



Prevalence of antimicrobial-resistant *Escherichia coli* and enterococcus species in pigs in northern Malawi, and the knowledge, attitude and practices amongst butchers

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DECLARATION

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DEDICATION

To my beloved parents, brother and sisters, for their unceasing moral support and encouragement during the entire period of my study; and

To my wife Catherine and my children, Ryan and Rhona, for their love, endurance and moral support.

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TABLE OF CONTENTS

DECLARATION.....	i
DEDICATION	ii
ACKNOWLEDGEMENT	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES.....	vii
DEFINITION OF ACRONYMS	viii
ABSTRACT	xi
CHAPTER 1: ORIENTATION AND GENERAL BACKGROUND	1
1.1. Introduction	1
1.2. Problem statement	2
1.3. Research objectives	3
1.4. Limitations of research	4
CHAPTER 2: LITERATURE REVIEW	5
2.1 OVERVIEW OF <i>ESCHERICHIA COLI</i>	5
2.2 OVERVIEW OF <i>ENTEROCOCCUS</i> SPECIES.....	11
2.3 FOOD SAFETY KNOWLEDGE, ATTITUDES AND PRACTICES AMONG PIG BUTCHERS	16
CHAPTER 3: MATERIALS AND METHODS	19

CHAPTER 4: RESULTS.....	24
CHAPTER 5: DISCUSSION.....	39
CHAPTER 8: CONCLUSION AND RECOMMENDATION.....	43
REFERENCES.....	44
APPENDICES.....	69

LIST OF TABLES

Table 1: Summary of the sociodemographic characteristics of the respondents.....	24
Table 2: Distribution of sampled slaughter places in northern Malawi.....	26
Table 3: Butchers' knowledge of good hygienic practices in slaughter places	26
Table 4: Food safety knowledge among butchers in the northern part of Malawi.....	27
Table 5: Food safety attitudes among pig butchers in northern Malawi.....	30
Table 6: Food safety practices among pig butchers in northern Malawi.....	32
Table 7: Correlation analysis to determine the direction and strength of the KAP sub-scales	34
Table 8: Distribution of <i>E. coli</i> and enterococcal species isolates in caecal samples	35
Table 9: Resistance patterns for <i>E. coli</i> and enterococcal species in the sampled districts	36
Table 10: Multidrug resistance patterns of <i>E. coli</i> and <i>Enterococcus</i> species in northern Malawi.....	37

LIST OF FIGURES

Figure 1: Map of Malawi showing population density (NSO, 2019)	19
Figure 2: Responses on zoonotic diseases posing occupational risk to butchers	29
Figure 3: Likert scale on respondent's attitudes towards food safety and occupational health risks	31
Figure 4: Likert scale on practices of the respondents	34
Figure 5: Resistance patterns of <i>E. coli</i> and <i>Enterococcus</i> species	37

DEFINITION OF ACRONYMS

CAC	Codex Alimentarius Commission
CDC	Centre for Disease Control
CTX-M-14	Cefotaximase 14-like subfamily
CTX-M-15	Cefotaximase 15-like subfamily
DAHLD	Department of Animal Health and Livestock Development
DEC	Diarrhoeagenic <i>Escherichia coli</i>
<i>dfrA</i>	dihydrofolate reductase type A encoding gene
<i>dfrB</i>	dihydrofolate reductase type B encoding gene
<i>E. coli</i>	<i>Escherichia coli</i>
<i>eae</i>	attaching and effacing factor
EAEC	Enter aggregative <i>Escherichia coli</i>
ECA	Enterobacterial common antigens
EFSA	European Food Safety Authority
EIEC	Enteroinvasive <i>Escherichia coli</i>
EPEC	Enteropathogenic <i>Escherichia coli</i>
ESBL	Extended β -Spectrum Lactamase
ESKAPE	<i>Enterococcus faecium</i> , <i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> , <i>Acinetobacter baumannii</i> , <i>Pseudomonas aeruginosa</i> , and <i>Enterobacter</i> species
ETEC	Enterotoxigenic <i>Escherichia coli</i>
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ExPEC	Extraintestinal <i>Escherichia coli</i>
FAO	Food and Agriculture Organisation

GMP	Good Management Practice
H-	Flagella
HACCP	Hazard Analysis and Critical Control Points
K-	Capsular
LMIC	Low- and middle-income countries
LT	Heat-labile
MALDI-TOF-MS	Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry
MDR	Multi-drug resistance
MLSA	Multi-locus sequence analyses
MLST	Multi-locus sequence typing
MNEC	Meningitis-associated <i>Escherichia coli</i>
MoA	Ministry of Agriculture
MoAIWD	Ministry of Agriculture, Irrigation and Water Development
NAAT	Nucleic acid amplification test
NSO	National Statistical Office
O-	Somatic
PFGE	Pulsed-field gel electrophoresis
PNA-FISH	Peptide nucleic acid fluorescent in situ hybridization
REC	Research Ethics Committee
ST	Heat-stable
STEC	Shiga toxin-producing <i>E. coli</i>
<i>Stx</i> ₁	Shiga toxin variant 1 encoding gene
<i>Stx</i> _{2a}	Shiga toxin variant 2a encoding gene

<i>Stx_{2c}</i>	Shiga toxin variant 2c encoding gene
<i>Stx_{2d}</i>	Shiga toxin variant 2d encoding gene
<i>Stx_{2e}</i>	Oedema associated shiga toxin
<i>sul1</i>	sulfonamide resistance type-1 encoding gene
<i>sul2</i>	sulfonamide resistance type-2 encoding gene
<i>sul3</i>	sulfonamide resistance type-3 encoding gene
TEM	Temoniera type encoding gene
UPEC	Uropathogenic <i>Escherichia coli</i>
VRE	Vancomycin-resistant <i>Enterococcus</i>
WHO	World Health Organisation

ABSTRACT

The prevalence of multidrug-resistant (MDR) opportunistic pathogens in many of the sub-Saharan countries has been one of the compelling reasons for proper mapping and management of the disease in hotspot areas. This study investigated the prevalence of antimicrobial-resistant *Escherichia coli* and enterococcus species isolated from pigs in the northern part of Malawi and analysed the knowledge, attitudes and practices (KAP) of butchers concerning good hygienic practices. The KAP questionnaire was administered to 75 participants and 232 caecal samples were collected from pigs slaughtered at randomly selected slaughter places in Nkhata Bay, Rumphi, Mzimba, Karonga, and Mzuzu city. Overall KAP scores for knowledge, attitude, and practices were 81%, 73% and 46%, respectively. A positive correlation between the butchers' knowledge and their attitude ($r = .46$, $p < 0.001$), knowledge and practices ($r = .38$, $p < 0.001$), and attitude and practices ($r = .76$, $p < 0.001$) was observed. Microbiological tests confirmed the presence of *Escherichia coli* and *Enterococcus* species by biochemical tests. *Escherichia coli* resistance to ampicillin was high (82.2%) and this was followed by trimethoprim-sulfamethoxazole (75.8%), gentamicin (22.5%), cefotaxime (13.1%), ciprofloxacin (7.9%), and tigecycline (0.5%). In terms of *Enterococcus* species, resistance to vancomycin, ampicillin and ciprofloxacin were at 79.7%, 50% and 23.4%, respectively. No resistance to tigecycline was observed in all the *Enterococcus* isolates. The study also found that 26.7% ($n=51$) and 10.1% ($n=16$) of the *E. coli* and *Enterococcus* isolates, respectively, were resistant to more than one class of antibiotics. The study revealed a high risk of cross-contamination with resistant pathogens. Therefore, good hygienic practices in the farm-to-fork continuum, enforcement of food safety regulations and capacity building are pivotal in reducing the dissemination of resistant genes in the pork chain. The importance of antimicrobial stewardship should not be underemphasized if optimal utilisation of antimicrobials is to be attained.

Keywords: butchers; KAP; food safety; *Escherichia coli*; enterococcus species; northern Malawi

CHAPTER 1: ORIENTATION AND GENERAL BACKGROUND

1.1. Introduction

Antimicrobial resistance (AMR) remains a worldwide threat due to globalisation, which has allowed the distribution of antimicrobial-resistant microbes (MacPherson et al., 2009). It occurs when microbes such as viruses, bacteria, fungi, and parasites demonstrate capabilities of adaptation and growth in the presence of antimicrobial drugs (Ramos-Martín & D'Amelio, 2023). Inappropriate and overuse of antibiotics in humans and animals as well as poor sanitation and hygiene have exacerbated the emergence of AMR (FAO, 2016; Shallcross & Davies, 2014). Acquired resistance has been linked to bacterial gene mutations or the acquisition of foreign genetic elements that encode resistance genes while intrinsic resistance is a natural phenomenon due to the genetic composition of bacteria (Morrison & Zembower, 2020).

The prevalence of multidrug-resistant (MDR) bacterial pathogens in many sub-Saharan countries is one of the compelling reasons for proper mapping and management of disease in hotspot areas (Kariuki et al., 2019). Mortalities in humans associated with antimicrobial resistance are estimated to be around 1.27 million people per year globally but the figure is likely to increase to 10 million by 2050, if no action is taken, surpassing common non-communicable diseases (CDC, 2022; WHO, 2019). Several studies documented resistance against commonly used antibiotics, such as tetracyclines, phenicols, polymyxins, penicillins, cephalosporins, fluoroquinolones, aminoglycosides, and/or trimethoprim-sulfamethoxazole (Alhashash et al., 2013; Chao et al., 2022; Choonara et al., 2022; Zhang et al., 2014). The presence of these multidrug-resistant pathogens signifies the need for further actions to reduce the current trend.

Bacteria can be characterised as MDR when they resistant to at least one agent in at least three or more key antimicrobial classes during *in vitro* antimicrobial susceptibility testing (Magiorakos et al., 2012). The terms Pan-drug resistance (PDR) and extensive or extreme drug resistance (XDR) have been used to define bacterial isolates which are lack susceptibility to all agents in all antimicrobial classes and those resistant to at

least one agent in all but two or fewer antimicrobial categories, respectively (Magiorakos et al., 2012). More recently, the term Difficult-to-treat resistance (DTR) has been proposed to define Gram-negative bacteria which are non-susceptible to all first-line antimicrobial agents resulting in the use of second-line agents which are characterised by poor pharmacokinetic properties and increased risk of toxicity (Cosentino et al., 2023).

The increase in the prevalence of antimicrobial-resistant bacterial pathogens is of major concern worldwide. Most of these resistant pathogens pose significant public health risks to humans through exposure to contaminated meat products. Overuse of antimicrobials continues to play a detrimental role in disruption of the intestinal microbiome leading to overgrowth of opportunistic pathogens which may be difficult to treat (Bhalodi et al., 2019). The development of resistance by the common opportunistic commensal bacteria, such as *Escherichia coli* and *Enterococcus* species, poses a huge risk in humans. The presence of *E. coli* and *Enterococcus* species in meat products are considered useful indicators of contamination and unhygienic slaughter conditions (Byappanahalli et al., 2012; Corbellini et al., 2016). Therefore, understanding the prevalence and distribution of such multidrug-resistant commensal bacteria is important.

1.2. Problem statement

Microbiological contamination of food and food products has been attributed as one of the major causes of morbidity and mortality hence a growing public health concern (Aidara-Kane et al., 2016). The food supply chain has been regarded as a crucial area which connects environmental, human and animal habitats for bacteria due to the usage of antibiotics in both agriculture and aquaculture (Bengtsson-Palme, 2017). Meat and meat products have been highlighted as one of the major food vehicles associated with foodborne disease outbreaks in household settings (EFSA, 2017). Both commensal and pathogenic bacteria have been linked with foodborne illnesses with the commensal bacteria contributing a higher proportion of bacteria in foods than the pathogenic strains (Huang et al., 2023).

Considering the role that livestock sector plays in people's livelihoods, the interaction between humans and livestock is inevitable. The spread of vancomycin-resistant enterococci and antimicrobial-resistant Enterobacteriaceae species is considered a global threat to public health (González-Fandos et al., 2022). Some of these Enterobacteriaceae species isolated from carcasses during the slaughtering process signify a high risk of microbial contamination hence the need to educate slaughterhouse personnel on good hygienic practices to reduce the risk (Biasino et al., 2018; Paz et al., 2023; Siluma et al., 2023). Containment of such antimicrobial-resistant pathogens may prove to be challenging once the pathogens disseminate in the community (Brolund, 2014).

Knowledge about the resistance patterns and epidemiology of *E. coli* and *Enterococcus* species is crucial in managing these diseases. Wilson et al. (2020) and Kalumbi et al. (2022) documented the prevalence of some Enterobacteriaceae species in pigs from Chikwawa and Bvumbwe, respectively, areas found in the southern region of Malawi. Studies conducted in other areas south of Malawi observed high colonisation rates (56.8%) by extended-spectrum beta-lactamase (ESBL) -producing *E. coli* in pigs (Cocker et al., 2022). Contamination of food sources has been linked to community foodborne disease outbreaks in some districts in Malawi though most of the cases are not thoroughly investigated and hence have been rendered inconclusive (Mithi et al., 2024). Despite the World Health Organisation (WHO) listing *E. coli* third-generation cephalosporin- and carbapenem-resistant and *Enterococcus faecium* vancomycin-resistant as priority pathogens (WHO, 2024), very few studies have been conducted on the prevalence of antimicrobial-resistant strains in pigs in other regions of the country. This study, therefore, investigated the prevalence of antimicrobial-resistant *E. coli* and *Enterococcus* species in pigs slaughtered in the northern region of Malawi and assessed the level of knowledge, attitudes and practices among butchers which may increase the risk of cross-contamination.

1.3. Research objectives

The study aimed at investigating the prevalence and risk factors of drug-resistant *E. coli* and *Enterococcus* species in pigs in the northern part of Malawi.

The study objectives were:

- To determine the prevalence of antimicrobial-resistant *E. coli* species in pigs in northern Malawi.
- To determine the prevalence of antimicrobial-resistant *Enterococcus* species in pigs in northern Malawi.
- To assess the knowledge, attitudes, and practices of pig carcass handlers related to zoonosis at the human-animal interface using a questionnaire.

1.4. Limitations of study

The study did not cover Chitipa and Likoma districts due to financial constraints. This also affected confirmation of the bacterial isolates using molecular techniques. Therefore, biochemical tests were used for confirmation of the isolated pathogens.

CHAPTER 2: LITERATURE REVIEW

2.1 OVERVIEW OF *ESCHERICHIA COLI*

2.1.1 Introduction

Escherichia coli is one of the four recognised species under the genus *Escherichia*, which belongs to the Enterobacteriaceae family (Yu et al., 2021). As an Enterobacteriaceae family member, *E. coli* is a facultatively anaerobic, non-spore-forming, Gram-negative rod, oxidase-negative bacterium (Janda & Abbott, 2021). It is usually motile with peritrichous flagella, possesses fimbriae and has the capability of both fermentative and respiratory metabolism (McClure, 2005). In addition, the bacterium is considered one of the commensal inhabitants of the lower gastrointestinal tract of warm-blooded animals (Strockbine et al., 2015). It can also be isolated from undercooked meat, vegetables, water, and soil (Maule, 2000).

Morphologically, *E. coli* contains a cell wall, cell membrane, cytoplasm, flagella, capsules, fimbriae, and enterobacterial common antigens (ECA) which is a key marker associated with all members of the Enterobacteriaceae family except *Erwinia chrysanthema* (Goździewicz et al., 2015; Holst, 2007; Kuhn et al., 1988). The exoskeleton of *E. coli* contains an envelope which has three layers namely the outer membrane, peptidoglycan, and inner or cytoplasmic membrane (Nanninga, 1998). The bacterium is devoid of cell structures when stained and examined under a light microscope (Farmer et al., 2010). Using a serotyping scheme, the organism can be classified into strains based on the presence of the outer membrane O (somatic), K (capsular), and H (flagellar) antigens (McClure, 2005). *Escherichia coli* genome is estimated to have between 4,000 to 5,000 genes with 3,000 genes shared by different isolates (Poirel et al., 2018). Phylogenetic grouping, using molecular techniques, has demonstrated a close relationship between *E. coli* and *Shigella* despite having distinct morphological and biochemical differences (Yu et al., 2021).

Escherichia coli is broadly categorised into three groups, based on clinical and genetic attributes, namely commensal *E. coli*, enteric (intestinal or diarrheagenic) pathogenic *E. coli*, and extraintestinal pathogenic *E. coli* (ExPEC) (Johnson & Russo, 2005). The presence of amphiphilic lipopolysaccharides, which are well-characterised for their

immunological, pathophysiological, and pharmacological effects, in the outer membrane of the cell wall acts as effective virulence factors in pathogenic strains (Holst, 2007). Serotypic characteristics of *E. coli* are determined by combining O- and H-antigens; more than 185 of the O- groups and 53 different H- groups have been documented (Smith & Fratamico, 2017). The pathogenic strains are further classified into pathotypes, or pathovars, depending on where they cause a disease (Poirel et al., 2018). Furthermore, each strain is unique in terms of the clinical, epidemiological, and pathological features of the disease it causes due to the presence of a unique set of virulence-associated features (Robins-Browne & Hartland, 2002).

The enteric *E. coli* are categorised into enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), enterotoxigenic *E. coli* (ETEC), and Shiga toxin-producing *E. coli* (STEC) strains (Strockbine et al., 2015). Extraintestinal pathogenic *E. coli* (ExPEC) strains, which cause disease at multiple anatomical sites outside the gastrointestinal tract, share the same phylogenetic backgrounds and virulence profiles which cannot be found in either commensal or intestinal pathogenic strains (Johnson & Russo, 2005). Currently, only three distinct groups of the ExPEC strains have been documented namely meningitis-associated *E. coli* (MAEC), avian pathogenic *E. coli* (APEC), and uropathogenic *E. coli* (UPEC) (Ramos et al., 2020). However, some disease-associated strains of ExPEC have not yet been classified into specific pathotypes (Strockbine et al., 2015).

Humans are regarded as the main reservoir of ETEC and EIEC strains and outbreaks associated with consuming water and food contaminated with human excreta and contact between individuals (McAuley & Fegan, 2022; Zhang et al., 2022). In pigs, ETEC strains are the most relevant and pathogenic strains expressing heat-stable (ST) and/or heat-labile (LT) enterotoxins, and they are usually associated with oedema and diarrhoea commonly found in colibacillosis (Barros et al., 2023). Beef have been implicated as the major source of STEC infections of food origin for humans with 2-8% of the infections progressing to the potentially fatal haemolytic uremic syndrome (Monte et al., 2024). Although certain subtypes of STEC of swine origin have been found in pigs, there is no empirical evidence implicating swine STEC isolates as a

cause of human illness except for consumption of contaminated pork-derived products (Arancia et al., 2019; Tseng et al., 2015). The *stx*_{2e} has commonly been isolated in STEC of swine-origin but human cases have been associated with *stx*₁, *stx*_{2a}, *stx*_{2c}, *stx*_{2d}, and *eae* virulence genes (Arancia et al., 2019). Atypical EPEC strains, a subgroup of EPEC besides the typical EPEC strains whose only reservoirs are humans, have also been reported to be well-adapted to both human and animal hosts (Trabulsi et al., 2002). Similarly, ExPEC strains associated with infections in animals have also demonstrated their potential to cause zoonosis (Bélanger et al., 2011).

2.1.2 Epidemiology of drug-resistant *Escherichia coli*

Antimicrobial resistance remains a global threat due to globalisation which has allowed the distribution of drug-resistant microbes (MacPherson et al., 2009). Cases of multidrug resistance among members of the Enterobacteriaceae family continue to rise worldwide. Some of the notable classes of antibiotics that have registered resistance include penicillins, tetracyclines, carbapenems, fluoroquinolones, and third-generation cephalosporins (Chen et al., 2013; Fowler et al., 2021; Mellou et al., 2021). *Escherichia coli*, like any other bacteria, can acquire resistance through vertical or horizontal transfer of mobile genetic elements (Ouchar Mahamat et al., 2021). Co-selection and persistence of resistance to antimicrobial agents in humans have been attributed to the massive use of antimicrobials in veterinary medicines (Poirel et al., 2018).F

Beta-lactams are antimicrobials that are widely prescribed and efficacious in the treatment of severe and life-threatening infections and for prophylaxis before surgery (Brolund, 2014; Bush & Bradford, 2016). Currently, the class consists of four main groups namely penicillins, cephalosporins, carbapenems, and monobactams (De Angelis et al., 2020). However, their usage does not often yield the intended results due to the development of resistance in the targeted bacteria. Some targeted bacteria have demonstrated the ability to inactivate drugs by producing beta-lactamases such as CTX-M-14, CTX-M-15, TEM and OXA, that hydrolyse the beta-lactam ring (Bush, 2013). Although carbapenems are not approved for use in food animals, the common use of ceftiofur and cefquinome (higher generation cephalosporins) in livestock has been associated with increased selective pressure that promotes the dissemination of

carbapenem resistance (Mollenkopf et al., 2018). The exact relationship between cephalosporin use and carbapenem resistance is not well understood (Mollenkopf et al., 2017). Similar observations were reported with the usage of enrofloxacin and oxytetracycline in food-producing animals selected for ESBL- and carbapenemase-producing *E. coli* (González-Fandos et al., 2022).

The prevalence of ESBL-producing Enterobacteriaceae has been documented in many countries such as South Africa, Kenya, Nigeria, Gabon, Cameroon, Senegal, Tanzania, Central African Republic, Sweden, Denmark, Norway, Thailand, USA, India, among others (Brolund, 2014). Cases of ESBL-resistant *E. coli* in bloodstream infection in Blantyre, Malawi, increased significantly from 0.7% to 30.3% between 2003 and 2016 (Musicha et al., 2017). In domestic pigs, various studies have demonstrated the presence of multidrug-resistant bacteria, including ESBL-producing Enterobacteriaceae species (Kimera et al., 2021; Ohene Larbi et al., 2022; Tsekouras et al., 2022). Brolund et al. (2014) indicated the possibility of ESBL-producing Enterobacteriaceae displaying multidrug resistance phenotypic characteristics thus compromising the number of options available in treating such pathogens. This shows the potential threat posed by ESBL-producing *E. coli* globally in both human and veterinary medicine.

Resistance against quinolones is also emerging in *E. coli* species worldwide. Acquisition of resistance among the Enterobacteriaceae species has been described to be complex and multifactorial; and the mechanisms of resistance include target site gene mutations, down-expression of porins, over-expression of AcrAB –tolC multidrug-resistant efflux pump, and plasmid-mediated resistance namely Qnr protection proteins, plasmid-encoded active efflux pumps such as OqxAB and QepA and acetylation by aminoglycoside acetyltransferase (Azargun et al., 2020). Selection pressure can occur due to resistance mutations resulting in highly resistant strains (Hooper & Jacoby, 2015). Ciprofloxacin-resistant *E. coli* has been documented in human blood cultures in Malawi (Musicha et al., 2017). However, there is limited information on the prevalence of ciprofloxacin resistant *E. coli* in pigs.

Escherichia coli has also been observed to exhibit high resistance against sulphonamides and trimethoprim which are synthetic antimicrobial agents that inhibit

the folic acid synthesis (Puangseree et al., 2021). Three *sul* genes, namely *sul1*, *sul2* and *sul3*, have been reported to confer resistance against sulphonamides while resistance against trimethoprim is conferred by *dfrA* and *dfrB* genes (Poirel et al., 2018). Studies conducted in Thailand, Estonia, Cameroon and South Africa have shown high resistance rates of *E. coli* against trimethoprim-sulfamethoxazole in pigs (Aasmäe et al., 2019; Founou et al., 2019; Puangseree et al., 2021).

2.1.3 Outbreaks associated with *Escherichia coli*

Members of the Enterobacteriaceae family are the major cause of foodborne enteritis and zoonotic infections in animals and humans (Janda & Abbott, 2021). According to Reilly (1998), an outbreak can be defined either as the occurrence of two or more epidemiologically linked cases of the same illness or an increase in the number of unexplained cases above the background level. Outbreaks associated with *E. coli* infections have been reported in both humans and livestock worldwide (Heiman et al., 2015; Wöchtel et al., 2017). Unfortunately, some outbreaks signify the risk of transmitting multidrug-resistant genes (Ahmed et al., 2005; Bumbangi et al., 2022).

Shiga toxin-producing *E. coli* (STEC) causes 2.8 million acute illnesses and 230 deaths worldwide (Majowicz et al., 2014). Studies conducted in South Africa implicated less characterised EAEC as the leading cause of DEC associated with food and waterborne infections in humans (Aijuka et al., 2018). In Malawi, an outbreak of haemorrhagic colitis due to a verotoxin-producing *E. coli* was reported in a Mozambican refugee camp and the illnesses were attributed to the consumption of cooked food from the market (Paquet et al., 1993). The occurrence of such less-characterised pathotypes during outbreaks suggests the risk of highly virulent strains emerging.

2.1.4 Diagnostic techniques

Various techniques, ranging from conventional microbiological tests based on morphological and biochemical characterisation to molecular techniques, can be employed to aid the identification of the Enterobacteriaceae family. However, phenotypic similarities between individual species have made it difficult to accurately identify bacteria (Zakrzewski et al., 2022). Despite not being 100% accurate,

conventional biochemical methods are widely used in many resource-constrained settings to differentiate bacterial strains.

Genotypic techniques, such as phylogenetic analyses, DNA-DNA hybridisation, elongation factor Tu, F-ATPase β sub-unit and *dnaJ* genes, and multi-locus sequence analyses (MLSA), can be used to classify bacterial isolates (Glaeser & Kämpfer, 2015; Hernandez-Alonso et al., 2021). The matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) continues to gain ground as a conventional molecular diagnostic technique due to its improved turnaround time and accuracy (Osa et al., 2021). Despite these molecular techniques being highly sensitive, their complexity, need for trained personnel as well as expensive and specialised equipment limit their usage in many countries (Zakrzewski et al., 2022).

2.1.5 Prevention, treatment and control

In veterinary science, prevention and control of *E. coli* depend on interventions such as vaccinations, diet and supplements, use of prebiotics and probiotics, bacteriophage therapy and genetic breeding for resistant herds (Luppi, 2017). The use of some of these bacteriophages and gut microbiota instead of antibiotics is gaining ground due to their effectiveness in the prevention and control of bacterial infection and in reducing the transmissibility of antibiotic-resistant genes (Kunishima et al., 2019; Nikkhahi et al., 2017). Zeolite, a porous alumina-silicate mineral, has proved to be effective in reducing bacteria due to its bactericidal properties (Prasai et al., 2017). However, it is important to understand the pathotypes, virotypes and risk factors that facilitate the occurrence of diseases to effectively manage *E. coli* infections (Luppi, 2017).

The use of antimicrobials in the treatment of infections caused by *E. coli* species varies worldwide (Luppi, 2017). Carbapenems have been considered a drug of choice for the empirical treatment of patients with ESBL-producing Enterobacteriaceae infections (Karaiskos & Giamarellou, 2020). Variations in the efficacy of some antibiotics such as enrofloxacin, apramycin, ceftiofur, neomycin, gentamicin, colistin, and amoxicillin/clavulanic acid in the treatment of *E. coli* infections have been reported (Kowalczyk et al., 2022; Luppi, 2017).

2.2 OVERVIEW OF *ENTEROCOCCUS* SPECIES

2.2.1 Introduction

Enterococcus is a Gram-positive, catalase-negative, non-spore-forming, non-motile, facultatively anaerobic bacteria that usually appears spherical and occurs as single cocci or chains (Arias & Murray, 2015). The organisms were previously considered streptococci until 1984 when they were reclassified as a separate genus (Schleifer & Kilpper-Bälz, 1984). The WHO has included *Enterococcus* as one of the priority pathogens in a group comprising *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.* (ESKAPE), which can escape the biocidal actions of antibiotics, due to rising cases of nosocomial and antimicrobial resistance (Mulani et al., 2019; Pendleton et al., 2013). Enterococcal species are generally classified into 5 physiological groups on the basis of hydrolysis of arginine and acid production from mannitol and sorbose (Teixeira et al., 2015). There are 58 recognised species so far with *E. faecalis* and *E. faecium* as the most clinically important and commonly found species (Arias & Murray, 2015). Surface components for members of this family include polysaccharide capsules, adhesins, pili, and aggregation substances (Hancock et al., 2014). *Enterococcus* species are capable of secreting virulence factors such as bacteriocins, hemolysins/cytolysin, gelatinase, and serine protease, enhancing their pathogenicity (García-Solache & Rice, 2019).

Enterococcus members can be found in water, sewage, soil, plants, and humans (Janda & Abbott, 2021). The organisms are quite resilient and capable of surviving hostile environments such as temperatures ranging from 5 to 50°C and a broad range in pH and hypotonic and hypertonic conditions (Huycke et al., 1998). In the food industry, certain strains of *Enterococcus* have been used to add taste and flavour to traditional cheese due to their high lipolytic and proteolytic activity, citrate utilisation, and production of aromatic volatile compounds (Taban et al., 2014). They can also be used in the production of some traditionally fermented food products and as probiotics (Fisher & Phillips, 2009; Foulquié Moreno et al., 2006).

Although most members of this family are relatively harmless, they have been associated with increased resistance to antimicrobial agents thus raising public

concerns (Silva et al., 2012). Similarities in terms of pathogenicity, antimicrobial resistance patterns, virulence gene profile and multilocus sequence typing/pulsed-field gel electrophoresis (MLST/PFGE) types between isolates obtained from humans and pigs have been reported (Larsen et al., 2011; Shankar et al., 2006). These pathogens have been implicated in causing urinary tract infections, infective endocarditis, intra-abdominal infections, soft tissue infections, and meningitis in humans (Arias & Murray, 2015).

2.2.2 Epidemiology of antimicrobial-resistant *Enterococcus* species

Enterococcus species are considered normal inhabitants of the intestines of humans and animals hence commonly isolated worldwide. These pathogens serve as reservoirs of resistant genes which can easily be transferred to humans and other animals (Badul et al., 2021). They have demonstrated intrinsic resistance to clindamycin, aminoglycosides, cephalosporins, sulphonamides and trimethoprim-sulfamethoxazole and increased acquired resistance to vancomycin (García-Solache & Rice, 2019). The emergence of multidrug-resistant species, which has led to high morbidity and mortality, remains a great public health concern (Herc et al., 2017). The use of tetracycline and enrofloxacin in the treatment of infectious diseases in food-producing animals has contributed to the selection of resistant enterococci which are often resistant to vancomycin (González-Fandos et al., 2022).

Studies conducted in South Africa found that the highest number (80.3%) of isolates were resistant to tetracycline and the least resistance (3.2%) was against nitrofurantoin (Badul et al., 2021). Vancomycin, ampicillin, erythromycin, tetracycline, ciprofloxacin, norfloxacin, and rifampin resistance have also been reported in Thailand (Chopjitt et al., 2024). Vancomycin resistance has also been registered in many African countries namely South Africa (74.8%), Egypt (37.2%), Uganda (9.8%), Morocco (8.2%), Ethiopia (7.9%), Tunisia (6.5%), Tanzania (6.1%), Nigeria (2.8%) and Algeria (2.8%) (Alemayehu & Hailemariam, 2020). High-level resistance to aminoglycosides, cephalosporins and semisynthetic penicillin has also been reported in other studies (Haslam & St. Geme, 2023; Lebreton et al., 2014).

2.2.3 Diagnostic techniques

Enterococcus species can present as individuals or as pairs, chains, or groups. Considering that biochemical tests cannot outrightly differentiate *Enterococcus* from other Gram-positive catalase-negative cocci, culture and molecular techniques have been employed to isolate and identify the bacteria (García-Solache & Rice, 2019). Colonies often appear cream, grey or white, 1-2 mm in diameter, and have a regular and circular shape with a smooth surface (PHE, 2021). Most members are capable of growing in the presence of sodium azide (up to 0.4%) and salt (as high as 6.5%); hydrolyses esculin in the presence of 40% bile salts; and produce pyrrolidonyl arylamidase and acetoin (Švec & Devriese, 2015). Furthermore, the production of acids from β -glucosidase, leucine arylamidase, salicin, N-acetylglucosamine, cellobiose, methyl β -D-glucoside, amygdalin, arbutin, β -gentiobiose, glucose, lactose, D-fructose, galactose, ribose, maltose, d-mannose, and trehalose is considered as a typical characteristic feature amongst most members (García-Solache & Rice, 2019). They are generally resistant to desiccation and urease-negative (Švec & Devriese, 2015). However, enterococcal species are not capable of producing acid from sugars D-arabinose, D- and L-fuctose, erythritol, and L-xylose and the glycoside methyl α -D-xyloside, (García-Solache & Rice, 2019). Selective media, consisting of sodium azide, bile salts, and/or antibiotics as selective components and tetrazolium or esculin as indicator substances, have been developed for the isolation of enterococcus based on their biochemical properties (Teixeira et al., 2015).

Commercially made diagnostic systems can be used as alternatives to conventional testing methods. These commercial systems include the Crystal Rapid Gram-Positive and the Crystal Gram-Positive identification systems (Becton Dickinson Microbiology Systems, Sparks, MD), the API Rapid ID32 STREP system and the API 20 Strep (bioMérieux, France), and the commercial identification panels on BD Phoenix systems (Becton Dickinson Microbiology Systems, Sparks, MD), Vitek (bioMérieux), and MicroScan (Dade MicroScan, West Sacramento, CA). However, these commercial testing systems may not be reliable when testing *E. faecium* hence the need for additional tests to improve accuracy (Teixeira et al., 2015).

Molecular diagnostic techniques have proved to be more reliable in identifying bacteria to the species level. Some of the molecular techniques commonly used in the classification and identification of enterococci include matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS), peptide nucleic acid fluorescent in situ hybridization (PNA-FISH), nucleic acid amplification tests (NAAT), and the multilocus sequence typing (MLST) (García-Solache & Rice, 2019).

The matrix-assisted laser desorption ionisation-time of flight mass spectrometry-based platforms, such as Microflex LT Biotyper (Bruker Daltonics, Germany) and Vitek MS System (bioMérieux, France), can be used in the identification of isolates to the species level, differentiation of closely related species, and antimicrobial resistance profiling (Teixeira et al., 2015). The NAAT methods can identify organisms' genetic material to genus or species level and detect antimicrobial-resistant genes. However, the technique cannot accurately differentiate species within a group of species (García-Solache & Rice, 2019). The MLST method has replaced the pulsed-field gel electrophoresis (PFGE) technique in understanding the molecular epidemiology of pathogens and investigating disease outbreaks due to high reproducibility and easier implementation (García-Solache & Rice, 2019).

2.2.4 Prevention, treatment and control

Considering that treatment options for *Enterococcus* infections are limited, implementing measures that minimise the dissemination of resistant organisms is imperative. Environmental cleaning, decontamination using antiseptics, adhering to barrier precautions and cohorting colonised and/or infected patients, active periodic surveillance cultures, and antimicrobial stewardship are key in preventing and controlling pathogens (Higuita & Huycke, 2014; Reyes et al., 2016). The inclusion of antimicrobial stewardship helps minimise inappropriate or excessive antibiotic prophylaxis and therapy which may exacerbate resistance (Muto et al., 2003).

Treatment of enterococcal infections usually involves combining different agents that can synergistically achieve bactericidal effects. Monomicrobial infections can be treated with β -lactams that demonstrate in vitro activity namely penicillin, ampicillin, and piperacillin alone and vancomycin (García-Solache & Rice, 2019). Polymicrobial infections require a combination of ampicillin and other antimicrobial agents (Higuita

& Huycke, 2014). Chloramphenicol, linezolid and daptomycin are recommended as drugs of choice in the treatment of vancomycin-resistant enterococcal infections (Chopjitt et al., 2024). Despite having bacteriostatic effects and associated toxicity, linezolid remains the only drug which has been approved by the United States Food and Drug Administration for use in the treatment of VRE bacteraemia (Khan et al., 2022). In other countries, daptomycin is commonly used as the treatment of choice for VRE bloodstream infections (García-Solache & Rice, 2019). However, resistance against daptomycin and linezolid have been reported (Fiore et al., 2019). Tigecycline has also been used in the treatment of complicated soft tissue, skin, and intra-abdominal infections (Santimaleeworagun et al., 2020).

2.3 FOOD SAFETY KNOWLEDGE, ATTITUDES AND PRACTICES AMONG PIG BUTCHERS

2.3.1 Introduction

Foodborne diseases are the major cause of morbidity and mortality worldwide hence a growing public health concern (Aidara-Kane et al., 2016). Food outbreaks have a huge impact on human health and can cause severe economic losses to people, businesses, and the country (Adley & Ryan, 2016). A foodborne disease outbreak can be described as the occurrence of two or more cases exhibiting similar clinical signs after ingestion of a common contaminated food or drink (WHO, 2017). These outbreaks in humans have mostly been attributed to failure or inability to control microorganisms in the food supply chain resulting in consumption of contaminated animal products (Jajere, 2019). In low- and middle-income countries (LMIC), food safety risks have been exacerbated by poor infrastructure, lack of regulatory oversight, and environmental conditions (Wallace et al., 2022). Approximately 2 billion illnesses and 1.09 million deaths reported in 2010 were attributed to foodborne diseases with the majority being under-five children from Africa and South East Asia (Kirk et al., 2015).

The World Health Organisation describes safe food as one which is not contaminated, at any point of production and distribution, by harmful microbes, parasites, toxins, chemicals or radionuclides (WHO, 2022). Viruses, bacteria and parasites have all been implicated as the main biological hazards which cause foodborne diseases worldwide (Todd, 2014). Dissemination of these pathogens has been attributed to improper food handling during handling, preparation and storage have been identified as some of the possible ways (C.A.C, 2003). Animal-source foods are considered as potential food risks associated with foodborne disease outbreaks usually in household settings (Li et al., 2019).

Although regulatory frameworks governing the meat sector exist, adherence has always been a challenge, especially in the informal sector (Grace et al., 2019). Most inspections in many slaughter places rely on visual assessments without consideration of microbiological tests thus putting the lives of consumers at risk (Rani et al., 2017). Implementation of highly formalised preventive risk-based approaches, such as

Hazard Analysis and Critical Control Points (HACCP) and Good Management Practices (GMPs) by smaller-scale operators or producers, have proved to be challenging in low-resource settings (Waldman et al., 2020). Although lack of awareness among food handlers and consumers has always been cited as the biggest setback, studies have not demonstrated any relationship between access to the right information and behavioural change or adoption (Waldman et al., 2020). Few studies on understanding the fundamental role meat handlers play in food safety have been conducted in Malawi. Therefore, assessing their knowledge, attitudes and practices concerning food safety and occupational hazards is crucial in dealing with zoonotic diseases.

2.3.2 Food safety knowledge

Knowledge about personal hygiene, cross-contamination, and foodborne pathogens is vital to ensuring food safety. Uzoama et al. (2023) indicated that the level of knowledge of food handlers has a positive correlation with the age, educational status, work experience, supportive supervision, and location of the slaughter. Studies conducted in Cameroon, Trinidad and Tobago, Iran and Ghana demonstrated that the educational status and experience of food handlers have no considerable influence on food safety knowledge (Annor & Baiden, 2011; Matchawe et al., 2019; Webb & Morancie, 2015; Yacoob & Tahir, 2016). Despite finding no correlation, most meat handlers have unsatisfactory level of knowledge about food safety (Tegegne & Phyo, 2017). To improve the level of knowledge, Raji et al. (2021) recommended training on food hygiene as an intervention to improve the knowledge gap existing among food handlers.

2.3.3 Food safety attitudes

Attitude is considered to have a strong effect on the level of practice among meat handlers (Yenealem et al., 2020). Meat handlers' level of knowledge has a significant effect on the level of attitude (Sarma et al., 2022). However, other factors such as cognitive dissonance theory and financial constraints may impede the translation of a positive attitude into good practice (Matchawe et al., 2019).

2.3.4 Food safety practices

Implementing good hygienic practices during slaughtering and proper carcass handling can significantly reduce the risk of cross-contamination of carcasses with foodborne pathogens (Ansari-Lari et al., 2010). Meat contamination can occur during evisceration due to inappropriate handling practices thus undermining the quality and safety of meat and consequently posing a health risk to consumers (Greig et al., 2007; Nyamakwere et al., 2017). Ansari-Lari et al. (2010) documented that improved knowledge and excellent attitudes do not always translate into good practices. Additionally, other factors such as sanitation and hygiene of the slaughtering establishment and personal hygiene have a negative association with incidences of foodborne diseases (WHO, 2006). The informal HACCP principles and Good Hygienic Practices must be adopted in slaughtering places to significantly reduce the risk of meat contamination (Mortimore & Wallace, 2013).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study location

The study was conducted in four districts in the northern part of Malawi namely Nkhata-Bay, Rumphu, Mzimba and Karonga (Figure 1). The northern region borders the central region in the south, Zambia in the west, Tanzania in the north and northeast, and Mozambique in the east. The majority of the inhabitants are engaged in agriculture; and indigenous chickens, pigs, goats and cattle are the dominant livestock species (MoAIWD, 2016). According to the country's Agricultural Production Estimates and Surveys figures for 2023, the northern region has 560,576 cattle, 1,003,273 goats, 35,436 sheep, 1,019,985 pigs and 11,736,415 chickens (MoA, 2023). The total human population of the northern region is estimated to be around 2,289,780 thus 1,114,497 males and 1,175,283 females (NSO, 2019).

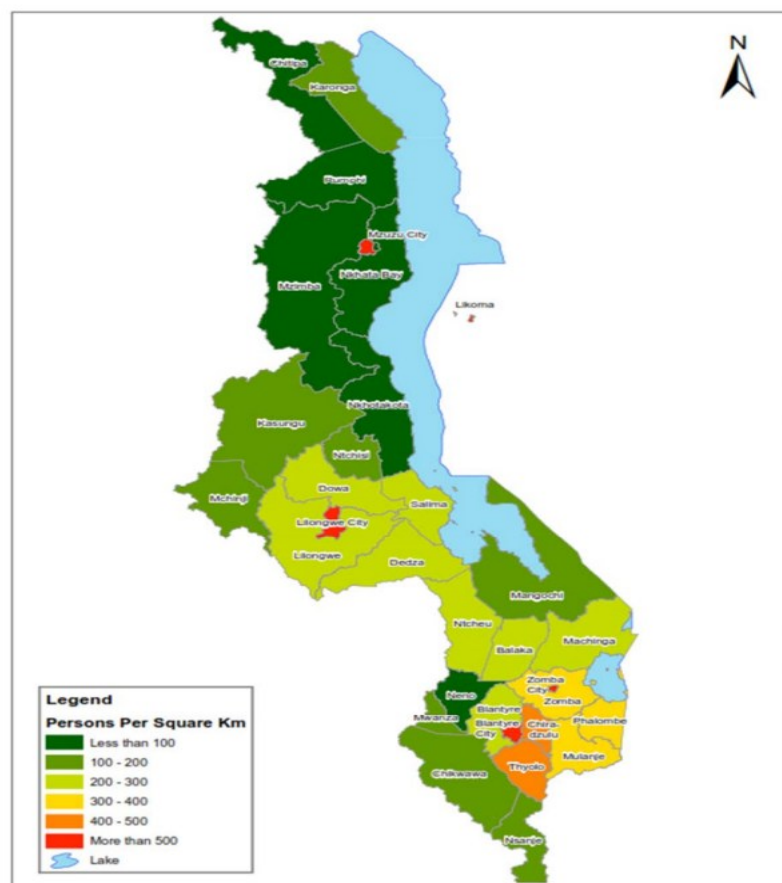


Figure 1: Map of Malawi showing population density (NSO, 2019)

3.2 Study design

A descriptive cross-sectional study was conducted to estimate the prevalence of antimicrobial-resistant *E. coli* and enterococcal species in slaughtered pigs in northern Malawi. The study involved isolation, identification and antimicrobial susceptibility testing of *E. coli* and *Enterococcus* species from pig caecal samples. Knowledge, attitudes and meat hygiene practices of the butchers were assessed using semi-structured questionnaire.

3.3 Sampling strategy: Inclusion and exclusion criteria

Healthy pigs slaughtered at selected establishments at the time of sample collection were included in the sampling unit: For respondents, only butchers above 18 years of age who consented to the study were included.

Clinically sick pigs and those not slaughtered at a selected establishment at the time of sample collection were excluded from the sampling unit: Butchers who were below 18 years of age or did not consent to the study were excluded.

3.4 Sampling methods and sample size

The stratified random sampling method was used during the selection of the sampled slaughtering establishments. The slaughter places were categorised into 3 groups namely slaughterhouse, slaughter slab and makeshifts (without structures) based on availability of structures and designs. Caecal samples from pigs, slaughtered at the time of sampling, were collected aseptically for a period of three months. The questionnaires were administered to butchers who were conveniently sampled and present at the time of sample collection.

The study enrolled 271 caecal samples from all the selected slaughtering establishments. The sample size was calculated based on the expected true proportion of 50% at 90% confidence level and 5% margin of error.

3.5 Sample collection and transportation

Pig caecal samples were collected from pigs slaughtered at each slaughter slab/house using sterile gloves and scalpel blades. The samples were placed in 2 ml of transport

media which were packed in Ziploc bags and then put in a cool box at a temperature ranging from 4 to 8°C. The samples were immediately taken to Mzuzu Regional Veterinary Laboratory for bacteriological analysis.

3.6 Data collection tools and procedure

Data was collected at all slaughtering establishments using a semi-structured questionnaire. The questionnaires included socio-demographic information and the butchers' knowledge, attitudes and meat handling practices. The questionnaire was translated into a local language by an interpreter and pre-tested to improve its reliability and validity. Trained veterinary paraprofessionals were used to administer the questionnaires face-to-face using KoboToolbox®.

To ensure data integrity is maintained, raw data was saved in Excel (.xlsx format) before processing. Backups were kept separately to avoid data loss. Data cleaning was performed to improve the accuracy and quality of the data.

3.7 Questionnaire Design

The Knowledge, Attitude and Practices (KAP) questionnaire was used to assess study participants' knowledge level, their attitude towards food safety, and conformity to hygienic practices. The questionnaire consisted of four parts namely sociodemographic, knowledge, attitude, and practices questions. There were 5 questions on knowledge, 6 on attitude, and 9 on practices. Three questions on knowledge were rated “Yes” = 1 or “No” = 0, except for two open-ended questions. On attitude, responses were rated “agree” = 2, “not sure” = 1, or “disagree” = 0 and “important” = 2, “not important” = 0, or “not sure” = 1. Practice question responses were rated “yes” = 2, “no” = 0, and “not sure” = 1, except for the question where participants were required to indicate the treatments used at least two weeks before bringing their pig(s) for slaughter. Cumulative scores of the respondents were classified into “good” if the score was above 80%, “moderate” if the score was between 60 – 79% and “poor” for scores below 60% in case of knowledge and practices. The level of the respondent's attitude was categorised into “positive”, “neutral” or “negative”. The cut-off points were based on Bloom's cut-off points which were used in previous studies (Karunamoorthi, 2004; Seid & Hussien, 2018).

3.8 Bacterial culture, isolation and identification

Samples were processed and tested following the recommended standard procedures. The swabs were removed from the transport media and inoculated on nutrient, blood, MacConkey, and XLD agar (Merck KGaA, Darmstadt, Germany). The agar plates were incubated aerobically for 24 hours at 37°C. Colony characteristics were observed before performing Gram staining. Cell morphology identification was performed using a 100x objective of a light microscope (Leica Microsystems GmbH, Germany). Single pink colonies from MacConkey agar, presumed to be *E. coli*, were subjected to conventional biochemical tests namely Triple Sugar Iron (TSI), Citrate, Nitrate, Urease broth, Methyl Red, and Motility Indole Ornithine (MIO) (Merck KGaA, Darmstadt, Germany) to confirm the bacterial isolates. Lactose positive isolates that were Indole positive were considered to be *E. coli*. Positive reaction to L-Pyrrolidonyl- β -naphthylamide (PYR) broth, Voges Proskauer (VP) and Bile Esculin Agar (from pure cultures on blood agar) and a negative catalase result were used to confirm *Enterococcus* colonies.

3.9 Antimicrobial Susceptibility Testing

The disk diffusion (modified Kirby-Bauer) method was performed to ascertain susceptibility against the antibiotic panel, recommended by WHO-AGISAR, in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Direct colony suspension method was used to suspend the colonies of the appropriate organism, cultured overnight on non-selective media, in saline to a density of 0.5 McFarland turbidity standard. The standardised inoculum was spread evenly on Mueller-Hinton agar (Oxoid, Hampshire, England). Seven commercially available antibiotic susceptibility disks (Oxoid, Hampshire, England) were used to determine their resistance profiles of the isolates.

Escherichia coli isolates were screened for resistance to 6 antibiotic agents namely ampicillin (10 μ g), trimethoprim sulfamethoxazole (10 μ g), cefotaxime (30 μ g), gentamicin (10 μ g), tigecycline (15 μ g), and ciprofloxacin (5 μ g). Resistance profile of *Enterococcus* species to ampicillin (2 μ g), vancomycin (30 μ g), tigecycline (15 μ g) and ciprofloxacin (5 μ g) was also determined. The inhibition zones were measured in millimetres, using a ruler, and interpreted as either susceptible or resistant following

the guidelines. Pathogens exhibiting resistance to at least one agent in three or more classes of antimicrobial categories were considered multidrug-resistant.

3.10 Quality control

The procedures were conducted according to manufacturers' instructions. Appropriate Quality Control (QC) reference strains namely *E coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used to check for growth and reaction.

3.11 Statistical analysis.

Data were analysed using R package version 4.3.2 (R Core Team, 2024) and presented using frequencies and percentages. The statistical differences between the socio-demographic profile of carcass handlers and their KAP levels were determined using Chi-square. Pearson correlation coefficient was used to measure the association between variables. The p-value of less than 0.05 was considered statistically significant.

3.12 Ethical considerations

Ethical approval was obtained from the Research Ethics Committee (Reference: REC 153-22; approved on 8 February 2023), Animal Ethics Committee (Reference: REC 153-22; approved on 6 March 2023) and the Human Ethics Committee (Reference: HUM006/0223; approved on 6 July 2023) of the University of Pretoria. The Animal Health Committee in the Department of Animal Health and Livestock Development also approved the study to be conducted in Malawi (Reference: DAHLD/01/2023/1; approved on 4 January 2023). Informed consent was obtained from all respondents before commencement of data collection.

CHAPTER 4: RESULTS

4.1 Knowledge, attitude and practices of pig butchers in northern Malawi

4.1.1 Sociodemographic characteristics of respondents

Respondents came from four districts in the northern part of Malawi and Mzuzu which is an urban area. Only 75 meat handlers consented to participate in the study. In terms of sex, males constituted 97% of the total respondents, while females constituted 3% only. The 18 – 35 age group was the majority, constituting 64%, followed by those in the range of 36 and 50 years (32%) and those above 50 years of age were the least at 4%. Differences in terms of the educational levels of the respondents were observed with the majority of them, comprising 53%, reporting not having gone beyond primary education, 43% having completed secondary education, and 4% managed to attain tertiary education qualifications. The majority of the respondents had less than 5 years of work experience (43%), followed by those with 5 to 10 years of experience (27%) while 17% were those with more than 5 years of experience (Table 1).

Table 1: Summary of the sociodemographic characteristics of the respondents

Variable	Category	Frequency	proportion (n = 75)
Location	Mzimba	13	17.33%
	Nkhata Bay	5	6.67%
	Mzuzu	29	38.67%
	Rumphi	19	25.33%
	Karonga	9	12.00%
Gender	Male	73	97.33%
	Female	2	2.67%

Age	18 - 35 years	48	64.00%
	36 - 50 years	24	32.00%
	>50 years	3	4.00%
Education	Primary and below	40	53.33%
	Secondary	32	42.67%
	Tertiary	3	4.00%
Experience	<5 years	32	42.67%
	5 - 10 years	20	26.67%
	>10 years	13	17.33%

The majority of the respondents operated from places without structures (73%), whereas 24% operated from slaughter slabs and 3% were using slaughterhouses (Table 2). In Nkhata Bay and Rumphu districts, all the respondents (25% and 7% respectively) utilised places without slaughter structures. Despite Mzuzu having a slaughterhouse, the majority of the respondents were utilising undesignated slaughter points (17%) or slaughter slabs (19%) where meat inspection was rarely done.

Table 2: Distribution of sampled slaughter places in northern Malawi

Type	Districts					Total
	Karonga	Mzimba	Mzuzu city	Nkhatabay	Rumphi	
Place without structures	6 (8%)	12 (16%)	13 (17%)	5 (7%)	19 (25%)	55 (73%)
Slaughterhouse	0 (0%)	0 (0%)	2 (3%)	0 (0%)	0 (0%)	2 (3%)
Slaughter slab	3 (4%)	1 (1%)	14 (19%)	0 (0%)	0 (0%)	18 (24%)
Total	9 (12%)	13 (17%)	29 (39%)	5 (7%)	19 (25%)	75 (100%)

4.1.2 Knowledge of the respondents about food safety

The study found that 59% of the 75 participants knew what occupational health hazards were. However, the majority of the respondents (55%) had little to very little knowledge of zoonotic diseases which can be disseminated during the slaughtering process. It was also noted that 80% of the respondents were not familiar with antimicrobial resistance and the role of food animals in AMR (Table 3).

Table 3: Butchers' knowledge of good hygienic practices in slaughter places

Factors of knowledge	Category	Frequency (n=75)	Proportion (%)	KAP Score
Knowledge of occupational health hazards	Yes	44	58.67	41%
	No	31	41.33	
Knowledge of zoonosis	Yes	34	45.33	54.67
	No	41	54.67	
Knowledge of AMR	Yes	15	20	80
	No	60	80	

In terms of overall scores, the majority of the respondents (81%) had poor knowledge of food safety and occupational health risks, and these were followed by 13% and 5% of the respondents with moderate and adequate, respectively. Differences were also observed when the five sites were compared in terms of their levels of knowledge. It was observed that Rumphi had the lowest score in terms of knowledge with 100% of the respondents falling within the poor knowledge category. Butchers from Mzuzu, Karonga and Mzimba also scored poorly with about 83%, 78% and 77% of them falling in the poor knowledge category. Nkhata Bay registered a higher proportion of respondents with good knowledge of food safety and occupational health risks. The mean score of the respondents on knowledge was 33%. There were statistically significant relationships ($p < 0.005$) among the four sites in terms of the level of knowledge (Table 4).

Table 4: Food safety knowledge among butchers in the northern part of Malawi

Parameter	Good knowledge	Moderate knowledge	Poor knowledge	Sum	p-value
District					
Karonga	1 (11%)	1 (11%)	7 (78%)	9 (100%)	$p < 0.005$
Mzimba	0 (0%)	3 (23%)	10 (77%)	13 (100%)	
Mzuzu	0 (0%)	5 (17%)	24 (83%)	29 (100%)	
Nkhata Bay	3 (60%)	1 (20%)	1 (20%)	5 (100%)	
Rumphi	0 (0%)	0 (0%)	19 (100%)	19 (100%)	
Age					
18 - 35	2 (4%)	5 (10%)	41 (85%)	48 (100%)	$p = 0.138$
36 - 50	1 (4%)	4 (17%)	19 (79%)	24 (100%)	
> 50 years	1 (33%)	1 (33%)	1 (33%)	3 (100%)	

Education					
Primary and below	1 (3%)	4 (10%)	35 (88%)	40 (100%)	p=0.118
Secondary	2 (6%)	5 (16%)	25 (78%)	32 (100%)	
Tertiary	1 (33%)	1 (33%)	1 (33%)	3 (100%)	
Experience					
<5 years	2 (6%)	6 (19%)	24 (75%)	32 (100%)	0.623
5 - 10 years	1 (3%)	2 (7%)	27 (90%)	30 (100%)	
>10 years	1 (8%)	2 (15%)	10 (77%)	13 (100%)	

The majority of the participants (85%) of youth (18 – 35 years of age) had poor knowledge about food safety and occupational health risks. The middle group (36 – 50 years) ranked second (79%) in terms of being less knowledgeable, and those above 50 years of age were the least (33%). However, there were no statistically significant differences ($p=0.138$) among the three age groups (Table 4).

In terms of education levels and knowledge, those who attained primary school level or below were rated high (88%) in terms of having poor knowledge of food safety and occupational health risks. The group who reached the secondary school level constituted 78% of the respondents with poor knowledge and 33% were those in the tertiary group. It was observed that there were no statistically significant differences ($p=0.118$) among the three categories (Table 4). Remarkably, most respondents who acknowledged knowing occupational zoonotic diseases indicated cysticercosis (37%, 16/43) and African Swine fever (30%, 13/43) as some of the major occupational health risks. About 4.7% ($n = 2/43$) of the respondents failed to mention any occupational zoonotic disease despite responding to be knowledgeable (Figure 2).

Responses on zoonotic diseases

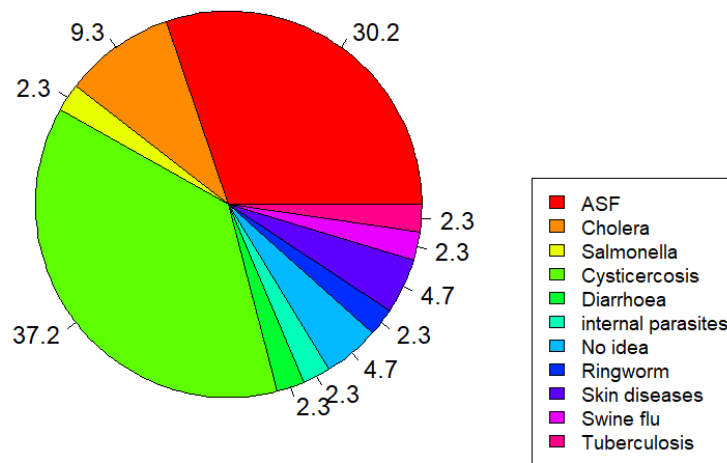


Figure 2: Responses on zoonotic diseases posing occupational risk to butchers

The study revealed that 90% of those in the range of 5 to 10 years of work experience had poor knowledge about food safety and occupational health risks, followed by 77% for those with more than 10 years of work experience, and those with less than 5 years constituted 75%. There were no statistical differences among the three work experience groups ($p = 0.623$) (Table 4).

4.1.3 Attitude towards good hygienic practices

The study revealed that 73% of the respondents in the districts had a good attitude towards good hygienic practices, followed by 23% who were neutral and 4% with a poor attitude. Karonga and Nkhata Bay had the largest proportion of respondents (100%), 97% in Mzuzu, 46% in Mzimba and 37% in Rumphi. Rumphi was the only district that registered a poor attitude (16%). There were significant differences among the districts in terms of the level of respondents' attitudes ($p < 0.005$) (Table 5).

Table 5: Food safety attitudes among pig butchers in northern Malawi

Parameter	Good attitude	Neutral attitude	Poor attitude	Sum	p-value
District					
Karonga	9 (100%)	0 (0%)	0 (0%)	9 (100%)	p<0.005
Mzimba	6 (46%)	7 (54%)	0 (0%)	13 (100%)	
Mzuzu	28 (97%)	1 (3%)	0 (0%)	29 (100%)	
Nkhata Bay	5 (100%)	0 (0%)	0 (0%)	5 (100%)	
Rumphi	7 (37%)	9 (47%)	3 (16%)	19 (100%)	
Age					
18 - 35	38 (79%)	9 (19%)	1 (2%)	48 (100%)	p=0.272
36 - 50	14 (58%)	8 (33%)	2 (8%)	24 (100%)	
> 50 years	3 (100%)	0 (0%)	0 (0%)	3 (100%)	
Education					
Primary and below	22 (55%)	15 (38%)	3 (8%)	40 (100%)	p=0.005
Secondary	30 (94%)	2 (6%)	0 (0%)	32 (100%)	
Tertiary	3 (100%)	0 (0%)	0 (0%)	3 (100%)	
Experience					
<5 years	27 (84%)	4 (13%)	1 (3%)	32 (100%)	p=0.031
5 - 10 years	16 (53%)	12 (40%)	2 (7%)	30 (100%)	
>10 years	12 (92%)	1 (8%)	0 (0%)	13 (100%)	

There were no statistical differences ($p = 0.272$) among the various age groups in terms of attitude. Most of the respondents with good attitudes (100%) were those above 50 years of age, with those within the range of 18 to 35 years of age constituting 79% while those between 36 and 50 constituted 58% of the respondents (Table 5).

The majority of the respondents (100%) who completed tertiary education had a good attitude towards good hygienic practices while those who attained primary education and below scored the least (55%). About 94% of respondents who reached the secondary school level registered a good attitude towards food safety and occupational health risks. The three groups were statistically different ($p = 0.005$) in terms of their level of attitude (Table 5).

The respondents from the category with more than 10 years of work experience constituted the largest proportion (92%) of those having a good attitude, and these were followed by those with less than 5 years of work experience who constituted 84% of the respondents. The category within the range of 5 to 10 years of work experience had 53% of the respondents. Furthermore, statistically significant differences ($p = 0.031$) were observed among the three groups (Table 5).

The majority of the respondents had a positive attitude towards good hygienic practices as depicted in Figure 4. Despite obtaining high scores, 32% of the total respondents were not sure whether their actions during the slaughtering process could potentially disseminate zoonotic diseases.

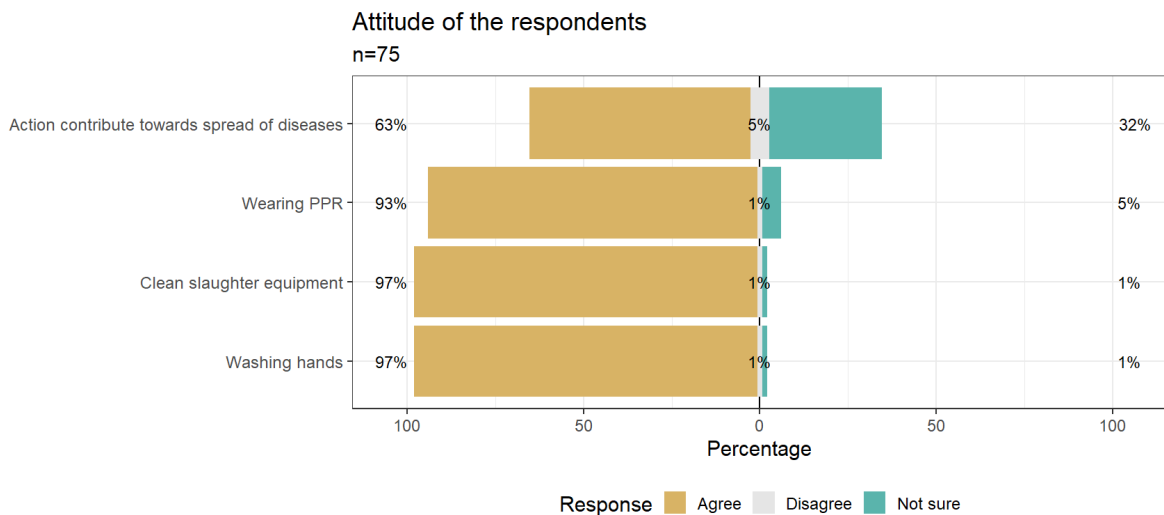


Figure 3: Likert scale on respondent's attitudes towards food safety and occupational health risks

4.1.4 Analysis of pig butcher's practices in relation to food safety

Of the 75 respondents, 46 (61%) were observed to have poor practices, compared to 24 (32%) and 5 (7%) whose practices were moderate and good, respectively. The majority of the respondents (100%) from Rumphi had poor practices followed by Mzimba (92% each). Respondents from Karonga and Mzuzu constituted 89% and 45% respectively in terms of having moderate practices. Statistically significant differences ($p < 0.005$) were observed among the sites (Table 6).

Table 6: Food safety practices among pig butchers in northern Malawi

Parameter	Good practice	Moderate	Poor practice	Sum	p-value
District					
Karonga	0 (0%)	8 (89%)	1 (11%)	9 (100%)	0
Mzimba	0 (0%)	1 (8%)	12 (92%)	13 (100%)	
Mzuzu	4 (14%)	13 (45%)	12 (41%)	29 (100%)	
Nkhata Bay	1 (20%)	2 (40%)	2 (40%)	5 (100%)	
Rumphi	0 (0%)	0 (0%)	19 (100%)	19 (100%)	
Age					
18 - 35	3 (6%)	18 (38%)	27 (56%)	48 (100%)	$p=0.686$
36 - 50	2 (8%)	5 (21%)	17 (71%)	24 (100%)	
> 50 years	0 (0%)	1 (33%)	2 (67%)	3 (100%)	
Education					
Primary and below	1 (33%)	10 (25%)	29 (73%)	40 (100%)	$p=0.099$
Secondary	3 (9%)	13 (41%)	16 (50%)	32 (100%)	
Tertiary	1 (33%)	1 (33%)	1 (33%)	3 (100%)	

Experience					
<5 years	1 (3%)	9 (30%)	20 (67%)	30 (100%)	p=0.085
5 - 10 years	3 (9%)	10 (31%)	19 (59%)	32 (100%)	
>10 years	1 (8%)	5 (38%)	7 (54%)	13 (100%)	

Poor slaughtering practices were registered (71%) among those between 36 and 50 years of age. About 67% of the respondents within the category of those above 50 years of age and 56% of those in the youthful group were noted to have poor practices. However, there were no statistically significant differences ($p = 0.686$) among the three groups (Table 6).

The study found that 73% of the respondents with a primary school certificate or below practised poor hygiene. This was followed by 50% of the respondents who attained the secondary school level and 33% in the tertiary group. Respondents who reached the secondary school level constituted 9% of those practising good hygiene. There were no significant differences ($p = 0.099$) among the three categories (Table 6).

Respondents with less than five years of work experience constituted the largest proportion (67%) of those practising poor hygiene. Butchers in the group of 5 to 10 years and those above 10 years of work experience constituted 59% and 54% respectively. There were no statistically significant differences ($p = 0.085$) among the groups (Table 6).

It was noted that 29% of the 75 respondents were using some form of protective clothing during butchering. However, none of the respondents wore gloves, facemasks, or hairnets. It was also worth noting that 57% of the respondents cleaned their slaughtering tools regularly. On the contrary, 24% did not bother cleaning them during butchering (Figure 4).

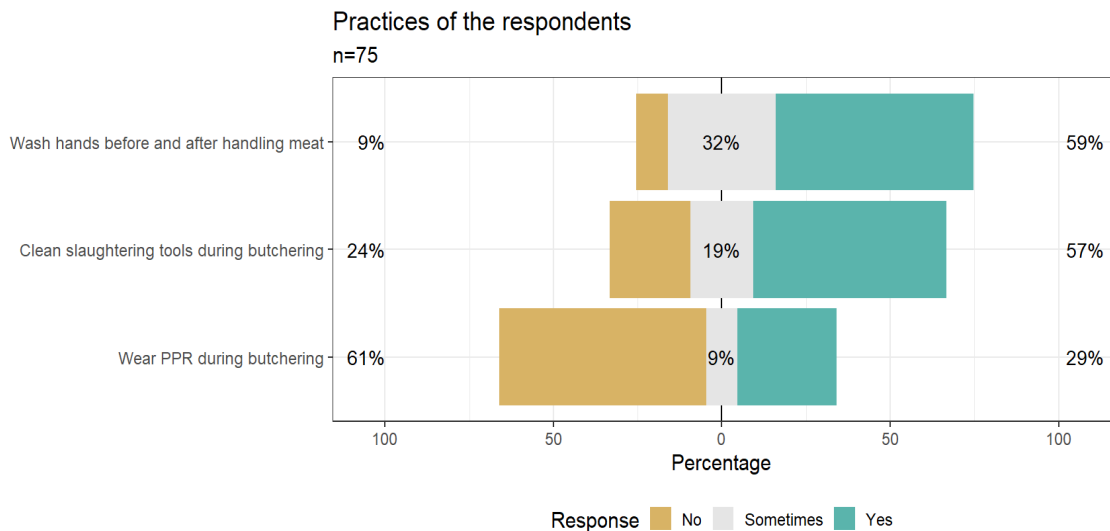


Figure 4: Likert scale on practices of the respondents

The results of the Pearson correlation analysis, illustrated in Table 7, revealed a positive correlation between the butchers' knowledge levels and attitude ($r = .46$, $p < 0.001$) and knowledge levels and their practices ($r = .38$, $p < 0.001$). Similarly, a positive correlation was observed between attitude and practices ($r = .76$, $p < 0.001$).

Table 7: Correlation analysis to determine the direction and strength of the KAP subscales

		Practice	Attitude	Knowledge
Practice	Pearson's correlation	1	0.756**	0.379**
	Sig. (2-tailed)		<0.001	<0.001
Attitude	Pearson's correlation	0.756**	1	0.460**
	Sig. (2-tailed)	<0.000		<0.000
Knowledge	Pearson's correlation	0.379**	0.460**	1
	Sig. (2-tailed)	0.000	0.000	

** indicates correlation is significant at $p \leq 0.01$

4.2 Prevalence of multidrug-resistant *E. coli* and *Enterococcus* species

A total of 349 isolates were obtained from 232 caecal samples of pigs collected from six sites in four districts. The samples were collected from Nkhata Bay (6.9%, n = 24) Rumphi (6.6%, n = 23), Mzimba (25.8%, n = 90), Mzuzu (35.2%, n = 123), and Karonga (25.5%, n = 89) (Table 8). *Escherichia coli* (82%, 191/232) and Enterococci species (68%, 158/232) were isolated from the samples during culture (Table 8).

Table 8: Distribution of *E. coli* and enterococcal species isolates in caecal samples

District/Site	<i>Enterococcus</i>	<i>E. coli</i>	Sum
Karonga	37 (10.6%)	52 (14.9%)	89 (25.5%)
Mzimba	43 (12.3%)	47 (13.5%)	90 (25.8%)
Mzuzu City	58 (16.6%)	65 (18.6%)	123 (35.2%)
Nkhata Bay	12 (3.4%)	12 (3.4%)	24 (6.9%)
Rumphi	8 (2.3%)	15 (4.4%)	23 (6.6%)
Sum	158 (45.3%)	191 (54.7%)	349 (100%)

The disk diffusion test results revealed that bacterial isolates were highly resistant to trimethoprim-sulfamethoxazole (87.7%), vancomycin (82.8%), and ampicillin (74.8%) in Mzuzu. Isolates from samples from Mzimba were also often resistant to vancomycin (72.1%), ampicillin (65.6%) and trimethoprim-sulfamethoxazole (63.8%). Similar findings were registered in Karonga where isolates were often resistant to vancomycin (78.4%), trimethoprim-sulfamethoxazole (75.0%), and ampicillin (73.0%) (Table 9).

Table 9: Resistance patterns for *E. coli* and enterococcal species in the sampled districts

Location	Antibiotic agent						
	AMP	GEN	CIP	TGC	SXT	VAN	CTX
Karonga	73.0%	0.0%	4.5%	0.0%	75.0%	78.4%	7.7%
Mzimba	65.6%	23.4%	11.1%	0.0%	63.8%	72.1%	19.1%
Mzuzu City	74.8%	36.9%	26.0%	0.8%	87.7%	82.8%	16.9%

Abbreviations: AMP: ampicillin, GEN: gentamicin, TGC: tigecycline; CIP: ciprofloxacin, SXT: trimethoprim-sulphamethoxazole, VAN: vancomycin; CTX: cefotaxime,

Escherichia coli resistance to ampicillin was common (82.2%) and this was followed by trimethoprim-sulfamethoxazole (75.8%), gentamicin (22.5%), cefotaxime (13.1%), ciprofloxacin (7.9%), and tigecycline (0.5%). In terms of *Enterococcus* species, resistance against vancomycin, ampicillin and ciprofloxacin were at 79.7%, 50% and 23.4%, respectively (Figure 5). No resistance against tigecycline was observed in any of the *Enterococcus* species. The study found that 26.7% (n=51) of *E. coli* isolates were resistant to more than one class of antibiotics and 10.1% (n=16) for enterococcal species with an overall frequency of 19.2% (n=67) (Table 10).

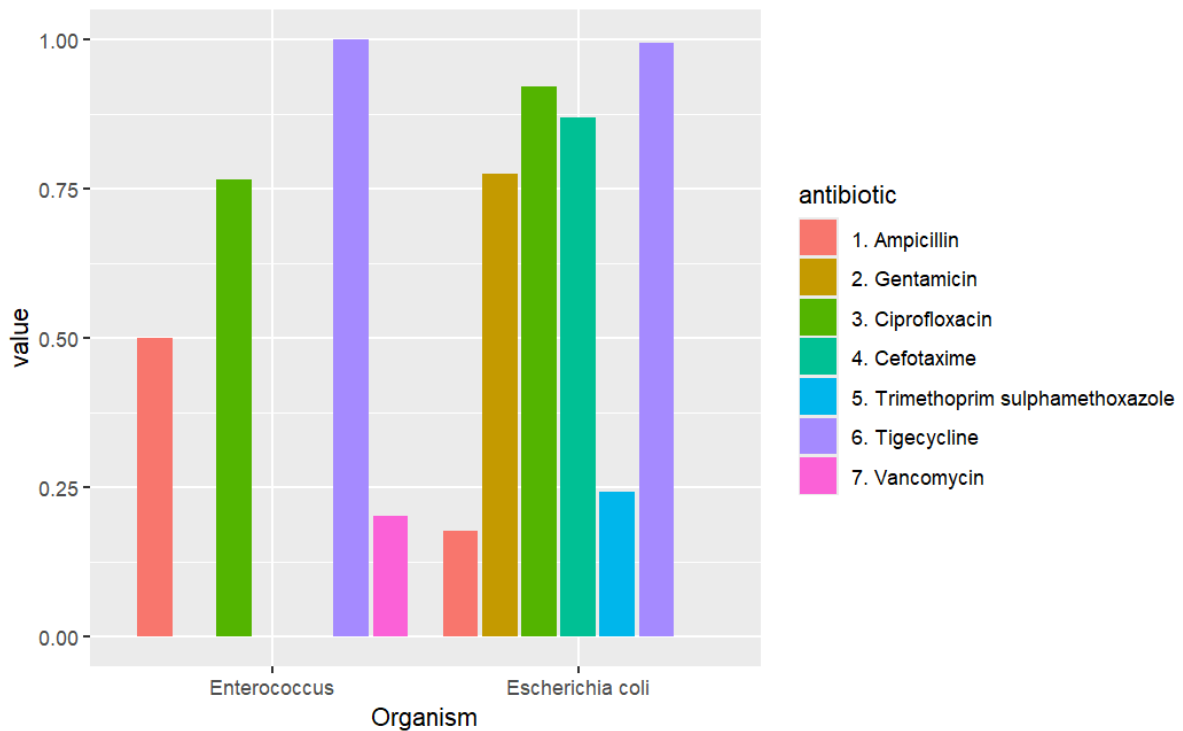


Figure 5: Resistance patterns of *E. coli* and *Enterococcus* species

Table 10: Multidrug resistance patterns of *E. coli* and *Enterococcus* species in northern Malawi

	Freq	% Valid	% Valid Cum.	% Total	% Total Cum.
Overall MDR					
Negative	0	0	0	0	0
Multidrug-resistant (MDR)	67	100	100	19.2	19.2
Extensively drug-resistant (XDR)	0	0	100	0	19.2
Pandrug-resistant (PDR)	0	0	100	0	19.2
<NA>	282			80.8	100
Total	349	100	100	100	100

Escherichia coli

Negative	0	0	0	0	0
Multidrug-resistant (MDR)	51	100	100	26.7	26.7
Extensively drug-resistant (XDR)	0	0	100	0	26.7
Pandrug-resistant (PDR)	0	0	100	0	26.7
<NA>	140			73.3	100
Total	191	100	100	100	100

Enterococcus species

Negative	0	0	0	0	0
Multidrug-resistant (MDR)	16	100	100	10.1	10.1
Extensively drug-resistant (XDR)	0	0	100	0	10.1
Pandrug-resistant (PDR)	0	0	100	0	10.1
<NA>	142			89.9	100
Total	158	100	100	100	100

CHAPTER 5: DISCUSSION

Antimicrobial resistance continues to be a major challenge across the human, animal and environmental sectors. The increased demand for livestock products has increased the risk of infection with resistant microbes of animal origin among consumers and meat handlers. Good hygienic practices play a crucial role in protecting butchers from zoonotic infections and minimising the chances of microbial contamination during meat processing. Thus, implementing interventions at the human, animal and environmental interface remains the only viable way of controlling zoonotic disease transmission. The study, therefore, aimed at assessing the level of knowledge, attitude and practices towards food safety and occupational hazards among butchers and determining the prevalence of multidrug-resistant *E. coli* and *Enterococcus* species in northern Malawi.

The study revealed that 81% of the respondents had limited knowledge about meat safety and occupational health hazards. Similar observations were reported by Gebeyehu and Tsegaye (2022) where 88.9% of the respondents did not have adequate knowledge of food safety. The mean score on meat safety knowledge of 33% was below what was reported in other studies conducted in Morocco (Bahir et al., 2022), Cameroon (Matchawe et al., 2019), and Ethiopia (Urgesa & Sorsa, 2023) where 39%, 49.02, and 56.8%, respectively, were the average scores. The findings were highly plausible since 53.3% of the respondents did not go beyond the primary school level. Educational level and experience have been reported to have a positive correlation with the level of knowledge manifested by meat handlers (Alemayehu et al., 2021; Nyamakwere et al., 2017). However, this has been disputed in other studies conducted in Cameroon and Ghana where educational levels did not significantly influence their knowledge of food safety (Annor & Baiden, 2011; Matchawe et al., 2019). Although Kainga et al. (2023) documented good knowledge of AMR (76.5%) among veterinary drug dispensers in the three main cities of Malawi, the level of knowledge among butchers in the study was poor. This shows the need for advocating antimicrobial stewardship in all spheres of life.

Despite registering low knowledge, it was observed that around 73% of the respondents had a good attitude towards good hygienic practices. The findings were

slightly higher than what was reported by Urgesa and Sorsa (2023) where 68.15% of the respondents had a good attitude on food safety. High positive attitude scores on handwashing (97.3%), cleaning slaughtering tools (97.3%), and wearing protective clothing (93%) were obtained. Similar observations on the attitude of meat handlers were made by Tegegne and Phyo (2017) on hand washing (93.4%) and cleaning of surfaces and utensils (91.2%). Although 73.3% of the respondents indicated that adherence to withdrawal periods was good practice, the study revealed that only 63% of the respondents believed that their actions could result in disease outbreaks. This was worrisome because this could cause laxity in their practices which could result in contamination with pathogenic microbes.

An average score of 46% was obtained when the respondents' practices were assessed. About 29% of the respondents were observed to maintain good food safety practices. The findings were considerably lower than 53.7% of the respondents who were reported to maintain good food safety practices in Ethiopia (Alemayehu et al., 2021). Contrary to the findings documented in Ethiopia (Urgesa & Sorsa, 2023), Morocco (Bahir et al., 2022) and South Africa (Nyamakwere et al., 2017) where all the respondents practised good hand hygiene before handling carcasses, the study found that only 59% practised regular hand hygiene. The use of protective clothing was also noted to be a challenge amongst the majority of the butchers with only 29% reportedly using some form of protective clothing. Studies conducted by Tanganyika et al. (2017) found that contamination on goat carcasses in Lilongwe, Malawi, exceeded acceptable levels due to poor hygiene practices. This shows that the possibility of contaminating pig carcasses during slaughter could not be ruled out. The absence of meat inspectors at such undesignated slaughter places exacerbated the risk of foodborne outbreaks. The Codex Alimentarius Commission (C.A.C), therefore, recommends the use of proper protective clothing and good hand hygiene to avert cross-contamination (C.A.C, 2003).

Pearson's correlation analysis showed a positive correlation between knowledge and attitude, knowledge and practice, and attitude and practice. The findings, however, revealed a discordance between attitude and practices/knowledge. Although the respondents scored high on attitude, it was found that they had poor knowledge of

food safety practices and their practices were contrary to what they believed should be done. High prevalence rates of households with ESBL-contaminated food have been documented in southern Malawi signifying poor hygiene practices (Cocker et al., 2022). This posed a high risk of contaminating food products and the environment.

Escherichia coli was the predominant isolate (82%) which was followed by *Enterococcus* species (68%). This was largely attributed to *E. coli* and some *Enterococcus* species being part of the normal microflora in animals (Guentzel, 1996; Lebreton et al., 2014). However, low detection of the pathogens could be attributed to the sensitivity of the laboratory method and antibiotic usage in pigs prior to slaughter. Studies conducted by Taulo et al. (2008) in Lungwena, Malawi, isolated *E. coli*, *Staphylococcus aureus* and *Salmonella* from 48%, 23% and 61% of the food samples. This shows the potential risk of foodborne outbreaks due to microbial contamination if the situation is not rectified. The translocation of these bacteria, which results in infections, can be exacerbated by several factors including antibiotic misuse and overuse (Vu & Carvalho, 2011).

The study revealed high *E. coli* resistance patterns against ampicillin (82.2%), trimethoprim-sulfamethoxazole (75.8%), gentamicin (22.5%), cefotaxime (13.1%), ciprofloxacin (7.9%), and tigecycline (0.5%). *Enterococcus* species displayed phenotypic resistance against vancomycin (79.7%), ampicillin (50%) and ciprofloxacin (23.4%). *Enterococcus* species did not exhibit any resistance against tigecycline. Studies conducted in pigs under an intensive production system in South Africa revealed almost similar highest and lowest *E. coli* resistance patterns to ampicillin (71.1%), trimethoprim-sulfamethoxazole (61.3%), cefotaxime (16.9%), gentamicin (15.5%) and tigecycline (0.9%) (Abdalla et al., 2022). Badul et al. (2021) observed 26% resistance to ciprofloxacin and 100% susceptibility to tigecycline in *Enterococcus* isolates from pigs under intensive farming. Absence and low tigecycline resistance in *Enterococcus* species and *E. coli* isolates, respectively, were quite encouraging considering that the drug remains a therapeutic option in the treatment of enterococcal and MDR-*E.coli* infections (Mohanty & Mahapatra, 2021; Santimaleeworagun et al., 2020). However, there is a high possibility of resistant pathogens ending up in the food chain or infecting butchers since the majority of them have poor hygiene practices.

The contribution of improper stewardship of antimicrobial agents towards growing resistance in the study cannot be underestimated (Reygaert, 2018). Studies conducted in Malawi revealed that oxytetracycline (27.8%), procaine penicillin (14.8%), sulphonamide (12.1%), trimethoprim-sulfamethoxazole (7.9%) were the most commonly dispensed veterinary drugs. Gentamicin, ampicillin and ciprofloxacin were the least commonly dispensed at 3%, 1.2% and 0.3%, respectively (Kainga et al., 2023). The usage of these antibiotics in food animals has contributed towards selective pressure for the development of resistance (Mollenkopf et al., 2018). Kainga et al. (2023) also found that 89.7% of the participants accessed veterinary drugs without a written prescription. This demonstrates the risk of acquired resistance building up due to either misuse or overuse of antibiotics.

Multidrug resistance among *E. coli* and *Enterococcus* isolates was 26.7% and 10.1% respectively. Similar observations were reported in studies conducted in the southern part of Malawi where the multidrug resistance prevalence rate for *E. coli* was 25% (Kalumbi et al., 2022). Other studies conducted in Uganda (Ikwap et al., 2021), South Africa (Abdalla et al., 2022), and Thailand (Nguyet et al., 2022) found higher *E. coli* multidrug resistance rates to be 47%, 73.83%, and 97.3% respectively. Discrepancies in the resistance rates can be attributed to a limited number of tested antibiotics. Multidrug resistance patterns in the isolates signify the potential transfer of these genes from pigs to humans along the farm-to-fork continuum.

CHAPTER 8: CONCLUSION AND RECOMMENDATION

Escherichia coli and *Enterococcus* species continue to be important pathogens associated with foodborne outbreaks. The study, being the first of its kind in the northern part of Malawi, revealed poor food safety knowledge and hygiene practices among butchers in the pork value chain. The presence of multidrug-resistant *E. coli* and enterococci isolates signifies the risk of exposure to consumers. It is highly recommended that the Department of Animal Health and Livestock Development should ensure that good hygienic practices in the farm-to-fork continuum are adhered to and the capacity of staff and farmers is built to reduce the dissemination of resistant genes in the pork value chain. There is also a need for multi-sectoral collaboration in enforcing food safety regulations. The importance of antimicrobial stewardship should not be underemphasized if optimal utilisation of antimicrobials is to be attained. Exploring microbial contamination in the whole pork value chain using the farm-to-fork approach and characterising resistant pathogens using molecular techniques be considered in future to inform policy on the prevalence of WHO bacterial priority pathogens in the country.

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APPENDICES

Appendix A: Knowledge, attitude and practices study questionnaire



Prevalence of antimicrobial-resistant *Escherichia coli* and enterococcus species in pigs in the northern part of Malawi, and the knowledge, attitude and practices (KAP) among butchers.

ID:

Principal Investigator: Michael Luwe

Supervisor: Dr Annelize Jonker

Introduction

*Thanks for accepting to participate in this study that will enable us understand the resistance patterns of Escherichia coli and enterococcus species in pigs in the northern part of Malawi and the knowledge, attitudes and practices among butchers. Your answers will be **treated with confidentiality** and will be entirely **anonymous** such that they cannot be linked to you in any way.*

*Please answer the questions indicated below which are likely to take about **15 minutes**. You have been selected because you have pigs which were presented for slaughter. However, you may **discontinue participation at any time**.*

Epidemiological Information	
1. District	1. Chitipa 2. Karonga 3. Rumphi 4. Nkhata Bay 5. Mzimba
2. Location of the slaughter point	
3. GPS Coordinates	Lat.: Long.:
4. Type of slaughter point	1. Abattoir 2. Slaughter house 3. Slaughter slab 4. Place without structures
5. How long have you been with the animals? days

Questionnaire v_3 (02/10/2024)

Socio-demographic Information	
6. Age Category	1. 18 – 35 2. 36 – 50 3. Above 50years
7. Level of education	1. Primary School and below 2. Secondary 3. Tertiary
8. Type of trade	1. Farmer 2. Butcher
9. How long have you been handling pigs	1. Less than 5years 2. 5 – 10 years 3. More than 10 years
10. Breeds presented for slaughter at the time of sampling and their quantities	1. Indigenous 2. Exotics
11. Places of origin of the pigs	1. 2. 3.
12. Production system of the pigs	1. Extensive 2. Semi-intensive 3. Intensive
13. Condition of the sampled pigs	1. Healthy 2. Emaciated 3. Others? (Specify)
Knowledge of the Respondents towards Occupational hazards and AMR	
14. Do you know occupational health hazards	1. Yes 2. No
15. Do you know of any diseases which can be spread when handling carcasses	1. Yes 2. No
16. Mention any two diseases which can be spread at a slaughter place ?	1. 2.
17. What do you understand by the term 'antimicrobial resistance'?

Questionnaire v 3 (02/10/2024)

19. Do you know how AMR can occur in pigs?	1. 2.
Attitudes of the Respondents towards Occupational hazards and AMR	
20. Do you think it's necessary to put on protective clothing when handling carcasses?	1. Agree 2. Disagree 3. Not sure
21. Do you think it's necessary to wash your hands after handling carcasses?	1. Agree 2. Disagree 3. Not sure
22. Do you think it's relevant to clean your slaughter equipment and hand tools?	1. Agree 2. Disagree 3. Not sure
23. How do you feel about adhering to withdraw periods when dealing with animals which were on treatment?	1. Important 2. Not Important 3. Not Sure
24. Do you believe your actions can spread diseases from animals to humans?	1. Agree 2. Disagree 3. Not sure
25. Do you think that AMR is a challenge?	1. Yes, both animals and humans 2. Yes, humans only 3. Yes, animals only 4. No
Practices of the Respondents towards Occupation hazards and AMR	
26. Do you put on protective clothing when handling carcasses?	1. Yes 2. No 3. Sometimes
27. Do you wash your hands after handling carcasses?	1. Yes 2. No 3. Sometimes
28. Do you hang the carcass during evisceration	1. Yes 2. No 3. Sometimes

Questionnaire v_3 (02/10/2024)



29. Do you clean knives during slaughtering process?	1. Yes 2. No 3. Sometimes
30. Do you clean knives after slaughtering process?	1. Yes 2. No 3. Sometimes
31. If 'Yes/sometimes', what do you use to clean your knives during and after slaughter?
32. If you have reared the pig(s) for at least 2 weeks, has it been on treatment during the period?	1. Yes 2. No 3. Others? (Specify).....
33. If "Yes", please provide the name of the medication used	1. 2. 3.
34. How can your actions contribute towards lessening the burden of AMR?
Thank you for taking time to complete this questionnaire	

Appendix B: DAHLD - Certificate of Ethical Approval

Tel: 01 766341
01 766348
Fax: 01 751349

E-mail:



Ministry of Agriculture
Department of Animal Health and
Livestock Development,
P.O. Box 2096
LILONGWE - MALAWI

Ref: DAHLD/AHC/01/2023/1

4th January, 2022

CERTIFICATE OF ETHICS APPROVAL

This is to certify that the Animal Health Committee under the Department of Animal Health and Livestock Development on 4th January 2022 received, reviewed and approved a study proposal entitled:

‘Prevalence and Resistance Patterns of non-typhoidal Salmonellosis in pigs in Northern Malawi’

By

Michael Luwe

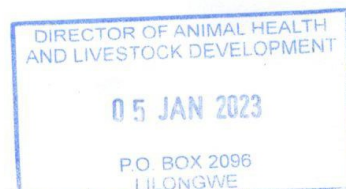
(U22899317)

During the implementation of the study, we would like you to adhere to International ethical guidelines, Malawi Animal welfare guidelines (2019) and all other requirements by the Animal Health committee stipulated herewith on the next page.


Dr Julius Chulu (PhD)

The Director of Animal Health and Livestock development.

Date: 4th January, 2023.



Appendix C: DAHLD – Study site authorisation

Government of Malawi

Tel: 01 766341
01 766348
Fax: 01 751349



Ministry of Agriculture
Department of Animal Health and
Livestock Development,
P. O. Box 2096,
LILONGWE - MALAWI

Ref: DAHLD/AHC/01/2023/3
Michael Luwe
Mzuzu ADD

4th January, 2023.

RE: STUDY SITE AUTHORISATION

The Department of Animal Health and Livestock Development (DAHLD) would like to congratulate you for successfully obtaining Ethical Clearance for your study titled **“Prevalence and Resistance Patterns of non-typhoidal Salmonellosis in pigs in Northern Malawi”**.

DAHLD has granted you Study Site Authorization in Mzuzu, Lilongwe and Blantyre districts as indicated in your submitted proposal. The department wishes you all the best as you will be conducting the study.

Yours sincerely


Dr. Julius Chulu (PhD)



The Director, Department of Animal Health and Livestock Development

Cc: Deputy Director of Animal Health and Livestock Development
Deputy Director Research and Investigation

Appendix D: Research Ethics Committee - Amendment letter of approval



Faculty of Veterinary Science

Research Ethics Committee

8 October 2024

AMENDMENT LETTER OF APPROVAL

Ethics Reference No	REC153-22 Line 3
Protocol Title	Knowledge, attitude and practices (KAP) and prevalence study of antimicrobial-resistant Escherichia coli and Enterococcus species in pigs in northern Malawi
Principal Investigator	Michael Luwe
Supervisors	Prof KH Keddy

Dear Michael Luwe,

We are pleased to inform you that the **Amendment** conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC153-22) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

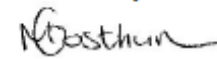
Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. Note: All FVS animal research applications for ethical clearance will be automatically rerouted to the Animal Ethics committee (AEC) once the applications meet the requirements for FVS ethical clearance. As such, all FVS REC applications for ethical clearance related to human health research will be automatically rerouted to the Health Sciences Research Ethics Committee, and all FVS applications involving a questionnaire will be automatically rerouted to the Humanities Research Ethics Committee. Also take note that, should the study involve questionnaires aimed at UP staff or students, permission must also be obtained from the relevant Dean and the UP Survey Committee. Research may not proceed until all approvals are granted.

Acknowledge Documentation

We wish you the best with your research.

Yours sincerely



PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

Appendix E: Animal Ethics Committee - Approval Certificate



Faculty of Veterinary Science
Animal Ethics Committee

07 August 2024

Approval Certificate Annual Renewal (EXT1)

AEC Reference No.: REC153-22 Line 2
Title: Knowledge, attitude and practices (KAP) and prevalence study of drug-resistant Enterobacteriaceae and Enterococcus species in pigs in northern Malawi
Researcher: Michael Luwe
Student's Supervisor: Annelize Jonker

Dear Michael Luwe,

The **Annual Renewal** as supported by documents received between 2024-07-15 and 2024-07-30 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2024-07-30.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Approved
Pigs – Domesticated (Malawi)	384
Samples	Approved
Caecum (sample from live animals)	384

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2025-08-07.
3. Please remember to use your protocol number (REC153-22) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. All incidents must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

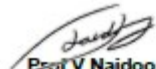
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Room 6-13, Arnold Thaler Building, Onderstepoort
Private Bag 204, Onderstepoort 9110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Kolapha la Diseense la Bongakadirulwa

We wish you the best with your research.

Yours sincerely


Prof V Naidoo

CHAIRMAN: UP-Animal Ethics Committee

Appendix F: Animal Ethics Committee - Approval Certificate (Amendment 1)



Faculty of Veterinary Science
Animal Ethics Committee

15 March 2024

Approval Certificate Amendment 1

AEC Reference No.: REC153-22 Line 1
Title: Knowledge, attitude and practices (KAP) and prevalence study of drug-resistant Enterobacteriaceae and Enterococcus species in pigs in northern Malawi
Researcher: Michael Luwe
Student's Supervisor: Annelize Jonker

Dear Michael Luwe,

The **Amendment** as supported by documents received between 2024-01-16 and 2024-02-27 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2024-02-27.

Please note the following about your ethics approval:

1. The new title is approved:

The new title is: "Knowledge, attitude and practices (KAP) and prevalence study of drug-resistant Enterobacteriaceae and Enterococcus species in pigs in northern Malawi"

(Old title: Prevalence and antimicrobial resistance patterns of non-typhoidal Salmonella species in pigs in Malawi)

2. Please note that the approved date(s) from the original application certificate / annual renewal certificate will be applicable to this amendment.
3. Please remember to use your protocol number (REC153-22) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee

We wish you the best with your research.

Yours sincerely



Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Appendix G: Animal Ethics Committee - Approval Certificate (Amendment 2)



Faculty of Veterinary Science
Animal Ethics Committee

Institution: The AEC is registered with the National Health Research Ethics Council (NHREC) of South Africa, under the National Health Act (Act 61 of 2003). The AEC, Faculty of Veterinary Science, University of Pretoria complies with NHREC, and SANS 10386 (2021)

Reg no: AREC-261110-001, registration certificate is valid until 21 December 2027.

30 October 2024

Approval Certificate Amendment 2

AEC Reference No.: REC153-22 Line 3
Title: Knowledge, attitude and practices (KAP) and prevalence study of antimicrobial-resistant *Escherichia coli* and *Enterococcus* species in pigs in northern Malawi
Researcher: Michael Luwe
Student's Supervisor: Prof KH Keddy

Dear Michael Luwe,

The **Amendment** as supported by documents received between 2024-09-16 and 2024-10-29 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2024-10-29.

Please note the following about your ethics approval:

- **The change in the Title has been approved** - The proposed title is: Knowledge, attitude and practices (KAP) and prevalence study of antimicrobial-resistant *Escherichia coli* and *Enterococcus* species in pigs in northern Malawi.
 - **The change in the Research team has been approved** - Prof Karen Keddy is the new supervisor after resignation of Dr Annelize Jonker.
1. Please note that the approved date(s) from the original application certificate / annual renewal certificate will be applicable to this amendment.
 2. Please remember to use your protocol number (REC153-22) on any documents or correspondence with the AEC regarding your research.
 3. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
 4. The AEC may select your research project for an audit (including documents/records) or a site visit.
 5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
 6. The committee also requests that you **record major procedures** undertaken during your study for self-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change the investigators, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely



Prof. V. Naidoo

CHAIRMAN: UP-Animal Ethics Committee

Appendix H: Faculty of Humanities - Research approval



Faculty of Humanities

Fakulteit Geesteswetenskappe
Lefapha la Bomotho



20 November 2024

Dear Michael Luwe,

Project Title: Prevalence and antimicrobial resistance patterns of non-typhoidal Salmonella species in pigs in Malawi
Researcher: Michael Luwe
Supervisor(s): Annelize Jonker
Department: Veterinary Tropical Diseases
Reference number: 22899317 (HUM008/0223 Line 2) (Amendment)
Degree: Masters

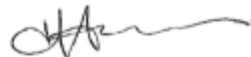
Thank you for the application to amend the existing protocol that was previously approved by the Committee.

The revised / additional documents were reviewed and **approved** on 20 November 2024 along these guidelines, further data collection may therefore commence (where necessary).

Please note that this approval is based on the assumption that the research will be carried out along the lines laid out in the amended proposal. Should your actual research depart significantly from the proposed research, it will be necessary to apply for a new research approval and ethical clearance.

We wish you success with the project.

Sincerely,



Prof Karen Harris
Chair: Research Ethics Committee
Faculty of Humanities
UNIVERSITY OF PRETORIA
e-mail: tracey.andrew@up.ac.za

Research Ethics Committee Members: Prof **KL Harris** (Chair); Dr S Abdoolca, Mr A Blos; Dr S Chigaza; Dr A-M de Beer; Dr A Dos Santos; Prof Salome Geertsema, Prof P Gutura; Ms KT Govinder Andrew; Dr D Krige; Mr A Mohamed; Dr T Nichola-Ramunenywa; Dr I Noomi; Dr C Puttgeril; Prof D Reyburn; Prof E Taljard

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