (58%), *sul3* (53%), *qnrB* (37%), *sul2* (33%), *TEM* (5%) and *aac(6)-lb-cr* (2%),. No *qnrS* were detected.
- ERIC-PCR grouped the isolates into 27 clusters using a 75% similarity. Of these, eight clusters had only isolates from one sample and two clusters had isolates from different samples with the same antibiogram.

3.2 Limitations

- Only one poultry house from a single farm was sampled in this study. Results therefore might not be representative of other farms.
- This study was done in August and September and results may differ during different times of the year, as disease incidence changes and therefore antibiotic use changes
- Due to the outbreak of Avian Influenza at the time of sampling, all samples were taken by farm personnel and pooled, this could have affected the randomness and variation in samples

3.3 Future recommendations

The following recommendations are made based on the findings of the study:

- Virulence factors should be investigated to evaluate the public health risk to farm and abattoir workers as well as the public who consume poultry meat. Investigation into virulence genes and determining the number of isolates that classify as DEC, ExPEC or APEC would help to quantify this risk.
- Other resistance mechanisms should be explored where resistance could not be attributed to the selected resistance genes investigated in this study.
- ESBLs should be definitively identified by DNA sequencing.

Additionally,

- Whole genome sequencing may help to detect further resistance mechanisms not tested for by PCR in this study.
- Comparison between farms and seasons would give a better overview of the resistance in poultry in KwaZulu Natal and South Africa.
- MLST and phylogenic testing would allow better comparison with studies done in other countries.

APPENDICES

Appendix 1: Animal Research Ethics Committee (AREC) approval letter

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

23 November 2016

Dr John Osei Sekyere
School of Health Sciences
Westville Campus

Dear Dr Osei Sekyere

Protocol reference number: AREC/073/016PD
Project title: Genomic insights into the Molecular Epidemiology, Evolution, Resistance Mechanisms, and persistence of Veterinary and Food Antibiotic-Resistant Bacteria isolated from South Africa

Full Approval – Research Application

With regards to your revised application received on 11 October 2016. The documents submitted have been accepted by the Animal Research Ethics Committee and **FULL APPROVAL** for the protocol has been granted.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 23 November 2017.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully [redacted]

.....

Prof S Isiam, PhD
Chair: Animal Research Ethics Committee

/ms

Cc Dean & Head of School:Dr Mahmoud Soliman
Cc Academic Leader Research: Professor Mershen Pillay
Cc Registrar: Mr Simon Mokoena
Cc NSPCA: Ms Jessica Light

Animal Research Ethics Committee (AREC)
Ms Mariette Snyman (Administrator)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 8350 **Facsimile:** +27 (0) 31 260 4609 **Email:** animalethics@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Animal-Ethics.aspx

Appendix 2: Biomedical Research Ethics Committee (BREC) approval letter

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

17 March 2017

Prof SY Essack
Department of Pharmaceutical Sciences
School of Health Sciences
essacks@ukzn.ac.za

Dear Prof Essack

Title: One Health approach to the containment of antibiotic resistance.
Degree: Non-degree
BREC Ref No: BCA444/16

CLASS APPROVAL

The Biomedical Research Ethics Committee (BREC) has considered the abovementioned application at a meeting held on 13 September 2016.

The study was provisionally approved by BREC pending appropriate responses to queries raised. Your responses dated 28 February 2017 to queries raised on 19 September 2016 have been noted and approved by the Biomedical Research Committee at a meeting held on 14 March 2017.

This approval is valid for one year from **17 March 2017.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

Pg. 2/...

Biomedical Research Ethics Committee
Professor J Tsoka-Gwegweni (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 2486 **Facsimile:** +27 (0) 31 260 4609 **Email:** brec@ukzn.ac.za

Appendix 3: Department of Agriculture, Forestry and Fisheries (DAFF) record

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/5 (879)

Professor Sabiha Yusuf Essack
Antimicrobial Research Unit
College of Health Sciences
University of KwaZulu-Natal
Tel: 031 260 7785
E-mail: ESSACKS@ukzn.ac.za

Dear Prof Essack,

RETROSPECTIVE APPLICATION UNDER SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984) FOR: "ANTIBIOTIC RESISTANCE AND ONE HEALTH", SPECIFICALLY PERTAINING TO THE STUDY CONDUCTED IN INTENSIVELY PRODUCED POULTRY

Your application received on 30 August 2018 for a Section 20 permit for the above mentioned study refers. Unfortunately, Section 20 approval cannot be given retrospectively for a study that has already proceeded or concluded, but we hereby take note of the information divulged in the application.

We take note of the letter of apology dated 28 August 2018, as well as the letter dated 13 September 2018 where you undertake to comply with Section 20 of the Animal Diseases Act, 1984 (Act No. 35 of 1984) and relevant processes in future.

Kind regards,

[redacted]

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2018 -09- 2 5

Appendix 4: Table of Raw Data: Antibiotic Susceptibility Test Results

Isolate	AMP	AMC	LEX	FOX	CRO	CAZ	FEP	CTX	NAL	CIP	IMP	MEM	TE	SXT	CHL	GEN	TGC	AZM	AMK	Antibiogram (intermediate denoted by *)
1T1	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	NAL*
1T2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
1T3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	CHL
1T4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
1T5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
1T7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
1T8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
1T9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
1T10	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	NAL*
1T11	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
1T12	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I	S	S	S	S	AMP*-TE-CHL*
1T13	S	S	S	S	S	S	S	S	I	S	S	S	R	S	R	S	S	S	S	NAL*-TE-CHL
1T14	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	NAL*
1T15	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
1T16	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
1T17	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
1T18	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
1T19	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
1T20	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	SXT
2F3	R	S	S	S	S	S	S	S	I	S	S	S	S	S	I	S	S	S	I	AMP-NAL*-CHL*-AMK*
2F6	R	S	S	S	S	S	S	S	I	S	S	S	S	S	R	S	S	S	S	AMP-NAL*-CHL
2F7	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*
2F10	R	S	R	S	S	S	S	S	I	S	S	S	S	S	R	S	S	S	S	AMP-LEX-NAL*-CHL
2F11	R	S	S	S	S	S	S	S	I	S	S	S	S	S	R	S	S	S	S	AMP-NAL*-CHL
2F13	R	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	AMP-NAL*

2F14	I	I	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	AMP*-AMC*-SXT-CHL
2F17	R	S	S	S	S	S	S	S	I	S	S	S	S	S	I	S	S	S	S	AMP-NAL*-CHL*
2F19	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*
2L1	I	I	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	AMP*-AMC*-CHL*
2L2	R	R	S	R	S	S	S	S	S	S	S	S	S	R	R	S	S	S	R	AMP-AMC-FOX-SXT-CHL-AMK
2L3	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	TE-SXT
2L4	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
2L5	R	S	S	S	S	S	S	S	S	S	S	S	I	S	R	S	S	S	S	AMP-TE*-CHL
2L6	I	S	S	S	S	S	S	S	I	S	S	S	S	R	R	S	S	S	S	AMP*NAL*-SXT-CHL
2L7	I	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*-LEX
2L8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
2L9	I	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	R	S	AMP*-NAL*-AZM
2L10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
2L11	I	S	R	I	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	AMP*-LEX-FOX*-NAL*
2L12	R	S	R	S	S	S	S	S	I	S	S	S	S	S	I	S	S	S	S	AMP-LEX-NAL*-CHL*
2L13	I	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*-AMC*
2L14	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
2L15	I	I	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	AMP*-AMC*-NAL*
2L17	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
2L18	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*
2L19	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
2L20	I	S	S	S	S	S	S	S	I	S	S	S	S	R	R	S	S	S	S	AMP*-NAL*-SXT-CHL
3F1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3F3	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	AMP*-TE
3F5	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R	S	S	S	AMP*-TE-GEN
3F6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3F7	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
3F8	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
3F9	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	AMP*-TE
3F10	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	AMP*-TE
3F12	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE

3F13	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
3F14	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	AMP*-TE
3F15	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*
3F16	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
3F17	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
3F18	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3F19	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3F20	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	AMP*-TE
3L1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3L3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3L4	R	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	AMP-NAL
3L5	R	S	R	S	R	S	I	R	R	I	S	S	R	S	R	S	S	S	S	AMP-LEX-CRO-FEP*-CTX-NAL-CIP*-TE-CHL
3L6	I	S	S	S	S	S	S	S	R	I	S	S	S	S	S	S	S	S	S	AMP*-NAL-CIP*
3L7	S	S	S	S	S	S	S	S	R	I	S	S	S	S	S	S	S	S	S	NAL-CIP*
3L8	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*
3L9	I	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	AMP*-SXT-CHL
3L10	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
3L11	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3L12	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3L13	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3L14	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3L15	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3L16	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
3L17	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3L18	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
3L19	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	SXT-CHL
3L20	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3L21	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
4F4	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
4F5	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	AMP*-TE

4F6	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	AMP*-TE
4F7	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	AMP*-TE
4F8	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
4F9	S	S	R	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	LEX-TE
4F10	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
4F11	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
4F12	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
4F13	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
4F14	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
4F15	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
4F16	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
4F17	R	S	R	S	R	S	S	R	S	S	S	S	S	S	R	S	S	S	I	AMP-LEX-CRO-CTX-CHL-AMK*
4F18	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	AMP*-TE
4F19	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
4F20	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
4L1	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
4L2	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
4L3	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
4L4	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
4L5	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
4L6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
4L7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
4L8	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
4L9	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
4L10	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
4L11	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
4L12	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
4L13	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
4L14	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
4L15	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
4L16	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP

4L17	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
4L18	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*
4L20	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	AMP-TE
4L21	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
5F1	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F2	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	AMP*-TE
5F3	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	AMP*-TE
5F8	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F9	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F10	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F11	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F12	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
5F13	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
5F14	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F15	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F16	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F17	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F18	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
5F19	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F20	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F21	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F22	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F23	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
5F24	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5F25	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5L1	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP-AMC*
5L2	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
5L3	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5L5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
5L7	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S	AMP-TE-SXT-CHL
5L9	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP

5L10	I	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*-AMC*
5L11	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5L13	R	S	S	S	S	S	S	S	S	S	S	S	R	R	I	S	S	S	S	AMP-TE-SXT-CHL*
5L14	R	S	S	S	S	S	S	R	S	S	S	S	R	R	R	S	S	S	S	AMP-CTX-TE-SXT-CHL
5L15	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5L17	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S	AMP-TE-SXT-CHL
5L19	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5L20	R	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	AMP-NAL
5L21	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5L22	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5L23	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
5L24	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP-LEX
5L25	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
CR1	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
CR2	S	S	S	S	S	S	S	S	S	I	S	S	R	S	S	S	S	S	S	CIP*-TE
CR3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
CR4	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
CR5	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	TE
CR6	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	SXT
CR7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
CR8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	CHL
CR9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
CR10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
CR11	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	NAL-TE
CR12	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
CR13	S	S	S	S	S	S	S	S	I	S	S	S	S	R	R	S	S	S	S	NAL*-SXT-CHL
CR14	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
CR15	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
CR16	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
CR17	S	S	S	S	S	S	S	S	I	S	S	S	R	S	S	S	S	S	S	NAL*-TE
CR18	R	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	AMP-NAL

CR19	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
RI1	R	S	S	S	S	S	S	S	R	S	S	S	S	S	S	R	S	S	S	AMP-NAL-GEN
RI2	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP-LEX
RI4	I	S	S	S	S	S	S	S	R	S	S		S	S	S	S	S	S	S	AMP*-NAL
RI5	I	S	S	S	S	S	S	S	I	S	S	S	R	I	S	S	S	S	S	AMP*-NAL*-TE-SXT*
RI6	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*
RI7	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	NAL
RI9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
RI10	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	NAL-TE
RI11	S	I	S	S		S	S	S	S	S	S	S	S	S	S	R	S	S	S	AMC*-GEN
RI12	I	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	AMP*-SXT
R115	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	NAL
RI16	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	R	S	S	S	NAL*-GEN
RI17	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
RI18	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R	S	S	S	NAL-CIP-GEN
RI20	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	NAL
CA3	I	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	AMP*-NAL-TE
CA4	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	TE-SXT
CA5	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	TE-SXT
CA7	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	NAL-TE
CA8	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
CA9	I	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	AMP-NAL-TE
CA10	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	AMP-TE-SXT
CA16	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	NAL-TE
CA20	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	NAL-TE
RC1	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*
RC2	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	NAL-TE
RC3	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	SXT
RC4	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	NAL-TE
RC5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
RC6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	

RC7	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*
RC9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
RC10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
RC11	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	NAL-TE
RC12	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	CIP
RC13	I	S	S	S	S	S	S	S	R	R	S	S	R	R	S	S	S	S	S	AMP*-NAL-CIP-TE-SXT
RC14	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	NAL
RC15	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	LEX
RC16	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
RC19	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	NAL-TE
RC20	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
TH1	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	NAL
TH2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
TH3	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	FEP*
TH4	S	S	S	S	S	S	S	S	R	I	S	S	R	R	I	S	S	S	S	NAL-CIP*-TE-SXT-CHL*
TH7	R	S	S	S	S	S	I	S	S	S	S	S	R	R	S	S	S	S	S	AMP-FEP*-TE-SXT
TH10	S	S	S	S	S	S	S	S	R	I	S	S	R	R	I	S	S	S	S	NAL-CIP*-TE-SXT-CHL*
TH11		S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	TE-SXT
TH12	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	NAL
TH13	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
TH14	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
TH15	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	SXT-CHL
TH16	R	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	AMP-NAL-TE
TH17	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*
TH18	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
TH19	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
TH20A	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
TH20B	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	TE-SXT
N1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
N2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
N4	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*

N5	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*
N6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
N7	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
N8	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*
N9a	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
N9b	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
N11	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	LEX
N12	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
N13	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
N14	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
N15	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
N16	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	AMP*-AZM
N17	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	AMP-SXT
N18	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
N19	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	SXT-CHL
N20	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP
WW1	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	AMP*
WW2	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	SXT-CHL
WW4	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	SXT
WW6	I	S	S	S	S	S	S	S	R	S	S	S	R	R	S	S	S	S	S	AMP*-NAL-TE-SXT
WW7	S	S	S	S	S	S	S	S	R	S	S	S	R	R	S	S	S	S	S	NAL-TE-SXT
WW9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
WW10	R	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	AMP-CHL*
WW11	R	R	R	R	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	AMP-AMC-LEX-FOX-NAL-CIP
WW13	S	S	S	S	S	S	S	S	R	S	S	S	R	R	S	S	S	S	S	NAL-TE-SXT

Appendix 5: Table of Raw Data: Isolates Selected for ERIC-PCR and Resistance Gene Screening showing Antibiograms, Resistance Genes and Clusters

Isolates	Antibiogram, intermediate resistance denoted with *	Resistance genes detected by PCR	Cluster	Isolates	Antibiogram, intermediate resistance denoted with *	Resistance genes detected by PCR	Cluster
1T3	CHL		*A*	**5L7**	AMP-TE-SXT-CHL	*Sul1, sul2, sul3, tetA*	*T*
1T8			*A*	**5L14**	AMP-CTX-TE-SXT-CHL	*sul1,tetA,ctx*	*Y*
1T10	NAL*		*A*	**5L15**	AMP		*D*
1T12	AMP*-TE-CHL*	*tetA*	*C*	**5L17**	AMP-TE-SXT-CHL	*sul1,sul3,tetA,tetB*	*Y*
1T13	NAL*-TE-CHL	*tetA*	*C*	**5L24**	AMP-LEX		
1T17			*A*	**CR2**	CIP*-TE	*tetA*	*R*
1T20	SXT		*A*	**CR4**	AMP		*R*
2F3	AMP-NAL*-CHL*-AMK*		*M*	**CR5**	TE	*tetA,tetB*	*L*
2F7	AMP*		*U*	**CR6**	SXT		*B*
2F10	AMP-LEX-NAL*-CHL		*M*	**CR8**	CHL		
2F11	AMP-NAL*-CHL		*T*	**CR11**	NAL-TE	*tetA,tetB*	*R*
2F13	AMP-NAL*		*M*	**CR13**	NAL*-SXT-CHL	*sul1,sul3,qnrB*	*B*
2F14	AMP*-AMC*-SXT-CHL	*shv,ctx*	*U*	**CR14**			*B*
2F17	AMP-NAL*-CHL*		*M*	**CR18**	AMP-NAL		*K*
2F19	AMP*		*T*	**RI1**	AMP-NAL-GEN		*Z*
2L1	AMP-AMC*-CHL*	*shv,ctx*	*U*	**RI2**	AMP-LEX		*A*
2L2	AMP-AMC-FOX-SXT-CHL-AMK	*sul1,shv,ctx*	*U*	**RI4**	AMP*-NAL		*K*
2L3	TE-SXT	*sul1*	*R*	**RI5**	AMP*-NAL*-TE-SXT*	*tetA,tetB*	*H*
2L4	TE	*tetB*	*O*	**RI6**	AMP*		*H*
2L5	AMP-TE-CHL*		*T*	**RI7**	NAL		
2L6	AMP*-NAL*-SXT-CHL	*sul1*	*U*	**RI9**			*R*
2L7	AMP*-LEX		*U*	**RI10**	NAL-TE	*qnrB,tetA,tetB*	*B*
2L8			*T*	**RI11**	AMC*-GEN	*shv,ctx*	*L*
2L9	AMP*-NAL*-AZM		*U*	**RI12**	AMP*-SXT	*sul1*	*B*
2L10	AMP*		*U*	**RI16**	NAL*-GEN		*Z*
2L11	AMP*-LEX-FOX*-NAL*	*ctx*	*U*	**RI18**	NAL-CIP-GEN		
2L12	AMP-LEX-NA*-CHL*		*X*	**CA3**	AMP*-NAL-TE	*tetA,tetB*	*W*
2L13	AMP*-AMC*	*shv,ctx*		**CA5**	TE-SXT	*sul1,tetA,tetB*	*S*
2L15	AMP*-AMC*-NAL*	*shv,ctx*	*U*	**CA8**	AMP		*S*
2L17		*shv,ctx*	*U*	**CA10**	AMP-TE-SXT	*sul1,sul2,sul3,tetA,tetB*	*S*
2L19	AMP		*P*	**CA16**	NAL-TE	*tetA,tetB,qnrB*	*W*
2L20	AMP*-NAL*-SXT-CHL	*sul3*	*U*	**CA20**	NAL-TE	*tetA,tetB,qnrB,aac(6)-lb-cr*	*W*
3F5	AMP*-TE-GEN	*tetB*	*F*	**RC1**	AMP*		*C*
3F7	AMP		*M*	**RC3**	SXT	*sul1*	*W*
3F9	AMP*-TE	*tetB*	*F*	**RC4**	NAL-TE	*tetA,tetB*	*AB*
3F13	TE	*tetB*	*F*	**RC11**	NAL-TE	*tetA,tetB,shv,ctx*	*AB*
3F14	AMP*-TE	*tetB,ctx*	*F*	**RC12**	CIP	*qnrB*	*X*

3F17	TE	*tetB,ctx*	*F*	**RC13**	AMP*-NAL-CIP-TE-SXT	*sul1,sul3,tetA,tetB,qnrB*	
3F18			*M*	**RC14**	NAL	*qnrB*	*J*
3F19			*M*	**RC15**	LEX		*J*
3L4	AMP-NAL		*Z*	**RC19**	NAL-TE	*tetA,tetB*	*AA*
3L5	AMP-LEX-CRO-FEP*-CTX-NAL-CIP*-TE-CHL	*tetA,tetB,shv,ctx*		**RC20**			*AA*
3L6	AMP*-NAL-CIP*		*U*	**TH1**	NAL	*qnrB*	
3L7	NAL-CIP*		*P*	**TH3**	FEP*	*ctx*	
3L8	AMP*		*P*	**TH4**	NAL-CIP*-TE-SXT-CHL*	*sul1,sul3,tetA,tetB,qnrB*	
3L9	AMP*-SXT-CHL		*B*	**TH7**	AMP-FEP-TE-SXT	*sul1,sul2,sul3,tetA,tetB,shv,ctx,tem*	
3L16	TE	*tetB*	*O*	**TH10**	NAL-CIP*-TE-SXT-CHL*	*sul1,sul2,tetA,tetB,qnrB*	*T*
3L17				**TH11**	TE-SXT	*sul1,sul3,tetA,tetB*	*A*
3L19	SXT-CHL	*sul1,sul3*	*B*	**TH13**	AMP		*I*
3L21			*T*	**TH15**	SXT-CHL	*sul1,sul2,sul3*	*E*
4F4	TE	*tetA*	*G*	**TH16**	AMP-NA-TE	*tetA,tetB,qnrB*	*Z*
4F5	AMP*-TE	*tetA,tetB*	*G*	**TH19**			
4F9	LEX-TE	*tetA*	*G*	**TH20B**	TE*-SXT*	*sul1,tetA,tetB*	*I*
4F17	AMP-LEX-CRO-CTX-CHL-AMK*	*ctx*	*E*	**N5**	AMP*		*E*
4F19	AMP		*G*	**N8**	AMP*		*E*
4F20			*N*	**N9a**			*E*
4L7			*D*	**N11**	LEX	*ctx*	*Q*
4L17	AMP		*M*	**N16**	AMP*-AZM		*E*
4L20	AMP-TE	*tetA,tetB*	*G*	**N17**	AMP-SXT*	*sul3*	
4L21	TE	*tetA*	*G*	**N19**	SXT-CHL	*sul1,sul2,sul3,tetA,tetB*	*E*
5F3	AMP*-TE	*tetA,tetB*	*N*	**WW1**	AMP*		*D*
5F10	AMP		*Q*	**WW2**	SXT-CHL	*sul1,sul2,sul3,tetA,tetB*	*Q*
5F12	TE	*tetA*	*N*	**WW4**	SXT	*sul1,sul2,sul3*	
5F14	AMP	*sul2, sul3*	*T*	**WW6**	AMP*-NAL-IMP*-TE-SXT	*sul1,sul2,tetA,tetB,qnrB*	*Q*
5F15	AMP			**WW7**	NAL-TE-SXT	*tetB,qnrB*	*E*
5F23			*D*	**WW9**			
5L1	AMP-AMC*	*shv,ctx*	*D*	**WW10**	AMP-CHL*		
5L2	TE	*tetA,tetB*	*G*	**WW11**	AMP-AMC-LEX-FOX-NAL-CIP	*tetA,qnrB,ctx*	
5L5			*B*	**WW13**	NAL-TE-SXT	*qnrB,sul1,sul2,sul3,tetA*	*Q*