Molecular Epidemiology of Antibiotic-Resistant *Escherichia coli* from Companion Animals attending Veterinary Practices in Durban, KwaZulu-Natal, South Africa

Nondumiso Ntuli

222127632

A dissertation submitted in fulfillment 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-supervisor

Dr. Joshua Mbanga

Dr. Luther King Abia Akebe

2023

Molecular Epidemiology of Antibiotic-Resistant *Escherichia coli* from Companion Animals attending Veterinary Practices in Durban, KwaZulu-Natal, South Africa

2023

Nondumiso Ntuli

222127632

A dissertation submitted to the School of Laboratory Medicine and Medical Sciences, College of Health Science, 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 this dissertation is the original research work of Miss Nondumiso Ntuli, supervised by:

Supervisor:

Signed [redacted] ……Name:…S Y Essack……Date: 16 April 2024……

Co-supervisor:

Signed [redacted] Name: .. J Mbanga ...Date: 15 April 2024

Co-supervisor:

Signed …… [redacted] ……. …Name: …LK Abia Akebe…… Date: ……*15 April 2024*……

DECLARATION

I, Nondumiso Lungile Ntuli declare that:

i. The research reported in this dissertation, except where otherwise indicated, is my original research.
ii. This dissertation has not been submitted for any degree, or examination at any other university.
iii. This dissertation does not contain other persons' data, pictures, graphs, or other information, unless specifically acknowledged as being sourced from other persons.
iv. This dissertation does not contain other persons' writing unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a. Their words have been re-written, but the general information attributed to them has been referenced.
    b. Where their exact words have been used, their writing has been placed inside quotation marks, and referenced.
v. Where I have reproduced a manuscript of which I am an author, co-author, or editor, I have indicated in detail which part of the publication was written by myself alone and have fully referenced such publications.
vi. This dissertation does not contain text, graphics, or tables copied, and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation/thesis, and in the References sections.

Signature: ____[redacted]_____

Student number: 222127632

Date: 15/04/2024

DEDICATION

I dedicate this project to my mother, Nancy Busisiwe Mathenjwa, my daughter, Lisakhanya Eyami Mlambo, and my late dog Ntchintchues who has incredibly inspired me.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation, and gratitude to the following individuals for their roles, and support during the research study:

- Professor Sabiha Yusuf Essack, for excellent, and distinguished supervision of my work, her extensive knowledge of the subject, publication experience, help in writing my work, and being an intellectual leader.
- My family, for their patience, and support throughout my academic journey.
- Dr Luther King Abia Akebe for assisting with laboratory protocols.
- Dr Joshua Mbanga for mentoring, and assisting me with the analysis of data, and the write-up.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ABR	Antibiotic-Resistance
AMC	Amoxicillin-Clavulanate
AMK	Amikacin
AMP	Ampicillin
AREC	Animal Research Ethics Committee
ARG	Antibiotic-Resistance Gene
ATCC	American Type Culture Collection
AWaRe	Access, Watch, and Reserve
AZM	Azithromycin
BLAST	Basic Local Alignment Search Tool
BLBLI	β-Lactams-β-Lactamase Inhibitors
BREC	Biomedical Research Ethics Committee
C1	First-Criterion
C2	Second Criterion
CAZ	Ceftazidime
CHL	Chloramphenicol
CIA	Critically Important Antimicrobial
CIP	Ciprofloxacin
CLSI	Clinical, and Laboratory Standards Institute
CRO	Ceftriaxone
CTX	Cefotaxime
DAFF	Department of Agriculture, Forestry, and Fisheries
DALARRD	Department of Agriculture, Land Reform, And Rural Development
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EMB	Eosin Methylene Blue
ERIC	Enterobacterial Repetitive Intergenic Consensus
ESBL	Extended-Spectrum Beta-Lactamase

EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EXPEC	Extraintestinal Pathogenic *E. coli*
FAO	Food, and Agriculture Organization of The United Nations
FEP	Cefepime
FOX	Cefoxitin
GEN	Gentamicin
GLASS	Global Antimicrobial Resistance Surveillance System
HGT	Horizontal Gene Transfer
HIA	Highly Important Antimicrobial
HPCIA	Highest Priority Critical Important
I	Intermediate-Resistance
IA	Important Antimicrobial
IMP	Imipenem
IPC	Infection Prevention, and Control
LEX	Cephalexin
LMIC	Low- And Middle-Income Countries
MDR	Multi-Drug Resistant
MEM	Meropenem
MIA	Medically Important Antimicrobial
MLST	Multilocus Sequence Typing
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MRSP	Methicillin-Resistant *Staphylococcus pseudintermedius*
NAL	Nalidixic Acid
NBBLI	Non-β-Lactam-β-Lactamase Inhibitors
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction
R	Resistance
RNA	Ribonucleic Acid
S	Susceptibility
SARCHI	South African Research Chairs Initiative
SAVC	South African Veterinary Council

SDD	Susceptible-Dose Dependent
TAE	Tris-Acetate-EDTA
TET	Tetracycline
TGC	Tigecycline
TSB	Tryptone Soy Broth
TZP	Piperacillin-Tazobactam
UDG	Uracil-DNA Glycosylase
UK	United Kingdom
UPEC	Uropathogenic *E. coli*
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
USA	United States of America
UTI	Urinary Tract Infections
VRE	Vancomycin-Resistant Enterococci
WHO	World Health Organization
WOAH	World Organization for Animal Health
XDR	Extensively Drug Resistant

ABSTRACT

Background: Companion animals are globally documented to harbour antibiotic-resistant *E. coli*. This study aimed to investigate the molecular epidemiology of antibiotic-resistant *E. coli* from companion animals presenting at veterinary practices in Durban, KwaZulu-Natal, South Africa. **Methods**: *E. coli* were isolated on selective media from rectal swabs sampled from dogs and cats attending veterinary practices in Durban, KwaZulu-Natal, South Africa. All isolates were confirmed using real-time polymerase chain reaction (PCR) of the *uidA* gene. Antibiotic susceptibility testing was done against 20 antibiotics using the Kirby-Bauer disk diffusion method. Selected antibiotic-resistance genes (ARGs) that confer resistance to third-generation cephalosporins (bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$), tetracycline (*tetA,* and *tetB*), and tigecycline (*tet*X/X2, *tet*X3, and *tet*X4), were detected using conventional PCR. PCR amplicons were confirmed by DNA sequencing and bioinformatics analysis. Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) was carried out to determine the clonality of *E. coli* (101) isolates that showed resistance to at least one antibiotic. **Results**: A total of 330 *E. coli* isolates from dogs (234) and cats (96) formed the study sample. Overall resistance was high in tetracycline (24.2%), ampicillin (18.8%), trimethoprim-sulfamethoxazole (14%), cephalexin (11.2%) and nalidixic acid (9.7%). Whilst lower resistance was observed in amikacin (0.3%), ceftazidime (0.3%), and piperacillin-tazobactam (0.6%). Third-generation cephalosporin-resistant *E. coli* retrieved from cats (26%) was more prevalent compared to dogs (9.8%). *E. coli* from dogs (2.1%) and cats (2%) were resistant to forth-generational cephalosporins. *E. coli* (3%) retrieved from dogs was resistant to tigecycline, which is regarded as a medically important antimicrobial (MIA) in human medicine. No resistance was observed against carbapenems. Thirty-five (10.6%) *E. coli* were multidrug-resistant (MDR) and exhibited twenty-two different phenotypic patterns. Amongst the *E. coli* that were not susceptible to third-generation cephalosporin, and tetracycline, it was observed that the $bla_{\text{CTX-M-15}}$ (8%), and *tetA* (24%) were the most prevalent resistance genes. Thirty-one (9.3%) isolates were non-susceptible to third-generation cephalosporins and had the corresponding extended-spectrum beta-lactamase (ESBL) genes. The $bla_{\text{CTX-M-15}}$ type gene was prevalent in all 25 *E. coli* isolates that tested positive for the $bla_{\text{CTX-M}}$. The $bla_{\text{TEM-1}}$ (17) was the second most prevalent β-lactamase gene. A total of 80/330 (24%) isolates were phenotypically not susceptible to tetracycline and carried either one, or both of *tet*A and *tet*B resistance genes. Only one tetracycline-resistant *E. coli* isolate did not harbour either *tet*A, or *tet*B genes. The bla_{SHV}, *tet*X/X2, *tet*X3, and *tet*X4 were not detected in all the isolates. Using a 75% similarity cut-off, forty-eight clusters with isolates from both dogs and cats were identified. The

ERIC-PCR types depicted a variety of clusters within veterinary practices in Durban, indicating that a high diversity of *E. coli* is in circulation in Durban, South Africa. **Conclusion**: Companion animals are reservoirs of antibiotic-resistant *E. coli* and ARGs. However, there was no evidence of transmission of antibiotic-resistant *E. coli* in Durban, South Africa. Resistance of *E. coli* from companion animals to MIA for humans is of particular concern and requires measures to control the spread of antibiotic-resistant bacteria, and ARGs between companion animals, veterinary practice personnel, and owners.

CHAPTER ONE

1.1 Introduction

Antibiotic resistance (ABR) is a global public health threat precepting from the alarming increase in the evolution and dissemination of antibiotic-resistant bacteria (Sobur et al., 2019) within humans, animals (food animals, companion animals, wildlife), and the environment interface (Massella et al., 2020).

Studies show that companion animals also referred to as pets are regarded as a family in many households, and they offer numerous benefits including physical, emotional, and social benefits such as stress, and depression management (Kogan et al., 2021). Companion animals include dogs, horses, and cats etc. (Goins & Hanlon, 2021). The use of antibiotics in companion and food animals plays a critical role in animal welfare and food security, however, it is one of the drivers of ABR (FAO, 2016). Companion animals are one of the reported carriers of antibiotic-resistant bacteria globally and animal-human co-existence results in the ease of cross-transmission of antibiotic-resistant bacteria between companion animals and humans (Gwenzi et al., 2021).

Escherichia coli is one of the pathogens responsible for infections such as urinary tract infections (UTI) in companion animals and is reported to be resistant to commercially available antibiotics including broad-spectrum, and last-resort antibiotics that are of critical importance to both animal and human medicine (Weese et al., 2011). Several *E. coli* strains are multidrug-resistant (MDR), i.e., resistant to one, or more antimicrobial agents belonging to three, or more different classes (Hadeel et al., 2022). MDR *E. coli* has been reported in companion animals, and similar strains have been detected in owners of companion animals (Saputra et al., 2017). However, research on surveillance and monitoring of antibiotic-resistant *E. coli* in companion animals, and the implication of dissemination of antibiotic-resistant *E. coli* from companion animals to humans is in its infancy in South Africa (Chipangura et al., 2017). This study investigated the prevalence of antibiotic-resistant *E. coli* in companion animals in veterinary practices in South Africa.

1.2. Literature review

1.2.1. Antibiotic-resistance

ABR is defined as the ability of bacterial cells to adapt, and grow in the presence of antibiotics (WHO, 2015). Moreover, resistance to antibiotics causes treatment failure (Shamsuddin et al., 2016). ABR was initially described in *E. coli*, which was then known as *Bacillus coli*, just after penicillin was discovered, and used to treat bacterial infections (Ventola, 2015). Since then, the overuse and misuse of antibiotics in humans and animals have resulted in an increased rate of ABR cases globally (Mehdi et al., 2018; Smith et al., 2019). Bacteria can either have intrinsic or acquired resistance. Intrinsic resistance occurs when bacteria are naturally resistant to certain antimicrobial agents due to inherent genetic characteristics. Acquired resistance refers to when bacteria obtain resistance through a variety of mechanisms, for example, though mutations (induced gene mutation, and spontaneous gene mutations), and horizontal gene transfer (HGT) (transformation, transduction, and conjugation (**Figure 1)** (FAO, 2016; Riedl et al., 2016).

There are several factors contributing to ABR, including inadequate regulation, or enforcement of regulations on antimicrobial use in animals and humans, poor stewardship, indiscriminate use of antibiotics in veterinary feed, sub-optimal infection prevention and control (IPC) in health care systems and facilities, poor biosecurity in agricultural settings, and poor water, sanitation, and hygiene in communities (WHO, 2015, 2019). These factors are actively eroding the efficacy of antibiotics (De Oliveira et al., 2020). The Global Antimicrobial Resistance Surveillance System (GLASS) developed by WHO, reports that there is a strong association between ABR and the use of antimicrobial agents. In addition, the WHO reports the emerging shift to broad-spectrum, and last-resort antibiotics, because of ABR (WHO, 2018b).

One of the routes of ABR to humans is through the food chain and environment (soil and water). ABR spreads between farms, and communities through food products, companion animals, and wildlife resulting in drug-resistant infections (Hong et al., 2019).

1.2.2. Important antibiotic groups in animal and human medicine

Considering the antimicrobial class importance in human medicine and the risk of ABR, various organizations such as the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (WOAH) and the World Health Organization (WHO) have taken important measures to address ABR and antibiotics use in both human and veterinary medicine. These organisations have compiled antibiotics based on their importance in human, and animal medicine (WOAH, 2021). The WHO medically important antimicrobial (MIA) list (previously known as the WHO critically important antimicrobial (CIA) list (**Table 1**), aims to categorize antimicrobial classes that are authorised for use in humans only (medically important), not authorised in humans (not medically important for humans) and authorised in both humans and animals to aid risk management of antimicrobial use (WHO, 2023).

Authorised antimicrobials for human use in humans only include antimicrobial agents that are of high priority and should not be used in veterinary medicine. Not medically important antimicrobials include antimicrobials that would not result in resistance to medically important antimicrobials, and hence are used in animals only. However, antimicrobials that meet the criterion formulated by WHO are authorised in both humans and animals (WHO, 2023).

The first-criterion (C1) relates to the limited antimicrobial therapies that treat serious bacterial infections in humans and the second-criterion (C2) is that the antimicrobial class is used to treat infections in people caused by either bacteria that may be transmitted to humans from non-human sources, or bacteria that may acquire resistance genes from non-human sources (WHO, 2018a). Antimicrobial agents that meet either one of C1 and C2 are considered highly important antimicrobials (HIA), whilst those that neither meet, C1 and C2 are considered important antimicrobial (IA) agents (WHO, 2023).

If both C1 and C2 are met, two prioritization factors are considered to distinguish between the highest priority critical important antimicrobials (HPCIA) and CIA. Prioritisation factor 1 is met when the antimicrobial class has an antimicrobial agent that is categorised as an essential medicine, and is classified as access, watch, and reserve on the AWaRe classification list. Prioritisation factor 2 is met when an antimicrobial class includes an antimicrobial agent that is used to treat invasive and life-threatening infections by resistant bacteria, or resistant genes transmittable from non-human sources. The antimicrobial agent is considered HPCIA when both prioritisation factors are met, and is considered CIA when one, or neither of prioritisation factors is met (WHO, 2023).

In veterinary medicine, antibiotics were categorised by two criteria. The first criterion relates to the importance of the antimicrobial class in animal health. The second criterion relates to compounds within the class that were identified as essential against specific infections, and where there was a lack of sufficient therapeutic alternatives (WOAH, 2021). Antimicrobial agents that meet both criteria are categorised as "critically important", antimicrobial agents that meet one of the criteria are categorised as "highly important", and antimicrobial agents that meet none of the two criteria are categorised as "important". Some examples are tabulated in **Table 1** (WOAH, 2021).

Table 1: Antibiotics used in both human and animal medicine (WHO, 2023; WOAH, 2021)

Antimicrobial class	Antimicrobial agent	Human medicine	Veterinary medicine
3rd, 4th, and 5th generation cephalosporins with β-lactamase inhibitor	Cefoperazone-sulbactam, ceftazidime-avibactam, ceftriaxone-sulbactam, -ceftolozane-tazobactam	MIA	-
5th generation cephalosporins	Ceftaroline, ceftobiprole	MIA	-
Siderophore cephalosporin	Cefiderocol		-
Fluorocycline	Eravacycline	MIA	-
Glycopeptides, and lipoglycopeptides	Dalbavancin, oritavancin, ramoplanin teicoplanin, telavancin, vancomycin	MIA	-
Glycylcyclines	Tigecycline	MIA	-
Ketolides	Telithromycin	MIA	-
Lipopeptides	Daptomycin	MIA	-
18 membered-ring macrolides	Fidaxomicin	MIA	-
Monobactams	Aztreonam, carumonam	MIA	-
Aminocoumarins	Novobiocin	Not-MIA	IA
Arsenicals	Roxarsone, nitarsone	Not-MIA	IA
Bicyclomycins	Bicozamycin	Not-MIA	IA
Orthosomycins	Bambermycin (flavomycin) flavophospholipol, moenomycin, avilamycin	Not-MIA	IA
Ionophores (including polyethers)	Laidlomycin, lasalocid, maduramicin monensin, narasin, salinomycin, semduramicin	Not-MIA	HIA
Quinoxalines	Carbadox, olaquindox	Not-MIA	IA

Amphenicols	Chloramphenicol, florfenicol thiamphenicol	HIA	CIA
Cephalosporins (1st, and 2nd Generation), and cephamycin	Cefacetrile, cefaclor, cefadroxil, cefalexin cefalonium, cefaloridine, cefalotin, cefamandole, cefapirin, cefatrizine, cefazedone, cefazolin, cefbuperazone, cefmetazole, cefminox, cefonicid, ceforanide, cefotetan, cefotiam, cefoxitin, cefprozil, cefradine, cefroxadine, ceftezole, cefuroxime, flomoxef, loracarbef	HIA	HIA
Lincosamides	Clindamycin, lincomycin, pirlimycin	HIA	HIA
Nitroimidazoles	Metronidazole, ornidazole, secnidazole, tinidazole	HIA	HIA
Penicillins (amidinopenicillins)	Mecillinam, pivmecillinam	HIA	CIA
Penicillins (aminopenicillins)	Amoxicillin, ampicillin, azidocillin, bacampicillin, epicillin, hetacillin, metampicillin, pivampicillin, sultamicillin, talampicillin, temocillin	HIA	CIA
Penicillins (aminopenicillins with betalactamase inhibitors)	Amoxicillin-clavulanic acid, ampicillin-sulbactam	HIA	CIA
Penicillins (anti-staphylococcal)	Cloxacillin, dicloxacillin, flucloxacillin, meticillin (methicillin), nafcillin, oxacillin	HIA	CIA
Penicillins (narrow spectrum)	Benzathine-benzylpenicillin, benethamine-benzylpenicillin benzylpenicillin (penicillin G), clometocillin, penamecillin, penethamate, hydriodide, pheneticillin	HIA	CIA
Streptogramins	Pristinamycin, quinupristin-dalfopristin, virginiamycin	HIA	IA
Sulfonamides, dihydrofolate reductase inhibitors, and combinations	Brodimoprim, formosulfathiazole, iclaprim, phthalylsulfathiazole, pyrimethamine, sulfadiazine, sulfadimethoxine, sulfadimidine, sulfafurazole (sulfisoxazole), sulfaisodimidine, sulfalene, sulfamazone sulfamerazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazi ne,	HIA	CIA

	sulfametomidine, sulfametoxydiazine, sulfametrole, sulfamoxole, sulphanilamide, sulfaperin, sulfaphenazole, sulfapyridine, sulfathiazole, sulfathiourea, tetroxoprim, trimethoprim		
Fusidane	Fusidic acid	HIA	IA
Tetracyclines	Chlortetracycline, clomocycline, demeclocycline, doxycycline, lymecycline, metacycline, minocycline, oxytetracycline, penimepicycline, rolitetracycline, sarecycline, tetracycline	HIA	CIA
Aminocyclitols	Spectinomycin	IA	CIA
Cyclic	Bacitracin	IA	HIA
Pleuromutilins	Lefamulin, retapamulin, tiamulin, valnemulin	IA	HIA
Aminoglycosides	Amikacin, apramycin, arbekacin, astromicin, bekanamycin, dibekacin, dihydrostreptomycin, framycetin, gentamicin, isepamicin, kanamycin, micronomicin, neomycin, netilmicin, paromomycin, ribostamycin, sisomicin, streptoduocin, streptomycin, tobramycin	CIA	CIA
Ansamycins	Rifabutin, rifampicin, rifamycin, rifapentine, rifaximin	CIA	HIA
Macrolides (14, 15, 16 membered-ring)	Azithromycin, cethromycin, clarithromycin, dirithromycin, erythromycin, flurithromycin, gamithromycin, josamycin, kitasamycin, midecamycin, miocamycin, oleandomycin, rokitamycin, roxithromycin, spiramycin, tildipirosin, tilmicosin, troleandomycin, tulathromycin, tylosin, tylvalosin	CIA	CIA
Cephalosporins (3rd, 4th generation)	Cefcapene, cefdinir, cefditoren, cefepime, cefetamet, cefixime, cefmenoxime, cefodizime, cefoperazone, cefoselis, cefotaxime, cefovecin, cefozopran, cefpiramide, cefpirome, cefpodoxime, cefsulodin, ceftazidime,	HPCIA	CIA

	ceftibuten, cefteram-pivoxil, ceftiofur, ceftizoxime, ceftolozane, ceftriaxone, latamoxef		
	Cefquinome	HPCIA	CIA
Quinolones	Besifloxacin, cinoxacin, ciprofloxacin, danofloxacin, delafloxacin, difloxacin, enoxacin, enrofloxacin, fleroxacin, flumequine, garenoxacin, gatifloxacin, gemifloxacin, grepafloxacin, ibafloxacin, lascufloxacin, levonadifloxacin, levofloxacin, lomefloxacin, marbofloxacin, moxifloxacin, nadifloxacin, nemonoxacin, norfloxacin, ofloxacin, orbifloxacin, ozenoxacin, pazufloxacin, pefloxacin, pipemidic acid, piromidic acid, pradofloxacin, prulifloxacin, rosoxacin, rufloxacin, sitafloxacin, sparfloxacin, temafloxacin, trovafloxacin	HPCIA	CIA
	Flumequin, Miloxacin, Nalidixic acid, Oxolinic acid	HPCIA	IA
Polymyxins	Colistin, polymyxin B	HPCIA	HIA
Phosphonic acid derivatives	Fosfomycin	HPCIA	HIA

Abbreviations: highest priority critically important antimicrobials -HPCIA, critically important antimicrobials -CIA, highly important antimicrobials -HIA, important antimicrobials -IA, medically important antimicrobials-MIA

The South African Veterinary Council (SAVC) enforces two Acts to regulate the use of antibiotics for animals in South Africa. The first is the Fertilizers, Farm Feeds, Agricultural Remedies, and Stock Remedies Act (Act 36 of 1947). The act regulates the use of over-the-counter antimicrobials which allows farmers to have direct access to antimicrobials, in places where there is no access to veterinary practices (Eagar & Naidoo, 2017). The second is the Medicines, and Related Substances Control Act (Act 101 of 1965), which requires veterinarians to produce a license to dispense veterinary medicine. Various antibiotics are registered in specific dosages in the form of injectable, water-soluble powders, feed premixes, tablets, enteral solutions, topical preparations, and intra-mammary suspensions for use in both food and companion animals. These are used to control infectious diseases both in food animals and companion animals (Eagar & Naidoo, 2017).

1.2.3. Antibiotic use in food animals

Antibiotics are intensively used in food-animal production systems to control the infectious disease. They are mainly used for prophylaxis (the use of antimicrobial agents in susceptible healthy animals for infectious disease prevention) (FAO, 2016). Examples include ionophores, and sulfonamides which are used to prevent coccidiosis mostly in poultry production systems (Thu et al., 2020). Antibiotics are also used for metaphylaxis, which is the administration of antimicrobial agents at known therapeutic doses to all animals in a group, including an individual animal that has exhibited infections, and those at risk of becoming infected (FAO, 2016).

Antibiotics are also used for non-medical purposes as growth promoters, and feed proficiency enhancers (Sobur et al., 2019). The use of antibiotics for non-medical purposes is related to economic gains such as meeting the increase in food demand (Thu et al., 2020). However, the use of growth promoters has been reported to interfere with gut microorganisms of the treated food animals and promote the dissemination of antibiotic-resistant bacteria, and residues to the environment in contact with humans presenting a threat to public health (FAO, 2016). Examples of growth promoters include ionophores, flavophospholipol (flavomycin), olaquindox, zinc bacitracin, and tylosin, but these are not of use in human medicine. However, there are numerous antibiotics used in food animals that are regarded as either MIA in human medicine, and these include macrolides, aminoglycosides, and third-

generation cephalosporins (Thu et al., 2020). Tetracycline and tylosin are registered growth promoters under the measures of Act 36 of 1947 in South Africa (NDoH, 2018).

In a study, undertaken in an intensive poultry production system in South Africa, 67% of the 266 *E. coli* isolates were MDR, and the *bla*$_{\text{CTX-M}}$, *sul*, *tetA*, and *tetB* resistance genes were detected in 19, 24, 39, and 36 of the *E. coli* isolates respectively. This study concluded that food animals (in poultry farms) carry, and can potentially transmit ABR genes (McIver et al., 2020).

The need to reduce the use of antimicrobials in food-producing animals was reviewed by the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA) In these reviews certain measures, such as the use of vaccines, probiotics, prebiotics, bacteriophages, and organic acids, and strategies to prevent the dissemination of infectious disease into farms without using antimicrobials were introduced. The European Union has implemented veterinary medicine regulations which include restrictions such as (I) the elimination of routine use of antimicrobial medicinal agents as growth promoters, (II) the elimination of antimicrobial medicinal agents use for prophylaxis except of use in individual animals at risk of infection and (III) use of antimicrobial medicinal agents for metaphylaxis only in the case of dissemination of infections in the absence of limited alternative measures (EFSA, 2017).

1.2.4. Antibiotic use and resistance in companion animals

Antibiotics are used as therapeutic and prophylactic agents in companion animals. There is, however, limited data on the use of antibiotics in companion animals in South Africa. It is, therefore, difficult to trace the antibiotic consumption trends, as these are usually inferred from antibiotic sales data which provides inadequate in-depth statistics on antibiotic consumption (NDoH, 2018). In the United Kingdom (UK) there are antimicrobial use and sales surveillance programs such as veterinary ABR, and the sales surveillance report (UK-VARSS, 2021). A study was carried out at the University of Liverpool, in which they, explored antibiotic prescription patterns in the UK for both dogs and cats. A total of 1,482,001 dogs and 765,312 cats from 300 veterinary practices that prescribed antibiotics between 2014, and 2020 formed the sample size. In 2020, 9.6% of dogs and 12% of cats had received prescribed antibiotics. Antibiotics prescribed included 54%, 13%, 11%, 7%, 6%, 3%, 1%, and 5% of amoxicillin with clavulanic acid, a first-generation cephalosporin, metronidazole, clindamycin, amoxicillin, fluoroquinolones, third-generation cephalosporins, and other (sulphonamides,

tetracyclines macrolides, aminoglycosides, and simple penicillins) respectively in dogs. For cats, antibiotic prescriptions included 44%, 33%, 11%, 5%, 2%, 2%, and 3% of a third-generation cephalosporins, amoxicillin with clavulanic acid, amoxicillin, clindamycin, fluoroquinolone, metronidazole, and other (tetracyclines, sulphonamides, macrolides, aminoglycosides, first/second-generation cephalosporins, simple penicillins, and combination products) respectively (UK-VARSS, 2021).

A study by Joosten et al. (2020), investigated antimicrobial use in companion animals that attended veterinary practices in Europe (Belgium, Italy, and the Netherlands) within one-year. Amoxicillin clavulanate (27%), amoxicillin (8%), cefovecin (8%), enrofloxacin (8%) and spiramycin metronidazole (7%) were the most prescribed antibiotics to companion animals (Joosten et al., 2020).

A study conducted by Buckland et al. (2016), investigated the use of antimicrobials on dogs and cats visiting companion animal veterinary practices, in the UK. The study revealed that antibiotics such as fluoroquinolones, macrolides, and third-generation cephalosporins were prescribed for companion animals. The quantity of antimicrobial agents used throughout the study period (two years) was 1,473, 910 kg for dogs and 58, 383 kg for cats. The study reported on the high usage of antimicrobial agents in companion animal practices (Buckland et al., 2016).

The emergence of MDR bacteria in companion animals has resulted in limited treatment options, resulting in the need to use human MIA. For example, in the case where the microbe is resistant to amoxicillin/clavulanate, sulphamethoxazole/trimethoprim, fluoroquinolones, and tetracycline, antibiotics such as carbapenems that are unlicensed for use in companion animals are prescribed with the potential of increasing AMR, and its transmission (Jessen et al., 2019). Like in humans, ABR in companion animals may result in severe infectious diseases that are hard to cure, and may result in treatment failure (Li et al., 2021). Some drug-resistant bacteria behave as opportunistic pathogens causing different types of infections, however, some only colonize healthy hosts, posing the risk that they silently transmit their drug-resistant bacteria to the community (EMA et al., 2015).

There are several examples of pathogenic bacteria that are resistant to antibiotics found in companion animals such as those reported in the study by Li et al. (2021), carried out in the Iberian Peninsula.

Antimicrobial resistance was investigated in bacteria isolated from cats (789 samples) and dogs (5,086 samples). The most common bacteria in companion animals (dogs and cats) included *Staphylococcus* spp., (31% in dogs, and 30% in cats), and *Streptococcus* spp. (19% in dogs and 17% in cats), *Pseudomonas* spp. (16% in dogs and 10% in cats), *E. coli* (8% in dogs and 5.6% in cats), and *Enterococcus* spp. (5.5% in dogs and 6.8% in cats) (Li et al., 2021), these bacteria are considered global priority pathogens by WHO (WHO, 2017).

A study by Constanca et al. (2017), characterized bacteria found in companion animals (dogs, cats, and horses) based on their risk of transmission to humans as direct microbiological hazards (bacteria that can disseminate from companion animals to humans, and cause zoonosis) and indirect micro-biological hazards (bacteria that harbour resistant genes that can be co-transmitted between companion animals and humans). Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) were grouped under direct hazards whilst, Extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales, carbapenem-resistant Gram-negative bacteria, colistin-resistant *E. coli*, and vancomycin-resistant Enterococci (VRE) pose an in-direct hazard (Constanca et al., 2017).

1.2.5. *E. coli* and its Drug-resistance in companion animals

E. coli is a Gram-negative Enterobacterium with the ability to colonise the gastrointestinal tract in animals and humans (Durrotul Faridah et al., 2020; Saputra et al., 2017). However, some *E. coli* strains are known as opportunistic pathogens causing infectious disease in companion animals. For example, *E. coli* is reported to be the most common causative agent of UTIs, bacterial pneumonia, granulomatous colitis, bronchitis, diarrhea in dogs, cats, and horses, pyometra in cats, and mastitis in horses

ABR is thought to be a health threat to companion animals which are considered reservoirs of antibiotic-resistant *E. coli*. However, there is limited data on the degree of carriage and dissemination of antibiotic-resistant *E. coli* and resistance genes between humans and companion animals in South Africa. Qekwana et al. (2018), conducted a study on AMR in *E. coli* isolates from dogs with UTIs treated at a veterinary teaching hospital in South Africa. Of the 755 urinary isolates, 168 (22.3%)

were identified as *E. coli*. Moreover, in 168 *E. coli* isolates, 99.4%, 100%, 95%, 84%, 70%,68%, and 63% were resistant to penicillin-G, clindamycin, tylosin, cephalothin, amoxicillin-clavulanic acid, doxycycline, and lincospectin respectively. The study highlighted a clinical and veterinary concern about the presence of MDR, extensively drug-resistant (XDR), and pan-drug resistance *E. coli* isolates in dogs (Qekwana et al., 2018).

In a study done in Mwanza, Tanzania, 600 *E. coli* were isolated from companion animals and domestic farm animals in 2014. Of these, 130 isolates were ESBL carriers, 51/130 ESBL isolates were from dogs, and 79/130 isolates were from domestic food animals including pigs, chickens, goats, and cattle. Of the 130 ESBL isolates, 87% (113), 61% (79), 32% (42), and 18% (24) were resistant to trimethoprim/sulphamethoxazole, tetracycline, ciprofloxacin and gentamicin, respectively. Twenty-five ESBL isolates were chosen for whole genome sequencing, and all 25 of the ESBL isolates carried $bla_{\text{CTX-M-15}}$ whilst 22 carried the aminoglycoside resistance genes including, *strA*, and *strB*, *aac(3)-IId* (6/25), *aac(3)-IIa(5/25)*,*aadA5* (9/25), *aadA1* (1/25), *aadA2* (1/25), and *aacA4* (1/25). The quinolone resistance genes included *aac (6)-Ib-cr* (12/25), and *qnrS1* (12/25). The study concluded that companion animals have high faecal carriage of $bla_{\text{CTX-M-15}}$, and hence, there's a need for more antimicrobial surveillance systems in veterinary and human medicine to delineate the transmission routes and potential reservoirs for ABR (Seni et al., 2016).

In another study carried out in Egypt, the distribution of antimicrobial genes in Shiga-toxigenic *E. coli* isolated from dogs with haemorrhagic diarrhoea was investigated. The *E. coli* isolates showed high resistance towards trimethoprim-sulfamethoxazole 100% (19), tetracycline 100% (19), amoxicillin 84.2% (16), amoxicillin-clavulanic acid 68.4% (13), ceftazidime 42.1% (8), cefotaxime 42.1% (8), and colistin sulfate 42.1% (8). *Tet*A and *sul*1genes were detected in all isolates, followed by bla_{TEM} (84.2%), $bla_{\text{CTX-M}}$ (42.1%), *tet*B (57.9%), *qnr*A (36.8%), and *qnr*S (10.5%). A total of six isolates (31.6%) were MDR and carried the bla_{TEM}, $bla_{\text{CTX-M}}$, *tet*A, *tet*B, and *sul*1 genes. This study was the first to report MDR-Shiga-toxin *E. coli* in dogs in Egypt (Algammal et al., 2022). The common outcome of these studies is that there is a need for regular surveillance systems for the emergence of MDR *E. coli* strains in companion animals that pose a health threat to animals and have the potential to be transmitted to humans.

In many cases, drug-resistant *E. coli* has been reported in companion animals, even the last-resort antibiotics such as colistin (Hamame et al., 2022a; Kumar et al., 2020). In one of the studies conducted by Hamame et al. (2022b), faecal samples were collected from dogs and cats to screen colistin-resistant bacteria. Three *E. coli* isolates from cats, and 10 from dogs were resistant to colistin and carried the *mcr-1* gene (Hamame et al., 2022b). As a result of the occurrence of mcr-*1* gene, SAVC has banned the use of colistin in animals (Eagar & Naidoo, 2017). MDR *E. coli* is commonly reported in humans, and companion animals.

1.2.6. HGT, antibiotic mode of action, and mechanisms of antibiotic-resistance

E. coli is amongst the genetically versatile microorganisms, allowing it to attain, and transmit ABR, and its determinants (Braz et al., 2020). *E. coli* can acquire ABR through HGT which involves three main gene transfer systems (**Figure 1A**). These include conjugation (the transfer of a plasmid, or any self-transmittable Deoxyribonucleic acid (DNA) element through direct contact by means of a sex pilus). Transduction (the transfer of DNA from bacterial donor to recipient cell through virus particles, or bacteriophage), and transformation, (the transfer of free DNA, in the form of plasmid, transposons, integrons, chromosomal DNA, from a donor cell into the extracellular environment, resulting in new expression of acquired characteristics in a recipient cell (Burmeister, 2015; Fouz et al., 2020).

As depicted in **figure 1(B**), antibiotics are subdivided into antibiotic groups based on their mode of action such as (1) cell wall synthesis inhibition (β-Lactams inhibit bacterial cell wall composed of peptidoglycan. However, some strains of *E. coli*, produce broad-spectrum β-lactamase enzymes (ESBL), AmpC β-Lactamases (AmpC) and carbapenemases, these have different hydrolytic activity against β-Lactam antibiotics, contributing to β-Lactamase resistance, (2) cell membrane depolarization (membrane structure) cell membrane, and macromolecular gets disrupted by daptomycin which acts by depolarizing calcium-dependent membrane (Etebu & Arikekpar, 2016), (3) protein synthesis inhibition (antibiotics such as tetracyclines, and aminoglycosides blocks the initiation phase by binding to 30S subunits, and antibiotics such as chloramphenicol interfere with elongation phase during protein synthesis by binding to the 50S ribosomal subunits (Etebu & Arikekpar, 2016)), (4) nucleic acid synthesis inhibition (quinolones disrupt the helicase enzymes which are responsible for unwinding DNA during transcription, resultantly the DNA replication, and

repair is disrupted. Quinolones also interfere with topoisomerase II, and topoisomerase IV of bacteria, affecting the ribonucleic acid (RNA) polymerase resulting in RNA synthesis prevention (Etebu & Arikekpar, 2016), and (5) metabolic pathway inhibition (folate mechanisms) (Sulphonamides and trimethoprim are some of the antibiotics that interfere with metabolic pathways, they are similar in structure to that of bacterial cell metabolism substrates such as tetrahydrofolate (Etebu & Arikekpar, 2016). During metabolic processes, enzymes bind to these antibiotics instead of the substrate. Metabolism of nucleic acid (DNA and RNA), and amino acid production requires folic acid, which is synthesized in the presence of tetrahydrofolate, therefore the sulphonamides interfere with nucleic acid and amino acid production by binding to enzymes (Reygaert, 2018).

Gram-negative bacteria such as *E. coli* can: (i) inactivate drugs such as aminoglycosides through drug degradation, and chemical group transfer, (ii) limit drug uptake such as β-Lactams, aminoglycoside, and tetracyclines by decreasing cell wall polarity, (iii) modify drug target such as DNA gyrase to obtain ABR towards fluoroquinolones, and (iv) *E. coli* have efflux on drugs to such as AcrAB-TolC pump resulting in resistance to chloramphenicol, macrolides, fluoroquinolones, tetracycline, and penicillin (**Figure 1(C)** (Reygaert, 2018).

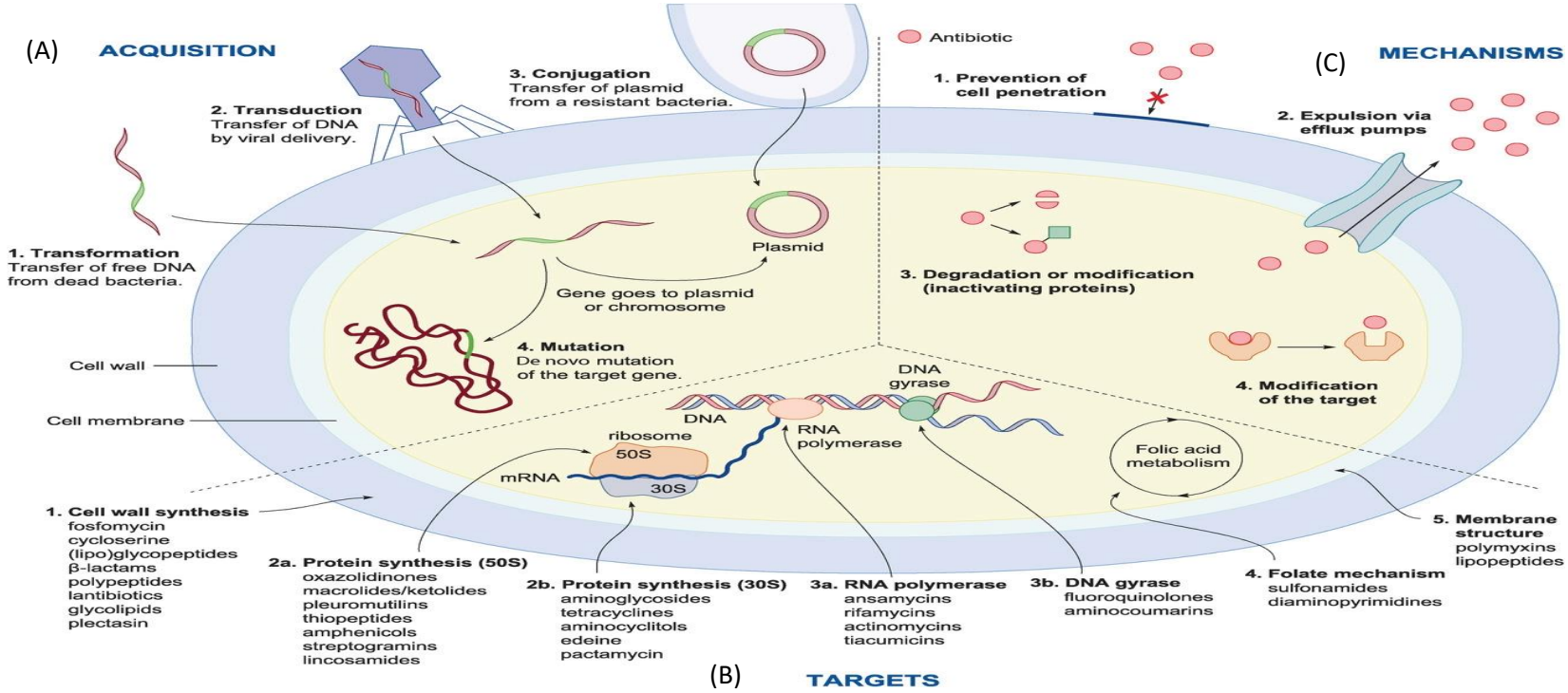


Figure 1: (**A**) HGT illustration (**B**) antibiotic mode of actions based on their targets in bacterial cells. (**C**) Mechanisms of antibiotic-resistance (Adapted from Riedl et al., 2016).

1.2.7. Antibiotic-resistant *E. coli* transmission

The co-existence of humans, and animals, results in infectious drug-resistant bacteria cross-transmission, hence, some human infections originate from animals (Rahman et al., 2020).

The amount of interaction between companion animals and humans provides several transmission routes for infectious bacteria. Cross-contamination could either be in-direct (contamination of food, and household environment), or direct (petting, biting, and licking) i.e., the spread of infectious bacteria to humans by handling contaminated food, contact with companion animal feed equipment, companion animal waste, and the contaminated household environment by companion animals (Hong et al., 2019).

Companion animals specifically, cats and dogs, have been reported to be potential reservoirs of pathogenic antibiotic-resistant *E. coli* (Hamame et al., 2022a). Extraintestinal pathogenic *E. coli* (ExPEC) strains cause infections in non-intestinal sites, i.e. urinary tract and bloodstream (Manges et al., 2019). They include uropathogenic *E. coli* (UPEC) strains that have evolved, or acquired virulence factors that allow them to persist in the urinary tract system (Chhaya et al., 2019). UPEC are common in humans, however, *E. coli* strains that resemble human UPEC strains have been identified in companion animals (Bourne et al., 2019). For example, the *E. coli* sequence type 131 (ST131) is the MDR UPEC responsible for extra-intestinal infections in humans and has also been isolated from companion animals. A study in Australia investigated dogs and cats as reservoirs of MDR EXPEC strains (ST131 and ST1193) over one year (2013 to 2014). Eleven of the ST131 isolates were retrieved from companion animals (nine from dogs and two from cats), of which six isolates were resistant to fluoroquinolones (FQs), it was then concluded that there are cases where human ExPEC strains are distributed in companion animals, suggesting that companion animals are reservoirs of drug-resistant *E. coli* (Kidsley et al., 2020).

Another study by Bourne et al. (2019), explored the genetic structure, AMR, and the prevalence of human-associated ExPEC sequence types in healthy dogs and cats in Canberra, Australia. This study discovered human ExPEC strains such as ST69 (8 in cats and 2 in dogs), ST73 (64 in cats and 8 in dogs), ST95 (24 in cats and 4 in dogs), ST127 (6 in cats and 5 in dogs), and ST131 (1 in cats and 2 in

dogs). This data is evidence that humans and companion animals share the same strains of pathogenic *E. coli*.

A study by Damborg et al. (2023) demonstrated the transmission of *E. coli* between dogs and their owners. The owner of the dog had a UTI, and the *E. coli* strain causing the UTI was isolated from the dog's faecal matter. This strain was also isolated in the same dog 10 months after the patient was diagnosed with the UTI, intimating that a dog was the carrier of the *E. coli* strain causing UTI (Damborg et al., 2023).

A study conducted by Schmiedel et al. (2014) in Germany, reported the dissemination of ESBL-producing MDR Enterobacterales strains between humans and companion animals. There were 513 Enterobacterales isolates composed of *E. coli* (74%), *K. pneumoniae* (17.5%), *Enterobacter cloacae* (4.2%), and 4.3% composed of *Klebsiella oxytoca*, *Enterobacter intermedius*, *Citrobacter freundii*, *Providencia stuartii*, *Morganella morganii* and *Proteus mirabilis*. Of these, a total of 361 isolates from both humans (183) and companion animals (178) were resistant to ampicillin and trimethoprim/sulfamethoxazole. All isolates carried at least one of the β-lactamase genes (bla_{TEM}, bla_{SHV}, $bla_{\text{OXA-1}}$, $bla_{\text{OXA-48}}$, and $bla_{\text{CTX-M}}$). The $bla_{\text{CTX-M-1}}$gene was carried by 23% of human isolates and 28.7% of companion animal isolates, and $bla_{\text{CTX-M-15}}$ was carried by 52.5% of the human isolates and 46.1% of companion animal isolates.

Tuerena et al. (2016) conducted a study associating antibiotic-resistant *E. coli* in hospitalised companion animals, and their veterinary hospital environments in Northwest, UK. In the study, faecal matter was obtained from hospitalised dogs and cats, and the hospital environment (ward floors; computer keyboards in kennel rooms and treatment areas; examination tables in treatment areas (not in consulting rooms) and the outside dog walking areas were sampled. Of the 333 faecal, and 257 environmental samples collected, 13% and 8.9% were found to have MDR *E. coli* respectively. Amoxicillin-resistant isolates were tested for $bla_{\text{CTX-M.}}$, while ceftazidime-resistant isolates were tested for bla_{TEM}, bla_{SHV}, and bla_{OXA} genes. All isolates showing quinolone resistance were tested for plasmid-mediated quinolone resistance genes (*qnrA*,*qnrB*, and *qnrS*). The $bla_{\text{CTX-M-15}}$ gene was the most dominant gene in both faecal (6%) and environmental samples (3%) followed by $bla_{\text{TEM-158}}$ detected in 3% faecal samples although it was less prevalent in environmental samples (0.3%). It was

concluded, based on the resistance profiles of faecal and environmental samples that there was cross-contamination between animals and the environment (Tuerena et al., 2016).

1.2.8. Rationale for the study

The rising incidence and prevalence of drug-resistant *E. coli* in companion animals, and the intense human-companion-animal interaction provide a potential ABR transmission route between humans and companion animals (Hong et al., 2019). Several ABR surveillance studies on companion animals have been conducted, and published internationally, however, in South Africa, there is still little information on the epidemiology of resistant *E. coli* in companion animals. It is, therefore, important to conduct surveillance studies and understand the molecular epidemiology of drug-resistant *E. coli* in companion animals.

The present study aimed to close the gap of information on drug-resistant *E. coli* in companion animals in South Africa. The study sought to provide the prevalence and clonality of drug-resistant *E. coli* retrieved from dogs and cats from different veterinary practices. The results will hopefully lead to the establishment of a surveillance system to monitor and control ABR in companion animals and within the human-animal-interaction phase in South Africa.

1.2.9. Aim

To elucidate the molecular epidemiology of antibiotic-resistant *E. coli* from companion animals presenting at veterinary practices in Durban, KwaZulu-Natal, South Africa.

1.2.10. Objectives

- To confirm the identity of *E. coli* isolates from rectal swab samples taken from companion animals using selective media, and real-time polymerase chain reaction (Real-time PCR).
- To determine the antibiotic susceptibility of *E. coli* against a selected antibiotic panel using the Kirby-Bauer disk diffusion test according to Clinical, and Laboratory Standards Institute (CLSI), and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, and further distinguish multidrug-resistance (MDR).
- To determine the prevalence of selected antibiotic-resistance genes (ARGs) by conventional PCR, and gene sequencing.

- To evaluate clonal similarities of *E. coli* by strain-typing using Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR).

1.2.11. Study outline

This study aimed to elucidate the molecular epidemiology of antibiotic-resistant *E. coli* from companion animals presenting at veterinary practices in Durban, KwaZulu-Natal, South Africa. The research is presented in three chapters as follows:

- **Chapter 1**: provides the background, literature review, rationale for the study as well as the aims, and objectives.
- **Chapter 2**: provides information about the investigations undertaken, and the findings presented in the form of a manuscript.
- **Chapter 3**: presents the conclusions, limitations, and recommendations for this study.

1.2.12. Summary of methodology

Ethical consideration: Ethical approval was obtained from the Animal Research Ethics Committee (Reference: AREC/007/019M), and the Biomedical Research Ethics Committee (Reference: BE135/19) of the University of KwaZulu-Natal (**Appendices 1**, and **2** respectively). Section 20A permission to conduct research on animals was obtained from the Department of Agriculture, Land Reform, and Rural Development (DALARRD) (**Appendix 3**). Permission to collect rectal swab samples was sought and granted by pet owners of companion animals attending veterinary practices in Durban, KwaZulu-Natal in South Africa **(Appendix 4).**

General methodology: *E. coli* isolates retrieved from rectal swabs collected from dogs and cats visiting veterinary practices across Durban, KwaZulu-Natal, South Africa constituted the study sample. *E. coli* isolates were confirmed by real-time PCR targeting the *uidA* gene. *E. coli* susceptibility profiles were determined using the Kirby-Bauer disk diffusion test according to Clinical and Laboratory Standards Institute (CLSI, 2020), and European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2022) guidelines for selected antibiotics. The presence of

antibiotic-resistance genes was detected using conventional-PCR and confirmed by gene sequencing. The clonal similarities of *E. coli* were evaluated by ERIC-PCR.

1.3. References

Algammal, A. M., Tarabili, R. M. El, Alfifi, K. J., Otaibi, A. S. Al, Hashem, M. E. A., Maghraby, M. M. El, & Mahmoud, A. E. (2022). Virulence determinant, and antimicrobial resistance traits of emerging MDR Shiga toxigenic *E. coli* in diarrheic dogs. *Applied Microbiology, and Biotechnology, Express*, *12*(34), 1–12.

Bourne, J. A., Chong, W. L., & Gordon, D. M. (2019). Genetic structure, antimicrobial resistance, and frequency of human-associated *Escherichia coli* sequence types among faecal isolates from healthy dogs and cats living in Canberra, Australia. *Public Library Of Science One*, *14*(3), 1–13.

Braz, V. S., Melchior, K., & Moreira, C. G. (2020). *Escherichia coli* as a multifaceted pathogenic, and versatile bacterium. *Frontiers in Cellular, and Infection Microbiology*, *10*(2020), 1–9.

Buck, and E. L., Neill, D. O., Summers, J., Mateus, A., Church, D., Redmond, L., & Brodbelt, D. (2016). Characterisation of antimicrobial usage in cats, and dogs attending UK primary care companion animal veterinary practices. *Veterinary Record*, *179*(19), 489–489.

Burmeister, A. R. (2015). Horizontal Gene Transfer. *Evolution, Medicine, and Public Health*, *2015*(1), 193–194.

Chhaya, Zs., Ratna, B., Bijay, B., Lok, B., Xu, X., & Mao, H. (2019). Virulence factors of uropathogenic *Escherichia coli* (UPEC), and correlation with antimicrobial resistance. *Bio Medical Central Microbiology*, *19*(204), 1–6.

Chipangura, J. K., Eagar, H., Kgoete, M., Abernethy, D., & Naidoo, V. (2017). An investigation of antimicrobial usage patterns by small animal veterinarians in South Africa. *Preventive Veterinary Medicine*, *136*(2017), 29–38.

Constanca, P., Duijkeren, E. Van, Mateus, A., Moreno, M. A., Pyo, S., Teale, C., Threlfall, E. J., Kunsagi, Z., Torren-edo, J., & Valle, C. (2017). Public health risk of antimicrobial resistance transfer from companion animals. *Journal of Antimicrobial Chemotherapy*, *72*(2016), 957–

968.

Damborg, P., Gumpert, H., Johansson, L., Jana, B., & Frimodt-Moller, N., and Guardabassi, L. (2023). Dogs as reservoirs of *Escherichia coli* strains causing urinary tract infection in Human Household Contacts. Antibiotics,12(1269), 1-10.

De Oliveira, D. M. P., Forde, B. M., Kidd, T. J., Harris, P. N. A., Schembri, M. A., Beatson, S. A., Paterson, D. L., & Walker, M. J. (2020). Antimicrobial resistance in ESKAPE pathogens. *Clinical Microbiology Reviews*, *33*(3), 1–49.

Durrotul Faridah, H., Kristiana Dewi, E., Helmi Effendi, M., & Plumeriastuti, H. (2020). A Review of antimicrobial resistance (AMR) of *Escherichia coli* on livestock, and animal products: public health importance. *Systematic Reviews in Pharmacy*, *11*(11), 1210–1218.

Eagar, H., & Naidoo, V. (2017). Veterinary antimicrobial stewardship in South Africa. *International Biology Review*, *1*(2), 1–14.

European Food Safety Authority (EFSA). (2017). *It ' s time to reduce, replace, and re-think the use of antimicrobials in animals EMA - EFSA joint opinion on EU measures to reduce antimicrobials use.* Parma Italy.

EMA, CVMP, & AWP. (2015). *Reflection paper on the risk of antimicrobial resistance transfer from companion animals*. http://www.fecava.org/sites/default/files/files/FECAVA%2520Recommendations%2520for%2520Appropriate%2520Antimicrobial%252. (Assessed July 2022).

Etebu, E., & Arikekpar, I. (2016). Antibiotics : Classification, and mechanisms of action with emphasis on molecular perspectives. *International Journal of Applied Microbiology, and Biotechnology Research*, *4*(2017), 90–101.

Food, and Agriculture Organization (FAO). (2016). *The FAO action plan on antimibrobial resistance 2016-2020*. United State of America.

Fouz, N., Pangesti, K. N. A., Yasir, M., Al-Malki, A. L., Azhar, E. I., Hill-Cawthorne, G. A., & El Ghany, M. A. (2020). The contribution of wastewater to the transmission of antimicrobial resistance in the environment: Implications of mass gathering settings. *Tropical Medicine, and Infectious Disease*. *5*(1). 1-8.

Goins, M., & Hanlon, A. J. (2021). Exotic pets in Irel, and: prevalence of ownership, and access to veterinary services. *Irish Veterinary Journal*, *74*(1), 1–7.

Gwenzi, W., Chaukura, N., Muisa-Zikali, N., Teta, C., Musvuugwa, T., Rzymski, P., & Abia, A. L. K. (2021). Insects, rodents, and pets as reservoirs, vectors, and sentinels of antimicrobial resistance. *Antibiotics*.10(1),1–42.

Hadeel, A., M, A. A., Alya, A., Thamer, A., Aseel, A., Daad, A., Aljohara, A., Aljohara, A., & Mushira, E. (2022). Multidrug-resistant, and extensively drug-resistant Enterobacteriaceae: prevalence, treatments, and outcomes – A retrospective cohort Study. *Infection, and Drug Resistance*, *13*(2020), 4653–4662.

Hamame, A., Davoust, B., Cherak, Z., Rolain, J., & Diene, S. M. (2022a). Mobile colistin resistance (*mcr*) genes in cats, and dogs, and their zoonotic transmission risks. *Pathogens*, *11*(698), 1–20.

Hamame, A., Davoust, B., Cherak, Z., Rolain, J., & Diene, S. M. (2022b). Screening of colistin-resistant bacteria in domestic pets from France. *Animals*, *12*(633), 1–10.

Hong, J. S., Song, W., Park, H., & Oh, J. (2019). Clonal spread of Extended-spectrum Enterobacteriaceae between companion animals, and humans in South Korea. *Frontiers in Cell, and Developmental Biology*, *10*(1371), 1–8.

Jessen, L. R., Damborg, P. P., Sorensen, T. M., Langhorn, R., Goericke-Pesch, S. K., Houser, G., Willesen, J., Schjærff, M., Eriksen, T., and V. F. J., & Guardabassi, L. (2019). Antibiotic use guidelines for companion animal practice (2nd edition). In . *The Danish Small Animal Veterinary Association, SvHKS, 2019,* (https://www.ddd.dk/sektioner/familiedyr/antibiotikavejledning/Documents/AB_uk_2019.pdf) (Accessed November 2020)

Joosten, P., Ceccarelli, D., Odent, E., Sarrazin, S., Gravel, and H., Van Gompel, L., Battisti, A., Caprioli, A., Franco, A., Wagenaar, J. A., Mevius, D., & Dewulf, J. (2020). Antimicrobial usage, and resistance in companion animals: A cross-sectional study in three European countries. *Antibiotics*, *9*(2), 1–17.

Kidsley, A. K., White, R. T., Beatson, S. A., Saputra, S., Schembri, M. A., Gordon, D., Johnson, J. R., Dea, M. O., Mollinger, J. L., Abraham, S., & Trott, D. J. (2020). Companion animals are spillover hosts of the multidrug-resistant human extraintestinal *Escherichia coli* p, andemic

clones ST131, and ST1193. *Frontiers in Microbiology*, *11*(2020), 1–10.

Kogan, L. R., Accornero, V. H., Gelb, E., & Slater, M. R. (2021). Community veterinary medicine programs : pet owners ' perceptions, and experiences. *Frontiers in Veterinary Science*, *8*(678595), 1–7.

Kumar, H., Chen, B., Kuca, K., Nepovimova, E., Kaushal, A., Nagraik, R., Bhatia, S. K., Dhanjal, D. S., & Kumar, V. (2020). Underst, anding of colistin usage in food animals, and available detection techniques : A review. *Animals*, *10*(1892), 1–19.

Li, Y., Fernández, R., Durán, I., Molina-lópez, R. A., Darwich, L., & Campo, R. Del. (2021). Antimicrobial resistance in bacteria isolated from cats, and dogs from the iberian Peninsula. *Frontiers in Microbiology*, *11*(621597), 1–12.

Manges, A. R., Geum, H. M., Guo, A., Edens, T. J., Fibke, C. D., & Pitout, J. D. D. (2019). Global extraintestinal pathogenic *Escherichia coli* (Expec) lineages. *Clinical Microbiology Reviews*, *32*(3), 1–25.

Massella, E., Reid, C. J., Cummins, M. L., Anantanawat, K., Zingali, T., Serraino, A., Piva, S., Giacometti, F., & Djordjevic, S. P. (2020). Snapshot study of whole genome sequences of *Escherichia coli* from healthy companion animals, livestock, wildlife, humans, and food in Italy. *Antibiotics*, *9*(782), 1–22.

McIver, K. S., Amoako, D. G., Abia, A. L. K., Bester, L. A., Chenia, H. Y., & Essack, S. Y. (2020). Molecular epidemiology of antibiotic-resistant *Escherichia coli* from farm-to-fork in intensive poultry production in Kwazulu-Natal, South Africa. *Antibiotics*, *9*(12), 1–16.

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

National Department of Health (NDoH). (2018). *Survaillance for resistance, and consumption of antibiotics in South Africa*. https://www.knowledgehub.org.za/elibrary/surveillance-antimicrobial-resistance-, and-consumption-antibiotics-south-africa#:~:text=Surveillance%20for%20Antimicrobial%20Resistance%20,and%20Consumption%20of%20Antibiotics,cultures%20for%20the%20ESKAPE1%20pathogens%20in%20the%20country. [Accessed

January 2023].

Qekwana, D. N., Phophi, L., Naidoo, V., Oguttu, J. W., & Odoi, A. (2018). Antimicrobial resistance among *Escherichia coli* isolates from dogs presented with urinary tract infections at a veterinary teaching hospital in South Africa. *Bio Medical Central Veterinary Research*, *14*(1), 1–6.

Rahman, M. T., Sobur, M. A., Islam, M. S., Ievy, S., Hossain, M. J., Zowalaty, M. E. E., Rahman, A. M. M. T., & Ashour, H. M. (2020). Zoonotic diseases: Etiology, impact, and control. *Microorganisms*, *8*(9), 1–34.

Reygaert, W. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *American Institute of Mathematical Science, Microbiology*, *4*(3), 482–501.

Riedl, R., Chellat, M. F., & Raguz, L. (2016). Targeting ABR. *Angewandte International Edition*, *55*(23), 6600–6626.

Saputra, S., Jordan, D., Mitchell, T., San, H., Abraham, R. J., Kidsley, A., Turnidge, J., Trott, D. J., & Abraham, S. (2017). Antimicrobial resistance in clinical *Escherichia coli* isolated from companion animals in Australia. *Veterinary Microbiology*, *211*(2017), 43–50.

Schmiedel, J., Falgenhauer, L., Domann, E., Bauerfeind, R., Prenger-Berninghoff, E., Imirzalioglu, C., & Chakraborty, T. (2014). Multi-resistant extended-spectrum β-lactamase-producing Enterobacteriaceae from humans, companion animals, and horses in central Hesse, Germany. *Bio Medical Central Microbiology*, *14*(187), 1–13.

Seni, J., Falgenhauer, L., Simeo, N., & Mirambo, M. M. (2016). Multiple ESBL-producing *Escherichia coli* sequence types carrying quinolone, and aminoglycoside resistance genes circulating in companion, and domestic farm animals in Mwanza, Tanzania, harbour commonly occurring plasmids. *Frontiers in Microbiology*, *7*(142), 1–8.

Shamsuddin, S., Akkawi, M. E., Zaidi, S. T. R., Ming, L. C., & Manan, M. M. (2016). Antimicrobial drug use in primary healthcare clinics: a retrospective evaluation. *International Journal of Infectious Diseases*, *52*(2016), 16–22.

Smith, P. W., Agbaje, M., LeRoux-Pullen, L., van Dyk, D., Debusho, L. K., Shittu, A., Sirdar, M. M., Fasanmi, O. G., Adebowale, O., & Fasina, F. O. (2019). Implication of the knowledge, and perceptions of veterinary students of antimicrobial resistance for future prescription of

antimicrobials in animal health, South Africa. *Journal of the South African Veterinary Association*, *90*(2019), 1–8.

Sobur, A., Al, A., Sabuj, M., Sarker, R., Rahman, A. M. M. T., & Kabir, S. M. L. (2019). Antibiotic-resistant *Escherichia coli*, and *Salmonella* spp . associated with dairy cattle, and farm environment having public health significance. *Veterinary Record*, *12*(7), 984–993.

Thu, T., Van, H., Yidana, Z., Smooker, P. M., & Coloe, P. J. (2020). Antibiotic use in food animals worldwide, with a focus on Africa : Pluses, and minuses. *Journal of Global Antimicrobial Resistance*, *20*(2020), 170–177.

Tuerena, I., Williams, N. J., Nuttall, T., & Pinchbeck, G. (2016). Antimicrobial-resistant *Escherichia col*i in hospitalised companion animals, and their hospital environment. *Journal of Small Animal Practice*, *57*(2016), 339–347.

United Kingdom-Veterinary Antimicrobial Resistance, and Sales Surveillance (UK-VARSS). (2021). *Veterinary ABR, and Sales Surveillance Report*. New Haw, Addlestone.

Ventola, C. (2015). The ABR crisis part 1: Causes, and Threats. *The ABR crisis*. 40(4), 277–283.

Weese, J. S., Blondeau, J. M., Boothe, D., Breitschwerdt, E. B., Guardabassi, L., Hillier, A., Lloyd, D. H., Papich, M. G., Rankin, S. C., Turnidge, J. D., & Sykes, J. E. (2011). Antimicrobial use guidelines for treatment of urinary tract disease in dogs and cats: Antimicrobial guidelines working group of the international society for companion animal infectious diseases. *Veterinary Medicine International*, *2011*(263768), 1–10.

World Health Organisation (WHO). (2015). G*lobal Action Plan on Antimicrobial Resistance.* Geneva, Switzerland.

World Health Organisation (WHO). (2017). *Global priority list of antibiotic resistant bacteria to guide research, discovery, and development new antibiotics*. Geneva, Switzerland.

World Health Organisation (WHO). (2018a). *Critically Important Antimicrobials for Human Medicine*. Geneva, Switzerland.

World Health Organisation (WHO). (2018b). *WHO report on surveillance of antibiotic consumption*. Geneva, Switzerland WHO. (2019). Geneva, Switzerland.

World Health Organisation (WHO). (2023). WHO Medically Important Antimicrobial list. In *World*

Health Organisation. Geneva, Switzerland.

World Organisation for Animal Health (WOAH). (2021). *WOAH list of antimicrobial agents of veterinary importance*. Paris, France.

CHAPTER TWO

This dissertation is in a manuscript format (as per the requirement of the College of Health Sciences at the University of KwaZulu-Natal) as follows:

Molecular Epidemiology of Antibiotic-Resistant *Escherichia coli* from Companion Animals attending Veterinary Practices in Durban, KwaZulu-Natal, South Africa

Nondumiso L. Ntuli, Akebe Luther King Abia, Joshua Mbanga, Sabiha Y. Essack

Contributions

- Ms Nondumiso Ntuli, as the principal investigator co-conceptualized the study, executed the laboratory work, and wrote the manuscript.
- Dr Joshua Mbanga, and Dr. Akebe Luther King Abia as co-supervisors, assisted with laboratory protocols, analysis of the study results, writing, and revision of the manuscript.
- Prof. Sabiha Y. Essack, as the principal supervisor, co-conceptualized the study, guided the literature review, and ethical clearance application, facilitated data collection, and analysis, and undertook a critical revision of the manuscript.

Molecular Epidemiology of Antibiotic-Resistant *Escherichia coli* from Companion Animals attending Veterinary Practices in Durban, KwaZulu-Natal, South Africa

N.L. Ntuli[1], A.L.K. Abia[1], J. Mbanga[1], S.Y. Essack[1]*

[1]Antimicrobial Research Unit, College of Health 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

Running title: Antibiotic-resistant *E. coli* from companion animals in Durban, South Africa.

ABSTRACT

Background: Companion animals are globally documented to harbour antibiotic-resistant *E. coli*. This study aimed to investigate the molecular epidemiology of antibiotic-resistant *E. coli* from companion animals presenting at veterinary practices in Durban, KwaZulu-Natal, South Africa. **Methods**: *E. coli* were isolated on selective media (Eosin methylene blue) from rectal swabs sampled from dogs and cats attending veterinary practices in Durban, KwaZulu-Natal, South Africa, and were confirmed using real-time polymerase chain reaction (PCR) of the *uidA* gene. Antibiotic susceptibility testing against 20 antibiotics was done using the Kirby-Bauer disk diffusion method. Selected antibiotic-resistance genes (ARGs) that confer resistance to third-generation cephalosporins (bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$), tetracycline (*tetA* and *tetB*), and tigecycline (*tet*X/X2, *tet*X3, and *tet*X4), were detected using conventional PCR. PCR amplicons were confirmed by DNA sequencing and bioinformatics analysis. Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) was carried out to determine the clonality of antibiotic-resistant *E. coli* (101 isolates). **Results**: A total of 330 *E. coli* isolates from dogs (234) and cats (96) formed the study sample. Overall resistance in dogs was high in tetracycline (25.2%), ampicillin (17.9%), trimethoprim-sulfamethoxazole (14.5%), and nalidixic acid (12.4%). Whilst lower resistance was observed in amikacin (0.4%) and piperacillin-tazobactam (0.4%). No resistance was observed in meropenem, imipenem, and ceftazidime. Overall resistance in cats was high in tetracycline (22.4%), ampicillin (20.8%), cephalexin (20.8%), cefoxitin (14.6%), cefotaxime (14.6%), amoxicillin-clavulanate (12.5%), trimethoprim-sulfamethoxazole (12.5%), and ceftriaxone (10.4%). Whilst lower resistance was observed in piperacillin-tazobactam (1.0%), and ceftazidime (1.0%). No resistance was observed in meropenem, imipenem, tigecycline, and amikacin. Thirty-five (10.6%) *E. coli* were multidrug-resistant (MDR) with twenty-two different phenotypic patterns. The most common MDR antibiogram was group A (resistance to AMP-AMC*-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT) exhibited by six isolates from both dogs (4), and cats (2). The $bla_{\text{CTX-M-15}}$ (8%) and *tetA* (24%) were the most prevalent resistance genes. The $bla_{\text{TEM-1}}$ was the second most prevalent β-lactamase gene. While the $bla_{\text{SHV,}}$ *tet*X/X2, *tet*X3, and *tet*X4 genes were not detected. Using a 75% similarity cut-off, forty-eight clusters with both dogs and cats were identified. The ERIC-PCR types depicted a variety of clusters within veterinary practices in Durban, indicating that a high diversity of *E. coli* is in circulation in Durban, South Africa. **Conclusion**: Companion animals are reservoirs of antibiotic-resistant *E. coli* and ARGs. However, there was no evidence of transmission of antibiotic-resistant *E.*

coli in Durban. Resistance of *E. coli* from companion animals to medically important antimicrobials for humans is of particular concern and requires measures to control the spread of antibiotic-resistant bacteria, and ARGs between companion animals, veterinary practice personnel, and owners.

Keywords: Antibiotic-resistance; antibiotic-resistance genes; *E. coli*; companion animals; veterinary practice; South Africa.

2. 1. Introduction

E. coli is a member of the Enterobacterales order (Darwich et al., 2021). It may exist as a commensal residing in the gastrointestinal tract of companion animals (Cui et al., 2022), or be pathogenic causing infections such as urinary tract infections (UTIs) (Nielsen et al., 2022). A wide range of antibiotics are used to treat *E. coli*-associated infections in companion animals including β-lactams, sulphonamides, chloramphenicols, and fluoroquinolones which are usually reserved as first-line antibiotics (Singleton et al., 2020). A major concern in veterinary medicine is the use of antibiotics that are categorised as medically important antibiotics in human medicine. These antibiotics include third-generation cephalosporins and tetracyclines (WHO, 2021). *E. coli* that is resistant to third-generation cephalosporins is generally suspected to produce extended-spectrum beta-lactamases (ESBL). ESBL-producing *E. coli* is recognised as a global priority pathogen, because of its frequent isolation. Therefore, it is regarded as an indicator microorganism for resistance development in Gram-negative bacteria (WHO, 2021).

There are approximately over 200 ESBL determinants, these include $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM} which are common in both companion animals and humans (Matos et al., 2023; Møller et al., 2016). Amongst these, the $bla_{\text{CTX-M-15}}$ variant has been widely documented in dogs and cats (Matos et al., 2023). β-Lactam-β-lactamase inhibitors (BLBLIs) such as clavulanic acid, and tazobactam, and non-B-Lactam-β-lactamase inhibitors (NBBLIs) such as avibactam (Duval & Grare, 2019) are known to hydrolyse $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM} (Lohani et al., 2019; Møller et al., 2016). Consequently, they are usually preferred for treating ESBL-mediated resistant *E. coli* infections in veterinary medicine (Ong et al., 2020; WHO, 2021). Serious infections associated with ESBL *E. coli* are managed using carbapenems in humans. Therefore, carbapenems are not recommended for use in companion animals (Møller et al., 2016). Besides β-lactams, tetracyclines are one of the common antibiotics used to treat *E. coli*-associated infections in companion animals. Tetracycline is a broad-spectrum antibiotic, however, its increasing resistance has resulted in its reduced use in humans in developed countries (Møller et al., 2016). However, in Nigeria tetracycline is still used in human medicine (Perewari et al., 2022).

Some of the known mechanisms that confer resistance to antibiotics include efflux pump activity, ribosomal protection, and enzymatic inactivation (Gholami-Ahangaran et al., 2021). The *tet*(A), *tet*(B), *tet*(C), *tet*(D), and *tet*(E) genes are among 40 *tet* genes that confer resistance to tetracyclines, and are reported to be associated with the efflux mechanism (Chang et al., 2015). These genes have been documented to be mobilizable among companion animals, the environment, and humans (Gholami-Ahangaran et al., 2021). The risk of transmission of antibiotic-resistant *E. coli*, and antibiotic-resistance genes (ARGs) between companion animals, and humans presents a challenge to infection management in veterinary medicine (Joosten et al., 2020). Damborg et al. (2023) reported that approximately 10% of patients with UTIs have shared an *E. coli* pathotype with their pet dogs (Damborg et al., 2023). Chang et al. (2015), suggested that analysis of bacterial antibiograms, and resistance genes provide important data for veterinary medicine in the management of bacteria responsible for persistent infections (Chang et al., 2015).

The rising incidence, and prevalence of drug-resistant *E. coli* in companion animals, and the intense human-companion-animal interaction provide a potential ABR transmission route between humans, and companion animals (Hong et al., 2019). Several ABR surveillance studies on companion animals have been conducted and published internationally.

A study that investigated the multi-drug resistant *E. coli* in dogs and cats by Teng at al. (2023), reported a high prevalence of MDR *E. coli* in dogs and cats including an MIA antibiotic such as carbapenem in China. However, in South Africa, there is still little information on the epidemiology of resistant *E. coli* in companion animals. For example, Qekwana et al. (2018) reported phenotypic characterisation of *E. coli* isolated from dogs only. This study, therefore, aimed to elucidate the molecular epidemiology of antibiotic-resistant *E. coli* from companion animals presenting at veterinary practices in Durban, KwaZulu-Natal, South Africa.

2.2 Methodology

2.2.1. Ethical considerations

Ethical approval was obtained from the Animal Research Ethics Committee (Reference: AREC/007/019M), the Biomedical Research Ethics Committee (Reference: BE135/19) of the University of KwaZulu-Natal (**Appendices 1**, and **2**), and Section 20A permission to conduct research

on animals was obtained from the Department of Agriculture, Land Reform, and Rural Development (DALARRD) (**Appendix 3**). Permission to collect rectal swab samples was sought and granted by pet owners attending veterinary practices in Durban, KwaZulu-Natal in South Africa (**Appendix 4**).

2.2.2. Study sample sites

Samples were collected at six veterinary practices, located in Durban, KwaZulu-Natal, South Africa in February 2020. The veterinary practices' names are withheld and therefore referred to as Vet A, Vet B, Vet C, Vet D, Vet E, and Vet F for non-disclosure purposes.

2.2.3. Sample collection

Rectal swabs were collected from non-medicated companion animals (44 dogs and 21 cats) by a practising veterinarian. One rectal swab was collected per each animal. The samples were labelled and transported on ice to the laboratory for analysis. The samples were processed on arrival in the laboratory, and were then stored in tryptone soy broth (TSB) (Sigma-Aldrich, Missouri, United States of America (USA) with 20% glycerol (Associated Chemical Enterprises, Johannesburg, South Africa) at -20℃.

2.2.4. Identification of *E. coli*

2.2.4.1. Phenotypic identification

Rectal swabs were cultured on Eosin methylene blue (EMB) agar (Sigma-Aldrich, Missouri, USA). Typical colonies exhibiting a metallic green sheen were further sub-cultured onto nutrient agar (Sigma-Aldrich, Missouri, USA) to obtain pure cultures. *E. coli* American type culture collection (ATCC) 25922 was used as a positive control. A maximum of ten pure cultures per sample were stored in tryptone soy broth (TSB) (Sigma-Aldrich, Missouri, USA) with 20% glycerol (Associated Chemical Enterprises, Johannesburg, South Africa). The samples were stored at -20°C until needed (Bourne et al., 2019).

2.2.4.2. Deoxyribonucleic acid (DNA) extraction

A heat lysis method was used for the extraction of DNA (Abrar et al., 2019). A loopful of bacterial culture was inoculated into 200 µL of sterile distilled water. The solution was vortexed and placed in a thermoblock for 15 minutes at 100 °C, followed by centrifugation at 13 000 rpm for 5 minutes. The supernatant containing the crude DNA was transferred to a sterile Eppendorf tube and was used for molecular confirmation of *E. coli* isolates. DNA to detect ARGs by conventional PCR was extracted using a bacterial genomic DNA purification kit (Tiangen Biotech Co. Ltd. Beijing, China) according to the manufacturer's guidelines (Abrar et al., 2019).

2.2.4.3. Genotypic confirmation of isolates

Molecular confirmation of *E. coli* was done using real-time PCR, targeting the *uidA* (β-D-glucuronidase) gene as described previously (Momtaz et al., 2013). The reactions were performed in a total volume of 10 µL consisting of 5 uL of Luna® universal qPCR master mix (New Engl, and Biolabs, Ipswich, MA, USA), 0.5 µL of each *uidA* primer (forward-AAAACGGCAAGAAAAAGCAG, and reverse-ACGCGTGGTTAACAGTCTTGCG) final concentration 0.5 µM (Inqaba Biotechnical Industries (Pty) Ltd. Pretoria, South Africa), 3 µL DNA, and 1 µL of nuclease-free water. Optimized thermal cycling conditions for *uidA* (β-D-glucuronidase) were an initial Uracil-DNA glycosylase (UDG) activation (50◦C for 2 min), Dual-LockTM polymerase activation (95 ◦C for 2 min), thirty-five 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. A high-resolution melting curve analysis was done on 65 ◦C to 95 ◦C continuous mode. After the final extension step, a melt curve was generated and analysed. All reactions were performed on a Quant Studio® 5 Real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). DNA from *E. coli* ATCC 25922 was used as a positive control, while the reaction mixture with no DNA (replaced with nuclease-free water) was used as a negative control.

2.2.5. Antibiotic Susceptibility Testing

Antibiotic susceptibility of *E. coli* was determined by Kirby–Bauer disk diffusion assay on Mueller Hinton Agar (Oxoid, Basingstoke, Hampshire, England), using a panel of twenty antibiotics. The results were interpreted according to the Clinical, and Laboratory Standards Institute (CLSI), and the

European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (CLSI, 2020; EUCAST, 2022). The antibiotic panel used for susceptibility testing is shown in **Table 1.** The inhibition zones were measured according to CLSI guidelines for all antibiotics except for cephalexin, and tigecycline which were measured according to EUCAST guidelines. *E. coli* ATCC 25922 was used as the control (CLSI, 2020; EUCAST, 2022).

2.2.6. Determination of Multidrug-Resistance (MDR)

E. coli isolates displaying resistance to ≥1 antibiotic in ≥ 3 antibiotic classes were assigned as MDR isolates (Kazemnia et al., 2014).

2.2.7. Detection of antibiotic-resistance genes

E. coli isolates that were resistant to antibiotics including third-generation cephalosporins, tetracycline, and tigecycline were further tested for the presence of ESBL (*bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$, and *bla*$_{\text{SHV}}$), tetracycline *(tetA*, and *tetB)*, and tigecycline (*tet*(X/X2), *tet*X3, and *tet*X4) resistance genes. Conventional PCR was used to detect antibiotic-resistance genes using the primers synthesized at Inqaba Biotechnical Industries (Pty) Ltd. Pretoria, South Africa (**Table S1).** Conventional PCR was conducted in a total volume of 25 µL, composed of 12.5 µL of Dream Taq Green PCR Master Mix (2X) (ThermoFisher Scientific Waltham, MA, USA), 0.5 µL each of 0.5 µM of forward, and reverse-primers, 8.5 µL nuclease-free water, and 3 µL of template DNA. The PCR was performed (for all resistance genes) following the optimised cycling conditions of initial activation at 95◦C for 5 min, 35 cycles of denaturation at 95 ◦C for 1 min, annealing at 55 ◦C for 30 sec, extension at 72 ◦C for 1 min, and a final extension at 72◦C for 5 min. All reactions were carried out using a BIO-RAD T100 Thermal Cycler (Thermo Fisher Scientific Waltham, MA, USA). Each PCR assay included a positive control (DNA of in-house isolates that were previously confirmed to have *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$, *tetA* and *tetB*), and negative template control (nuclease-free water was used to substitute DNA) as displayed in **Appendix 6**. The PCR products were electrophoresed at 100 V on a 1% agarose gel containing 5µl Ethidium Bromide for 45 min in a 1X Tris-acetate-EDTA (TAE) buffer (BioConcept Ltd., Basel, Switzerland). Gels were viewed using the Gel Doc™ a+ imaging system (Bio-Rad, Hercules, California, USA), and photographed.

2.2.8. Sequencing of amplicons, and bioinformatics analysis

PCR products from conventional PCR were sent to Inqaba Biotechnology Industries (Pty) Ltd (South Africa) for DNA sequencing using the Sanger sequencing method to identify ESBL genes (*bla*CTX-M (25), and *bla*TEM (27), and *tetA (10),* and *tetB (10)* genes (**Appendix 8**). Chromas (http://technelysium.com.au/wp/chromas/) were used for quality control of sequences including trimming, base calling, and retrieval of DNA sequences for sequence analysis. Sequence analysis was done using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) in the National Center for Biotechnology Information (NCBI) database.

2.2.9. ERIC-PCR

Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) was carried out to determine the clonality of *E. coli* isolates (101/330) that were non-susceptible to at least one antibiotic. DNA was extracted using a bacterial genomic DNA purification kit (Tiangen Biotech Co. Ltd. Beijing, China) according to the manufacturer's guidelines. Conventional PCR was conducted in a total volume of 20 µL, composed of 12.5 µL of Dream Taq Green PCR Master Mix (2X) (ThermoFisher Scientific Waltham, MA, USA), 0.5 µL each of 1 µM of ERIC-1 primer 5'-3'(ATGTAAGCTCCTGGGGATTCAC), and ERIC-2 primer 5'-3'(AAGTAAGTGACTGGGGTGAGCG), 3.5 µL nuclease-free water, and 3 µL of template DNA. The PCR cycling conditions used were as follows: initial denaturation at 95°C for 3 minutes followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 1 minute, extension at 65°C for 8 minutes, and a final extension at 65°C for 16 minutes (Ardakani et al., 2016). Amplicons were subjected to electrophoresis on a 1.5% agarose gel at 100 V for 45 minutes in 1 X Tris-acetate-EDTA (TAE) buffer (BioConcept Ltd., Basel, Switzerland). Quick-load® 1 kb DNA ladder (Biolabs, New England, Hertfordshire, UK) was used as the molecular weight standard marker. The gels were stained in 0.5 µg/ml ethidium bromide solution and visualized using the Gel Doc™ XR imaging system (Bio-Rad, Hercules, California, USA) (example displayed on **Appendix 7**). The resultant electrophoretic patterns were analysed using GelJ software version 2.3 (Department of Mathematics, and Computer Science of University of LaRioja, and Mysic, Logrono, Spain) using dice coefficient, and clustering analysis through unweight pair group with arithmetic averages (Unweighted Pair Group Method with Arithmetic Mean (UPGMA)) using 1% tolerance. A 75% similarity cut-off was used to determine clusters (Heras et al., 2015).

2.3. Results

2.3.1. Distribution of resuscitated *E. coli* isolates across veterinary practices

E. coli isolates were retrieved in all companion animals that participated in the study. A total of 352 presumptive *E. coli* isolates from different veterinary practices constituted the study sample. A total of 251 (71%) *E. coli* were isolated from dogs and 101 (29%) from cats (**Appendix 5**). Three hundred and thirty (94%) were confirmed as *E. coli* isolates by real time-PCR, 55.6% (234) from dogs, and 27.3% (96) from cats.

2.3.2. Antibiotic Susceptibility Testing

A total of 101 *E. coli* isolates were resistant to one or more antibiotics (**Appendix 9**). The highest ABR of *E. coli* isolates retrieved from companion animals was observed against tetracycline (24.2%), ampicillin (18.8%), trimethoprim-sulfamethoxazole (14.0%), and cephalexin (11.2%). The highest resistance was observed against tetracycline in both dogs (25.2%), and cats (21.9%). Amongst the cephalosporins, high resistance was observed against first-generation cephalosporins (11.2%) followed by third-generation cephalosporins (cefotaxime (7.6%), and ceftriaxone (6.7%)). Intermediate resistance was highest against ampicillin (11.1%) in *E. coli* isolated from dogs, whilst ceftazidime (8.3%), and nalidixic acid (8.3%) topped in cats. All *E. coli* isolates were susceptible to imipenem, and meropenem. A few *E. coli* isolates were susceptible dose-dependent (SDD) toward cefepime, suggesting that, using higher doses of cefepime would be effective, according to CLSI guidelines (**Table 2**). Tigecycline resistance was only observed in *E. coli* isolated from dogs (3%). A total of 35/330 (10.6%), *E. coli* isolates were MDR, consisting of 25/35 (71.4%) from dogs, and 10/35 (28.6%) from cats. Twenty-two different antibiograms were displayed by MDR isolates (**Table 3).** The most common MDR antibiogram was group A (resistance to AMP-AMC*-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT) exhibited by 6 isolates from both dogs (4), and cats (2).

2.3.3. Detection of antibiotic-resistant genes

ESBL (*bla*$_{CTX-M}$, *bla*$_{SHV}$, and *bla*$_{TEM}$), tetracycline (*tetA*, and *tetB*), and tigecycline (*tet*X/X2, *tet*X3, and *tet*X4) resistance genes were screened in *E. coli* isolates that were phenotypically-resistant, and intermediately-resistant to third generational cephalosporins, tetracyclines, and tigecycline. The most prevalent resistance gene observed in this study across all companion animals was *tetA* 24% (80). A total of 80/330 (24%) isolates were phenotypically not susceptible to tetracycline and carried either one, or both of *tet*A, and *tet*B resistance genes. There was concordance between the phenotypic resistance to tetracycline and the detection of the assayed tetracycline resistance genes. From the total *E. coli* isolates, 52 (16%) were found to harbour the *tet*B resistance gene; more *E. coli* isolates harbouring the *tetB* genes were from dogs 41 (18%) than cats 11 (11%). In addition, t*et*A, and *tet*B were carried by an intermediate-resistant *E. coli* isolate retrieved from cats.

Thirty-one (9.3%) isolates were non-susceptible to third-generation cephalosporins and had the corresponding ESBL genes. The *bla*$_{CTX-M-15}$ type gene was prevalent in all *E. coli* (25) isolates that tested positive for the presence of *bla*$_{CTX-M}$. The *bla*$_{TEM-1}$ (17) was the second most prevalent β-lactamase gene (**Table 4**). Cats (15%) were observed to prominently harbour ESBL resistance genes compared to dogs (5%). The *bla*$_{SHV,}$ along with tigecycline resistance genes (*tet*X/X2, *tet*X3, and *tet*X4) were not detected in all *E. coli* isolates.

2.3.5. Clonality of *E. coli* isolates from companion animals

The ERIC type and profiles of *E. coli* isolates from both dogs and cats are depicted in **Figure 1**. Using a 75% similarity cut-off, forty-eight clusters were identified. All clusters were unique amongst different veterinary practices. Cluster K was observed to be a major cluster, consisting of eight *E. coli* isolates retrieved from dogs that attended the same veterinary practice. However, some clusters were shared between dogs and cats of the same veterinary practice. For example, cluster L, Q, Z, AE, AF, AG, and AK. At 100% similarity cut-off, two *E. coli* isolates from dogs that attended the same veterinary practice, had the *tet*A, and *tet*B genes in common, and two other isolates from cats harboured *bla*$_{TEM-1}$.

2.4. Discussion

This study provides the context of antibiotic susceptibility of *E. coli* isolated from companion animals that attended veterinary practices in Durban, KwaZulu-Natal, South Africa. The companion animals used in this study were not subjected to drug therapy before sampling. A total number of 330 isolates were identified as *E. coli* (234 isolates from dogs and 96 isolates from cats). There was a generally high concordance between phenotypic, and genotypic results for both tetracycline, and third-generation cephalosporins investigated in this study. This suggested that the observed resistance towards third-generation cephalosporins, and tetracycline was directly linked to transmittable resistance genes, however, the expression of these resistance genes was not investigated in this study.

Thirty-one (9.3%) *E. coli* isolates that were resistant to one, or more of the third-generation cephalosporins, were assumed to be producers of ESBLs as suggested by Gholami-Ahangaran et al. (2021). The isolates were further observed to carry one, or more of the $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} genes. These results are lower compared to 51% (185/362) ESBLs obtained from dogs (120), and cats (65) in a study from India that investigated MDR, and $bla_{\text{CTX-M}}$ variants in ESBL-producing *E. coli* isolated from companion animals (Banerjee et al., 2022).

In this study, 25 third-generation cephalosporin non-susceptible *E. coli* harboured the $\text{bla}_{\text{CTX-M}}$ gene. All of $\text{bla}_{\text{CTX-M}}$ gene positive *E. coli* isolates were identified to be $\text{bla}_{\text{CTX-M -15}}$ type. These results are comparable to a study in Switzerland, which aimed to characterise ESBL-producing *E. coli*, identify ESBL, and virulence genes, and analyse phylogroups. The study observed $bla_{\text{CTX-M -15}}$ being the most prevalent gene in ESBL-producing *E. coli* (8/8) isolated from both dogs and cats that attended a clinic for small animals at the University of Zurich, Switzerland between 2010, and 2011, (Huber et al., 2013). This is evidence that the $\text{bla}_{\text{CTX-M-15}}$ remains a dominant resistance gene in companion animals. This was also observed in a study that investigated MDR *E. coli* in dogs from the USA which reported $bla_{\text{CTX-M-15}}$ 10/19 (553%) variant as one of the predominant genes amongst $bla_{\text{CTX-M}}$ genes in companion animals, (Ortiz et al., 2022).

Of the 25 *E. coli*, 21 isolates carried $bla_{\text{CTX-M}}$ type combined with bla_{TEM}. The combination of $bla_{\text{CTX-M-15,}}$ and $bla_{\text{TEM-1}}$ was highly prevalent compared to other co-carried ESBL genes. This study revealed

low *bla*$_{TEM}$ prevalence in *E. coli* (4%) retrieved from dogs (**Table 4**) compared to a study that investigated the virulence, and AMR determinants in dogs attending small animal clinics in Ismailia province, Egypt, which reported a much higher prevalence 16/19 (84.2%) of the *bla*$_{TEM}$ gene in *E. coli* isolated from dogs (Algammal et al., 2022). The *bla*$_{TEM}$ prevalence in cats (18%) in our study was low compared to *bla*$_{TEM}$ 6/12 (50%) detected from ESBL *E. coli* in a study that evaluated MDR, and ESBL-producing *E. coli* in cats that visited a veterinary clinic in the Eastern Province of Saudi Arabia (Fayez et al., 2023). In this study, molecular characterisation of ESBL-resistance genes showed a diversity of *bla*$_{TEM}$ variants including, *bla*$_{TEM-1}$, *bla*$_{TEM-166}$, and *bla*$_{TEM-249}$, which dominated both in dogs and cats (**Table 4)**. The total prevalence of *bla*$_{TEM}$ (8%) in both dogs and cats contrasts with *bla*$_{TEM}$ 3/5 (60%) observed in *E. coli* retrieved from companion animals that attended governmental, and private pet animals clinics in Damietta, and Dakahlia Governorates, Egypt (Enany et al., 2021). No *bla*$_{SHV}$ genes were detected in the investigated *E. coli* isolates (**Table 4**). In addition, similar results were obtained in a study that investigated the prevalence of ESBL-producing *E. coli* 0/219 from dogs and cats that visited Animal Hospitals in Changchun, Jilin Province in North-eastern China from 2015 to 2021 (Zhou et al., 2022).

A study by Salgando-caxito et al. (2021) suggested that the carriage of third-generation cephalosporin-resistant *E. coli* isolated from companion animals can last several months after antibiotic use (Salgado-caxito et al., 2021). There are no recent reports on antibiotic use in companion animals from South Africa, resultantly, it is difficult to link third-generation cephalosporin use to resistance.

None of the *E. coli* isolates from this study showed resistance toward carbapenems (**Table 2**). This was expected as carbapenems are not used in veterinary medicine (Moreira et al., 2022). These results are consistent with the 100% carbapenem susceptibility of *E. coli* obtained from dogs and cats that attended a Veterinary Teaching Hospital in Taipei, Taiwan. The study investigated ABR determinants of ESBL-producing *E. coli* (Huang et al., 2020).

In this study, the ratio of resistance towards quinolone: fluoroquinolone was approximately 2:1. For example, resistance to nalidixic acid 32 (9.7%) was more than twice as much as resistance to ciprofloxacin 14 (4.2%), (**Table 2**). Nalidixic acid (resistance), and ciprofloxacin (susceptible)

phenotype is common in veterinary medicine, for example, a study in Argentina that investigated, companion dogs as reservoirs of antibiotic resistant *E. coli*, showed that this phenotype was frequent in *E. coli* isolates (12/46) from companion dogs that visited a veterinary clinic, and had not received antibiotics three months before to sampling (Marchetti et al., 2021).

The 27 % of tetracycline non-susceptible *E. coli* isolates retrieved from dogs in this study is proportionately higher compared to 16% reported in *E. coli* (7709) isolated in dogs in Cornell University Animal Health Diagnostic Center between 2007, and 2020 in New York (Osman et al., 2022). Of the 81 *E. coli* isolates from both dogs (59), and cats (22) that showed non-susceptibility to tetracycline, 98.8% carried the *tet*A, and 64.2% had the *tet*B gene. These results are high, compared to those reported in a study from China where the prevalence in dogs and cats that visited Animal Hospitals in Changchun, Jilin Province in China was *tet*A 154/181 (85%), and *tet*B 39/181 (22%) respectively (Zhou et al., 2022). Also, *tet*A was observed to be more prevalent than *tet*B in companion animals, which was also observed in tetracycline-resistant *E. coli* recovered from dogs (*tet*A 21/25, and *tet*B 13/25) that visited a Veterinary Clinical Complex in India (Mustapha et al., 2020). Fifty-two isolates that were resistant to tetracycline co-carried the *tet*A and *tet*B genes (**Table 4**). One *E. coli* isolate that had intermediate resistance to tetracycline also co-carried *tet*A and *tet*B. The high prevalence of *tet*A and *tet*B in *E. coli* from companion animals may implicate active efflux as the possible mechanism of resistance to tetracycline in *E. coli* of animal origin (Zhou et al., 2022). In addition, one tetracycline-resistant *E. coli* isolate was negative for the *tet*A and *tet*B genes, implying that other resistance mechanisms could be involved in tetracycline resistance.

Seven *E. coli* isolates from dogs were resistant to tigecycline. This is alarming and may present a possible risk to public health considering that tigecycline is unauthorised for use in veterinary medicine along with other glycylcyclines (Sun et al., 2019). Tigecycline is a last-resort antibiotic in human medicine due to its broad-spectrum antimicrobial activity in both Gram-negative, and Gram-positive bacteria (Yulin et al., 2020; Zeng et al., 2021). Although phenotypic resistance was observed against tigecycline-resistant isolates, none of the essayed *tet*X genes were detected (**Table 4**). However, this could not be verified as we did not have positive controls for tigecycline resistance. Further work needs to be done on the isolates to confirm the mechanism of resistance.

E. coli isolates did not show any clonal relatedness between dogs and cats across the veterinary practices in Durban. In addition, ERIC-typing of *E. coli* isolates revealed a variety of *E. coli* clusters, some of which were shared by isolates from both dogs and cats. For example, cluster, AK, AD, AE, AF, AG, Z, L, and Q (**Figure 1**). These results show that there are no clones of *E. coli* in circulation within the veterinary practices in Durban, KwaZulu-Natal, South Africa. A study in Turkey, that characterized ESBL-producing *E. coli* isolated from dogs that attended Van/Turkey Metropolitan Municipality Animal Care, and Rehabilitation Center between 2020, and 2021, revealed a variety of clusters (11) amongst 50 *E. coli* isolates (Kaplan, and Gulaydin, 2023) which contrast our study as *E. coli* from dogs had same cluster for example cluster K, M, N, O, AD, AL, AM, AP, AQ, and AR (**Figure 1**). The results mostly showed that there is a diversity of *E. coli* isolates that contribute to the burden of ABR. Some of these closely related isolates are present, and possibly circulating in both dogs and cats. However, more resolute typing approaches, such as MLST, and whole-genome sequencing, are recommended for assessing the lineages of *E. coli* isolates retrieved from dogs and cats.

The co-carriage of antibiotic-resistant *E. coli* by domestic dogs and cats, serves as a potential source for humans to acquire resistant bacterium which can contribute to ARGs spread. Therefore, our findings call for further surveillance to determine whether companion animals are reservoirs of antibiotic-resistant *E. coli* within households. This can be achieved by frequent sampling, and molecular characterising of non-susceptible *E. coli* from populations of dogs and cats, in addition to companion animal owners. It is also important for companion animal owners to follow prudent antibiotic use protocols.

2.5. Conclusion

In this study, all ESBL isolates harboured $bla_{\text{CTX-M-15}}$. This suggests that there is a rapid transmission of $bla_{\text{CTX-M-15}}$ in Durban. The prevalence of antibiotic-resistant, and MDR *E. coli* strains observed in this study, portray companion animals as possible reservoirs of antibiotic-resistant *E. coli*, ESBLs, and tetracycline resistance genes. Moreover, based on the clonality of *E. coli* results, there was no evidence of the transmission of antibiotic-resistant bacteria, and ARGs between veterinary practices situated in Durban, KwaZulu-Natal, South Africa. This study demonstrated the importance of continuous examination of antibiograms in companion animals to periodically depict the trend of

ABR, ARGs, and MDR bacteria. In addition, the close relationship between humans, their dogs and cats reinforce the need for continuous ABR surveillance studies to address the risks of ABR transmission.

Author contributions: Co-conceptualised the study: SYE & NLN.

Performed the laboratory work: NLN.

Analysed the data: All vetting of the results: All. Wrote the paper: NLN.

Undertook critical revision of the manuscript: NLN, ALKA, JM, and SYE.

Acknowledgment: The research reported in this publication was funded by the South African Research Chairs Initiative (SARChI).

Disclaimer: Any opinion, finding, conclusion, or recommendation expressed in this material is that of the author(s).

General Disclaimer: Any opinions, findings, conclusions, or recommendations expressed in this material are those of the author(s), and do not necessarily reflect the views of the organisations, or agencies that provided support for the project. The funders had no role in the study design, nor the decision to submit the work for publication.

Transparency Declaration: Professor Sabiha Essack is the chairperson of the Global Respiratory Infection Partnership, and a member of the Global Hygiene Council, both sponsored by unrestricted educational grants from Reckitt (Pty.) Ltd.UK.

Table 1: Antibiotic panel for susceptibility testing

Antibiotic class	Antibiotic agents	Concentration (µ/g)
β-lactams	Ampicillin	10
	Penicillin-inhibitor combinations (amoxicillin-clavulanate)	20/10
	Penicillin-inhibitor combination (piperacillin-tazobactam)	30/6
Cephalosporins	Ceftriaxone	5
	Cefotaxime	10
	Cefepime	30
	Ceftazidime	10
	Cefoxitin	10
Carbapenems	Meropenem	10
	Imipenem	10
Macrolides	Azithromycin	15
Aminoglycosides	Gentamicin	10
	Amikacin	30
Tetracyclines	Tetracycline	30
Glycylcycline	Tigecycline	15
Amphenicols	Chloramphenicol	30
Sulphonamides	Trimethoprim-sulfamethoxazole	1.25/23.7
Fluoroquinolones	Ciprofloxacin	5
Quinolones	Nalidixic acid	20

Table 2: Prevalence of non-susceptible *E. coli* from dogs (234), and cats (96) attending veterinary practices in Durban, KwaZulu-Natal, South Africa.

	Susceptibility profile											
	Dogs (234)				**Cats (96)**				**Total companion animals (330)**			
	S	**SSD**	**I**	**R**	**S**	**SDD**	**I**	**R**	**S**	**SDD**	**I**	**R**
AMP	166(70,9)	-	26(11, 1)	42(17,9)	73(76,0)	-	3(3,1)	20(20,8)	239(72,4)	-	29(8,8	62(18,8)
AMC	203(86,6)	-	17(7,23)	14(6.0)	77(80,2)	-	7(7,3)	12(12,5)	280(84,8)	-	24(7,2)	26(7,9)
TZP	231(98,7)	-	2(0,9)	1(0,4)	95(99,0)	-	0(0)	1(1,0)	326(98,8)	-	2(0,6)	2(0,6)
LEX*	217(92,)	-	0(0)	17(7,)	76(79,2)	-	-	20(20,8)	293(88,8)	-	0(0)	37(11,2)
FOX	229(97,9)	-	0(0)	5(2,1)	81(84,4)	-	1(1,0)	14(14,6)	310(93,9)	-	1(0,3)	19(5,8)
CTX	221(94,4)	-	2(0,9)	11(5.0)	77(80,2)	-	5(5,2)	14(14,6)	298(90,3)	-	7(2,1)	25(7,6)
CRO	221(94,4)	-	1(0,4)	12(5,1)	79(82,3)	-	7(7,3)	10(10,4)	300(90,9)	-	8(2,4)	22(6,7)
CAZ	231(98,7)	-	3(1,3)	0(0)	87(90,6)	-	8(8,3)	1(1,0)	318(96,3)	-	11(3,33)	1(0,3)
FEP	225(96,2)	4(1,7)	0(0)	5(2,1)	93(96,9)	1(1,0)	0(0)	2(2,0)	318(96,4)	5(1,5)	0(0)	7(2,1)
IMP	234(100)	-	0(0)	0(0)	96(100)	-	0(0)	0(0)	330(100)	-	0(0)	0(0)
MEM	234(100)	-	0(0)	0(0)	96(100)	-	0(0)	0(0)	330(100)	-	0(0)	0(0)
NAL	193(82,5)	-	12(5,1)	29(12,4)	85(88,5)	-	8(8,3)	3(3,1)	278(84,2)	-	20(6,1)	32(9,7)
CIP	214(91,)	-	8(3,4)	12(5,1)	93(96,9)	-	1(1,0)	2(2,1)	307(93,0)	-	9(2,7)	14(4,2)
AMK	225(96,2)	-	8(3,4)	1(0,4)	96(100)	-	0(0)	0(0)	321(97,3)	-	8(2,4)	1(0,3)
GEN	222(94,9)	-	4(1,7)	8(3,4)	92(95,8)	-	2(2,0)	2(2,0)	314(95,2)	-	6(1,8)	10(3,0)
AZM	221(94,4)	-	0(0)	13(5, 6)	94(97,9)	-	0(0)	2(2,0)	315(95,5)	-	0(0)	15(5.0)

TET	175(74,8)	-	0(0)	59(25, 2)	74(77,1)	-	1(1,0)	21(21,8)	249(75,5)	-	1(0,3)	80(24,2)
TGC*	227(97,0)	-	0(0)	7(3.0)	96(100)	-	-	0(0)	323(97,9)	-	0(0)	7(2,1)
CHL	213(91,0)	-	7(3.0)	14(6.0)	92(95,8)	-	1(1,0)	3(3,1)	305(92,4)	-	8(2,4)	17(5,2)
SXT	200(85,5)	-	0(0)	34(14,5)	84(87,5)	-	0(0)	12(12,5)	284(86,1)	-	0(0)	46(14.0)

* The inhibition zones were measured according to EUCAST guidelines

Abbreviations: AMP-Ampicillin, AMC-Amoxicillin-clavulanate, TZP-Piperacillin-tazobactam, LEX-Cephalexin, FOX-Cefoxitin, CTX-Cefotaxime, CRO-Ceftriaxone, CAZ-Ceftazidime, FEP-Cefepime, NAL-Nalidixic acid, CIP-Ciprofloxacin, IMP-Imipenem, MEM-Meropenem, AMK-Amikacin, GEN-Gentamicin, AZM-Azithromycin, TET-Tetracycline, TGC-Tigecycline, CHL-Chloramphenicol, SXT-Trimethoprim-sulfamethoxazole, SDD- susceptible-dose dependent, S-Susceptibility, I-intermediate-resistance, R-Resistance.

Table 3: Multidrug-resistance profiles of *E. coli* isolated from companion animals from different veterinary practices in Durban, KwaZulu-Natal, South Africa.

Pattern	Antibiogram	Dogs (234)	Cats (96)	Total companion animals (330)
A	AMP-AMC*-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT	4	2	6
B	AMP-AMC-TZP*-LEX-CTX-CRO-NAL-CIP-GEN-TET-CHL-SXT	1		1
C	AMP-AMC*-LEX-CTX-CRO-FEP-NAL-CIP-GEN-TET-CHL-SXT	1		1
D	AMP-AMC-LEX-FOX-CRO-CAZ*-NAL*-CIP*-TET-SXT		1	1
E	AMP-AMC-LEX-FOX-CTX-CRO*-CAZ*-NAL*-TET-SXT		4	4
F	AMP-AMC-LEX-CTX-CRO-NAL-CIP-GEN-TET-CHL-SXT	1		1
G	AMP-AMC*-LEX-NAL-CIP-GEN-AZM-TET-CHL-SXT	1		1
H	AMP-AMC-TZP-LEX-FOX-CTX-CRO-CAZ*-TET-SXT	1		1
I	AMP-AMC-LEX-FOX-CTX-CRO-CAZ*-CIP*-TET-SXT	1		1
J	AMP-AMC-LEX-FOX-CTX*-CRO-CAZ*-TET-SXT	1	1	2
K	AMP-AMC-LEX-FOX-CTX-CAZ*-NAL*-TET-SXT		1	1
L	AMP-AMC-LEX-CTX-CRO-NAL-CIP-TET-SXT	2		2
M	AMP-AMC-LEX-FOX-CTX-CRO*-TET-SXT		1	1
N	AMP-NAL*-AMK*-AZM-TET-TGC-SXT	1		1
O	AMP-AMC*-NAL*-TET-SXT	1		1
P	NAL-CIP*-TET-CHL-SXT	3		3
Q	AMP-AMC*-TET-SXT	1		1
R	AMP-AMC-NAL-TET	1		1
S	AMP-NAL*-TGC-SXT	1		1

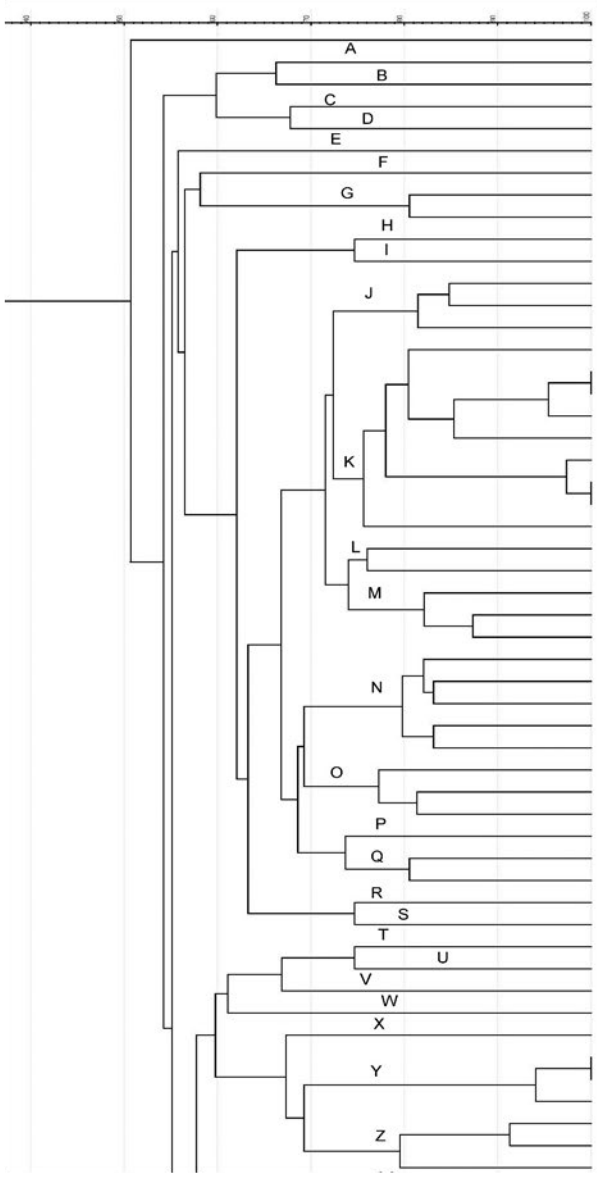
T	NAL*-TET-CHL-SXT	2		2	
U	AMP-NAL-CIP-TET	1		1	
V	NAL-AZM-TET	1		1	
	TOTAL NUMBER OF *E. COLI* ISOLATES	**25 (10.7)**	**10 (10.4)**		**35 (10.6)**

* Denotes intermediate resistance

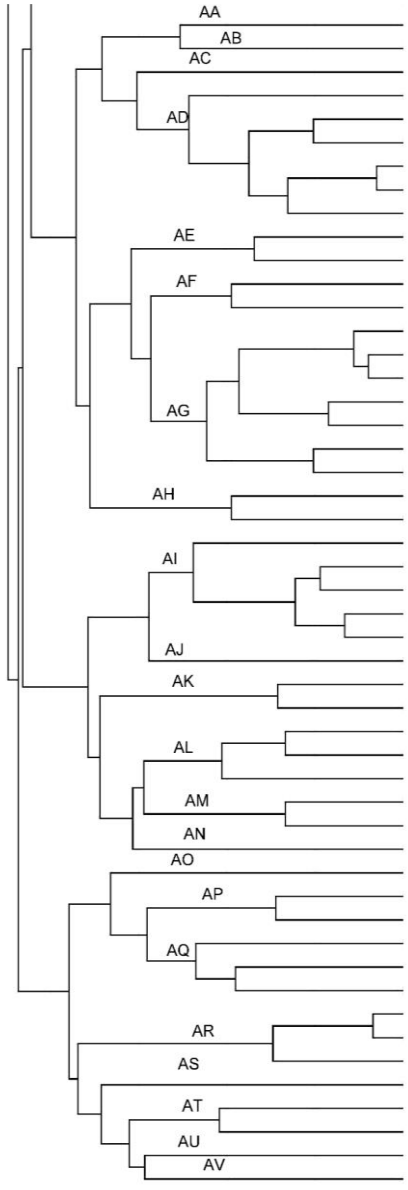
Abbreviations: AMP-Ampicillin, AMC-Amoxicillin-clavulanate, TZP-Piperacillin-tazobactam, LEX-Cephalexin, FOX-Cefoxitin, CTX-Cefotaxime, CRO-Ceftriaxone, CAZ-Ceftazidime, FEP-Cefepime, NAL-Nalidixic acid, CIP-Ciprofloxacin, AMK-Amikacin, GEN-Gentamicin, AZM-Azithromycin, TET-Tetracycline, TGC-Tigecycline, CHL-Chloramphenicol, SXT-Trimethoprim-sulfamethoxazol

Table 4: Distribution of resistance genes retrieved from *E. coli* isolates recovered from dogs and cats.

Resistance genes	**Companion animals n (%)**		**Total companion animals n (%)**
	Dog (234)	**Cat (96)**	**(330)**
*bla*CTX-M-15	11 (5%)	14 (15%)	25 (8%)
*bla*TEM-1	5 (2%)	12 (13%)	17 (5%)
*bla*TEM-166	3 (1%)	2 (2%)	5 (1.5%)
*bla*TEM-249	3 (1%)	2 (2%)	5 (1.5%)
*bla*SHV	0 (0)	0 (0)	0 (0)
tetA	59 (25%)	21 (24%)	80 (24%)
tetB	41(18%)	11(11%)	52 (16%)
tet(X/X2)	0 (0)	0 (0)	0 (0)
tetX3	0 (0)	0 (0)	0 (0)
tetX4	0 (0)	0 (0)	0 (0)



Key	Companion animal	Antibiogram	ARG's
ATCC 25922			
BD4.8	Dog	AMP-AMC-LEX-TET	tetA
RC3.9	Cat	AMP-AMC-LEX-FOX-CTX-CRO-CAZ*-NAL*-TET-SXT	TEM-1, tetA
VD2.5	Dog	AMP-NAL*-TGC-SXT	
VD1.4	Dog	TET-SXT	tetA, tetB
RD1.2	Dog	TET-CHL*	tetA
VD4.9	Dog	AMP-TET-SXT	tetA
VD2.9	Dog	NAL*-TET-CHL-SXT	tetA, tetB
VD2.7	Dog	NAL*-TET-CHL-SXT	tetA
WD8.1	Dog	AZM-TET	
WD13.10	Dog	AMP*-AMK*	
WD10.8	Dog	NAL-TET	tetA, tetB
WD10.6	Dog	NAL-TET	tetA, tetB
WD8.7	Dog	TET	tetA, tetB
WD15.6	Dog	AMP-NAL-CIP*-TET-CHL-SXT	tetA
WD10.5	Dog	NAL-TET	tetA, tetB
WD10.4	Dog	NAL-AZM-TET	tetA, tetB
WD10.3	Dog	AMP-AMC-NAL-TET	tetA, tetB
WD10.2	Dog	NAL-TET	tetA, tetB
WD8.8	Dog	AMP-AMC*-TET	tetA, tetB
WD8.6	Dog	TET	tetA, tetB
WD8.3	Dog	AMK*-TET	tetA, tetB
WD15.5	Dog	AMP	
WC2.8	Cat	AMP-AMC*-TZP-LEX-CTX*	CTX-M-15, TEM-166
WD4.9	Dog	TET	tetA, tetB
WD5.5	Dog	AMK*-AZM-TET	tetA, tetB
WD13.3	Dog	AMP*	
WD13.2	Dog	AMP*	
WD15.10	Dog	AMK*-TET	tetA
WD14.10	Dog	AMP-NAL*-AMK*-AZM-TET-TGC-SXT	tetA, tetB
WD16.7	Dog	AMP*-NAL*-CHL*	
WD1.9	Dog	AMP-GEN*	
WD4.4	Dog	TET	tetA, tetB
WD4.6	Dog	AZM-TET-TGC	tetA
WD10.10	Dog	NAL-TET	tetA
WC2.5	Cat	AMP*-NAL*-CHL	
WC1.10	Cat	TET	tetA, tetB
WD1.6	Dog	AMP	
WC2.6	Cat	AMP*	
WD14.1	Dog	AMP-TGC	
VD1.2	Dog	TET	tetA, tetB
UD4.8	Dog	AMP	
UD3.4	Dog	AMP*-NAL	
NC4.9	Cat	AMP-LEX-FOX*-CRO*-SXT	CTX-M-15, TEM-1
NC4.2	Cat	AMP-AMC-LEX-FOX-CTX	CTX-M-15, TEM-1
ND1.6	Dog	NAL*-CHL*-SXT*	
NC4.8	Cat	AMP-AMC-LEX-FOX-CRO*	TEM-1
NC4.7	Cat	AMP-AMC-LEX-FOX-CTX*-CRO-CAZ*	TEM-1
NC4.5	Cat	AMP-AMC-CTX-CRO*	CTX-M-15
NC4.4	Cat	AMP-AMC*-LEX-FOX-CTX-CRO*	CTX-M-15, TEM-1
NC4.3	Cat	AMP-AMC*-LEX-FOX-CTX-CRO*-CAZ*	CTX-M-15, TEM-1
ND3.4	Dog	AMP-AMC-FOX-CTX*	CTX-M-15, TEM-1



BD4.9	Dog	AMP-NAL-CIP-TET	tetA
BD6.9	Dog	AMP-AMC-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT	CTX-M-15, tetA, tetB
BC2.5	Cat	TET	tetA, tetB
BD6.8	Dog	AMP-AMC-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT	CTX-M-15, TEM-1,tetA, tetB
BD6.10	Dog	AMP-AMC-LEX-CTX-CRO-NAL-CIP-GEN-TET-CHL-SXT	CTX-M-15, tetA, tetB
BD6.6	Dog	AMP-AMC-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT	CTX-M-15, TEM-166, tetA, tetB
BC1.2	Cat	AMP-AMC-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT	CTX-M-15, TEM-1, tetA, tetB
BC1.1	Cat	AMP-AMC*-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT	CTX-M-15, TEM-166, tetA, tetB
BD6.1	Dog	AMP-AMC*-LEX-CTX-CRO-FEP-NAL-CIP-GEN-TET-CHL-SXT	CTX-M-15, TEM-1, tetA, tetB
BC3.6	Cat	TET	tetA, tetB
BD6.7	Dog	AMP-AMC*-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT	CTX-M-15, TEM-1, tetA, tetB
BC3.1	Cat	TET	tetA
BD5.7	Dog	AMP*-NAL*-CIP*-TET-CHL*-SXT	tetA
BC3.7	Cat	TET	tetA, tetB
BC4.2	Cat	TET	tetA
BC3.4	Cat	TET	tetA, tetB
BC4.7	Cat	TET*	tetA, tetB
BC3.3	Cat	TET	tetA, tetB
BC3.2	Cat	TET	tetA, tetB
BD5.3	Dog	AMP-AMC-TZP*-LEX-CTX-CRO-NAL-CIP-GEN-TET-CHL-SXT	CTX-M-15, TEM-166, tetA, tetB
BD3.6	Dog	AMP-AMC-LEX-CTX-CRO-NAL-CIP-TET-SXT	CTX-M-15, TEM-249, tetA
BD3.1	Dog	AMP-AMC-LEX-CTX-CRO-NAL-CIP-TET-SXT	CTX-M-15, TEM-249, tetA
RC3.4	Cat	AMP-AMC-LEX-FOX-CTX-CRO-CAZ-NAL*-TET-SXT	CTX-M-15, TEM-249, tetA
RC3.7	Cat	AMP-AMC-LEX-FOX-CTX-CAZ*-NAL*-TET-SXT	CTX-M-15, TEM-249, tetA
RC3.6	Cat	AMP-AMC-LEX-FOX-CTX-CRO*-TET-SXT	TEM-166, tetA
RC3.5	Cat	AMP-AMC-LEX-FOX-CTX-CRO*-CAZ*-NAL*-TET-SXT	CTX-M-15, TEM-1, tetA
RC3.3	Cat	AMP-AMC-LEX-FOX-CTX-CRO-CAZ*-NAL*-TET-SXT	CTX-M-15, TEM-1, tetA
RC3.2	Cat	AMP-AMC-LEX-FOX-CRO-CAZ*-NAL*-CIP*-TET-SXT	CTX-M-15, TEM-1, tetA
RC3.1	Cat	AMP-AMC-LEX-FOX-CTX-CRO-CAZ*-TET-SXT	CTX-M-15, TEM-1, tetA
RD6.10	Dog	AMP-AMC-TZP-LEX-FOX-CTX-CRO-CAZ*-TET-SXT	CTX-M-15, TEM-166, tetA, tetB
RD6.1	Dog	AMP-AMC-FOX-CRO*	
RD5.8	Dog	AMP*	
RD1.3	Dog	AMP*	
RD3.10	Dog	AMP*	
RD6.2	Dog	AMP-AMC-LEX-FOX-CTX*-CRO-CAZ*-TET-SXT	TEM-249, tetA, tetB
RD1.6	Dog	TET	tetA, tetB
UD4.4	Dog	AMC*	
UD3.10	Dog	AMC*-NAL	
UD3.8	Dog	AMP*-NAL	
UD4.2	Dog	TET	tetA
UD3.1	Dog	AMP*-NAL	
UD4.7	Dog	AMP-AMC*-TGC	
VD1.9	Dog	TET-SXT	tetA, tetB
VD1.8	Dog	TET-SXT	tetA, tetB
VD1.3	Dog	TET	tetA, tetB
RC1.3	Cat	TET	tetA
VD2.3	Dog	AMP-AMC*-TET-SXT	tetA
VD1.7	Dog	AMP*-TET-SXT	tetA, tetB
RC4.2	Cat	NAL*-TET	tetA
VD1.1	Dog	TET-SXT	tetA, tetB

Figure 1: Dendrogram displaying ERIC-PCR genotype of *E. coli* recovered from dogs and cats.

* A-AU: ERIC types of *E. coli* isolates based on a 75% similarity index. *E. coli* ATCC 25922 was used as a quality control isolate.

Abbreviations: AMP-Ampicillin, AMC-Amoxicillin-clavulanate, TZP-Piperacillin-tazobactam, LEX-Cephalexin, FOX-Cefoxitin, CTX-Cefotaxime, CRO-Ceftriaxone, CAZ-Ceftazidime, FEP-Cefepime, NAL-Nalidixic acid, CIP-Ciprofloxacin, AMK-Amikacin, GEN-Gentamicin, AZM-Azithromycin, TET-Tetracycline, TGC-Tigecycline, CHL-Chloramphenicol.

2.6. References

Abrar, S., Ain, N. U., Liaqat, H., Hussain, S., Rasheed, F., & Riaz, S. (2019). Distribution of *bla* $_{CTX-M}$, bla_{TEM}, *bla*$_{SHV}$, and *bla*$_{OXA}$ genes in Extended-spectrum-β-lactamase-producing clinical isolates: A three-year multi-center study from Lahore, Pakistan. *Antimicrobial Resistance, and Infection Control*, *8*(80), 1–10.

Algammal, A. M., Tarabili, R. M. El, Alfifi, K. J., Otaibi, A. S. Al, Hashem, M. E. A., Maghraby, M. M. El, & Mahmoud, A. E. (2022). Virulence determinant, and antimicrobial resistance traits of emerging MDR Shiga toxigenic *E. coli* in diarrheic dogs. *Applied Microbiology, and Biotechnology, Express*, *12*(34), 1–12.

Ardakani, M. A., Ranjbar, R., Branch, S., & Biology, M. (2016). Molecular typing of uropathogenic *E. coli* strains by the ERIC-PCR method. *Electronic Physician*, *8*(4), 2291–2296.

Banerjee, A., Pal, S., Goswami, P., Batabyal, K., B, andyopadhyay, S., & Samanta, I. (2022). Docking analysis of circulating CTX-M variants in multidrug-resistant, beta-lactamase, and biofilm-producing *E. coli* isolated from pet animals, and backyard livestock. *Microbial Pathogenesis*, *170*(2022), 1–9.

Bourne, J. A., Chong, W. L., & Gordon, D. M. (2019). Genetic structure, antimicrobial resistance, and frequency of human associated *Escherichia col*i sequence types among faecal isolates from healthy dogs and cats living in Canberra, Australia. *Public Library Of Science One*, *14*(3), 1–13.

Chang, S. K., Lo, D. Y., Wei, H. W., & Kuo, H. C. (2015). Antimicrobial resistance of *Escherichia coli* isolates from canine urinary tract infections. *Journal of Veterinary Medical Science*, *77*(1), 59–65.

Clinical, and Laboratory Standards Institute (CLSI). (2020). *Performance standards for antimicrobial susceptibility testing M100*. 27th Ed. Wayne, Pennsylvania.

Cui, L., Zhao, X., Li, R., Han, Y., Hao, G., Wang, G., & Sun, S. (2022). Companion animals as potential reservoirs of antibiotic-resistant diarrheagenic *Escherichia coli* in Shandong, China. *Antibiotics*, *11*(6), 1–14.

Damborg, P., Pirolo, M., Poulsen, L. S., Frimodt-møller, N., & Guardabassi, L. (2023). Dogs can be reservoirs of *Escherichia coli* strains causing urinary tractinfection in human household contacts. *Antibiotics*, *12*(1269), 1–10.

Darwich, L., Seminati, C., Burballa, A., Nieto, A., Durán, I., Tarradas, N., & Molina-López, R. A. (2021). Antimicrobial susceptibility of bacterial isolates from urinary tract infections in companion animals in Spain. *Veterinary Record*, *188*(9), 1–8.

Duval, R. E., & Grare, M. (2019). Fight against antimicrobial resistance : we always need new antibacterials but for the right bacteria. *Molecules*, *24*(3152), 1–9.

Enany., M., Wahdan., A., Marwa., E., & Wafaa., M. (2021). Bacterial causes of hemorrhagic gastroenteritis in dogs and cats with detection of some virulence, and β - lactamase resistance genes in *Escherichia coli*, and *Salmonella* by multiplex PCR. *Suez Canal Veterinary Medical Journal*, *1*(2021), 31–59.

European Committee on Antimicrobial Susceptibility Testing (EUCAST). (2022). Breakpoint tables for interpretation of MICs, and zone diameters. In *The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs, and zone diameters. Version 12.0, 2022.* http://www.eucast.org.

Fayez, M., Elmoslemany, A., Romaihi, A. A. Al, Azzawi, A. Y., Almubarak, A., & Elsohaby, I. (2023). Prevalence, and risk factors associated with multidrug resistance, and Extended-spectrum β -lactamase producing *E . coli* isolated from healthy, and diseased cats. *Antibiotics*, *12*(229), 1-14.

Fu, Y., Liu, D., Song, H., Liu, Z., Jiang, H., & Wang, Y. (2020). Development of a Multiplex Real-Time PCR assay for rapid detection of tigecycline resistance gene *tet* (X) Variants from. *Antimicrobial Agents, and Chemotherapy*, *64*(4), 3–8.

Gholami-Ahangaran, M., Karimi-Dehkordi, M., Miranzadeh-Mahabadi, E., & Ahmadi-Dastgerdi, A. (2021). The frequency of tetracycline resistance genes in *Escherichia coli* strains isolated from healthy, and diarrheic pet birds. *Iranian Journal of Veterinary Research*, *22*(4), 337–341.

Heras, J., Domínguez, C., Mata, E., Pascual, V., Lozano, C., Torres, C., & Zarazaga, M. (2015).

GelJ - a tool for analyzing DNA fingerprint gel images. *Bio Medical Central Bioinformatics*, *16*(1), 1–8.

Hong, J. S., Song, W., Park, H., & Oh, J. (2019). Clonal spread of Extended-spectrum Enterobacteriaceae between companion animals, and humans in South Korea. *Frontiers in Cell, and Developmental Biology*, *10*(1371), 1–8.

Huang, Y. H., Kuan, N. L., & Yeh, K. S. (2020). Characteristics of Extended-Spectrum β-Lactamase–producing *Escherichia coli* From dogs and cats admitted to a Veterinary Teaching Hospital in Taipei, Taiwan from 2014 to 2017. *Frontiers in Veterinary Science*, *7*(2020), 1–9.

Huber, H., Zweifel, C., Wittenbrink, M. M., & Stephan, R. (2013). ESBL-producing uropathogenic *Escherichia col*i isolated from dogs and cats in Switzerl, and. *Veterinary Microbiology*, *2(162)*, 992–996.

Joosten, P., Ceccarelli, D., Odent, E., Sarrazin, S., Gravel, and H., Van Gompel, L., Battisti, A., Caprioli, A., Franco, A., Wagenaar, J. A., Mevius, D., & Dewulf, J. (2020). Antimicrobial usage, and resistance in companion animals: A cross-sectional study in three European countries. *Antibiotics*, *9*(2), 1–17.

Kaplan, B.,1 & Gulaydin, O. (2023). Characterization of extended-spectrum β-lactamase producing *Escherichia coli* strains isolated from the urogenital system of dogs in Van province of Turkey *Iranian Journal of Veterinary Research.* 24(82), 22-29.

Kazemnia, A., Ahmadi, M., & Dilmaghani, M. (2014). ABR Pattern of Different *Escherichia coli* phylogenetic groups isolated from human urinary tract infection, and avian Colibacillosis. *Iranian Biomedical Journal*, *18*(4), 219–224.

Lohani, B., Thapa, M., Sharma, L., Adhikari, H., Sah, A. K., Khanal, A. B., Basnet, R. B., & Aryal, M. (2019). Predominance of CTX-M Type Extended spectrum β-lactamase (ESBL) producers among clinical isolates of Enterobacteriaceae in a tertiary care hospital, Kathmandu, Nepal. *The Open Microbiology Journal*, *13*(1), 28–33.

Marchetti, L., Buldain, D., Castillo, L. G., Buchamer, A., Chirino-trejo, M., & Mestorino, N. (2021). Pet, and stray dogs asreservoirs of antimicrobial-resistant *Escherichia coli*. *International Journal of Microbiology*, *2021*, 1–8.

Matos, A., Cunha, E., Baptista, L., Tavares, L., & Oliveira, M. (2023). ESBL-Positive Enterobacteriaceae from dogs of Santiago, and Boa Vista Islands, Cape Verde: A public health concern. *Antibiotics*, *12*(3), 1–14.

Moller, T. S. B., Overgaard, M., Nielsen, S. S., Bortolaia, V., Sommer, M. O. A., Guardabassi, L., & Olsen, J. E. (2016). Relation between *tet*R, and *tet*A expression in tetracycline-resistant *Escherichia coli*. *Bio Medical Central Microbiology*, *16*(1), 1–8.

Momtaz, H., Dehkordi, F. S., Rahimi, E., & Asgarifar, A. (2013). Detection of *Escherichia coli, Salmonella* species, and *Vibrio cholerae* in tap water, and bottled drinking water in Isfahan, Iran. *Bio Medical Central Public Health*, *13*(556), 1–7.

Moreira, J., Menezes, J., Marques, C., & Pomba, C. F. (2022). Companion animals — An overlooked, and misdiagnosed reservoir of carbapenem resistance. *Antibiotics*, *11*(533), 1–18.

Mustapha, M., Goel, P., Mittal, D., & Maan, S. (2020). Molecular investigations of tetracycline resistance genes in *Escherichia coli* strains from dogs affected with urinary tract infections. *Alex, Andria Journal of Veterinary Sciences*, *64*(1), 17–25.

Nielsen, S. S., Bicout, D. J., Calistri, P., Canali, E., Rojas, L. G., Gort, C., Drewe, J. A., Garin-bastuji, B., Herskin, M., Michel, V., Angel, M., Chueca, M., Padalino, B., Pasquali, P., Roberts, H. C., Spoolder, H., St, K., Velarde, A., Viltrop, A., Garin-bastuji, B. (2022). Assessment of listing, and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016 / 429): antimicrobial-resistant *Escherichia coli* in dogs and cats, horses, swine, poultry, cattle, sheep, and goats. *Europian Food Safety Authority*, *5*(2022), 1–93.

Ong, K. H., Khor, W. C., Quek, J. Y., Low, Z. X., Arivalan, S., Humaidi, M., Chua, C., Seow, K. L. G., Guo, S., Tay, M. Y. F., Schlundt, J., Ng, L. C., & Aung, K. T. (2020). Occurrence, and antimicrobial resistance traits of *Escherichia coli* from wild birds, and rodents in Singapore. *International Journal of Environmental Research, and Public Health*, *17*(15), 1–17.

Ortiz, D. A., Legenza, L. M., Olson, B. J., Knapp, C. C., Killian, S. B., Meece, J. K., Hall, M. C., & Fritsche, T. R. (2022). Surveillance for multidrug-resistant *Escherichia coli* carriage in cattle, dogs, and humans reveals predominance of CMY-2, CTX-M-15, and CTX-M-9 groups of β-lactamases. *Comparative Immunology, Microbiology, and Infectious Diseases*, *89*(2022), 1–6.

Osman, M., Albarracin, B., Altier, C., Gröhn, Y. T., & Cazer, C. (2022). Antimicrobial resistance trends among canine *Escherichia col*i isolated at a New York veterinary diagnostic laboratory between 2007 and 2020. *Preventive Veterinary Medicine*, *208*(2022), 1–15.

Perewari, D. O., Otokunefor, K., & Agbagwa, O. E. (2022). Tetracycline-resistant genes in *Escherichia coli* from clinical, and nonclinical sources in Rivers State, Nigeria. *International Journal of Microbiology*, *20*(2022), 1–5.

Salgado-caxito, M., Benavides, J. A., Munita, J. M., Rivas, L., García, P., Listoni, F. J. P., Moreno-switt, A. I., & Paes, A. C. (2021). Risk factors associated with faecal carriage of extended-spectrum cephalosporin-resistant *Escherichia coli* among dogs in Southeast Brazil. *Preventive Veterinary Medicine*, *190*(2020), 1–8.

Singleton, D. A., Pinchbeck, G. L., Radford, A. D., Arsevska, E., Dawson, S., Jones, P. H., Noble, P. M., Williams, N. J., & Sánchez-vizcaíno, F. (2020). Factors associated with prescription of antimicrobial drugs for dogs and cats, United Kingdom, 2014 – 2016. *Emerging Infectious Diseases*, *26*(8), 2014–2016.

Sun, C., Cui, M., Zhang, S., Wang, H., Song, L., Zhang, C., Zhao, Q., Liu, D., Wang, Y., Shen, J., Xu, S., & Wu, C. (2019). Plasmid-mediated tigecycline-resistant gene *tet*(X4) in *Escherichia coli* from food-producing animals, China, 2008–2018. *Emerging Microbes, and Infections*, *8*(1), 1524–1527.

Teng, L., Feng, M., Liao, S., Zheng, Z., Jia, C., Zhou, X., Nambiar, R. B., Ma, Z., & Yue, M. (2023). A Cross-Sectional study of companion animal-derived multidrug-resistant *Escherichia coli* in Hangzhou, China. *American Society for Microbiology*, *11*(2), 1–12.

World Health Organisation (WHO). (2021). *WHO integrated global surveillance on ESBL-producing E. coli using a "One Health" approach: Implementation, and opportunities*. Geneva, Switzerland

Yulin, F., Liu D, H, S., Z, L., H, J., & Wang Y. (2020). Development of a multiplex real-time PCR assay for rapid detection of tigecycline resistance gene *tet* (X) Variants from. *American Society for Microbiology*, *64*(4), 1–6.

Zeng, Y., Lu, J., Liu, C., Ling, Z., Sun, Q., Wang, H., Zhou, H., Hu, Y., Chen, G., & Zhang, R.

(2021). A method for screening tigecycline-resistant gene *tet* (X) from the human gut. *Integrative Medicine Research*, *24*(2021), 29–31.

Zhou, Y., Ji, X., Liang, B., Jiang, B., Li, Y., Yuan, T., Zhu, L., Liu, J., Guo, X., & Sun, Y. (2022). Antimicrobial resistance, and prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* from dogs and cats in Northeastern China from 2012 to 2021. *Antibiotics*, *11*(1506), 1–13.

SUPPLEMENTARY DATA

Table S1: Forward, and reverse primers for resistance genes used in this study.

Resistance description	Target genes	Primer sequences (5'-3')	Product size (pb)	Reference
ESBL	*bla*$_{TEM}$	F-ATAAAATTCTTGAAGACGAAA	643	(Cui et al., 2022)
		R- GACAGTTACCAATGCTTAATC		
	bla$_{SHV}$	F- TTATCTCCCTGTTAGCCACC	860	(Cui et al., 2022)
		R-GATTTGCTGATTTCGCTCGG		
	bla$_{CTX-M}$	F-CGCTTTGCGATGTGCAG	550	(Cui et al., 2022)
		R- ACCGCGATATCGTTGGT		
Tetracycline	*tetA*	F-GCTACATCCTGCTTGCCTTC	210	(Mustapha et al., 2020)
		R -CATAGATCGCCGTGAAGAGG		
	tetB	F-TTGGTTAGGGGCAAGTTTTG	659	(Mustapha et al., 2020)
		R-GTAATGGGCCAATAACACCG		
Tigecycline	*tet*(X/X2)	F-TGCGGCTAATGGCATCTCAC	227	(Fu et al., 2020)
		R-GCTGCTACACATGACAACGTCGT		
	*tet*X3	F-GTGGATGCTTTGCTATTGTCTGA	125	(Fu et al., 2020)
		R-TCTGTTGATTCGTCCTGCGTAT		
	*tet*X4	F-TCGCTACAAAGAACTGATTCGTG	93	(Fu et al., 2020)

	R- GGTCGCTTACTTCTCCAAGACTTAC

CHAPTER THREE

This study describes the antibiotic non-susceptibility profiles, and multidrug-resistance profiles, and examines the genetic relatedness of *E. coli* isolates retrieved from dogs and cats, from different veterinary practices, in Durban, KwaZulu-Natal, South Africa.

3.1. Conclusions

The following are conclusions with reference to the objectives of the study:

- **To confirm the identity of *E. coli* isolates from rectal swab samples taken from companion animals using selective media, and real-time polymerase chain reaction (Real-time PCR):** Three hundred, and fifty-two *E. coli* isolates were identified, using selective media (EMB), and later stored in Tryptic Soy Broth (TSB), with 20% glycerol. The bacterial isolates used in this study had been previously isolated from dogs and cats in February 2020 from six veterinary practices. Of these, 330 isolates were confirmed as *E. coli* using real-time polymerase chain reaction (Real-time PCR).
- **To determine the antibiotic susceptibility of *E. coli* against a selected antibiotic panel using the Kirby-Bauer disk diffusion test according to Clinical, and Laboratory Standards Institute (CLSI), and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, and further distinguish multidrug-resistance (MDR**):

- (i) *E. coli* retrieved from dogs, showed a resistance profile against the following antibiotics: tetracycline (25.2%), ampicillin (17.9%), trimethoprim-sulfamethoxazole (14.5%), nalidixic acid (12.4%), cephalexin (7%), amoxicillin-clavulanic acid (6%), chloramphenicol (6%), azithromycin (5.6%), ceftriaxone (5.1%), ciprofloxacin (5.1%), cefotaxime (5%), gentamicin (3.4%), tigecycline (3%), cefoxitin (2.1%) cefepime (2.1%), piperacillin-tazobactam (0.4%), amikacin (0.4%).
- (ii) *E. coli* retrieved from cats, showed a resistant profile against the following antibiotics: tetracycline (21.8%), ampicillin (20.8%), cefoxitin (14.6%), cefotaxime (14.6%), amoxicillin-clavulanic acid (12.5%), trimethoprim-sulfamethoxazole (12.5 %), ceftriaxone (10.4%), chloramphenicol (3.1%), nalidixic acid (3.1%), ciprofloxacin (2.1%), gentamicin (2%), azithromycin (2%), cefepime (2%), piperacillin-tazobactam (1, %), ceftazidime (1%)
- (iii) Twenty-two antibiograms were depicted on MDR*E. coli* isolates.

- **Determine the prevalence of selected antibiotic-resistance genes (ARGs) by conventional PCR, and gene sequencing**: Of the 234, and 96 *E. coli* from dogs and cats, respectively, the prevalence of resistant genes was as follows in dogs: $bla_{\text{CTX-M-15}}$ (5%), bla_{TEM-1} (2%), $bla_{\text{TEM-166}}$ (1%), $bla_{\text{TEM-249}}$ (1%), *tetA* (25%), and *tetB* (18%). In cats, $bla_{\text{CTX-M-15}}$ (15%), $bla_{\text{TEM-1}}$ (13%), $bla_{\text{TEM-166}}$ (2%), $bla_{\text{TEM-249}}$ (2%), *tetA* (24%), and *tetB* (11%). No $bla_{\text{SHV,}}$ *tet*X/X2, *tet*X3, and *tet*X4 genes were detected in *E. coli* isolates retrieved from both dogs and cats.
- **To evaluate clonal similarities of *E. coli* by strain-typing using Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR):** Strain typing of *E. coli* against selected antibiotics using Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) produced 48 ERIC-PCR genotypes using a 75% similarity index. There were no clonality similarities in *E. coli* isolates retrieved from companion animals in Durban, KwaZulu-Natal.

3.2. Limitations

- Limited veterinary practices were considered in this study hence, this study does not represent the situation in all veterinary practices in South Africa.
- One bacterial species (*E. coli*) was considered for this study.
- Only a limited number of ARGs were investigated.

3.3. Future recommendations

- Larger sample size covering an increased number of veterinary practices, to better depict ABR in companion animals in South Africa.
- Samples should be collected, upon the visit to the veterinary practice before companion animals undergo antibiotic treatment, or more than a month after taking the last dose of drug treatment.
- More ABR mechanisms should be investigated to gain insights into the non-susceptibility of *E. coli* that was not investigated in this study.
- Expression of resistance genes should be studied, to confirm that the present genes are functionally expressed as they confer resistance toward specific antibiotics.
- Virulence genes should be studied to gain more insight into the full virulence potential of isolates.

- Clonal typing methods such as multilocus sequence typing (MLST) can be used to obtain a clearer picture of the clonal transmission (if any), because it is unambiguous, and can depict allelic profiles which can be linked, or compared to a large central database.
- Whole-genome sequencing should be undertaken to gain more insight into the genomic profiles of bacterial isolates retrieved from dogs and cats.

APPENDICES

Appendix 1: Biomedical Research Ethics Committee (BREC) approval letter

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

29 May 2019

Dr SY Mangera (218047311
School of Laboratory Medicine and Medical Sciences
College of Health Sciences
saadiyadevet@gmail.com

Dear Dr Moodley

Protocol: Molecular Epidemiology of antibiotic resistant E.coli in companion animals and veterinary personnel in KwaZulu-Natal, South Africa
Degree: MMedSc
BREC Ref No: BE135/19

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received 03 March 2019.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 18 April 2019 to BREC letter dated 10 April 2019 has been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have been met and the study is given **full ethics approval** and may begin as from 29 May 2019. **Please ensure that site permissions are obtained and forwarded to BREC for approval before commencing research at a site.**

This approval is valid for one year from **29 May 2019.** 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).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on **11 June 2019.**

Yours sincerely

[redacted]

Prof V Rambiritch
Chair: Biomedical Research Ethics Committee

cc: Postgrad administrator: dudhrajhp@ukzn.ac.za
Supervisor: essacks@ukzn.ac.za abiaakabel@ukzn.ac.za

Biomedical Research Ethics Committee
Professor V Rambiritch (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
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

1910 - 2010
100 YEARS OF ACADEMIC EXCELLENCE

Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

Appendix 2: Animal Research Ethics Committee (AREC) approval letter

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

05 September 2019

Dr Saadiya Yusuf Mangera (218047311)
School of Health Sciences
Westville

Dear Dr Mangera,

Protocol reference number: AREC/007/019M
Project title: Molecular Epidemiology of Antibiotic Resistant E.coli from Companion Animals and Veterinary Personnel in KwaZulu-Natal, South Africa

Full Approval – Research Application

With regards to your revised application received on 05 March 2019. 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 27 August 2020.

Attached to the Approval letter is a template of the Progress Report that is required at the end of the study, or when applying for Renewal (whichever comes first). An Adverse Event Reporting form has also been attached in the event of any unanticipated event involving the animals' health / wellbeing.

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

Yours faithfully

[redacted]

.....
Dr Dalene Veslbo
Deputy Chair: Animal Research Ethics Committee

/kr

cc Supervisor: Professor Sabiha Essack
cc BRU Manager: Dr Jaca

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 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 (1187)

Dr Saadiya Yusuf Mangera
Antimicrobial Research Unit
College of Health Sciences
Westville campus
University of KwaZulu Natal
Email: saadiyadevet@gmail.com ; 218047311@stu.ukzn.ac.za

Dear Dr Mangera,

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Your application sent with the email on 10 June 2019 requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that permission is hereby granted to perform the following study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. The study is approved as per the application form received on 10 June 2019 and the correspondence thereafter. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this study under this Section 20 permit. Please apply in writing to HerryG@daff.gov.za;
3. No part of the research may commence until valid ethical approval has been obtained, in writing, from the relevant authority;
4. The study must be conducted in compliance with the Veterinary and Para-Veterinary Professions Act 1982 (Act No. 19 of 82);

Appendix 4: Consent form for companion animal's owner

Consent form for Companion Animal

I, (Person's name), the owner of (pet's name) have been informed about the study entitled "Molecular Epidemiology of Antibiotic Resistant *E.coli* from Companion Animals and Veterinary Personnel" by Dr Saadiya Mangera.
I understand the purpose of the study is to create awareness about antibiotic resistance.
I have been given an opportunity to ask questions about the study and have received answers to my satisfaction.
I declare that my participation in this study is entirely voluntary and that I may withdraw at any time.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher at saadiyadevet@gmail.com or 0789386182.

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

ANIMAL RESEARCH ETHICS COMMITTEE

Research Office, Westville Campus
Tel: +27 31 2608350 – Fax: +27 31 2603093
Email: animalethics@ukzn.ac.za

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: +27 31 2604769 – Fax: +27 31 2604609
Email: BREC@ukzn.ac.za

Signature and Name of Animal Owner _____

Date_____

Appendix 5: Antibiotic susceptibility data for *E. coli* isolated from companion animals.

Source	*E. coli* ID	AMP	AMC	TZP	LEX	FOX	CTX	CRO	CAZ	FEP	IMP	MEM	NAL	CIP	AMK	GEN	AZM	TET	TGC	CHL	SXT
Dog	VD1.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R
Dog	VD1.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Dog	VD1.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Dog	VD1.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R
Dog	VD1.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Dog	VD1.7	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R
Dog	VD1.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R
Dog	VD1.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R
Dog	VD1.10	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Dog	VD2.1	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R
Dog	VD2.2	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R
Dog	VD2.3	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R
Dog	VD2.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD2.5	R	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	R	S	R
Dog	VD2.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD2.7	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	R	S	R	R
Dog	VD2.9	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	R	S	R	R
Dog	VD2.10	R	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S	R
Dog	VD3.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD3.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD3.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD3.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD4.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Dog	VD4.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD4.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD4.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD4.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD4.9	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R
Dog	VD4.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD5.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD5.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S
Dog	VD5.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S
Dog	VD5.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD5.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD5.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD5.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD5.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Dog	VD5.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD6.1	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD6.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD6.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD6.5	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R
Dog	VD6.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD6.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD6.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	VD6.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD1.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD1.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	I	S
Dog	RD1.3	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD1.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD1.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Dog	RD1.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD1.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	s	S	S	S	S
Dog	RD1.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD1.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD2.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD2.3	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD2.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD2.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD2.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD2.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD4.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD4.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD4.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD4.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Dog	RD4.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD3.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD3.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD3.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD3.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD3.8	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S
Dog	RD3.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD3.10	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD5.2	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD5.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD5.8	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD5.9	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD6.1	R	R	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD6.2	R	R	S	R	R	I	R	I	S	S	S	S	S	S	S	S	R	S	S	R

Dog	RD6.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	I	S
Dog	RD6.4	R	R	S	R	R	R	R	I	S	S	S	S	I	S	S	S	R	S	S	R
Dog	RD6.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD6.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	s	S	S
Dog	RD6.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	RD6.10	R	R	R	R	R	R	R	I	S	S	S	S	S	S	S	S	R	S	S	R
Dog	BD1.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD1.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD1.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD1.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD1.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD1.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD1.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD1.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Dog	BD1.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD2.3	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD2.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD2.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD2.6	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD2.7	I	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD2.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD2.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD2.10	I	I	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S
Dog	BD3.1	R	R	S	R	S	R	R	S	S	S	S	R	R	S	S	S	S	S	S	S
Dog	BD3.6	R	R	S	R	S	R	R	S	S	S	S	R	R	S	S	S	R	S	S	R
Dog	BD3.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R
Dog	BD4.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD4.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Dog	BD4.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD4.8	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Dog	BD4.9	R	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	R	S	S	S
Dog	BD5.3	R	R	I	R	S	R	R	S	S	S	S	R	R	S	R	S	R	S	R	R
Dog	BD5.7	I	S	S	S	S	S	S	S	S	S	S	I	I	S	S	S	R	S	I	R
Dog	BD5.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	BD5.9	S	S	S	S	S	S	S	S	S	S	S	R	I	S	S	S	R	S	R	R
Dog	BD5.10	S	S	S	S	S	S	S	S	S	S	S	R	I	S	S	S	R	S	R	R
Dog	BD6.1	R	I	S	R	S	R	R	S	R	S	S	R	R	S	R	S	R	S	R	R
Dog	BD6.2	S	S	S	S	S	S	S	S	S	S	S	I	I	S	S	S	R	S	R	R
Dog	BD6.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Dog	BD6.6	R	R	S	R	S	R	R	S	R	S	S	R	R	S	R	R	R	S	R	R
Dog	BD6.7	R	I	S	R	S	R	R	S	R	S	S	R	R	S	R	R	R	S	R	R
Dog	BD6.8	R	R	S	R	S	R	R	S	R	S	S	R	R	S	R	R	R	S	R	R

Dog	BD6.9	R	R	S	R	S	R	R	S	R	S	S	R	R	S	R	R	R	S	R	R
Dog	BD6.10	R	R	S	R	S	R	R	S	SDD	S	S	R	R	S	R	S	R	S	R	R
Dog	ND1.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	ND1.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	ND1.6	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	I	I
Dog	ND1.9	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S
Dog	ND2.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	ND2.2	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S
Dog	ND3.4	R	R	S	S	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	ND3.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	ND3.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD1.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD1.3	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Dog	UD1.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Dog	UD1.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD1.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD1.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD1.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD1.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD2.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD2.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD2.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD2.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD2.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD2.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD2.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD2.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD2.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Dog	UD2.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD3.1	I	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
Dog	UD3.2	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
Dog	UD3.3	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD3.4	I	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
Dog	UD3.5	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
Dog	UD3.7	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
Dog	UD3.8	I	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
Dog	UD3.9	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
Dog	UD3.10	S	I	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
Dog	UD4.1	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Dog	UD4.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Dog	UD4.3	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD4.4	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Dog	UD4.5	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD4.6	S	I	I	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S
Dog	UD4.7	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
Dog	UD4.8	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD4.9	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD5.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD5.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD5.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD5.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	UD5.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD1.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S
Dog	WD1.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD1.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD1.5	R	I	S	R	S	S	S	S	SD D	S	S	R	R	S	R	R	R	S	R	S

Dog	WD1.6	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD1.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD1.9	I	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S
Dog	WD1.10	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD2.1	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S
Dog	WD2.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S
Dog	WD2.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD2.7	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD2.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
Dog	WD2.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD4.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD4.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD4.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S
Dog	WD4.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S

Dog	WD4.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S
Dog	WD4.7	S	S	S	S	S	S	S	S	S	S	S	S	S	R	I	S	S	R	S	S
Dog	WD4.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Dog	WD5.5	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	R	R	S	S	S
Dog	WD7.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD8.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S
Dog	WD8.2	I	I	S	S	S	S	S	S	S	S	S	S	S	S	I	S	R	S	S	S
Dog	WD8.3	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	R	S	S	S
Dog	WD8.4	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Dog	WD8.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD8.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Dog	WD8.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Dog	WD8.8	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Dog	WD8.10	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S

Dog	WD10.2	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S
Dog	WD10.3	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S
Dog	WD10.4	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	R	R	S	S	S
Dog	WD10.5	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S
Dog	WD10.6	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S
Dog	WD10.8	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S
Dog	WD10.10	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S
Dog	WD13.2	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD13.3	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Dog	WD13.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD13.5	I	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD13.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD13.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD13.9	I	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	I	S	
Dog	WD13.10	I	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	
Dog	WD14.1	R	S	S	S	S	S	S	S	SD	S	S	S	S	S	S	S	S	R	S	S	
Dog	WD14.2	R	I	S	S	S	S	S	S	S	S	S	I	S	S	S	S	R	S	S	R	
Dog	WD14.10	R	S	S	S	S	S	S	S	S	S	S	I	S	I	S	R	R	R	S	R	

Dog	WD15.3	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD15.5	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD15.6	R	S	S	S	S	S	S	S	SDD	S	S	R	I	S	S	S	R	S	R	R
Dog	WD15.8	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S
Dog	WD15.10	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	R	S	S	S
Dog	WD16.1	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S
Dog	WD16.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD16.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dog	WD16.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Dog	WD16.7	I	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	I	S
Dog	WD16.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	VC1.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	VC1.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	VC1.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	VC1.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	VC1.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	VC1.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	VC1.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	VC1.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	VC1.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	RC1.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Cat	RC1.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Cat	RC1.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	RC1.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	RC1.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	RC1.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	RC1.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	RC3.1	R	R	S	R	R	R	R	I	S	S	S	S	S	S	S	S	R	S	S	R
Cat	RC3.2	R	R	S	R	R	S	R	I	S	S	S	I	I	S	S	S	R	S	S	R
Cat	RC3.3	R	R	S	R	R	R	R	I	S	S	S	I	S	S	S	S	R	S	S	R
Cat	RC3.4	R	R	S	R	R	R	R	R	S	S	S	I	S	S	S	S	R	S	S	R
Cat	RC3.5	R	R	S	R	R	R	I	I	S	S	S	I	S	S	S	S	R	S	S	R
Cat	RC3.6	R	R	S	R	R	R	I	S	S	S	S	S	S	S	S	S	R	S	S	R
Cat	RC3.7	R	R	S	R	R	R	S	I	S	S	S	I	S	S	S	S	R	S	S	R
Cat	RC3.9	R	R	S	R	R	R	R	I	S	S	S	I	S	S	S	S	R	S	S	R
Cat	RC4.2	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	R	S	S	S

Cat	BC1.1	R	I	S	R	S	R	R	S	R	S	S	R	R	S	R	R	R	S	R	R
Cat	BC1.2	R	R	S	R	S	R	R	S	R	S	S	R	R	S	R	R	R	S	R	R
Cat	BC1.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC1.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC1.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC1.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC1.9	R	I	S	R	S	R	R	S	SD D	S	S	S	S	S	I	S	S	S	S	S
Cat	BC1.1 0	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC2.1	R	I	S	R	S	R	R	S	S	S	S	S	S	S	I	S	S	S	S	S
Cat	BC2.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC2.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC2.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Cat	BC2.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Cat	BC2.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC3.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Cat	BC3.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Cat	BC3.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Cat	BC3.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Cat	BC3.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Cat	BC3.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Cat	BC3.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC4.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Cat	BC4.6	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC4.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S
Cat	BC4.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC4.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC4.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Cat	BC5.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC5.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	BC5.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC2.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC2.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC2.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC2.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC2.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC3.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC3.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC3.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC3.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC3.5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC3.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC3.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

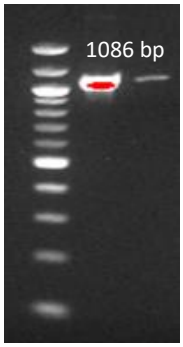
Cat	NC3.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC3.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC3.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC4.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC4.2	R	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC4.3	R	I	S	R	R	R	I	I	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC4.4	R	I	S	R	R	I	I	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC4.5	R	I	S	S	S	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC4.6	S	S	S	R	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC4.7	R	R	S	R	R	I	R	I	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC4.8	R	R	S	R	R	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	NC4.9	R	S	S	R	I	S	I	S	S	S	S	S	S	S	S	S	S	S	S	R
Cat	NC4.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	WC1.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

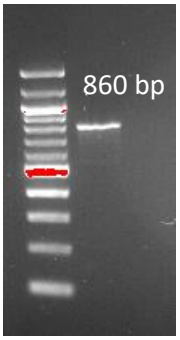
Cat	WC1.4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	WC1.5	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	I	R
Cat	WC1.6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	WC1.7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	WC1.8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	WC1.9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	WC1.10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Cat	WC2.1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	WC2.2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	WC2.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	WC2.4	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	WC2.5	I	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	R	S
Cat	WC2.6	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cat	WC2.8	R	I	R	R	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Cat	WC15.3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

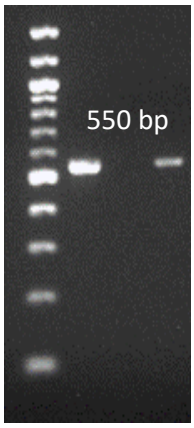
Abbreviations: AMP-Ampicillin, AMC-Amoxicillin-clavulanate, TZP-Piperacillin-tazobactam, LEX-Cephalexin, FOX-Cefoxitin, CTX-Cefotaxime, CRO-Ceftriaxone, CAZ-Ceftazidime, FEP-Cefepime, IMP-Imipenem, MEM-Meropenem NAL-Nalidixic acid, CIP-Ciprofloxacin, AMK-Amikacin, GEN-Gentamicin, AZM-Azithromycin, TET-Tetracycline, TGC-Tigecycline, CHL-Chloramphenicol, SXT-Trimethoprim-sulfamethoxazole, SDD-susceptible-dose dependent.

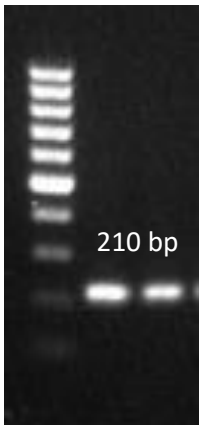
Appendix 6: Agarose gel electrophoresis and patterns of conventional PCR amplification of antibiotic-resistance genes





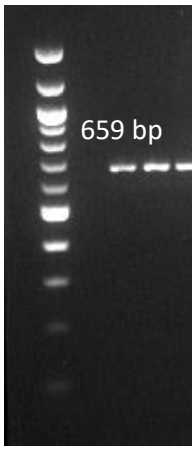
B.





C

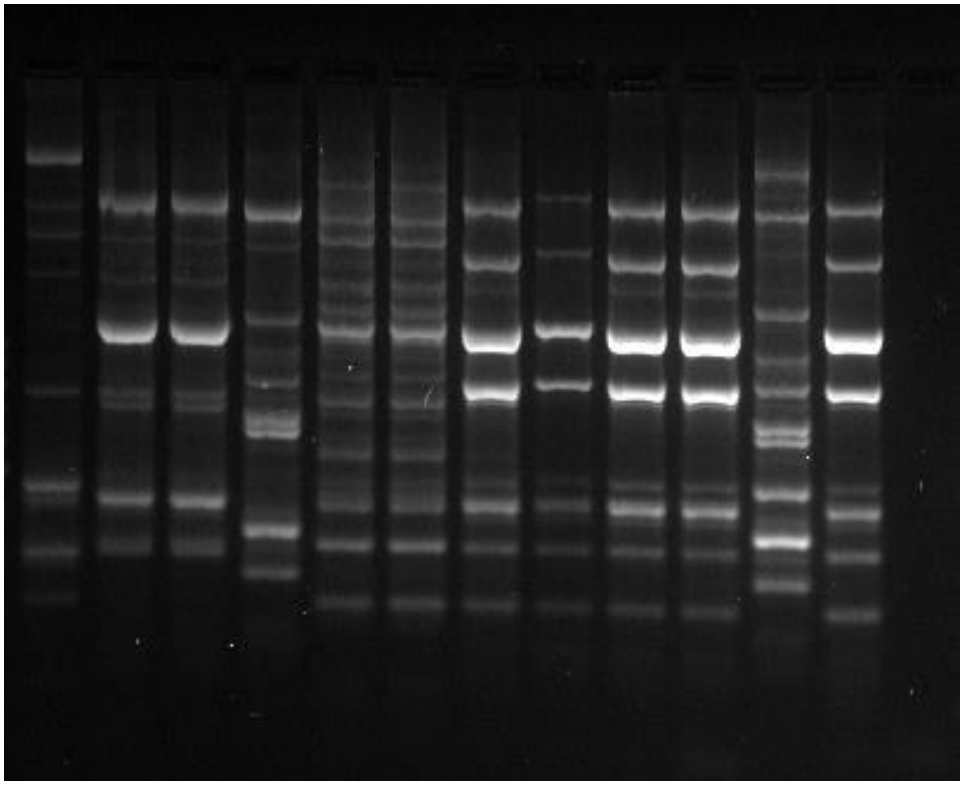
D.



E.

A: *bla*$_{TEM}$ amplified from in-house strain-positive control (lane 2) and samples (lane 3-8). **B**: *bla*$_{SHV}$ amplified from in-house strain-positive control (lane 2) and samples (lane 3-8). **C**: *bla*$_{CTX-M}$ amplified from in-house strain-positive control (lane 2) and samples (lane 4-5). **D**: *tetA* amplified from in-house strain-positive control (lane 2) and samples (lane 3-13). E: *tetB* amplified from in-house strain-positive control (lane 3) and samples (lane 4-13).

Appendix 7: ERIC-PCR agarose gel electrophoresis image



A: Lane 1: 1 kb DNA molecular weight marker (NEB Quick-Load®, Massachusetts, USA), **Lane 11**: *E. coli* ATCC 25922, **Lane 2** to **10** samples

Appendix 8: Gene bank ID from NCBI

Isolate ID	***bla*$_{\text{CTX-M-15}}$**	**bla$_{\text{TEM-1}}$**	***bla*$_{\text{TEM-166}}$**	***bla*$_{\text{TEM-249}}$**	***tetA***	***tetB***
ND3.4	ACQ42051.1	AKJ66803.1	-	-	-	-
NC4.2	ACU00080.1	AMQ45728.1	-	-	-	-
NC4.3	BCM94848.1	AXH80245.1	-	-	-	-
NC4.4	BCM94848.1	AXH80245.1	-	-	-	-
NC4.7	-	AXH80245.1	-	-	-	-
NC4.8	-	AXH80245.1	-	-	-	-
NC4.9	BCM94848.1	AXH80245.1	-	-	-	-
RD6.2	-	-	-	WP_262697193.1	-	-
RD6.4	-	AMQ45728.1	-	-	-	-
RD6.10	AGI41341.1	-	WP_063864874.1	-	-	-
RC3.1	AGI41341.1	AMQ45728.1	-	-	MBZ5869529.1	-
RC3.2	AGI41341.1	XH80245.1	-	-	SBN85062.1	-

RC3.3	ADR66026.1	AMQ45728.1	-	-	SBN85062.1	-
RC3.4	ACU00080.1	-	-	WP_262697193.1	MCA7079179.1	-
RC3.5	BCM94848.1	AMQ45728.1	-	-	SBN85062.1	-
RC3.6	-	-	WP_063864874.1	-	SBN85062.1	-
RC3.7	AGI41341.1	-	-	WP_262697193.1	QBF53387.1	-
RC3.9	-	AXH80245.1	-	-	SBN85062.1	-
BD3.1	AGI41341.1	-	-	WP_262697193.1	-	-
BD3.6	AGI41341.1	-	-	WP_262697193.1	-	-
BD5.3	AGI41341.1	-	WP_063864874.1	-	-	-
BD6.1	AGI41341.1	AEQ59621.1	-	-	-	-
BD6.6	AGI41341.1	-	WP_063864874.1	-	-	-
BD6.7	AGI41341.1	AMQ45728.1	-	-	-	-
BD6.8	AGI41341.1	AMQ45728.1	-	-	-	WP_169005818.1
BC1.2	AGI41341.1	AXH80245.1	-	-	-	-
WC2.8	AGB07536.1	-	WP_063864874.1	-	-	-

NC4.5	AGE61864.1	-	-	-	-	-
BD6.9	AGI41341.1	-	-	-	-	QBF53395.1
BD6.10	AGI41341.1	-	-	-	-	-
BC1.1	AGI41341.1	-	-	-	-	-
RD1.2	-	-	-	-	QBF53387.1	-
VD2.7	-	-	-	-	MBZ5869529.1	-
VD1.2	-	-	-	-	-	WP_169005818.1
VD1.3	-	-	-	-	-	WP_218343866.1
VD1.4	-	-	-	-	-	WP_169005818.1
VD1.6	-	-	-	-	-	WP_218343866.1
VD1.7	-	-	-	-	-	WP_218343866.1
VD1.8	-	-	-	-	-	WP_218343866.1
VD1.9	-	-	-	-	-	WP_169005818.1
VD1.1	-	-	-	-	-	WP_169005818.1

Appendix 9: Antibiogram and clusters of *E. coli*

Isolate ID	Antibiogram	ARG's	Clusters
BD4.8	AMP-AMC-LEX-TET	tetA	A
BD4.9	AMP-NAL-CIP-TET	tetA	AA
BD6.9	AMP-AMC-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT	CTX-M-15, tetA, tetB	AB
BC2.5	TET	tetA, tetB	AC
BD6.1	AMP-AMC*-LEX-CTX-CRO-FEP-NAL-CIP-GEN-TET-CHL-SXT	CTX-M-15, TEM-1, tetA, tetB	AD
BD6.6	AMP-AMC-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT	CTX-M-15, TEM-166, tetA, tetB	AD
BD6.8	AMP-AMC-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT	CTX-M-15, TEM-1, tetA, tetB	AD
BD6.10	AMP-AMC-LEX-CTX-CRO-NAL-CIP-GEN-TET-CHL-SXT	CTX-M-15, tetA, tetB	AD
BC1.1	AMP-AMC*-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT	CTX-M-15, TEM-166, tetA, tetB	AD
BC1.2	AMP-AMC-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT	CTX-M-15, TEM-1, tetA, tetB	AD
BD6.7	AMP-AMC*-LEX-CTX-CRO-FEP-NAL-CIP-GEN-AZM-TET-CHL-SXT	CTX-M-15, TEM-1, tetA, tetB	AE
BC3.6	TET	tetA, tetB	AE

BD5.7	AMP*-NAL*-CIP*-TET-CHL*-SXT	tetA	AF
BC3.1	TET	tetA	AF
BD5.3	AMP-AMC-TZP*-LEX-CTX-CRO-NAL-CIP-GEN-TET-CHL-SXT	CTX-M-15, TEM-166, tetA, tetB	AG
BC3.2	TET	tetA, tetB	AG
BC3.3	TET	tetA, tetB	AG
BC3.4	TET	tetA, tetB	AG
BC3.7	TET	tetA, tetB	AG
BC4.2	TET	tetA	AG
BC4.7	TET*	tetA, tetB	AG
BD3.1	AMP-AMC-LEX-CTX-CRO-NAL-CIP-TET-SXT	CTX-M-15, TEM-249, tetA	AH
BD3.6	AMP-AMC-LEX-CTX-CRO-NAL-CIP-TET-SXT	CTX-M-15, TEM-249, tetA	AH
RC3.3	AMP-AMC-LEX-FOX-CTX-CRO-CAZ*-NAL*-TET-SXT	CTX-M-15, TEM-1, tetA	AI
RC3.4	AMP-AMC-LEX-FOX-CTX-CRO-CAZ-NAL*-TET-SXT	CTX-M-15, TEM-249, tetA	AI
RC3.5	AMP-AMC-LEX-FOX-CTX-CRO*-CAZ*-NAL*-TET-SXT	CTX-M-15, TEM-1, tetA	AI
RC3.6	AMP-AMC-LEX-FOX-CTX-CRO*-TET-SXT	TEM-166, tetA	AI

RC3.7	AMP-AMC-LEX-FOX-CTX-CAZ*-NAL*-TET-SXT	CTX-M-15, TEM-249, tetA	AI
RC3.2	AMP-AMC-LEX-FOX-CRO-CAZ*-NAL*-CIP*-TET-SXT	CTX-M-15, TEM-1, tetA	AJ
RD6.10	AMP-AMC-TZP-LEX-FOX-CTX-CRO-CAZ*-TET-SXT	CTX-M-15, TEM-166, tetA, tetB	AK
RC3.1	AMP-AMC-LEX-FOX-CTX-CRO-CAZ*-TET-SXT	CTX-M-15, TEM-1, tetA	AK
RD1.3	AMP*		AL
RD5.8	AMP*		AL
RD6.1	AMP-AMC-FOX-CRO*		AL
RD3.10	AMP*		AM
RD6.2	AMP-AMC-LEX-FOX-CTX*-CRO-CAZ*-TET-SXT	TEM-249, tetA, tetB	AM
RD1.6	TET	tetA, tetB	AN
UD4.4	AMC*		AO
UD3.8	AMP*-NAL		AP
UD3.10	AMC*-NAL		AP
UD3.1	AMP*-NAL		AQ
UD4.2	TET	tetA	AQ

UD4.7	AMP-AMC*-TGC		AQ
VD1.3	TET	tetA, tetB	AR
VD1.8	TET-SXT	tetA, tetB	AR
VD1.9	TET-SXT	tetA, tetB	AR
RC1.3	TET	tetA	AS
VD1.7	AMP*-TET-SXT	tetA, tetB	AT
VD2.3	AMP-AMC*-TET-SXT	tetA	AT
RC4.2	NAL*-TET	tetA	AU
VD1.1	TET-SXT	tetA, tetB	AV
RC3.9	AMP-AMC-LEX-FOX-CTX-CRO-CAZ*-NAL*-TET-SXT	TEM-1, tetA	B
VD2.5	AMP-NAL*-TGC-SXT		C
VD1.4	TET-SXT	tetA, tetB	D
RD1.2	TET-CHL*	tetA	E
VD4.9	AMP-TET-SXT	tetA	F
VD2.7	NAL*-TET-CHL-SXT	tetA	G

VD2.9	NAL*-TET-CHL-SXT	tetA, tetB	G
WD8.1	AZM-TET		H
WD13.10	AMP*-AMK*		I
WD8.7	TET	tetA, tetB	J
WD10.6	NAL-TET	tetA, tetB	J
WD10.8	NAL-TET	tetA, tetB	J
WD8.3	AMK*-TET	tetA, tetB	K
WD8.6	TET	tetA, tetB	K
WD8.8	AMP-AMC*-TET	tetA, tetB	K
WD10.2	NAL-TET	tetA, tetB	K
WD10.3	AMP-AMC-NAL-TET	tetA, tetB	K
WD10.4	NAL-AZM-TET	tetA, tetB	K
WD10.5	NAL-TET	tetA, tetB	K
WD15.5	AMP		K
WD15.6	AMP-NAL-CIP*-TET-CHL-SXT	tetA	K
WD4.9	TET	tetA, tetB	L
WC2.8	AMP-AMC*-TZP-LEX-CTX*	CTX-M-15, TEM-166	L
WD5.5	AMK*-AZM-TET	tetA, tetB	M
WD13.2	AMP*		M

WD13.3	AMP*		M
WD1.9	AMP-GEN*		N
WD4.4	TET	tetA, tetB	N
WD14.10	AMP-NAL*-AMK*-AZM-TET-TGC-SXT	tetA, tetB	N
WD15.10	AMK*-TET	tetA	N
WD16.7	AMP*-NAL*-CHL*		N
WD4.6	AZM-TET-TGC	tetA	O
WD10.10	NAL-TET	tetA	O
WC2.5	AMP*-NAL*-CHL		O
WC1.10	TET	tetA, tetB	P
WD1.6	AMP		Q
WC2.6	AMP*		Q
WD14.1	AMP-TGC		R
VD1.2	TET	tetA, tetB	S
UD4.8	AMP		T
UD3.4	AMP*-NAL		U
NC4.9	AMP-LEX-FOX*-CRO*-SXT	CTX-M-15, TEM-1	V
NC4.2	AMP-AMC-LEX-FOX-CTX	CTX-M-15, TEM-1	W
ND1.6	NAL*-CHL*-SXT*		X
NC4.5	AMP-AMC-CTX-CRO*	CTX-M-15	Y
NC4.7	AMP-AMC-LEX-FOX-CTX*-CRO-CAZ*	TEM-1	Y
NC4.8	AMP-AMC-LEX-FOX-CRO*	TEM-1	Y

ND3.4	AMP-AMC-FOX-CTX*	CTX-M-15, TEM-1	Z
NC4.3	AMP-AMC*-LEX-FOX-CTX-CRO*-CAZ*	CTX-M-15, TEM-1	Z
NC4.4	AMP-AMC*-LEX-FOX-CTX-CRO*	CTX-M-15, TEM-1	Z

*Denote intermediate resistance

Abbreviations: AMP-Ampicillin, AMC-Amoxicillin-clavulanate, TZP-Piperacillin-tazobactam, LEX-Cephalexin, FOX-Cefoxitin, CTX-Cefotaxime, CRO-Ceftriaxone, CAZ-Ceftazidime, FEP-Cefepime, NAL-Nalidixic acid, CIP-Ciprofloxacin, AMK-Amikacin, GEN-Gentamicin, AZM-Azithromycin, TET-Tetracycline, TGC-Tigecycline, CHL-Chloramphenicol