ANTIBIOTIC RESISTANCE AND VIRULENCE PROFILES OF *Staphylococcus aureus* AND *Escherichia coli* FROM RODENTS, HUMANS AND CHICKEN COEXISTING IN KARATU, TANZANIA

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EXTENDED ABSTRACT

Antimicrobial resistance (AMR) is a rapidly growing multifaceted problem which threatens global security, public health and the economy. Currently, about 700 000 humans worldwide lose lives annually due to AMR infections that are difficult to treat which are also associated with higher health care costs, elongated time spent in hospitals and increased animal production costs. This study was conducted in Karatu district, northern Tanzania, to investigate antibiotic resistance and virulence profiles of *Staphylococcus aureus* and *Escherichia coli* isolated from humans, rodents, chicken and soil in households.

Interaction of rodents with humans and livestock in households' environment has frequently been reported in Karatu, facilitating wide spread of resistant bacterial infections among different hosts in the community. The main objective of this study was to determine the antibiotic resistance and virulence profiles of *Staphylococcus aureus* and *Escherichia coli* isolated from rodents, chicken, humans and their surrounding environment in Karatu District.

S. aureus were isolated from 284 human nasal swabs, 101 rodents' deep pharyngeal swabs, 286 chicken cloaca swabs and 285 household soil samples. Specimens were plated into Mannitol Salt Agar (Oxoid, Basingstoke, UK) and incubated aerobically at 37 °C for 24 h. Presumptive colonies of *S. aureus* were subjected to Gram staining, catalase, deoxyribonuclease (DNAse) and coagulase tests for identification. Antibiotic susceptibility testing (AST) was performed by using Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (Oxoid, Basingstoke, UK). The antibiotics tested were tetracycline (30 µg), erythromycin (15 µg), gentamicin (10 µg), ciprofloxacin (5 µg),

clindamycin (2 µg) and amoxicillin-clavulanate (20 µg/10 µg). *S. aureus* strain American Type Culture Collection (ATCC) 25923 was used as a standard organism. Results were interpreted according to Clinical and Laboratory Standard Institute (CLSI) guideline of 2020.

The samples used for *E. coli* isolation included 288 chicken cloaca swabs, 281 human stool, 101 rodents' intestinal contents and 290 household soils. The specimens were plated onto MacConkey agar (Oxoid Ltd., Detroid, Michigan, USA) and incubated aerobically at 37 ºC for 24 h. Presumptive *E. coli* colonies were subjected to motility test and later indole, methyl red, Voges-Proskauer and citrate utilization (IMViC) tests for identification. *E. coli* strain ATCC 29522 was used as a reference organism. AST was performed by using Kirby-Bauer disc diffusion method on Mueller-Hinton Agar plates (Oxoid, Basingstoke, UK). The antibiotics tested were; tetracycline (30 µg), imipenem (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg) cefotaxime (30 µg) and amoxicillin-clavulanate (20 µg/10 µg). The results were interpreted by using CLSI (2020) guideline.

Results of this investigation revealed high frequencies of isolation for *S. aureus* and *E. coli* in rodents, humans, and chicken and soil samples. For *S. aureus,* the isolation frequencies were 52.1%, 66.5%, 74.3% and 24.5% in samples from chicken, human, rodent and soil, respectively. The isolation frequencies of *E. coli* from chicken, humans, rodents and soil were 81.6 %, 86.5 %, 80.2 % and 31.0 %, respectively. Based on AST phenotypic results, *S. aureus* isolates displayed resistance to clindamycin (51%), erythromycin (50.9%) and tetracycline (62.5%) while *E. coli* isolates showed high resistance against tetracycline (73.7%), imipenem (79.8%) and cefotaxime (79.7%). MDR *E. coli* (n=50) and *S. aureus* (n=57) isolates that exhibited high levels of phenotypic resistance to various classes of antibiotics were subjected to molecular analysis using

multiplex polymerase chain reaction (PCR) technique to detect presence of antibiotic resistance (ARGs) and virulence genes (VGs). ARGs detected in MDR *E. coli* were; *blaTEM* (46%), *blaCTX-M* (26%), *blaSHV* (22%), *tetA* (46%), *tetB* (14%), *qnrA* (24%), *qnrB* (8%), *blaOXA-48* (12%) and *blaKPC* (6%) while VGs detected included; *ompA* (72%), *traT* (26%), *east* (18%), *bfp* (10%), *eae* (2%) and *stx-1* (4%). For MDR *S. aureus*, ARGs were; *tetK* (31.6%), *tetL* (8.9%), *ermC* (1.8%) and *mecA* (28.1%) while VGs detected were; *clfB* (10.5%), *coa* (14.0%), *clfA* (1.8%), *hlg* (1.8%), *ebpS* (3.5%), *fnbB* (3.5%), *luk-PV* (10.5%) and *tst* (1.8%).

Positive and negative correlations between resistance and virulence genes were observed. For MDR *E. coli*, positive correlations were found between *blaTEM* and *traT* genes (r=0.51) and *qnrB* and *bfp* genes (r=0.63), while negative correlations were found between *blaOXA-48* and *ompA* (r= -0.05), *blaSHV* and *traT* (r=-0.44) and *tetA* and *east* (r=-0.10). For *S. aureus*, positive correlations were found between resistance (*ermC*) and *clfA* (r=0.57), *hlg* (r=1.00) and *clfB* (r=0.43), *tetK* and *clfB* (r=0.39); *tetK* and *coa* (r=0.36). The principal component analysis (PCA) results for *S. aureus* showed that, resistance genes (*tetK* and *mecA*) and virulence determinants (*clfB, coa* and *luk-PV*) were common in all sample sources. The PCA also revealed that, MDR *E. coli* and S. *aureus* isolates from rodents and chicken had more ARGs and VGs compared to isolates from soil and humans. Besides, MDR *E. coli* isolates harboured *traT, east, eae, stx-1, bfp* and *ompA* genes indicating ability of isolates to cause various infections.

Based on findings, this study documents high levels of antimicrobial resistance including MDR in *E. coli* and *S. aureus* isolated from chicken, humans, rodents and soil samples in Karatu, northern Tanzania. According to PCA results, *E. coli* isolates from rodents had more ARGs and VGs while for *S. aureus* these genes were found more in rodents and soil

environment implying that both subjects are potential reservoirs and can be sources of transmission. The increased prevalence of both resistance and virulence genes in the isolates suggests the ability of the pathogens to cause infections that are difficult to treat.

Comprehensive one health interventions, are urgently needed and should include improving; i) improving hygiene and control of rodents in household environments. ii) Future studies should base on adequate understanding of the human-livestock-environment interphase using well-designed genomic studies such as whole genomic sequencing (WGS) which provides a comprehensive picture on the pattern and magnitude of AMR and virulence genes spread. The advances and accessibility of genomic sequencing and analytical methods are essential in improving our understanding of AMR transmission dynamics at the human-livestock/animal-environment interface.

Genomic studies should be coupled with behavioural, epidemiological, clinical and modelling using One Health approaches. This will ensure that the key drivers of resistance and virulence transmission between human-livestock-environment are accurately identified and the most appropriate interventions adopted. It is important to understand the importance of each component of the human-livestock/animal-environment. A One Health approach should be deployed to ensure involvement of relevant multisectoral and multidisciplinary to attain an optimal public health and ensure a safe environment.

DECLARATION

I, **Valery Silvery Sonola,** do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work done within the period of registration and that it has neither been submitted nor concurrently submitted for degree award in any other institution.

_____ _____

Valery Silvery Sonola Date

(PhD. Candidate)

The above declaration is confirmed by;

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Prof. Gerald Misinzo Date

(Supervisor)

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Prof. Mecky Matee Date

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DEDICATION

This work is dedicated to my parents, my father Silvery Sonola and my mother Laurencia Luhemeja, for identifying my talents and nurturing them with love and guidance since early childhood to a person I am today.

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LIST OF ABBREVIATIONS

µg	Microgram
ACEIRPM &BTD	Africa Centre of Excellence for Innotive Rodent Pest management and Biosensor Technology Development
AMC	Amoxicillin Clavulanate
AMR	Antimicrobial Resistance
AMU	Antimicrobial Use
AR	Antimicrobial Residues
ARGs	Antibiotic Resistance Genes
AST	Antibiotic Susceptibility Testing
ATCC	American Type Culture Collection
CD	Clindamycin
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standard Institute
CN	Gentamycin
CSOs	Civil Services Organizations
CTX	Cefotaxime
DNA	Deoxyribonucleic Acid
DNAse	Deoxyribonuclease
E	Erythromycin
IMP	Imipenem
IMViC	Indole, Methyl red, Voges –Proskauer and Citrate Utilization Tests
MDR	Multidrug Resistance
N	Total number of samples
n	Number of Isolates

NGOs	Non Governmental Organization
NIMR	National Institute for Medical Research
^{O}C	Degree Centigrade
OH	One Health
PC_1	Principal Component 1
PC_2	Principal Component 2
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
SUA	Sokoine University of Agriculture
TE	Tetracycline
TSST	Toxic Shock Syndrome Toxin – 1
TVLA	Tanzania Veterinary Laboratory Agency
USA	United States of America
UTI	Urinary Tract Infection
VGs	Virulence Genes
WGS	Whole Genome Sequencing

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Escherichia coli and *Staphylococcus aureus* are the most common species of gram-negative and gram-positive members of the bacterial community, respectively (Taylor and Unakal, 2022). These bacteria are commensal inhabitants in humans, livestock, wild animals and the environment (Aworh *et al.*, 2021; Gibson *et al.*, 2021), but also pathogens causing different life threatening infections to humans and food animals (Pakbin *et al.*, 2021; Preda *et al.*, 2021). For example, pathogenic *E. coli* is a major cause of urinary tract infections (UTI), diarrhea, sepsis, meningitis and wound infections in humans (Martinez-Medina, 2021). A wide range of *E. coli* prevalence in humans with UTI has been reported to be 40-72.2% in Tanzania (Odoki *et al.*, 2019; Letara *et al.*, 2021 and Ali *et al.*, 2022). In poultry, the bacterium is associated with avian colibacillosis and swollen head syndrome (Ahmad *et al.*, 2022; Thabet *et al.*, 2022). A range of 53.4% to 88.2% of *E. coli* isolation frequencies from chicken with avian colibacillosis has been reported (El-Sukhon *et al.*, 2002 and Ibrahim *et al.*, 2019). Colibacillosis causes higher mortality and morbidity in chicken resulting into reduced meat and egg production, decreased hatchability rates and increased condemnation of carcasses at slaughter (Xing *et al.*, 2021). On the other hand, *S. aureus* is a leading cause of various diseases in humans, ranging from skin and soft tissues to life threatening infections that include; pneumonia, endocarditis, osteomyelitis and sepsis (Taylor and Unakal, 2022). Studies in Tanzania have reported isolation of *S. aureus* from different infections in humans ranging from 8.7% to 71.4% (Kazimoto *et al.*, 2018; Seni *et al.*, 2019; Silago *et al.*, 2020). Infections such as arthritis, omphalitis, gangrenous dermatitis and bumble foot which are common in chicken are associated with *S. aureus* (Tsai *et al.*, 2015; Amer *et al.*, 2017; Szafraniec *et al.*, 2022). *S. aureus* is also a leading

cause of mastitis in dairy animals (Silva *et al.*, 2021). *E. coli* and *S. aureus* are both included in the priority list of 12 most drug resistant bacteria that deserve attention as declared by the World Health Organization (WHO, 2017). The Food and Agriculture Organization (FAO), World Health Organization (WHO) and World Organization for Animal Health (OIE) agreed that One Health approach is the best approach to combalt the global antibiotic resistance problem, emphasizing that the best public health relies on healthy animals, humans and the environment (Badau, 2021 and FAO, 2021).

The pathogenicity of *E. coli* and *S. aureus* are due to their ability to produce several genes that encode for virulence and antimicrobial resistance (Abd El-Baky *et al.*, 2020; Chai *et al.*, 2022). These genes are transmitted through horizontal gene transfer, mainly by conjugation process involving exchange of plasmids (Li *et al.*, 2022; Marincola *et al.*, 2021). Important AMR genes in *E. coli* include; *blaTEM*, *blaSHV* and *blaCTX-M*, which encode for extended spectrum beta-lactamases (ESBL)-production (Muriuki *et al.*, 2022), *blaVIM, blaIMP* and *blaNDM,* which code for carbapenem resistance (Abdelaziz, 2022), *qnrA, qnrB and qnrC* for quinolone resistance (Roshani *et al.*, 2022) and *tetA, tetB, tetC and tetK* for resistance to tetracycline (Aworh *et al.*, 2021; Chai *et al.*, 2022). The virulence factors with clinical relevance in *E. coli* are encoded by several genes including; locus enterocyte effacement (LEE), intimin, bundle forming (*bae, bfpA*) (Razaghi *et al.*, 2017), Shiga toxins, adhesins (*stx1, stx2, eae, ehxA and bfp*) (Shen *et al.*, 2022), heat labile, heat stable and colonization factors (*elt, est*) (Mondal *et al.*, 2022).

AMR genes for *S. aureus* include; tetracycline (*tetK, tetL, tetM, tetO*) plasmid mediated resistance genes coding for efflux pumping mechanism; erythromycin (*ermA, ermB* and *ermC*) resistance genes which encode the ribosomal modification of 23rRNA methylase enzymes (Mbindyo *et al.*, 2021); methicillin resistance gene (*mecA*) codes for production

of a penicillin-binding protein (PBP2a) with lower affinity to β-lactam antibiotics (Ali *et al.*, 2021) and quinolone resistance (*qnrA, qnrB* and *qnrC*) genes encoding for proteins of the pentapeptide repeat family that protects DNA gyrase and topoisomerase IV from quinolone inhibition resulting into drug resistance (Saleh *et al.*, 2022). For *S aureus*, the most important virulence genes include; *ebpS, clfA, clfB and fnbB* which facilitate bacterial adherence to host epithelial cells (Zamani *et al.*, 2022), *luk-PV* and *hlg* genes confer production of toxins (Khokhlova *et al.*, 2015) and *tst* gene responsible for production of toxic shock syndrome toxin-1 (TSST-1) protein (Sadat *et al.*, 2022). Studies have shown that, these genes can spread from humans to animals via the environment influenced largely by human activities (Aworh *et al.*, 2021; Silva *et al.*, 2021). Surveillance of AMR and virulence genes requires application of molecular techniques that include PCR, DNA microarray, whole genome sequencing (WGS) and metagenomics (Shen *et al.*, 2020). In principle, the application of these techniques has shown interactions between humans, rodents and poultry to be associated with spread of resistomes and virulence genes among them (Desvars-Larrive *et al.*, 2019; Himsworth *et al.*, 2016; Raafat *et al.*, 2022). Due to the reported interactions, it is possible that genes encoding for AMR and virulence could be transmitted between humans, rodents, chicken and soil as reported in other studies where, resistance genes*; blaTEM, blaOXA, blaSHV, ermA, ermB, ermC, tetA, tetB, tetC,qnrA, qnrB, msrE* (Desvars-Larrive *et al.*, 2019; Monecke *et al.*, 2016; Rosengren *et al.*, 2009) and virulence genes; *clfA, clfB, fnbA, hla,, eae, stx-1, stx-2 and traT* (Paudel *et al.*, 2021) were detected.

Of relevance to this study, different studies conducted in Karatu have documented significant interaction of rodents with humans in households with transmission of specific zoonotic bacterial infections that are threat to humans (Kilonzo *et al.*, 2006; Makundi *et al.*, 2008; Ziwa *et al.*, 2013; Makundi *et al.*, 2015). Most of these studies in Karatu were

focused on *Yersinia pestis*, the causative agent of plague (Makdasi *et al.*, 2022). However, none of these studies focused on the spread and distribution of AMR and virulence genes among them. Different studies have shown that interactions between humans, rodents and environment have the potential for transmitting AMR and virulence genes (Guenther *et al.*, 2012; Himsworth *et al.*, 2016; Furness *et al.*, 2017; Le Huy *et al.*, 2020; Gwenzi *et al.*, 2021). In such studies, *S. aureus* and *E. coli*, which are One Health (OH) pathogens, are commonly used to determine the flow of antimicrobial resistant and virulence genes between humans, animals and the environment (Poudel *et al.*, 2019; Le Huy *et al.*, 2020; Islam *et al.*, 2021). The information on AMR phenotypes, genotypic profiles and virulence factors of *E. coli* and *S. aureus* from humans, chicken, rodents and household soils will help to combat the widespread of AMR pathogens in communities and ensure optimum public health.

This study was conducted in Karatu District where interaction between rodents, humans, and chicken has been frequently reported (Kilonzo *et al.*, 2006; Makundi *et al.*, 2008; Ziwa *et al.*, 2013; Haule *et al.*, 2013; Makundi *et al.*, 2015). Karatu district has been recognized as a plague focus area for years which is associated with the interaction between rodents and humans in households. The study hypothesis is that such interactions also facilitate spread of resistomes and virulence genes, which can be tracked through surveillance of One Health pathogens such as *S. aureus* and *E. coli.*

The methodology used in this cross-sectional study included i) collection of samples in the field from chicken (cloaca swabs), humans (nasal swabs and stool samples), rodents (deep pharyngeal swabs and intestinal contents) ii) culture and biochemical identification of *E. coli* and *S aureus* iii) phenotypic antibiotic susceptibility testing using Clinical and Laboratory Standards Institute guidelines (CLSI, 2020) and iii) multiplex PCR

amplification for detection of resistance and virulence genes for each organism (Yao *et al.*, 2019). This thesis follows the following lay out; i) phenotypic antibiotic resistance profile of *S. aureus* from rodents, humans, chicken and soil in Karatu district (chapter two), ii) phenotypic antibiotic resistance profile of *E. coli* isolated from rodents, humans, chicken and soils in selected households of Karatu district (chapter three) iii) genotypic resistance and virulence profile of MDR *E. coli* isolated from rodents, humans, chicken and soil in Karatu district (chapter four), genotypic resistance and virulence profile of *S. aureus* isolated from chickens, humans, rodents and soil in Karatu, Northern Tanzania (chapter five) and discussion, conclusions and recommendations (chapter six). At the end, the following appendices are attached; i) a blank copy of an informed consent form for participants that was used during the study, ii) A map of Karatu district showing the study locations and iii) photographs showing some laboratory procedures.

1.2 Statement of the Problem

The interaction of rodents with humans in households has a long history in Karatu district with the first report being documented in early 1980s by then the place was part of Mbulu district (Kilonzo and Mtoi, 1983). Since then, different studies have been reporting the invasion of rodents in households with subsequent interaction with humans facilitating spread of rodent-borne zoonotic infections (Kilonzo *et al.*, 2006; Makundi *et al.*, 2008; Ziwa *et al.*, 2013; Haule *et al.*, 2013; Makundi *et al.*, 2015). Most of these studies were focused on occurrence of *Yersinia pestis* bacterium which is a leading cause of plague disease (Yang and Anisimov, 2016). Karatu is currently a plague focus area following the unique interaction of rodents with other hosts with ability to spread bacterial infections. Therefore, it is time now to investigate the possibility of these rodents to carry and spread antibiotic resistant organisms including the most common infectious and drug resistant *E. coli* and *S. aureus in* Karatu district. Rodents that invade human households in Karatu

have been reported to interact with wild rodents in nearby forests and parks with a possibility of exchanging bacterial zoonotic infections and transmit them to humans (Makundi *et al.*, 2008; Haule *et al.*, 2013). Such kind of interaction can enhance wide spread of antibiotic resistance and affect public health in the Karatu community and in the surrounding parks.

Several studies worldwide have associated rodents that invade households with transmission of different antibiotic resistant bacteria (Guenther *et al.*, 2010; Himsworth *et al.*, 2016; Ribas *et al.*, 2016; Dahmana *et al.*, 2020), suggesting the influence of anthropogenic factors in households. Following their unique ability of interacting with humans and other animals in the environment, rodents have been considered as important bio sentinels which can help in tracking the widespread of antimicrobial resistance in communities (Furness *et al.*, 2017; Kmeť *et al.*, 2018; Dahmana *et al.*, 2020; Gwenzi *et al.*, 2021). However, studies on occurrence and patterns of multidrug resistant (MDR) *S. aureus* and *E. coli,* the leading cause of various infections in humans and livestock are missing in Karatu district. The status of soil contamination in terms of antibiotic resistance is not known in Karatu, which can also be a reservoir and good source of transmission. Therefore, this study aimed to determine the prevalence of *S. aureus* and *E. coli* with their resistance and virulence genes in humans, rodents, chickens and soils in households of Karatu in northern Tanzania.

1.2.1 Rationale of the study

Most studies in Karatu have recognized rodents as carriers and transmission vehicles of important zoonotic pathogens that cause disease outbreaks (Ziwa *et al.*, 2013; Makundi *et al.*, 2015). Despite the potential interaction between humans, food animals and rodents there is no information about transmission of resistant bacteria with their resistance and

virulence genes across different host species in Karatu. Furthermore, no study has been conducted in this area to determine the type of resistant genes in the soil environment in households. Both *S. aureus* and *E. coli* are One Health (OH) pathogens that are commonly used as indicator organisms to determine the flow of resistant genes between compartments (Poudel *et al.*, 2019). These microbes are also in the ist of 12 most common infectious pathogens with multiple drug resistance properties declared by WHO (2017). The application of molecular techniques such as multiplex PCR to explore genotypic profiles of resistant isolates from various hosts provides useful insight in the possible cause of resistance and elucidate its transmission pathways. Specifically, the information on prevalence and patterns of antibiotic resistance and virulence genes in *E. coli* and *S. aureus,* will help to determine the magnitude of antibiotic resistance spread and provide stakeholders with baseline data for planning purposes and develop innovative strategies to address the problem.

1.3 Objectives of the Study

1.3.1 General objective

The main objective of this study was to determine the antibiotic resistance and virulence profiles of *Staphylococcus aureus* and *Escherichia coli* isolated from rodents, chicken, humans and their surrounding environment in Karatu District.

1.3.2 Specific objectives

i. To determine the phenotypic antibiotic resistance pattern of *S. aureus* isolated from rodents, humans, chicken and soil in households of Karatu district.

ii. To determine the phenotypic antibiotic resistance pattern of *E. coli* isolated from rodents, humans, chicken and soil in households of Karatu district.

iii. To determine the molecular epidemiology of antibiotic resistance and virulence genes of *E. coli* isolated from rodents, humans, chicken and soil in Karatu district.

iv. To determine the molecular epidemiology of antibiotic resistance and virulence genes of *S. aureus* isolated from chicken, humans, rodents and soil in Karatu district.

1.4 Research Question

i. What are the isolation frequencies and phenotypic and genotypic resistance profiles of MDR *S. aureus* and *E. coli* isolated from rodents, chickens and humans and the surrounding environment in Karatu District?

ii. Which virulence genes are found in MDR *S. aureus* and *E. coli* isolated from rodents, chickens and humans and the surrounding environment in Karatu District?

1.5 Hypothesis

The interaction between rodents, humans, chickens and their surrounding soil environment facilitates spread of resistomes and virulence genes among MDR strains of *S. aureus* and *E. coli.*

REFERENCES

Abd El-Baky, R. M., Ibrahim, R. A., Mohamed, D. S., Ahmed, E. F. and Hashem, Z. S. (2020). Prevalence of virulence genes and their association with antimicrobial resistance among pathogenic *E. coli* isolated from Egyptian patients with different clinical infections. *Infection and Drug Resistance* 13: 12 - 21.

Abdelaziz, N. A. (2022). Phenotype-genotype correlations among carbapenem-resistant Enterobacterales recovered from four Egyptian hospitals with the report of SPM carbapenemase. *Antimicrobial Resistance and Infection Control* 11(1): 1 - 10.

Ahmad, T., Fiaz, M., Sharif, A., Nadeem, M. and Umer, M. (2022). 6. In vitro and in vivo evaluation of antimicrobials in *Escherichia coli* infection in broilers and evaluation of ciprofloxacin in induced colibacillosis. *Pure and Applied Biology (PAB)* 11(3): 744 - 754.

Ali, T., Basit, A., Karim, A. M., Lee, J. H., Jeon, J. H. and Lee, S. H. (2022). Mutation-based antibiotic resistance mechanism in methicillin-resistant *Staphylococcus aureus* clinical isolates. *Pharmaceuticals* 14(5): 420.

Amer, M., ELbayoumi, K. M., Amin Girh, Z., Mekky, H. M. and Rabie, N. S. (2017). A study on bacterial contamination of dead in shell chicken embryos and culled one day chicks. *International Journal of Pharm Phytopharmacol Research* 7(2): 5-11.

Aworh, M. K., Kwaga, J. K., Hendriksen, R. S., Okolocha, E. C. and Thakur, S. (2021). Genetic relatedness of multidrug resistant *Escherichia coli* isolated from humans,

chickens and poultry environments. *Antimicrobial Resistance and Infection Control* 10(1): 1 - 13.

Chai, M., Sukiman, M. Z., Kamarun Baharin, A. H., Ramlan, I., Lai, L. Z., Liew, Y. and Ghazali, M. F. (2022). Methicillin-Resistant *Staphylococcus aureus* from Peninsular Malaysian Animal Handlers: Molecular Profile, Antimicrobial Resistance, Immune Evasion Cluster and Genotypic Categorization. *Antibiotics* 11(1): 103 - 114.

Chai, M., Sukiman, M., Jasmy, N., Zulkifly, N., Yusof, N. M., Mohamad, N. and Ghazali, M. (2022). Molecular Detection and Antibiogram of ESBL-Producing and Carbapenem-Resistant *Escherichia coli* from Rabbit, Swine and Poultry in Malaysia. *Tropical Animal Science Journal* 45(1): 16-23.

Clinical and Laboratory Standards Institute. (2020). *CLSI-Performance Standards for Antimicrobial Susceptibility Testing.* Pennsylvania, USA. Pp. 56.

Dahmana, H., Granjon, L., Diagne, C., Davoust, B., Fenollar, F. and Mediannikov, O. (2020). Rodents as hosts of pathogens and related zoonotic disease risk. *Pathogens* 9(3): 202 - 224.

Desvars-Larrive, A., Ruppitsch, W., Lepuschitz, S., Szostak, M. P., Spergser, J., Feßler, A. T. and Walzer, C. (2019). Urban brown rats (Rattus norvegicus) as possible source of multidrug-resistant Enterobacteriaceae and meticillin-resistant *Staphylococcus* spp., Vienna, Austria, 2016 and 2017. *Eurosurveillance* 24(32): 19 - 149.

FAO (2021). The FAO Action Plan on Antimicrobial Resistance 2021–2025. Rome. 23pp.

Furness, L. E., Campbell, A., Zhang, L., Gaze, W. H. and McDonald, R. A. (2017). Wild small mammals as sentinels for the environmental transmission of antimicrobial resistance. *Environmental Research* 154: 28 - 34.

Furness, L. E., Campbell, A., Zhang, L., Gaze, W. H. and McDonald, R. A. (2017). Wild small mammals as sentinels for the environmental transmission of antimicrobial resistance. *Environmental Research* 154: 28 - 34.

Gibson, J. F., Pidwill, G. R., Carnell, O. T., Surewaard, B. G., Shamarina, D., Sutton, J. A. and Siggins, M. K. (2021). Commensal bacteria augment *Staphylococcus aureus* infection by inactivation of phagocyte-derived reactive oxygen species. *PLoS pathogens* 17(9): e1009880.

Guenther, S., Bethe, A., Fruth, A., Semmler, T., Ulrich, R. G., Wieler, L. H. and Ewers, C. (2012). Frequent combination of antimicrobial multiresistance and extraintestinal pathogenicity in *Escherichia coli* isolates from urban rats (*Rattus norvegicus*) in Berlin, Germany. *PLoS One* 7(11): e50331.

Guenther, S., Grobbel, M., Heidemanns, K., Schlegel, M., Ulrich, R. G., Ewers, C. and Wieler, L. H. (2010). First insights into antimicrobial resistance among faecal *Escherichia coli* isolates from small wild mammals in rural areas. *Science of the total environment* 408(17): 3519 - 3522.

Gwenzi, W., Chaukura, N., Muisa-Zikali, N., Teta, C., Musvuugwa, T., Rzymski, P. and Abia, A. L. K. (2021). Insects, rodents, and pets as reservoirs, vectors and sentinels of antimicrobial resistance. *Antibiotics* 10(1): 68 - 98.

Haule, M., Lyamuya, E. E., Hang'ombe, B. M. and Matee, M. I. (2013). Investigation of fleas as vectors in the transmission of plague during a quiescent period in North-Eastern, Tanzania. pp. 67.

Himsworth, C. G., Zabek, E., Desruisseau, A., Parmley, E. J., Reid-Smith, R., Leslie, M. and Cox, W. (2016). Avian pathogenicity genes and antibiotic resistance in *Escherichia coli* isolates from wild Norway rats (Rattus norvegicus) in British Columbia, Canada. *Journal of Wildlife Diseases* 52(2): 418 - 421.

Islam, M., Sobur, M., Rahman, S., Ballah, F. M., Ievy, S., Siddique, M. P. and Rahman, M. (2021). Detection of blaTEM, blaCTX-M, blaCMY and blaSHV Genes Among Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* Isolated from Migratory Birds Travelling to Bangladesh. *Microbial Ecology* 6: 1 - 9.

Khokhlova, O. E., Hung, W. C., Wan, T. W., Iwao, Y., Takano, T., Higuchi, W. and Kotlovsky, Y. V. (2015). Healthcare-and community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) and fatal pneumonia with pediatric deaths in Krasnoyarsk, Siberian Russia: unique MRSA's multiple virulence factors, genome, and stepwise evolution. *PLoS One* 10(6): e0128017.

Kilonzo, B. and Mtoi, R. (1983). Entomological, bacteriological and serological observations after the 1977 plague outbreak in Mbulu District, Tanzania. *East African Medical Journal* 60(2): 91 - 97.

Kilonzo, B., Mbise, T., Mwalimu, D. and Kindamba, L. (2006). Observations on the endemicity of plague in Karatu and Ngorongoro, northern Tanzania. *Tanzania Journal of Health Research* 8(1): 1 - 6.

Kimera, Z. I., Mgaya, F. X., Misinzo, G., Mshana, S. E., Moremi, N. and Matee, M. I. (2021). Multidrug-resistant, including extended-spectrum beta lactamase-producing and quinolone-resistant, *Escherichia coli* isolated from poultry and domestic pigs in dar es salaam, Tanzania. *Antibiotics* 10(4): 406.

Kmeť, V., Čuvalová, A. and Stanko, M. (2018). Small mammals as sentinels of antimicrobial-resistant staphylococci. *Folia microbiologica* 63(5): 665 - 668.

Kinabo, G. D., van der Ven, A., Msuya, L., Shayo, A., Schimana, W., Ndaro, A. and Hermans, P. (2013). Dynamics of nasopharyngeal bacterial colonisation in HIVexposed young infants in T anzania. *Tropical medicine and international health* 18(3): 286 - 295.

Le Huy, H., Koizumi, N., Ung, T. T. H., Le, T. T., Nguyen, H. L. K., Hoang, P. V. M. and Haga, T. (2020). Antibiotic-resistant *Escherichia coli* isolated from urban rodents in Hanoi, Vietnam. *Journal of Veterinary Medical Science* 82(5): 653 - 660.

Li, C., Zhu, L., Wang, D., Wei, Z., Hao, X., Wang, Z. and Long, M. (2022). T6SS secretes an LPS-binding effector to recruit OMVs for exploitative competition and horizontal gene transfer. *The ISME Journal* 16(2): 500 - 510.

Makdasi, E., Atiya-Nasagi, Y., Gur, D., Zauberman, A., Schuster, O., Glinert, I. and Weiss, S. (2022). An Improvement in Diagnostic Blood Culture Conditions

Allows for the Rapid Detection and Isolation of the Slow Growing Pathogen Yersinia pestis. *Pathogens* 11(2): 255 - 345.

Makundi, R. H., Massawe, A. W., Borremans, B., Laudisoit, A. and Katakweba, A. (2015). We are connected: flea–host association networks in the plague outbreak focus in the Rift Valley, northern Tanzania. *Wildlife Research* 42(2): 196 - 206.

Makundi, R. H., Massawe, A. W., Mulungu, L. S., Katakweba, A., Mbise, T. J. and Mgode, G. (2008). Potential mammalian reservoirs in a bubonic plague outbreak focus in Mbulu District, northern Tanzania, in 2007. 97pp.

Marincola, G., Jaschkowitz, G., Kieninger, A. K., Wencker, F. D., Feßler, A. T., Schwarz, S. and Ziebuhr, W. (2021). Plasmid-Chromosome Crosstalk in *Staphylococcus aureus*: A Horizontally Acquired Transcription Regulator Controls Polysaccharide Intercellular Adhesin-Mediated Biofilm Formation. *Frontiers in Cellular and Infection Microbiology* 11: 2 - 21.

Martinez-Medina, M. (2021). Pathogenic *Escherichia coli*: Infections and Therapies. In Multidisciplinary Digital Publishing Institute. 10: 112.

Mbindyo, C. M., Gitao, G. C., Plummer, P. J., Kulohoma, B. W., Mulei, C. M. and Bett, R. (2021). Antimicrobial resistance profiles and genes of Staphylococci isolated from mastitic cow's milk in Kenya. *Antibiotics* 10(7): 772 – 754.

Mondal, I., Bhakat, D., Chowdhury, G., Manna, A., Samanta, S., Deb, A. K. and Chatterjee, N. S. (2022). Distribution of virulence factors and its relatedness

towards the antimicrobial response of enterotoxigenic *Escherichia coli* strains isolated from patients in Kolkata, India. *Journal of Applied Microbiology* 132(1): 675 - 686.

Monecke, S., Jatzwauk, L., Müller, E., Nitschke, H., Pfohl, K., Slickers, P. and Ehricht, R. (2016). Diversity of SCC mec elements in *Staphylococcus aureus* as observed in south-eastern Germany. *PLoS One* 11(9): e0162654.

Muriuki, C. W., Ogonda, L. A., Kyanya, C., Matano, D., Masakhwe, C., Odoyo, E. and Musila, L. (2022). Phenotypic and genotypic characteristics of uropathogenic *Escherichia coli* isolates from Kenya. *Microbial Drug Resistance* 28(1): 31 - 38.

Odoki, M., Almustapha, A. A., Tibyangye, J., Nyabayo, M. J., Wampande, E., Drago, K. C. and Bazira, J. (2019). Prevalence of bacterial urinary tract infections and associated factors among patients attending hospitals in Bushenyi district, Uganda. *International Journal of Microbiology* 2(1): 37 - 48.

Pakbin, B., Brück, W. M. and Rossen, J. W. (2021). Virulence factors of enteric pathogenic *Escherichia coli*: A review. *International Journal of Molecular Sciences* 22(18): 9922.

Paudel, A., Panthee, S., Hamamoto, H., Grunert, T. and Sekimizu, K. (2021). YjbH regulates virulence genes expression and oxidative stress resistance in *Staphylococcus aureus*. *Virulence* 12(1): 470 - 480.

Poudel, A., Hathcock, T., Butaye, P., Kang, Y., Price, S., Macklin, K. and Adekanmbi, F. (2019). Multidrug-resistant *Escherichia coli*, Klebsiella pneumoniae and *Staphylococcus* spp. in houseflies and blowflies from farms and their environmental settings. *International Journal of Environmental Research and Public Health* 16(19): 35 - 83.

Preda, M., Mihai, M. M., Popa, L. I., Dițu, L. M., Holban, A. M., Manolescu, L. S. C. and Chifiriuc, C. M. (2021). Phenotypic and genotypic virulence features of staphylococcal strains isolated from difficult-to-treat skin and soft tissue infections. *PLoS One* 16(2): e0246478.

Raafat, M., Kamel, A. A., Shehata, A. H., Ahmed, A. S. F., Bayoumi, A., Moussa, R. A. and El-Daly, M. (2022). Aescin Protects against Experimental Benign Prostatic Hyperplasia and Preserves Prostate Histomorphology in Rats via Suppression of Inflammatory Cytokines and COX-2. *Pharmaceuticals* 15(2): 130 - 156.

Razaghi, M., Tajeddin, E., Ganji, L., Alebouyeh, M., Alizadeh, A. H. M., Sadeghi, A. and Zali, M. R. (2017). Colonization, resistance to bile and virulence properties of *Escherichia coli* strains: unusual characteristics associated with biliary tract diseases. *Microbial pathogenesis* 111: 262 - 268.

Ribas, A., Saijuntha, W., Agatsuma, T., Prantlová, V. and Poonlaphdecha, S. (2016). Rodents as a source of Salmonella contamination in wet markets in Thailand. *Vector-Borne and Zoonotic Diseases* 16(8): 537 - 540.

Rosengren, L. B., Waldner, C. L. and Reid-Smith, R. J. (2009). Associations between antimicrobial resistance phenotypes, antimicrobial resistance genes and virulence

genes of fecal *Escherichia coli* isolates from healthy grow-finish pigs. *Applied and Environmental Microbiology* 75(5): 1373 - 1380.

Roshani, M., Goodarzi, A., Hashemi, A., Afrasiabi, F., Goudarzi, H. and Arabestani, M. (2022). Detection of qnrA, qnrB and qnrS genes in Klebsiella pneumoniae and *Escherichia coli* isolates from leukemia patients. *Reviews and Research in Medical Microbiology* 33(1): 45 – 67.

Sadat, A., Shata, R. R., Farag, A. M., Ramadan, H., Alkhedaide, A., Soliman, M. M. and Awad, A. (2022). Prevalence and Characterization of PVL-Positive *Staphylococcus aureus* Isolated from Raw Cow's Milk. *Toxins* 14(2): 97 – 109.

Saleh, N. M., Moemen, Y. S., Mohamed, S. H., Fathy, G., Ahmed, A. A., Al-Ghamdi, A. A. and El Sayed, I. E.T. (2022). Experimental and Molecular Docking Studies of Cyclic Diphenyl Phosphonates as DNA Gyrase Inhibitors for Fluoroquinolone Resistant Pathogens. *Antibiotics* 11(1): 53 -72.

Shen, C., Zhong, L. L., Yang, Y., Doi, Y., Paterson, D. L., Stoesser, N. and Huang, S. (2020). Dynamics of mcr-1 prevalence and mcr-1-positive *Escherichia coli* after the cessation of colistin use as a feed additive for animals in China: a prospective cross-sectional and whole genome sequencing-based molecular epidemiological study. *The Lancet Microbe* 1(1): e34 - e43.

Silva, V., Vieira-Pinto, M., Saraiva, C., Manageiro, V., Reis, L., Ferreira, E. and Poeta, P. (2021). Prevalence and characteristics of multidrug-resistant livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) CC398 isolated from

quails (*Coturnix Japonica*) slaughtered for human consumption. *Animals* 11(7): 2038 - 2045.

Szafraniec, G. M., Szeleszczuk, P. and Dolka, B. (2022). Review on skeletal disorders caused by *Staphylococcus spp*. in poultry. *Veterinary Quarterly* (just-accepted). pp.1-33.

Taylor, T. A. and Unakal, C. G. (2022). *Staphylococcus Aureus*. In *StatPearls*. Treasure Island (FL): StatPearls Publishing Copyright © 2022, StatPearls Publishing LLC. 99pp.

Thabet, S., Souissi, N. and Khazri, I. (2022). Assessment of antimicrobial resistance in avian pathogenic *Escherichia coli* Strains isolated over four years in Tunisian poultry. *African Journal of Bacteriology Research* 14(1): 1 - 7.

Tsai, S.S., Chen, L. J., Shih, C. Y., Chang, T. C., Chiou, M. T. and Chuang, K. P. (2015). Joint Lesions in Taiwan native colored broiler chicken with natural and experimental *Staphylococcus aureus* or S. cohnii Infection. *Taiwan Veterinary Journa* 41(04): 237 - 244.

WHO (2017). World Health Organization. Critically important antimicrobials for human medicine: ranking of antimicrobial agents for risk management of antimicrobial resistance due to non-human use. pp. 87.

Xing, Z., Li, H., Li, M., Gao, R., Guo, C. and Mi, S. (2021). Disequilibrium in chicken gut microflora with avian colibacillosis is related to microenvironment damaged by antibiotics. *Journal of Science of the total environment* 762: 143058 -143058.

Yang, R. and Anisimov, A. (2016). *Yersinia pestis: retrospective and perspective*: *Springer* 6 (1): 35 – 98.

Yao, M., Zhang, X., Gao, Y., Song, S., Xu, D. and Yan, L. (2019). Development and application of multiplex PCR method for simultaneous detection of seven viruses in ducks. *BMC Veterinary Research* 15(1): 1 - 10.

Zamani, S., Mohammadi, A., Hajikhani, B., Abiri, P., Fazeli, M., Nasiri, M. J. and Haghighi, M. (2022). Mupirocin-Resistant *Staphylococcus aureus* in Iran: A Biofilm Production and Genetic Characteristics. *BioMed Research International* 2022.

Ziwa, M. H., Matee, M. I., Kilonzo, B. S. and Hang'ombe, B. M. (2013). Evidence of Yersinia pestis DNA in rodents in plague outbreak foci in Mbulu and Karatu Districts, Northern Tanzania. *Tanzania Journal of Health Research* 15(3): 14- 22.

CHAPTER TWO

PAPER I

International Journal of *Environmental Research and Public Health*

MDPI

Article

Occurrence of Multidrug-Resistant *Staphylococcus aureus* among Humans, Rodents, Chickens, and Household Soils in Karatu, Northern Tanzania

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Abstract: We conducted this study to investigate the isolation frequency and phenotypic antibiotic resistance pattern of *Staphylococcus aureus* isolated from rodents, chickens, humans, and household soils. Specimens were plated onto mannitol salt agar (Oxoid, Basingstoke, UK) and incubated aerobically at 37 °C for 24 h. Presumptive colonies of *S. aureus* were subjected to Gram staining, as well as catalase, deoxyribonuclease (DNAse), and coagulase tests for identification. Antibiotic susceptibility testing was performed by using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Oxoid, Basingstoke, UK). The antibiotics tested were tetracycline (30 µg), erythromycin (15 µg), gentamicin (10 µg), ciprofloxacin (5 µg), clindamycin (2 µg), and amoxicillin-clavulanate (20 µg/10 µg). The *S. aureus* strain American Type Culture Collection (ATCC) 25,923 was used as the standard organism. We found that 483 out of 956 (50.2%) samples were positive for *S. aureus*. The isolation frequencies varied significantly between samples sources, being 52.1%, 66.5%, 74.3%, and 24.5%, respectively, in chickens, humans, rodents, and soil samples ($p < 0.001$). *S. aureus* isolates had high resistance against clindamycin (51.0%), erythromycin (50.9%), and tetracycline (62.5%). The overall prevalence of multidrug-resistant (MDR) *S. aureus* isolates was 30.2%, with 8.7% resistant to at least four different classes of antibiotics.

Keywords: *Staphylococcus aureus*; antibiotic resistance; humans; chickens; rodents; soil

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1. Introduction

Staphylococcus aureus is both an opportunistic pathogen and a commensal microbe that colonizes a wide range of hosts, including humans, livestock, wild ungulates, and the environment [1–3]. *S. aureus* is also a leading cause of different infections in humans that range from minor skin infections to life-threatening diseases, such as pneumonia, osteomyelitis, endocarditis, and sepsis [2,4,5]. In farm animals, *S. aureus* causes mastitis in dairy animals [6,7] and septic arthritis in chickens [8], resulting in economic losses due to mortality and reduced production [9]. The pathogenicity of *S. aureus* is influenced by two important features: its ability to resist more than three classes of antibiotics [10] and the capacity to produce several toxins [2,11]. Multidrug-resistant bacteria have increased worldwide, resulting in the sharing of their genes with commensal microorganisms in humans, animals, and the environment and endangering public health [12]. Rodents have been extensively documented to carry and transmit different zoonotic pathogens, including

S. aureus, to humans and livestock [3,13,14]. Commensal rodents colonized with pathogens have been widely reported to invade chicken [15,16] and human houses [17–19], exposing them to bacterial infections. Different studies in Tanzania have documented the interaction of rodents with humans in households, predisposing them to rodent-borne zoonotic diseases [20–22]. Rodent infestation in human settlements has been frequently reported in Karatu, where interactions of rodents with humans and livestock are very common, making it a plague focus area [20,21,23–25]. However, studies on the occurrence and pattern of multidrug-resistant (MDR) *S. aureus* among humans, rodents, and the environment in the area are missing. Therefore, this study aimed to determine the occurrence of MDR *S. aureus* isolates in humans, rodents, chickens, and soils in the households of Karatu in northern Tanzania.

2. Materials and Methods

2.1. Study Area

The study was conducted in the Karatu district in the northern zone of Tanzania between June 2020 and March 2021. Karatu is located between latitudes 3°10′ and 4°00′ S and longitude 34°47′ to 59.99′ E. The district has a population of 230,166 people comprised of 117,769 men and 112,397 women, with an average of five people per household. Karatu has an altitude range of 1000 to 1900 m above sea level with two wet seasons annually (short rains between October and December and long rains from March to June).

2.2. Sampling Strategy

The study population comprised of households keeping local chickens, while the sampling frame was the list of these households. Five wards, Karatu, Endabash, Endamarariek, Mbulumbulu, and Rhotia, were purposively selected based on the population density (at least 16,000 people), number of households with chickens, and household size of at least five people. Households were randomly selected from a list provided by a livestock field officer at the ward level by using a table of random numbers. At the household level, permission from the head of the household was granted first before trapping the rodents where areas for trapping in the surrounding environments relied on signs of rodents' activities. For each household, one adult human (18 years and above) and one mature (seven months) scavenging chicken were involved in microbiological sampling to get one nasal swab and one cloaca swab, respectively. Furthermore, at least one rodent (in-house rat, peri-domestic rat, or both) could be captured, and one soil sample was collected per household. The selection of adult humans and mature chickens was based on the assumption that old individuals have been exposed to the interaction with rodents for a longer time than young ones, and hence are more likely to facilitate the sharing of infections.

2.3. Trapping of Rodents for Sample Collection

Live trapping of rodents was carried out using modified Sherman traps baited with peanut butter. An average of 100 traps (50 in houses and 50 in outside environments) were deployed per trap night for five consecutive nights in each ward. Each captured rodent was subjected to humane killing by using di-ethyl-ether and deep pharyngeal swabs, and the intestines were aseptically collected from the carcasses.

2.4. Collection of Samples from Humans, Chickens, and Soil

A total of 956 samples were collected from 286 households in the Karatu district wards. Of these, 286 were from chickens, 284 from humans, and 285 from soil (Table 1). Sterile cotton swabs were used to collect cloaca swabs from randomly picked scavenging chickens and human nasal swabs in households. Soil samples were randomly collected from five points in the household yards and mixed to compose one pooled soil sample [26]. Thereafter, cloaca and human nasal swabs were stored in sterile containers at −4°C and transported using Cary Blair transport medium and trypticase soy broth medium (Oxoid,

Basingstoke, UK), respectively, to the Tanzania Veterinary Laboratory Agency (TVLA)–Arusha laboratory for processing within four hours after collection.

2.5. Culture, Isolation, and Identification of S. aureus Isolates

Specimens were plated onto mannitol salt agar (Oxoid, Basingstoke, UK) and incubated aerobically at 37 °C for 24 h. Presumptive colonies of *S. aureus* were subjected to Gram staining, as well as catalase, deoxyribonuclease (DNAse), and coagulase tests for identification.

2.6. Antibiotic Susceptibility Testing of S. aureus Isolates

An antibiotic susceptibility test was performed by using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Oxoid, Basingstoke, UK) with commercially available discs, as described by [27]. The antibiotics tested were tetracycline (30 µg), erythromycin (15 µg), gentamicin (10 µg), ciprofloxacin (5 µg), clindamycin (2 µg), and amoxicillin-clavulanate (20 µg/10 µg). Pure colonies of the identified lactose fermenters were emulsified into 5 mL of sterile saline. The suspensions were adjusted to achieve a turbidity equivalent to 0.5 McFarland standard solutions, emulsified using sterile cotton swabs onto a Mueller–Hinton agar plate, and incubated at 37 °C for 16 to 18 h. After incubation, the inhibition zone of each antimicrobial agent was measured, and the results were interpreted according to the standards of [27]. *S. aureus* strain American Type Culture Collection (ATCC) 25,923 was used as the standard organism. An isolate was considered to be multidrug-resistant (MDR) if it was non-susceptible to three or more drugs from different classes of antibiotics [28].

2.7. Statistical Analyses

Isolation frequencies of *S. aureus* and the antibiotic resistance pattern of isolates were entered into Microsoft Excel version 2010 (Microsoft Corporation, Redmond, WA, USA) and their percentages were calculated by descriptive statistics. The association between categorical variables was analysed by using a chi-squared (Fisher's exact and Pearson's) test. Statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Isolation of Staphylococcus aureus from the Samples

Overall, 483 samples out of 956 (50.5%) had *S. aureus*. Significant variation in isolation frequencies was observed between the types of samples, being higher in rodents (74.3%) compared to soil (24.5%) samples ($p < 0.001$) (Table 1).

Table 1. Isolation frequencies of *Staphylococcus aureus* from different sample sources.

Types of Sample Sources	Number of Samples *n* (%)	Positive Samples *n* (%)	Chi-Squared	*p*-Value
Chickens	286 (29.9)	149 (52.1)		
Humans	284 (29.7)	189 (66.5)		
Rodents	101 (10.6)	75 (74.3)	$X^2 = 83.849$, df = 3	<0.001
Soil	285 (29.8)	70 (24.5)		
Total	956 (100.0)	483 (50.5)		

3.2. Antibiotic Susceptibility Testing (AST) Results of the S. aureus Isolates

The overall resistance rates were 51.0% to clindamycin, 50.9% to erythromycin, 6.9% to ciprofloxacin, 62.5% to tetracycline, 2.2% to gentamycin, and 10.7% to amoxicillin-clavulanate. The specific resistance rates are shown in Table 2 and Figure 1.

Table 2. Antibiotic resistance pattern of *Staphylococcus aureus* isolates from chicken, human, rodent, and soil samples.

Sample Type	Antibiotics, *n* (%)						Overall R	Chi-Squared Test
	Clindamycin	Erythromycin	Ciprofloxacin	Tetracycline	Gentamycin	Amoxicillin-Clavulanate		
Overall R Chickens	51.0 %	50.9 %	6.9 %	62.5 %	2.2 %	10.7 %		
R	102 (62.2)	94 (57.3)	6 (3.7)	108 (65.9)	1 (0.6)	15 (9.1)	33.1 %	247.61, df = 5, $p < 0.001$
I	16 (9.8)	21 (12.8)	17 (10.4)	13 (7.9)	0 (0.0)	2 (1.2)		
S	46 (28.0)	49 (29.9)	141 (86.0)	43 (26.2)	163 (99.4)	147 (89.6)		
Subtotal Humans	164 (100.0)	164 (100.0)	164 (100.0)	164 (100.0)	164 (100.0)	164 (100.0)		
R	93 (51.7)	113 (62.8)	21 (11.7)	134 (74.4)	2 (1.1)	22 (12.2)	35.7 %	243.1, df = 5, $p < 0.001$
I	12 (6.7)	16 (8.9)	18 (10.0)	13 (7.2)	9 (5.0)	6 (3.3)		
S	75 (41.7)	51 (28.3)	141 (78.3)	33 (18.3)	169 (93.9)	152 (84.4)		
Subtotal Rodents	180 (100.0)	180 (100.0)	180 (100.0)	180 (100.0)	180 (100.0)	180 (100.0)		
R	30 (40.0)	39 (52.0)	1 (1.3)	40 (53.3)	4 (5.3)	8 (10.7)	27.1 %	79.74, df = 5, $p < 0.001$
I	10 (13.3)	11 (14.7)	8 (10.7)	9 (12.0)	1 (1.3)	5 (6.7)		
S	35 (46.7)	25 (33.3)	66 (88.0)	26 (34.7)	70 (93.3)	62 (82.7)		
Subtotal Soil	75 (100.0)	75 (100.0)	75 (100.0)	75 (100.0)	75 (100.0)	75 (100.0)		
R	32 (50.0)	20 (31.3)	7 (10.9)	36 (56.3)	1 (1.6)	7 (10.9)	26.8 %	61.21, df = 5, $p < 0.001$
I	6 (9.4)	7 (10.9)	4 (6.3)	6 (9.4)	0 (0.0)	1 (1.6)		
S	26 (40.6)	37 (57.8)	53 (82.8)	22 (34.4)	63 (98.4)	56 (87.5)		
Subtotal	64 (100.0)	64 (100.0)	64 (100.0)	64 (100.0)	64 (100.0)	64 (100.0)		

R = resistant, I = intermediate, and S = susceptible.

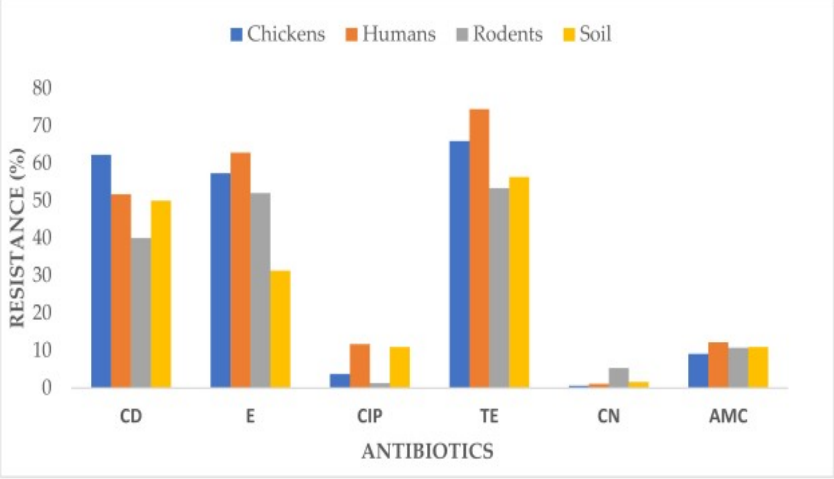

Figure 1. Resistance of *S. aureus* isolates against the antibiotics; CD = clindamycin, E = erythromycin, CIP = ciprofloxacin, TE = tetracycline, CN = gentamycin, and AMC = amoxicillin-clavulanate.

3.3. Prevalence of Multidrug-Resistant Isolates of S. aureus in Different Types of Samples

About 146 out of 483 isolates (30.2%) were resistant to at least three different classes of antibiotics. The population of MDR *S. aureus* was composed of 70 (14.5%), 51 (10.6%), 15 (3.1%), and 10 (2.1%) isolates from chicken, human, rodent, and soil samples, respectively (Table 3). The MDR rates varied significantly for isolates from chickens, humans, rodents, and soil ($p < 0.001$). In all types of samples, none of the MDR *S. aureus* isolates were resistant to all six classes of antibiotics.

Table 3. MDR rates of *S. aureus* isolates from different types of samples.

Type of Sample Source	Number of Antibiotic Classes to Which the Isolates Were Resistant, *n* (%)									Chi-Squared	*p*-Value
	0	1	2	3	4	5	6	Total Isolates	MDR Isolates (3–6 Classes)		
Overall	81 (16.8)	74 (15.3)	182 (37.7)	104 (21.5)	32 (6.6)	10 (2.1)	0 (0.0)	483 (100.0)	146 (30.2)		
Chickens	34 (42.0)	15 (20.3)	45 (24.7)	61 (58.7)	9 (58.7)	0 (0.0)	0 (0.0)	164 (34.0)	70 (14.5)	143.66 df = 3	$p < 0.001$
Humans	12 (14.8)	31 (41.9)	86 (47.3)	30 (28.8)	14 (43.8)	7 (70.0)	0 (0.0)	180 (37.3)	51 (10.6)	195.12 df = 3	$p < 0.001$
Rodents	19 (23.5)	16 (21.6)	25 (13.7)	8 (7.7)	7 (21.9)	0 (0.0)	0 (0.0)	75 (15.5)	15 (3.1)	51.47 df = 6	$p < 0.001$
Soil	16 (19.8)	12 (16.2)	26 (14.3)	5 (4.8)	2 (6.3)	3 (30.0)	0 (0.0)	64 (13.3)	10 (2.1)	57.84 df = 6	$p < 0.001$

3.4. Prevalence of MDR *S. aureus* in Samples from Different Wards in the Study Area

Most of the MDR *S. aureus* isolates (43.2%) were found in samples from Endamarariek, followed by Karatu (21.9%) and Endabash (15.8%), while a few MDR isolates were observed in samples from Mbulumbulu (11.0%) and Rhotia (8.2%) (Figure 2). The occurrence of MDR isolates varied significantly in samples from the Endabash, Karatu, Endamarariek ($p < 0.001$), Mbulumbulu ($p < 0.006$), and Rhotia ($p < 0.005$) wards (Table 4).

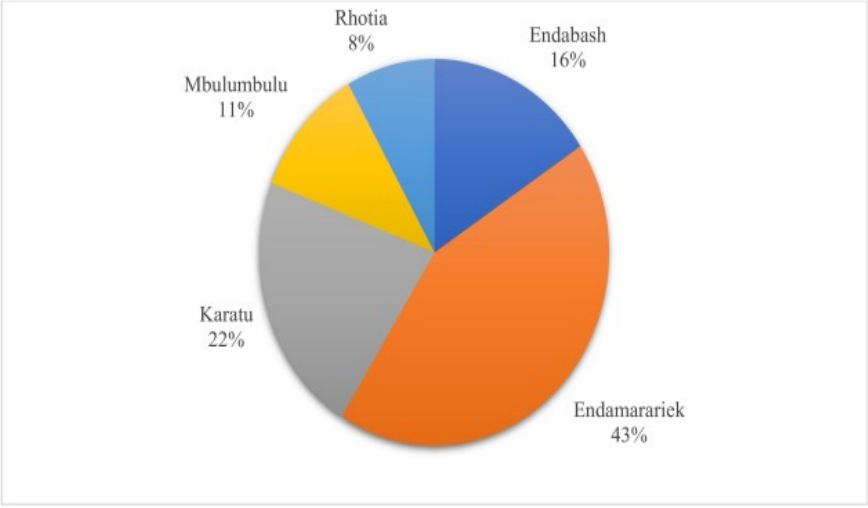

Figure 2. Distribution of MDR *S. aureus* isolates in different wards of Karatu.

Table 4. Prevalence of MDR *S. aureus* isolates in different samples by wards.

Wards	MDR Isolates from Different Sample Sources *n* (%)					Chi-Squared	*p*-Value
	Chickens	Humans	Rodents	Soil	Total		
Overall MDR	70 (14.5)	51 (10.6)	15 (3.1)	10 (2.1)	146 (30.2)		
Endabash	16 (3.3)	3 (0.6)	3 (0.6)	1 (0.2)	23 (4.8)	24.826 df = 3	<0.001
Endamarariek	18 (3.7)	30 (6.2)	10 (2.1)	3 (0.6)	61 (12.6)	29.508 df = 3	<0.001
Karatu	20 (4.1)	8 (1.7)	0 (0.0)	4 (0.8)	32 (6.6)	28 df = 3	<0.001
Mbulumbulu	8 (1.7)	7 (1.4)	1 (0.2)	1 (0.2)	17 (3.5)	12.5 df = 3	0.0059
Rhotia	8 (1.7)	3 (0.6)	1 (0.2)	1 (0.2)	13 (2.7)	12.667 df = 3	0.0054
Chi-squared	9.1429	55.962	22	8.25			
p-Value	0.0576	<0.001	0.0002	0.0828			

3.5. Phenotypic Patterns of MDR S. aureus Isolates

As shown in Table 5, MDR *S. aureus* isolates displayed variable resistance patterns, where CD-E-TE was the most common that appeared in chicken (34.2%), human (14.4%), rodent (3.4%), and soil (1.4%) isolates. CD-E-TE-AMC was also common in chicken (6.2%), human (6.2%), and rodent (2.7%) isolates, but not in the soil isolates. Patterns showing resistance to five different classes of antibiotics were CD-E-TE-CN-AMC found in soil and rodent (0.7%) samples and CD-E-CIP-TE-AMC in soil (1.4%) and human (4.8%) samples. However, none of the MDR *S. aureus* isolates were resistant to all antibiotic classes.

Table 5. Phenotypic resistance patterns of MDR *S. aureus* isolates from chickens, humans, rodents, and soil samples.

Source of Samples (*N* = 146)	Number of Isolates (*n*)	Occurrence (%)	Antibiotic Resistance Patterns	Number of Antibiotic Classes
Chickens (*n* = 70)	50	34.2	CD, E, TE	3
	3	2.1	CD, CIP, TE	
	1	0.7	E, CIP, TE	
	5	3.4	E, TE, AMC	
	9	6.2	CD, E, TE, AMC	4
	1	0.7	CD, E, CIP, TE	
	1	0.7	CD, E, TE, CN	
Humans (*n* = 51)	21	14.4	CD, E, TE	3
	3	2.1	CD, CIP, TE	
	2	1.4	CD, E, CIP	
	1	0.7	CD, E, AMC	
	1	0.7	CD, TE, AMS	
	1	0.7	E, TE, AMC	
	9	6.2	CD, E, TE, AMC	4
	1	0.7	E, CIP, TE, CN	
	3	2.1	CD, E, CIP, TE	
	2	1.4	CD, CIP, TE, AMC	
	7	4.8	CD, E, CIP, TE, AMC	5
Rodents (*n* = 15)	5	3.4	CD, E, TE	3
	1	0.7	CD, E, AMC	
	1	0.7	CIP, CN, AMC	
	1	0.7	CD, TE, AMC	
	4	2.7	CD, E, TE, AMC	4
	2	1.4	CD, E, TE, CN	
	1	0.7	CD, E, TE, CN, AMC	5
Soil (*n* = 10)	2	1.4	CD, E, TE	3
	1	0.7	E, TE, AMC	
	1	0.7	CD, TE, AMC	
	1	0.7	CD, CIP, TE	
	2	1.4	CD, E, CIP, TE	4
	2	1.4	CD, E, CIP, TE, AMC	5
	1	0.7	CD, E, TE, CN, AMC	
Total	146	100.0		

AMC = amoxicillin-clavulanate, TE = tetracycline, E = erythromycin, CD = clindamycin, CIP = ciprofloxacin, and CN = gentamycin.

4. Discussion

This is the first study to investigate the carriage of *S. aureus* in chickens, humans, rodents, and soils in a household environment in Tanzania. Overall, the isolation frequency of *S. aureus* was 50.5%. We observed significant variations in isolation frequencies among sample sources, where rodents had more *S. aureus* (74.3%), and soil had the lowest (24.5%). The presence of drug-resistant bacteria in soil serves as a potential reservoir of antibiotic resistomes, which encompasses all types of antibiotic resistance genes (ARGs) that can spread to humans and animals and to a wider environment [29,30]. Rodents carrying

different zoonotic pathogens have been frequently reported to invade human residences in Karatu [20–22]. Overall, the isolates exhibited high resistance to clindamycin (51.0%), tetracycline (62.5%), and erythromycin (50.9%). These antibiotics are commonly used in humans and poultry production in the study area, and their frequent use and misuse can significantly contribute to increased resistance [6,18,31–33]. In this community, there is frequent use and misuse of the drugs in food animals, including poultry, mainly tetracycline and erythromycin [34,35]. Farmers in rural areas of Tanzania have been treating their chickens with antibiotics without diagnosis or prescriptions from veterinarians [35].

Our study observed that 146 out of 483 (30.2 %) isolates were MDR, including 14.5% chicken, 10.6% human, 3.1% rodent, and 2.1% soil isolates. The higher prevalence of MDR *S. aureus* in humans and poultry can be associated with the extensive use of drugs in human medicine and poultry in the community [36]. Lower multidrug resistance rates in rodents could be because these animals are not direct consumers of antibiotics, as is the case for humans and chickens. Their exposure to drugs is indirect, depending on contact with human and chicken wastes when dropped in the household environment, as explained in other studies [37,38]. Our findings are in keeping with those of Vitale et al. [32], showing that *S. aureus* derived from humans were more resistant to antibiotics compared with those of animal origin.

In our study, most MDR isolates were found in the Endamarariek ward (12.6%), which is basically a rural area compared to Karatu (6.6%), an urban and district headquarter. These variations could be due to differences in the levels of awareness and use of antibiotics between the wards. Endamarariek is a rural area with a scarcity of veterinarians, where farmers mostly treat their chickens based on experiences using home-stored antibiotics and those purchased from village shops with a low level of control. Such variations can also explain why we found more MDR *S. aureus* isolates (2.1%) in rodent samples from Endamarariek compared to Karatu samples (0%). Among MDR patterns, CD-E-TE, standing for clindamycin, erythromycin, and tetracycline, was displayed in most of the isolates (34.2 %), and the pattern is identical for humans, chickens, and rodents. Different studies on the resistance profiles of *S. aureus* have reported similar patterns as well [7,18,32,33,39,40]. Erythromycin and tetracycline are the most commonly used antibiotics in this area, since they are cheap and can be purchased over the counter without a prescription [41].

Limitation of the Study

Despite our findings being useful in the control of antimicrobial resistance in Tanzania, a genetic characterization of antibiotic resistance and virulence factors of *S. aureus* could provide additional information to compliment the phenotypic approach.

5. Conclusions

These results suggest a potential role of the interaction of humans, chickens, and rodents in cross-transmission of MDR *S. aureus* among them, with the possibility of causing human and animal infections that are difficult to treat. Unfortunately, treatment alternatives are very limited due to the few types of antibiotics in the studied area and the economic reality. Therefore, necessary interventions, such as continuous educative campaigns on effective cleanliness in households, safe disposal of animal wastes, and rodent control strategies, are urgently needed.

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Informed Consent Statement: Informed verbal consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gao, J.; Ferreri, M.; Yu, F.; Liu, X.; Chen, L.; Su, J.; Han, B. Molecular types and antibiotic resistance of *Staphylococcus aureus* isolates from bovine mastitis in a single herd in China. *Ir. Vet. J.* **2012**, *192*, 550–552. [CrossRef]
2. Wang, W.; Baloch, Z.; Jiang, T.; Zhang, C.; Peng, Z.; Li, F.; Fanning, S.; Ma, A.; Xu, J. Enterotoxigenicity and antimicrobial resistance of *Staphylococcus aureus* isolated from retail food in China. *Front. Microbiol.* **2017**, *8*, 2256. [CrossRef] [PubMed]
3. Khalaf, S.K.; Nagham, M.; Abdulkarim, J.; Jenan, M. Isolation of methicillin resistant *Staphylococcus aureus* (MRSA) from Rattus rattus from Adhamiyah district in Baghdad governorate. *MRVSA* **2015**, *4*, 9–23.
4. Tong, S.Y.; Davis, J.S.; Eichenberger, E.; Holland, T.L.; Fowler Jr, V.G. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clin. Microbiol. Rev.* **2015**, *28*, 603–661. [CrossRef] [PubMed]
5. Rodríguez-Lázaro, D.; Ariza-Miguel, J.; Diez-Valcarce, M.; Fernández-Natal, I.; Hernández, M.; Rovira, J. Foods confiscated from non-EU flights as a neglected route of potential methicillin-resistant *Staphylococcus aureus* transmission. *Int. J. Food Microbiol.* **2015**, *209*, 29–33. [CrossRef]
6. Cvetnić, L.; Samardžija, M.; Duvnjak, S.; Habrun, B.; Cvetnić, M.; Jaki Tkalec, V.; Đuričić, D.; Benić, M. Multi Locus Sequence Typing and spa typing of *Staphylococcus aureus* isolated from the milk of cows with subclinical mastitis in Croatia. *Microorganisms* **2021**, *9*, 725. [CrossRef]
7. Massawe, H.; Mdegela, R.; Kurwijila, L. Antibiotic resistance of *Staphylococcus aureus* isolates from milk produced by smallholder dairy farmers in Mbeya Region, Tanzania. *Int. J. One Health* **2019**, *5*, 31–37. [CrossRef]
8. Marcon, A.V.; De Oliveira, G.; Caldara, F.R.; Garcia, R.G.; Matins, R.; Marcon, A.; Crone, C.; Assunción, A.S.d.A. Bacteriological and Histopathological Evaluation of Articulations of Chickens Diagnosed with Arthritis. *Rev. Bras.* **2019**, *21*. [CrossRef]
9. Nazia, M.K.; Durrani, N.; Kamboh, A.; Lakho, S.; Rind, R.; Abro, S.; Soomro, N. Prevalence of septic arthritis caused by *Staphylococcus aureus* in poultry birds at Tandojam. *Trop Anim. Health Prod.* **2015**, *3*, 73–77.
10. Jackson, C.R.; Davis, J.A.; Barrett, J.B. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from retail meat and humans in Georgia. *J. Clin. Microbiol.* **2013**, *51*, 1199–1207. [CrossRef]
11. Kmieciak, W.; Szewczyk, E.M. Coagulase-positive species of the genus Staphylococcus–taxonomy, pathogenicity. *Postępami Mikrobiol.* **2019**, *56*, 233–244. [CrossRef]
12. Subramanya, S.H.; Bairy, I.; Metok, Y.; Baral, B.P.; Gautam, D.; Nayak, N. Detection and characterization of ESBL-producing Enterobacteriaceae from the gut of subsistence farmers, their livestock, and the surrounding environment in rural Nepal. *Sci. Rep.* **2021**, *11*, 2091. [CrossRef]
13. Himsworth, C.G.; Miller, R.R.; Montoya, V.; Hoang, L.; Romney, M.G.; Al-Rawahi, G.N.; Kerr, T.; Jardine, C.M.; Patrick, D.M.; Tang, P. Carriage of methicillin-resistant *Staphylococcus aureus* by wild urban Norway rats (Rattus norvegicus). *PLoS ONE* **2014**, *9*, e87983. [CrossRef] [PubMed]
14. Ge, J.; Zhong, X.-s.; Xiong, Y.-q.; Qiu, M.; Huo, S.-t.; Chen, X.-j.; Mo, Y.; Cheng, M.-j.; Chen, Q. Methicillin-resistant *Staphylococcus aureus* among urban rodents, house shrews, and patients in Guangzhou, southern China. *BMC Vet. Res.* **2019**, *15*, 1–8. [CrossRef] [PubMed]
15. Henzler, D.; Opitz, H. The role of mice in the epizootiology of Salmonella enteritidis infection on chicken layer farms. *Avian Dis.* **1992**, *36*, 625–631. [CrossRef] [PubMed]
16. Lapuz, R.; Tani, H.; Sasai, K.; Shirota, K.; Katoh, H.; Baba, E. The role of roof rats (Rattus rattus) in the spread of Salmonella Enteritidis and S. Infantis contamination in layer farms in eastern Japan. *Epidemiol. Infect.* **2008**, *136*, 1235–1243. [CrossRef]
17. Morzunov, S.P.; Rowe, J.E.; Ksiazek, T.G.; Peters, C.J.; St. Jeor, S.C.; Nichol, S.T. Genetic analysis of the diversity and origin of hantaviruses in Peromyscus leucopus mice in North America. *J. Virol.* **1998**, *72*, 57–64. [CrossRef]
18. Rabiee, M.H.; Mahmoudi, A.; Siahsarvie, R.; Kryštufek, B.; Mostafavi, E. Rodent-borne diseases and their public health importance in Iran. *PLOS Negl. Trop. Dis.* **2018**, *12*, e0006256. [CrossRef] [PubMed]
19. Witmer, G.W.; Shiels, A.B. Ecology, impacts, and management of invasive rodents in the United States. In *Ecology and Management of Terrestrial Vertebrate Invasive Species in the United States*; CRC Press: Boca Raton, FL, USA, 2017; pp. 193–220.
20. Kilonzo, B.; Mbise, T.; Mwalimu, D.; Kindamba, L. Observations on the endemicity of plague in Karatu and Ngorongoro, northern Tanzania. *Tanzan. J. Health Res.* **2006**, *8*, 1–6. [CrossRef] [PubMed]
21. Makundi, R.H.; Massawe, A.W.; Mulungu, L.S.; Katakweba, A.; Mbise, T.J.; Mgode, G. Potential mammalian reservoirs in a bubonic plague outbreak focus in Mbulu District, northern Tanzania, in 2007. *Mammalia* **2008**, *72*, 253–257. [CrossRef]

22. Ziwa, M.; Matee, M.; Hang'ombe, B.; Lyamuya, E.; Kilonzo, B.S. Plague in Tanzania: An overview. *Tanzan. J. Health Res.* **2013**, *15*, 252–258. [CrossRef]
23. Makundi, R.H.; Massawe, A.W.; Borremans, B.; Laudisoit, A.; Katakweba, A. We are connected: Flea–host association networks in the plague outbreak focus in the Rift Valley, northern Tanzania. *Wildl. Res.* **2015**, *42*, 196–206. [CrossRef]
24. Theonest, N.O.; Carter, R.W.; Amani, N.; Doherty, S.L.; Hugho, E.; Keyyu, J.D.; Mable, B.K.; Shirima, G.M.; Tarimo, R.; Thomas, K.M.; et al. Molecular detection and genetic characterization of Bartonella species from rodents and their associated ectoparasites from northern Tanzania. *PLoS ONE* **2019**, *14*, e0223667. [CrossRef] [PubMed]
25. Haule, M.; Lyamuya, E.E.; Hang'ombe, B.M.; Matee, M.I. Investigation of Fleas as Vectors in the Transmission of Plague during a Quiescent Period in North-Eastern, Tanzania. 2013. Available online: http://www.suaire.sua.ac.tz/handle/123456789/1037 (accessed on 20 June 2021).
26. Lupindu, A.M.; Dalsgaard, A.; Msoffe, P.L.; Ngowi, H.A.; Mtambo, M.M.; Olsen, J.E. Transmission of antibiotic-resistant Escherichia coli between cattle, humans and the environment in peri-urban livestock keeping communities in Morogoro, Tanzania. *Prev. Vet. Med.* **2015**, *118*, 477–482. [CrossRef]
27. Clinical and Laboratory Standards Institute. *CLSI-Performance Standards for Antimicrobial Susceptibility Testing*; CLSI: Wayne, PA, USA, 2020; Volume 40, p. 293.
28. Magiorakos, A.-P.; Srinivasan, A.; Carey, R.t.; Carmeli, Y.; Falagas, M.t.; Giske, C.t.; Harbarth, S.; Hindler, J.t.; Kahlmeter, G.; Olsson-Liljequist, B. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* **2012**, *18*, 268–281. [CrossRef] [PubMed]
29. D'Costa, V.M.; Griffiths, E.; Wright, G.D. Expanding the soil antibiotic resistome: Exploring environmental diversity. *Curr. Opin. Microbiol.* **2007**, *10*, 481–489. [CrossRef] [PubMed]
30. Woolhouse, M.; Ward, M.; Van Bunnik, B.; Farrar, J. Antimicrobial resistance in humans, livestock and the wider environment. *Philos. Trans. R. Soc. B Biol. Sci.* **2015**, *370*, 20140083. [CrossRef]
31. Akanbi, O.E.; Njom, H.A.; Fri, J.; Otigbu, A.C.; Clarke, A.M. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from recreational waters and beach sand in Eastern Cape Province of South Africa. *Int. J. Environ. Res. Public Health* **2017**, *14*, 1001. [CrossRef]
32. Vitale, M.; Galluzzo, P.; Buffa, P.G.; Carlino, E.; Spezia, O.; Alduina, R. Comparison of antibiotic resistance profile and biofilm production of *Staphylococcus aureus* isolates derived from human specimens and animal-derived samples. *Antibiotics* **2019**, *8*, 97. [CrossRef] [PubMed]
33. Amoako, D.G.; Somboro, A.M.; Abia, A.L.; Molechan, C.; Perrett, K.; Bester, L.A.; Essack, S.Y. Antibiotic resistance in *Staphylococcus aureus* from poultry and poultry products in uMgungundlovu District, South Africa, using the "Farm to Fork" approach. *Microb. Drug Resist.* **2020**, *26*, 402–411. [CrossRef]
34. Caudell, M.A.; Quinlan, M.B.; Subbiah, M.; Call, D.R.; Roulette, C.J.; Roulette, J.W.; Roth, A.; Matthews, L.; Quinlan, R.J. Antimicrobial use and veterinary care among agro-pastoralists in Northern Tanzania. *PLoS ONE* **2017**, *12*, e0170328. [CrossRef]
35. Rugumisa, B.; Call, D.R.; Mwanyika, G.O.; Subbiah, M.; Buza, J. Comparison of the Prevalence of Antibiotic-Resistant Escherichia Coli Isolates from Commercial-Layer and Free-Range Chickens in Arusha District, Tanzania. 2016. Available online: http://dspace.nm-aist.ac.tz/handle/123456789/457 (accessed on 20 June 2021).
36. Odsbu, I.; Khedkar, S.; Lind, F.; Khedkar, U.; Nerkar, S.S.; Orsini, N.; Tamhankar, A.J.; Stålsby Lundborg, C. Trends in resistance to extended-spectrum cephalosporins and carbapenems among Escherichia coli and Klebsiella spp. isolates in a district in Western India during 2004–2014. *Int. J. Environ. Res. Public Health* **2018**, *15*, 155. [CrossRef]
37. Gómez, P.; González-Barrio, D.; Benito, D.; García, J.T.; Viñuela, J.; Zarazaga, M.; Ruiz-Fons, F.; Torres, C. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the mecC gene in wild small mammals in Spain. *J. Antimicrob. Chemother.* **2014**, *69*, 2061–2064. [CrossRef]
38. Silva, V.; Gabriel, S.I.; Borrego, S.B.; Tejedor-Junco, M.T.; Manageiro, V.; Ferreira, E.; Reis, L.; Caniça, M.; Capelo, J.L.; Igrejas, G. Antimicrobial Resistance and Genetic Lineages of *Staphylococcus aureus* from Wild Rodents: First Report of mecC-Positive Methicillin-Resistant S. aureus (MRSA) in Portugal. *Animals* **2021**, *11*, 1537. [CrossRef] [PubMed]
39. Abdeen, E.E.; Mousa, W.S.; Abdelsalam, S.Y.; Heikal, H.S.; Shawish, R.R.; Nooruzzaman, M.; Soliman, M.M.; Batiha, G.E.; Hamad, A.; Abdeen, A. Prevalence and Characterization of Coagulase Positive Staphylococci from Food Products and Human Specimens in Egypt. *Antibiotics* **2021**, *10*, 75. [CrossRef] [PubMed]
40. Oladipo, A.O.; Oladipo, O.G.; Bezuidenhout, C.C. Multi-drug resistance traits of methicillin-resistant *Staphylococcus aureus* and other Staphylococcal species from clinical and environmental sources. *J. Water Health* **2019**, *17*, 930–943. [CrossRef] [PubMed]
41. Mdegela, R.H.; Mwakapeje, E.R.; Rubegwa, B.; Gebeyehu, D.T.; Niyigena, S.; Msambichaka, V.; Nonga, H.E.; Antoine-Moussiaux, N.; Fasina, F.O. Antimicrobial Use, Residues, Resistance and Governance in the food and agriculture sectors, Tanzania. *Antibiotics* **2021**, *10*, 454. [CrossRef] [PubMed]

CHAPTER THREE

PAPER II

antibiotics

MDPI

Article

Occurrence of Multi-Drug-Resistant *Escherichia coli* in Chickens, Humans, Rodents and Household Soil in Karatu, Northern Tanzania

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Abstract: We investigated antibiotic resistance profiles of *Escherichia coli* among 960 samples obtained from chickens (236), humans (243), rodents (101) and soil (290). *E. coli* was isolated from 650 (67.7%) samples. Isolation frequency varied significantly between chickens, humans, rodents and soil samples, being 81.6%, 86.5%, 79.2% and 31.0%, respectively ($p < 0.001$). Resistance rates were particularly higher against imipenem (79.8%), cefotaxime (79.7%) and tetracycline (73.7%) and moderate against amoxicillin-clavulanate (49.4%). Overall, 78.8% of the isolates were multidrug-resistant (MDR) among which, 38.8%, 25.1%, 12.9% and 2.5% exhibited resistance to three, four, five and six different classes of antibiotics, respectively. Multidrug-resistant *E. coli* were observed in 27.7%, 30.3%, 10.8% and 10.0% of the isolates from chickens, humans, rodents and soil samples, respectively. Our results show high levels of antimicrobial resistance including MDR in *E. coli* isolated from chickens, humans, rodents and soil samples in Karatu, Northern Tanzania. Comprehensive interventions using a one-health approach are needed and should include improving (i) awareness of the community on judicious use of antimicrobial agents in humans and animals, (ii) house conditions and waste management and (iii) rodent control measures.

Keywords: *Escherichia coli*; antibiotic resistance; humans; rodents; chickens; soil; isolates

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1. Introduction

Antibiotic resistance is currently a serious problem worldwide that threatens human, animal and environmental health [1]. If the situation remains unmanaged by 2050, higher human mortalities, severe economic losses and a significant drop in livestock production are expected [2]. *Escherichia coli* is the major cause of urinary tract infections and neonatal meningitis in humans [3], and it also causes avian colibacillosis, a serious infectious disease in poultry [4]. Other conditions caused by *E. coli* in chickens include yolk sac infections, pericarditis, peritonitis and osteomyelitis [5]. *E. coli* is a commensal microbe in humans and chickens that carries and spreads resistance genes to other pathogens [6], threatening public health. Rodents that invade human habitats carry and transmit different zoonotic pathogens including MDR *E. coli*, threatening human health [7,8]. The interaction between rodents, humans, livestock and their environment in households can facilitate sharing of antibiotic-resistant bacteria and their resistance genes [9]. Studies have documented that,

in rural areas where poultry farming is common, household soils are contaminated with higher antibiotic residues from humans and animals [10–13], leading to an increase in and spread of antibiotic resistance genes, involving contamination of the environment [14]. Previous studies have pointed out the potential role of peridomestic rodents in the spread of resistant bacteria to humans and domestic animals, either directly or through the environment [7,14,15]. Thus, the interaction between humans, livestock and peridomestic rodents is of public health concern, since it has the potential to cause infections that are difficult to treat [9]. In Karatu district in Northern Tanzania, studies have shown intense interactions between rodents, humans and animals, leading to infectious disease epidemics [16–20]. However, none of these studies screened bacteria for antimicrobial resistance. We hypothesize these interactions can cause the transmission of resistant bacteria, fueling the spread of antimicrobial resistance (AMR) in the community. We conducted this study in Karatu district in the northern zone of Tanzania to isolate and phenotypically screen for antimicrobial resistant *E. coli* among humans, chickens, rodents and the soil in households that keep indigenous chickens. This cross-sectional study determined phenotypic AMR patterns of *E. coli* isolated from rodents, humans, chickens, and their environment in households in an area where their interaction is intense.

2. Results

2.1. Isolation of Escherichia coli from the Samples

E. coli was isolated from 650 (67.7%) samples (Table 1). Isolation of *E. coli* varied significantly between chickens, humans, rodents and soil samples at 81.9%, 86.5%, 80.2% and 31.0%, respectively ($p < 0.001$).

Table 1. Isolation frequencies of *Escherichia coli* from different samples.

Type of Samples	Total Number of Samples n (%)	Positive Samples n (%)	Chi-Square	*p*-Value
Chickens	288 (30.0)	236 (81.9)		
Humans	281 (29.3)	243 (86.5)		
Rodents	101 (10.5)	81 (80.2)	$X^2 = 147.58$, df = 3	<0.001
Soil	290 (30.2)	90 (31.0)		
Total	960 (100.0)	650 (67.7)		

2.2. Antibiotic Resistance of E. coli Isolates from Chickens, Humans, Rodents and Soil

Overall, the *E. coli* isolates were resistant to tetracycline (73.7%), amoxicillin-clavulanate (49.4%), imipenem (79.8%), ciprofloxacin (40.2%), cefotaxime (79.7%) and gentamycin (9.7%), as shown in Figure 1. The overall resistance rates were 34.5%, 38.6%, 13.7% and 13.1% for isolates from chickens, humans, rodents and soil, respectively. Increased resistance of isolates to imipenem, cefotaxime and tetracycline was observed in all types of samples (Figure 1).

2.3. Multidrug-Resistance (MDR) of Escherichia coli Isolates from All Samples

A total of 512 out of 650 isolates (78.8%), were resistant to three and above different classes of antibiotics. Most of the isolates (38.3%) were resistant to three classes of antibiotics, and 16 isolates (2.5%) were resistant to all classes where most of them were from chicken (seven isolates) and human (five isolates) samples (Table 2). We observed significant variation in the occurrence of MDR isolates from different types of samples being higher in humans (30.3%) and chickens (27.7%) compared to rodents (10.8%) and soils (10.0%) ($p < 0.001$).

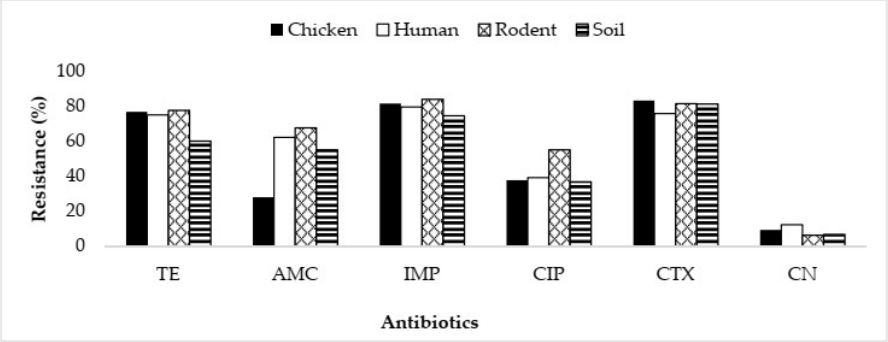

Figure 1. Resistance rates of *E. coli* isolates to different classes of antibiotics. TE = tetracycline; AMC = amoxicillin-clavulanate; IMP = imipenem; CIP = ciprofloxacin; CTX = cefotaxime; CN = gentamycin.

Table 2. Antibiotic resistance and MDR rates of *Escherichia coli* isolates from chickens, humans, rodents and soil samples.

Types of Sample Sources	Number of Antibiotic Classes to Which the Isolates Were Resistant, n (%)							Total Number of Isolates	MDR Isolates (3–6 Classes)	Chi-Square	*p*-Value
	0	1	2	3	4	5	6				
Overall n (%)	5 (0.8)	24 (3.7)	109 (16.8)	249 (38.3)	163 (25.1)	84 (12.9)	16 (2.5)	650 (100.0)	512 (78.8)		
Chickens	0 (0.0)	10 (1.5)	46 (7.1)	103 (15.8)	55 (8.5)	15 (2.3)	7 (1.1)	236 (36.3)	180 (27.7)	129.75 df = 3	<0.001
Humans	5 (0.8)	8 (1.2)	33 (5.1)	88 (13.5)	63 (9.7)	41 (6.3)	5 (0.8)	243 (37.4)	197 (30.3)	75.47, df = 3	<0.001
Rodents	0 (0.0)	2 (0.3)	9 (1.4)	25 (3.8)	20 (3.1)	22 (3.4)	3 (0.5)	81 (12.5)	70 (10.8)	16.16, df = 3	0.001
Soil	0 (0.0)	4 (0.6)	21 (3.2)	33 (5.1)	25 (3.8)	6 (0.9)	1 (0.2)	90 (13.8)	65 (10.0)	42.75, df = 3	<0.001

2.4. Prevalence of MDR Isolates of E. coli in Different Locations of Karatu

Among 512 MDR isolates, 74, 81, 153, 137 and 116 were isolated from the samples collected in Endabash, Endamarariek, Karatu, Mbulumbulu and Rhotia wards, respectively (Figure 2).

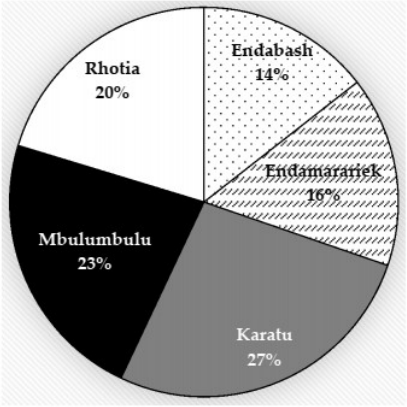

Figure 2. Distribution of MDR *E. coli* isolates in different areas of Karatu district.

All MDR *E. coli* isolates from chickens, humans, rodents and soil samples were distributed in the wards as shown in Table 3. Significant variation in the prevalence of MDR isolates was observed between the wards, being higher in samples from Karatu (21.1%) compared to those from Endabash (11.4%) ward ($p < 0.001$) (Table 3).

Table 3. Prevalence of MDR *E. coli* isolates from all types of samples in different wards in Karatu district.

Ward	Types of Sample Sources, n (%)				MDR Isolates	Chi-Square	*p*-Value
	Chickens	Humans	Rodents	Soil			
Overall n (%)	180 (27.7)	197 (30.3)	70 (10.8)	65 (10.0)	512 (78.8)		
Endabash	20 (3.1)	22 (3.4)	17 (2.6)	15 (2.3)	74 (11.4)	1.6571, df = 3	0.647
Endamarariek	32 (4.9)	27 (4.2)	10 (1.5)	12 (1.8)	81 (12.5)	18.532, df = 3	<0.001
Karatu	40 (6.2)	61 (9.4)	24 (3.7)	12 (1.8)	137 (21.1)	49.118, df = 3	<0.001
Mbulumbulu	50 (7.7)	42 (6.5)	8 (1.8)	16 (2.5)	116 (17.8)	43.571, df = 3	<0.001
Rhotia	38 (5.8)	45 (6.9)	11 (1.7)	10 (1.5)	104 (16.0)	39.44, df = 3	<0.001
Chi-Square	24.82, df = 4	25.03, df = 4	13.077, df = 4	2.00, df = 4			
p-value	<0.001	<0.001	0.011	0.736			

As shown in Figure 3, along principal component 1 (PC1), the arrow for gentamycin (CN) is very close to 0 (*X*-axis), followed by that of ciprofloxacin (CIP), indicating their respective lower variances compared to other drugs, indicating that isolates were more susceptible to CN followed by CIP. The arrow for amoxicillin–clavulanate (AMC) is far (high deviation) from the PC1 axis (Susceptibility), indicating relatively higher resistance rates of isolates to AMC compared to CN and CIP. Along the principal component 2 (PC2), the arrows for tetracycline (TE), imipenem (IMP), cefotaxime (CTX) and AMC are close to PC2 (*Y*-axis), showing higher variances compared to other drugs, which implies higher resistance rates of isolates to these drugs. The large positive loadings for TE, IMP and CTX indicate a greater and positive correlation between them in terms of resistance patterns. The overlapping of ellipses indicates that the proportions of resistant isolates did not vary significantly across sample sources.

2.5. Phenotypic Patterns of MDR E. coli Isolates from Chickens, Humans, Rodents and Soil

MDR *E. coli* isolates from chickens displayed different resistance patterns where TE-IMP-CTX (7.4%), TE-IMP-CIP-CTX (3.5%) and TE-AMC-CIP-CTX (3.1%) were the most common as shown in Table 4.

MDR isolates from human samples displayed TE-IMP-CTX (4.5%), TE-AMC-IMP-CTX (4.9%) and TE-AMC-IMP-CIP-CTX (5.1%) as the common patterns of resistance (Table 5).

Among 70 MDR *E. coli* isolates from rodent samples, 8 isolates (4.5%), 9 isolates (1.4%) and 22 isolates (3.4%) displayed TE-IMP-CTX, TE-AMC-IMP-CTX and TE-AMC-IMP-CIP-CTX as common patterns of resistance, respectively (Table 6).

For MDR isolates from soil samples, TE-IMP-CTX (2.0%) and TE-AMC-IMP-CTX (1.8%) were the common resistance patterns, as shown in Table 7 below. Overall, the combination TE-IMP-CTX was the most common pattern appearing in isolates from all types of samples.

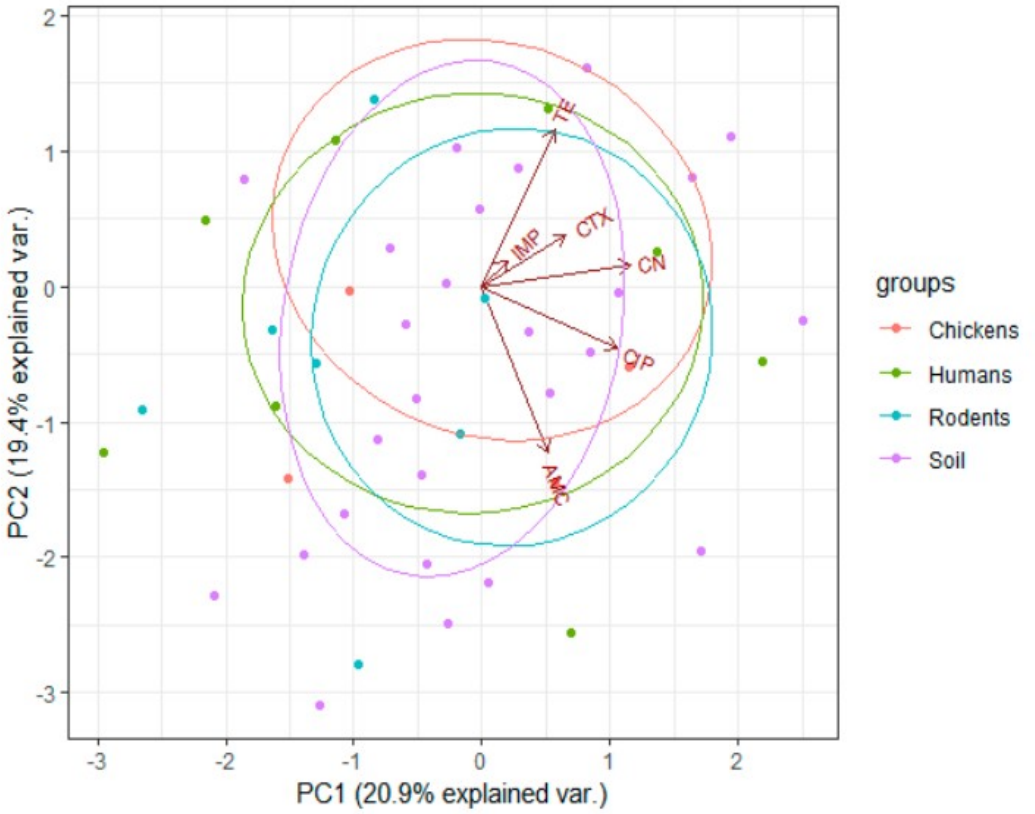

Figure 3. Principal component analysis (PCA) biplots of the antibiotic resistance profiles of *E. coli* isolated from chickens, humans, rodents and soil samples. For principal component 1 (PC1), the *X*-axis expresses susceptibility of the isolates to the drugs, while for principal component 2 (PC2), the *Y*-axis expresses resistance of the isolates. Arrows indicate the antibiotics that were used during resistance screening. The dots represent the *E. coli* isolates that were resistant to the tested antibiotics with respect to their sample sources. The ellipses indicate a 95% confidence interval of the respective isolates from the same sample source.

Table 4. Resistance patterns of 180 MDR *E. coli* isolates from chicken samples.

Chicken Samples	Number of Isolates (n)	Occurrence (%)	Antibiotic Resistance Patterns	Number of Antibiotic Classes
(n = 180)	10	1.5	AMC, IMP, CTX	3
	9	1.4	IMP, CIP, CTX	
	1	0.2	AMC, IMP, CIP	
	2	0.3	AMC, CIP, CTX	
	3	0.5	TE, AMC, CTX	
	2	0.3	TE, AMC, IMP	
	4	0.6	TE, CIP, CTX	
	48	7.4	TE, IMP, CTX	
	1	0.2	TE, IMP, CIP	
	2	0.3	TE, AMC, CTX	
	12	1.8	TE, CIP, CTX	
	2	0.3	TE, IMP, CIP	
	3	0.5	TE, CIP, CTX	
	2	0.3	TE, AMC, IMP	
	1	0.2	TE, IMP, CN	
	1	0.2	TE, AMC, CIP	

Table 4. *Cont.*

Chicken Samples	Number of Isolates (n)	Occurrence (%)	Antibiotic Resistance Patterns	Number of Antibiotic Classes
	1	0.2	TE, CIP, CTX, CN	4
	2	0.3	AMC, IMP, CTX, CN	
	4	0.6	AMC, IMP, CIP, CTX,	
	23	3.5	TE, IMP, CIP, CTX	
	1	0.2	TE, IMP, CIP, CN	
	20	3.1	TE, AMC, CIP, CTX	
	2	0.3	TE, AMC, IMP, CTX	
	2	0.3	TE, IMP, CTX, CN	
	10	1.5	TE, AMC, IMP, CIP, CTX	5
	3	0.5	TE, IMP, CIP, CTX, CN	
	1	0.2	TE, AMC, IMP, CTX, CN	
	1	0.2	TE, IMP, CIP, CTX, CN	
	7	1.1	TE, AMC, IMP, CIP, CTX, CN	6
Total	180	27.7		

AMC = amoxicillin-clavulanate; TE = tetracycline; IMP = imipenem; CTX = cefotaxime; CIP = ciprofloxacin; CN = gentamycin.

Table 5. Resistance patterns of 197 MDR *E. coli* isolates from human samples.

Human Samples	Number of Isolates (n)	Occurrence (%)	Antibiotic Resistance Patterns	Number of Antibiotic Classes
(n = 197)	17	2.6	AMC, IMP, CTX	3
	5	0.8	AMC, IMP, CIP	
	2	0.3	AMC, CIP, CTX	
	29	4.5	TE, IMP, CTX	
	10	1.5	TE, AMC, CTX	
	1	0.2	IMP, CIP, CTX	
	8	1.2	TE, AMC, IMP	
	7	1.1	TE, IMP, CIP	
	2	0.3	TE, AMC, CIP	
	2	0.3	TE, CIP, CTX	
	2	0.3	TE, CTX, CN	
	1	0.2	TE, IMP, CTX	
	1	0.2	TE, CIP, CTX	
	1	0.2	TE, IMP, CN	
	6	0.9	TE, AMC, IMP, CIP	4
	1	0.2	IMP, CIP, CTX, CN	
	1	0.2	TE, AMC, IMP, CN	
	1	0.2	TE, AMC, CTX, CN	
	1	0.2	AMC, IMP, CTX, CN	
	1	0.2	AMC, IMP, CIP, CN	
	32	4.9	TE, AMC, IMP, CTX	
	4	0.6	TE, AMC, CIP, CTX	
	9	1.4	TE, IMP, CIP, CTX	
	7	1.1	AMC, IMP, CIP, CTX	
	33	5.1	TE, AMC, IMP, CIP, CTX	5
	1	0.2	AMC, IMP, CIP, CTX, CN	
	4	0.6	TE, AMC, IMP, CTX, CN	
	2	0.3	TE, IMP, CIP, CTX, CN	
	1	0.2	TE, AMC, CIP, CTX, CN	
	5	0.8	TE, AMC, IMP, CIP, CTX, CN	6
Total	197	30.3		

AMC = amoxicillin-clavulanate; TE = tetracycline; IMP = imipenem; CTX = cefotaxime; CIP = ciprofloxacin; CN = gentamycin.

Table 6. Resistance patterns of 70 MDR *E. coli* isolates from rodent samples.

Rodent Samples	Number of Isolates (n)	Occurrence (%)	Antibiotic Resistance Patterns	Number of Antibiotic Classes
(n = 70)	3	0.5	AMC, IMP, CIP	3
	4	0.6	AMC, CIP, CTX	
	1	0.2	AMC, IMP, CTX	
	1	0.2	IMP, CIP, CTX	
	8	1.2	TE, IMP, CTX	
	1	0.2	TE, AMC, IMP	
	1	0.2	TE, CIP, CTX	
	1	0.2	TE, AMC, CTX	
	1	0.2	TE, AMC, IMP	
	3	0.5	TE, IMP, CIP	
	2	0.3	AMC, IMP, CIP, CTX	4
	9	1.4	TE, AMC, IMP, CTX	
	1	0.2	TE, AMC, CIP, CN	
	3	0.5	TE, IMP, CIP, CTX	
	5	0.8	TE, AMC, IMP CIP	
	22	3.4	TE, AMC, IMP, CIP, CTX	5
	1	0.2	TE, AMC, IMP, CIP, CN	
	3	0.5	TE, AMC, IMP, CIP, CTX, CN	6
Total	70	10.8		

AMC = amoxicillin-clavulanate; TE = tetracycline; IMP = imipenem; CTX = cefotaxime; CIP = ciprofloxacin; CN = gentamycin.

Table 7. Resistance patterns of 65 MDR *E. coli* isolates from soil samples.

Source of Samples	Number of Isolates (n)	Occurrence (%)	Antibiotic Resistance Patterns	Number of Antibiotic Classes
(n = 512)	1	0.2	AMC, IMP, CIP	3
	7	1.1	AMC, IMP, CTX	
	3	0.5	AMC, IMP, CTX	
	1	0.2	IMP, CIP, CTX, CN	
	1	0.2	IMP, CTX, CN	
	3	0.5	TE, AMC, CTX	
	1	0.2	TE, AMC, CIP	
	1	0.2	TE, CIP, CTX	
	2	0.3	TE, IMP, CIP	
	13	2.0	TE, IMP, CTX	
	12	1.8	TE, AMC, IMP, CTX	4
	4	0.6	TE, IMP, CIP, CTX	
	4	0.6	TE, AMC, CIP, CTX	
	4	0.6	AMC, IMP, CIP, CTX	
	1	0.2	TE, IMP, CTX, CN	
	1	0.2	AMC, IMP, CIP, CTX, CN	5
	1	0.2	TE, IMP, CIP, CTX, CN	
	4	0.6	TE, AMC, IMP, CIP, CN	
	1	0.2	TE, AMC, IMP, CIP, CTX, CN	6
Total	65	10.0		

AMC = amoxicillin-clavulanate; TE = tetracycline; IMP = imipenem; CTX = cefotaxime; CIP = ciprofloxacin; CN = gentamycin.

3. Discussion

We conducted this study in Karatu because of high and frequent interactions among rodents, humans and chickens that have been reported in previous studies and shown to cause the spread of infections [16–20]. To the best of our knowledge, this is the first study in Tanzania to simultaneously screen MDR *E. coli* in chickens, humans, rodents

and soil. In total, 650 out of 960 samples (67.7%) were positive for *E. coli*. We found high resistance of isolates against imipenem (79.8%), cefotaxime (79.7%), tetracycline (73.7%) and amoxicillin-clavulanate (49.4%) compared to ciprofloxacin (40.2%) and gentamicin (9.7%). Indeed, the principal component 1 (PC1) indicated that isolates were more susceptible to gentamycin followed by ciprofloxacin, and relatively higher resistance rates of isolates to amoxicillin-clavulanate. The principal component 2 (PC2), indicated that tetracycline, imipenem, cefotaxime and amoxicillin-clavulanate were close to PC2 (*Y*-axis) showing higher variances compared to other drugs, which implies higher resistance rates of isolates to these drugs. Interestingly, large positive loadings for tetracycline, imipenem and cefotaxime indicated greater and positive correlation between them in terms of resistance patterns. The overlapping of ellipses indicates that the proportions of resistant isolates did not vary significantly across sample sources, indicating widespread presence of AMR *E.coli*. Most of the highly resisted antibiotics are readily available and can be obtained over the counter without prescription [21–23].

We observed that 78.8% of all isolates were MDR *E. coli*, and that chicken (27.7%) and human (30.3%) isolates had significantly higher MDR isolates as compared to those recovered from rodents (10.8%) and soil (10.0%). Higher occurrence rates of MDR isolates in chickens and humans can be influenced by the frequent use and misuse of antibiotics in humans and poultry in Karatu [21,22,24,25]. The presence of MDR *E. coli* isolates in rodents indicates their potential role as hosts or vectors that can spread MDR *E. coli* to humans and chickens in Karatu and corresponds with other studies in Kenya, Germany, Canada and Vietnam [14,26–28] that associated rodents with carriage and spread of MDR and virulent *E. coli* strains in communities. The isolation frequency of MDR *E. coli* from soil was 10.0%, which is close to the 12.6% reported in Bangladesh [29]. This indicates that household soils are potential reservoirs of MDR *E. coli*, possibly due to poor disposal of sewages and poultry manure in households. Previous studies in Tanzania have revealed that mismanagement of human and livestock wastes in households contaminates the soil with *E. coli* [10,30,31]. In our study, MDR isolates exhibited different resistance patterns. However, the combination of tetracycline, imipenem and cefotaxime (TE-IMP-CTX) was the most common in all types of samples, highlighting their frequent use in humans and poultry. The increased use of cefotaxime and tetracycline during disease treatment and prevention in poultry has been widely documented worldwide, resulting in widespread antibiotic resistance [32–35]. Interestingly, we found a higher isolation frequency of MDR *E. coli* in samples from Karatu (21.1%), which is an urban area and district headquarter compared to Endabash ward (11.4%), a rural area. Concomitant with this, we found a higher level of interaction between humans, chickens, rodents and their environment in urban households, coupled with a higher level of antimicrobial use and low levels of waste management, which may have facilitated the spread of MDR *E. coli* [9,36]. Finally, we acknowledge that our findings provide preliminary insight on the magnitude and pattern of AMR *E. coli* in humans, chickens, rodents and their environment and not transmission dynamics. Molecular studies of AMR genes of isolates from humans, chickens, rodents and soil will be required to determine cross-transmission of superbugs among different hosts and the environment in the area. Nonetheless, these findings should alert public health officials to take the necessary interventions, including raising public awareness, on the appropriate use of antimicrobial agents and proper hygiene measures, including waste disposal.

4. Materials and Methods

4.1. Study Location

The study was conducted in Karatu district in the Northern zone of Tanzania between June 2020 and March 2021. Karatu is located between latitudes 3°10′ and 4°00′ S and longitude 34°47′ E. The district has a population of 230,166 people comprised of 117,769 men and 112,397 women with an average of 5 people per household [37]. Karatu has an altitude

range of 1000 to 1900 m above sea level with two wet seasons annually (short rains between October and December and long rains from March to June).

4.2. Sampling Strategy

The study population included all households keeping local chickens, while the sampling frame was the list of these households. Five wards (Karatu, Endabash, Endamarariek, Mbulumbulu and Rhotia) were purposively selected based on population density (at least 16,000), number of households with chickens, and household size of at least 5 people. Households were randomly selected from a list provided by a livestock field officer at ward level by using a table of random numbers. At the household level, house owners' permission was used in trapping the rodents where areas for trapping in the surrounding environments relied on signs of rodents' activities. For each household, one adult (18 years and above) resident and one mature (7 months) scavenging chicken participated in microbiological sampling (1 fecal and 1 nasal swab). Additionally, at least one rodent (in-house rat, peridomestic rat or both) could be captured, as well as one soil sample collected per household. The selection of adult humans and mature chickens was based on the assumption that old individuals have been exposed to the interaction with rodents for a long time than young ones and hence are more likely to facilitate sharing of infections.

4.3. Trapping of Rodents for Sample Collection

Live trapping of 101 rodents was carried out using Modified-Sherman traps baited with peanut butter. Each captured rodent was euthanized by using di-ethyl-ether. Deep pharyngeal swabs and intestines were aseptically collected from the carcasses.

4.4. Collection of Samples from Humans, Chickens and Soil

A total of 960 samples was obtained from chickens (236), humans (243), rodents (101) and soil (290). Cloacal swabs were collected from randomly picked scavenging local chickens and human stools in households. Additionally, soil samples were randomly collected from five points in the household yards and mixed to compose 1 pooled soil sample [10]. Thereafter, all samples were stored in sterile containers at −4 °C and transported using Cary Blair transport medium to Tanzania Veterinary Laboratory Agency (TVLA) laboratory in Arusha for bacteriological analyses.

4.5. Culture, Isolation and Identification of E. coli Isolates

The specimens were plated onto MacConkey agar (Oxoid ltd., Detroid, MI, USA) and incubated aerobically at 37 °C for 24 h. Presumptive colonies of *E. coli* were subjected to a combination of four biochemical tests—indole, methyl red, Voges–Proskauer and citrate utilization (IMViC)—as well as motility tests for identification as per Quinn et al. [38]. *E. coli* strain American Type Culture Collection (ATCC) 29,522 was used as a standard organism.

4.6. Antibiotic Susceptibility Testing of E. coli Isolates

An antibiotic susceptibility test was performed by using Kirby–Bauer disc diffusion method on Mueller–Hinton Agar plates (Oxoid, Basingstoke, UK) with commercially available discs as described by [39]. The antibiotics tested were tetracycline (30 µg), imipenem (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), cefotaxime (30 µg) and amoxicillin-clavulanate (20 µg/10 µg). Pure colonies of the identified lactose fermenters were emulsified into 5 mL of sterile saline. The suspensions were adjusted to achieve turbidity equivalent to 0.5 McFarland standard solutions, emulsified using sterile cotton swabs onto Mueller–Hinton Agar plate, and incubated at 37 °C for 16 to 18 h. After incubation the inhibition zone of each antimicrobial agent was measured, and results were interpreted according to the CLSI standards [39]. *E. coli* strain American Type Culture Collection (ATCC) 29,522 was used as standard organism. An isolate was considered to be multidrug-resistant if it was non-susceptible to three or more drugs from different classes of antibiotics [40].

4.7. Statistical Analyses

Isolation frequencies of *E. coli* and antibiotic resistance profiles of isolates were entered into Microsoft Excel version 2010 (Microsoft Corporation, Redmond, WA, USA) and their percentages calculated by descriptive statistics. Association between categorical variables was analyzed using Chi-square (Fisher's exact and Pearson's) test. Principal component analysis (PCA) was used to describe the distribution of *E. coli* resistant isolates with respect to their sample sources and antibiotic resistance profiles. PCA was performed using R Statistical Package Windows version 3.4.2. Statistical significance was accepted at $p < 0.05$.

5. Conclusions

The level of antimicrobial resistance, including multi-drug-resistant *E. coli* seen in isolates from humans, chickens, rodents and soil raises the possibility of widespread transmission of resistance genes and bacteria in the studied area, with the possibility of causing infections that are difficult to treat. The antibiotics used in this study are the ones that are commonly used in the area for treating both human and animal infections, implying that they have limited success in their intended use. Comprehensive interventions, using a one-health approach, would be required to control the situation. Such measures should include improving (i) awareness of the community on judicious use of antimicrobial agents in humans and animals, (ii) house conditions and waste management and (iii) rodent control measures.

Author Contributions: Collection of samples, laboratory processing and data analysis (V.S.S.), supervision and verification of the analytical methods (M.I.N.M., A.S.K. and G.M.). Manuscript developed by V.S.S. with input from M.I.N.M., G.M. and A.S.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The ethical clearance for the study was issued by the National Institute for Medical Research (NIMR) of Tanzania (NIMR/HQ/R.8a/Vol.IX/3386). NIMR is the national health research coordinating body that ensures all health research follows the national health ethics requirements. Sokoine University of Agriculture (SUA) Institutional Animal Care and Use Ccommittee (IACUC) approved the use of animals in this study. The permission to work in the study area was sought from the Regional Administrative Office, Arusha.

Informed Consent Statement: Informed verbal consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Islam, M.; Nayeem, M.; Hasan, M.; Sobur, M.; Ievy, S.; Rahman, S.; Kafi, M.; Ashour, H.M.; Rahman, M. Virulence Determinants and Multidrug Resistance of *Escherichia coli* Isolated from Migratory Birds. *Antibiotics* **2021**, *10*, 190. [CrossRef]
2. Orubu, E.S.F.; Zaman, M.H.; Rahman, M.T.; Wirtz, V.J. Veterinary antimicrobial resistance containment in Bangladesh: Evaluating the national action plan and scoping the evidence on implementation. *J. Glob. Antimicrob. Resist.* **2020**, *21*, 105–115. [CrossRef] [PubMed]
3. Etienne, M.; Lefebvre, E.; Frebourg, N.; Hamel, H.; Pestel-Caron, M.; Caron, F. Antibiotic treatment of acute uncomplicated cystitis based on rapid urine test and local epidemiology: Lessons from a primary care series. *BMC Infect. Dis.* **2014**, *14*, 1–8. [CrossRef]
4. Nolan, L.K.; Vaillancourt, J.P.; Barbieri, N.L.; Logue, C.M. Colibacillosis. In *Diseases of Poultry*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2020; pp. 770–830. [CrossRef]
5. Subramanya, S.H.; Bairy, I.; Metok, Y.; Baral, B.P.; Gautam, D.; Nayak, N. Detection and characterization of ESBL-producing Enterobacteriaceae from the gut of subsistence farmers, their livestock, and the surrounding environment in rural Nepal. *Sci. Rep.* **2021**, *11*, 1–13.

6. Ievy, S.; Islam, M.; Sobur, M.; Talukder, M.; Rahman, M.; Khan, M.F.R. Molecular detection of avian pathogenic *Escherichia coli* (APEC) for the first time in layer farms in Bangladesh and their antibiotic resistance patterns. *Microorganisms* **2020**, *8*, 1021. [CrossRef]
7. Desvars-Larrive, A.; Ruppitsch, W.; Lepuschitz, S.; Szostak, M.P.; Spergser, J.; Feßler, A.T.; Schwarz, S.; Monecke, S.; Ehricht, R.; Walzer, C. Urban brown rats (*Rattus norvegicus*) as possible source of multidrug-resistant Enterobacteriaceae and meticillin-resistant *Staphylococcus spp.*, Vienna, Austria, 2016 and 2017. *Eurosurveillance* **2019**, *24*, 1900149. [CrossRef]
8. Gwenzi, W.; Chaukura, N.; Muisa-Zikali, N.; Teta, C.; Musvuugwa, T.; Rzymski, P.; Abia, A.L.K. Insects, Rodents, and Pets as Reservoirs, Vectors, and Sentinels of Antimicrobial Resistance. *Antibiotics* **2021**, *10*, 68. [CrossRef]
9. Hassell, J.M.; Ward, M.J.; Muloi, D.; Bettridge, J.M.; Robinson, T.P.; Kariuki, S.; Ogendo, A.; Kiiru, J.; Imboma, T.; Kang'ethe, E.K.; et al. Clinically relevant antimicrobial resistance at the wildlife-livestock-human interface in Nairobi: An epidemiological study. *Lancet Planet. Health* **2019**, *3*, e259–e269. [CrossRef]
10. Lupindu, A.M.; Dalsgaard, A.; Msoffe, P.L.; Ngowi, H.A.; Mtambo, M.M.; Olsen, J.E. Transmission of antibiotic-resistant *Escherichia coli* between cattle, humans and the environment in peri-urban livestock keeping communities in Morogoro, Tanzania. *Prev. Vet. Med.* **2015**, *118*, 477–482. [CrossRef] [PubMed]
11. Zhang, Y.-J.; Hu, H.-W.; Gou, M.; Wang, J.-T.; Chen, D.; He, J.-Z. Temporal succession of soil antibiotic resistance genes following application of swine, cattle and poultry manures spiked with or without antibiotics. *Environ. Pollut.* **2017**, *231*, 1621–1632. [CrossRef] [PubMed]
12. Sanderson, H.; Brown, R.S.; Hania, P.; McAllister, T.A.; Majury, A.; Liss, S.N. Antimicrobial resistant genes and organisms as environmental contaminants of emerging concern: Addressing global public health risks. In *Management of Emerging Public Health Issues and Risks*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 147–187.
13. Touati, M.; Hadjadj, L.; Berrazeg, M.; Baron, S.A.; Rolain, J.M. Emergence of *Escherichia coli* harbouring *mcr-1* and *mcr-3* genes in North West Algerian farmlands. *J. Glob. Antimicrob. Resist.* **2020**, *21*, 132–137. [CrossRef] [PubMed]
14. Guenther, S.; Bethe, A.; Fruth, A.; Semmler, T.; Ulrich, R.G.; Wieler, L.H.; Ewers, C. Frequent combination of antimicrobial multiresistance and extraintestinal pathogenicity in *Escherichia coli* isolates from urban rats (*Rattus norvegicus*) in Berlin, Germany. *PLoS ONE* **2012**, *7*, e50331. [CrossRef]
15. Feng, A.Y.; Himsworth, C.G. The secret life of the city rat: A review of the ecology of urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). *Urban Ecosyst.* **2014**, *17*, 149–162. [CrossRef]
16. Kilonzo, B.; Mbise, T.; Mwalimu, D.; Kindamba, L. Observations on the endemicity of plague in Karatu and Ngorongoro, northern Tanzania. *Tanzan. J. Health Res.* **2006**, *8*, 1–6. [CrossRef]
17. Makundi, R.H.; Massawe, A.W.; Borremans, B.; Laudisoit, A.; Katakweba, A. We are connected: Flea-host association networks in the plague outbreak focus in the Rift Valley, northern Tanzania. *Wildl. Res.* **2015**, *42*, 196–206. [CrossRef]
18. Ziwa, M.H.; Matee, M.I.; Hang'ombe, B.M.; Lyamuya, E.F.; Kilonzo, B.S. Plague in Tanzania: An overview. *Tanzan. J. Health Res.* **2013**, *15*. [CrossRef]
19. Haule, M.; Lyamuya, E.E.; Hang'ombe, B.M.; Matee, M.I. *Investigation of Fleas as Vectors in the Transmission of Plague during a Quiescent Period in North-Eastern, Tanzania*; Sokoine University of Agriculture: Morogoro, Tanzania, 2013.
20. Makundi, R.H.; Massawe, A.W.; Mulungu, L.S.; Katakweba, A.; Mbise, T.J.; Mgode, G. Potential mammalian reservoirs in a bubonic plague outbreak focus in Mbulu District, northern Tanzania, in 2007. *Mammalia* **2008**, *72*, 253–257. [CrossRef]
21. Rugumisa, B.; Call, D.R.; Mwanyika, G.O.; Subbiah, M.; Buza, J. Comparison of the Prevalence of Antibiotic-Resistant Escherichia coli Isolates from Commercial-Layer and Free-Range Chickens in Arusha District, Tanzania. 2016. Available online: https://dspace.nm-aist.ac.tz/handle/20.500.12479/457 (accessed on 20 June 2021).
22. Lyimo, B.; Buza, J.; Subbiah, M.; Smith, W.; Call, D.R. Comparison of antibiotic resistant *Escherichia coli* obtained from drinking water sources in northern Tanzania: A cross-sectional study. *BMC Microbiol.* **2016**, *16*, 254. [CrossRef]
23. Caudell, M.A.; Mair, C.; Subbiah, M.; Matthews, L.; Quinlan, R.J.; Quinlan, M.B.; Zadoks, R.; Keyyu, J.; Call, D.R. Identification of risk factors associated with carriage of resistant *Escherichia coli* in three culturally diverse ethnic groups in Tanzania: A biological and socioeconomic analysis. *Lancet Planet. Health* **2018**, *2*, e489–e497. [CrossRef]
24. Kimera, Z.I.; Mgaya, F.X.; Misinzo, G.; Mshana, S.E.; Moremi, N.; Matee, M.I. Multidrug-Resistant, Including Extended-Spectrum Beta Lactamase-Producing and Quinolone-Resistant, *Escherichia coli* Isolated from Poultry and Domestic Pigs in Dar es Salaam, Tanzania. *Antibiotics* **2021**, *10*, 406. [CrossRef] [PubMed]
25. Mgaya, F.X.; Matee, M.I.; Muhairwa, A.P.; Hoza, A.S. Occurrence of multidrug resistant *Escherichia coli* in raw meat and cloaca swabs in poultry processed in slaughter slabs in Dar es Salaam, Tanzania. *Antibiotics* **2021**, *10*, 343. [CrossRef]
26. Gakuya, F.; Kyule, M.; Gathura, P.; Kariuki, S. Antimicrobial susceptibility and plasmids from *Escherichia coli* isolated from rats. *East Afr. Med. J.* **2001**, *78*, 518–522. [CrossRef]
27. Himsworth, C.G.; Zabek, E.; Desruisseau, A.; Parmley, E.J.; Reid-Smith, R.; Jardine, C.M.; Tang, P.; Patrick, D.M. Prevalence and characteristics of *Escherichia coli* and *Salmonella* spp. in the feces of wild urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) from an inner-city neighborhood of Vancouver, Canada. *J. Wildl. Dis.* **2015**, *51*, 589–600. [CrossRef]
28. Le Huy, H.; Koizumi, N.; Ung, T.T.H.; Le, T.T.; Nguyen, H.L.K.; Hoang, P.V.M.; Nguyen, C.N.; Khong, T.M.; Hasebe, F.; Haga, T. Antibiotic-resistant *Escherichia coli* isolated from urban rodents in Hanoi, Vietnam. *J. Vet. Med. Sci.* **2020**, *82*, 653–660. [CrossRef]

29. Montealegre, M.C.; Roy, S.; Böni, F.; Hossain, M.I.; Navab-Daneshmand, T.; Caduff, L.; Faruque, A.; Islam, M.A.; Julian, T.R. Risk factors for detection, survival, and growth of antibiotic-resistant and pathogenic *Escherichia coli* in household soils in rural Bangladesh. *Appl. Environ. Microbiol.* **2018**, *84*, e01978-18. [CrossRef] [PubMed]
30. Badowski, N.; Castro, C.M.; Montgomery, M.; Pickering, A.J.; Mamuya, S.; Davis, J. Understanding household behavioral risk factors for diarrheal disease in Dar es Salaam: A photovoice community assessment. *J. Environ. Public Health* **2011**, *2011*, 130467. [CrossRef]
31. Pickering, A.J.; Julian, T.R.; Marks, S.J.; Mattioli, M.C.; Boehm, A.B.; Schwab, K.J.; Davis, J. Fecal contamination and diarrheal pathogens on surfaces and in soils among Tanzanian households with and without improved sanitation. *Environ. Sci. Technol.* **2012**, *46*, 5736–5743. [CrossRef] [PubMed]
32. Sadiq, M.; Syed-Hussain, S.; Ramanoon, S.; Saharee, A.; Ahmad, N.; Zin, N.M.; Khalid, S.; Naseeha, D.; Syahirah, A.; Mansor, R. Knowledge, attitude and perception regarding antimicrobial resistance and usage among ruminant farmers in Selangor, Malaysia. *Prev. Vet. Med.* **2018**, *156*, 76–83. [CrossRef] [PubMed]
33. Teklu, D.S.; Negeri, A.A.; Legese, M.H.; Bedada, T.L.; Woldemariam, H.K.; Tullu, K.D. Extended-spectrum beta-lactamase production and multi-drug resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia. *Antimicrob. Resist. Infect. Control* **2019**, *8*, 39. [CrossRef]
34. Mdegela, R.H.; Mwakapeje, E.R.; Rubegwa, B.; Gebeyehu, D.T.; Niyigena, S.; Msambichaka, V.; Nonga, H.E.; Antoine-Moussiaux, N.; Fasina, F.O. Antimicrobial Use, Residues, Resistance and Governance in the food and agriculture sectors, Tanzania. *Antibiotics* **2021**, *10*, 454. [CrossRef]
35. Sindato, C.; Mboera, L.E.; Katale, B.Z.; Frumence, G.; Kimera, S.; Clark, T.G.; Legido-Quigley, H.; Mshana, S.E.; Rweyemamu, M.M.; Matee, M. Knowledge, attitudes and practices regarding antimicrobial use and resistance among communities of Ilala, Kilosa and Kibaha districts of Tanzania. *Antimicrob. Resist. Infect. Control* **2020**, *9*, 194. [CrossRef] [PubMed]
36. Arnold, K.E.; Williams, N.J.; Bennett, M. 'Disperse abroad in the land': The role of wildlife in the dissemination of antimicrobial resistance. *Biol. Lett.* **2016**, *12*, 20160137. [CrossRef] [PubMed]
37. URT (United Republic of Tanzania). *2012 Population and Housing Census—Population Distribution by Administrative Areas*; URT: Dodoma, Tanzania, 2013; p. 244.
38. Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W.J.; Leonard, F.C. *Veterinary Microbiology and Microbial Disease: Pathogenic Bacteria Blackwell Scientific Publications*; Oxford: London, UK, 2002; pp. 113–115.
39. CLSI. *Clinical and Laboratory Standards Institute-Performance Standards for Antimicrobial Susceptibility Testing*; CLSI: Wayne, PA, USA, 2020.
40. Magiorakos, A.-P.; Srinivasan, A.; Carey, R.; Carmeli, Y.; Falagas, M.; Giske, C.; Harbarth, S.; Hindler, J.; Kahlmeter, G.; Olsson-Liljequist, B. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* **2012**, *18*, 268–281. [CrossRef] [PubMed]

CHAPTER FOUR

PAPER III

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Article

Molecular Epidemiology of Antibiotic Resistance Genes and Virulence Factors in Multidrug-Resistant *Escherichia coli* Isolated from Rodents, Humans, Chicken, and Household Soils in Karatu, Northern Tanzania

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Abstract: The interaction of rodents with humans and chicken in the household environment can facilitate transmission of multidrug-resistant (MDR) *Escherichia coli* (*E. coli*), causing infections that are difficult to treat. We investigated the presence of genes encoded for carbapenem, extended spectrum beta-lactamases (ESBL), tetracycline and quinolones resistance, and virulence among 50 MDR *E. coli* isolated from human (n = 14), chicken (n = 12), rodent (n = 10), and soil (n = 14) samples using multiplex polymerase chain reaction (PCR). Overall, the antimicrobial resistance genes (ARGs) detected were: *blaTEM* 23/50 (46%), *blaCTX-M* 13/50 (26%), *tetA* 23/50 (46%), *tetB* 7/50 (14%), *qnrA* 12/50 (24%), *qnrB* 4/50 (8%), *blaOXA-48* 6/50 (12%), and *blaKPC* 3/50 (6%), while *blaIMP*, *blaVIM*, and *blaNDM-1* were not found. The virulence genes (VGs) found were: *ompA* 36/50 (72%), *traT* 13/50 (26%), *east* 9/50 (18%), *bfp* 5/50 (10%), *eae* 1/50 (2%), and *stx-1* 2/50 (4%), while *hlyA* and *cnf* genes were not detected. Resistance (*blaTEM*, *blaCTX-M*, *blaSHV*, *tetA*, *tetB*, and *qnrA*) and virulence (*traT*) genes were found in all sample sources while *stx-1* and *eae* were only found in chicken and rodent isolates, respectively. Tetracycline resistance phenotypes correlated with genotypes *tetA* (r = 0.94), *tetB* (r = 0.90), *blaKPC* (r = 0.90; *blaOXA-48* (r = 0.89), and *qnrA* (r = 0.96). ESBL resistance was correlated with genotypes *blaKPC* (r = 0.93), *blaOXA*-48 (r = 0.90), and *qnrA* (r = 0.96) resistance. Positive correlations were observed between resistance and virulence genes: *qnrB* and *bfp* (r = 0.63) also *blaTEM*, and *traT* (r = 0.51). Principal component analysis (PCA) indicated that *tetA*, *tetB*, *blaTEM*, *blaCTX-M*, *qnrA*, and *qnrB* genes contributed to tetracycline, cefotaxime, and quinolone resistance, respectively. While *traT stx-1*, *bfp*, *ompA*, *east*, and *eae* genes contributed to virulence of MDR *E. coli* isolates. The PCA ellipses show that isolates from rodents had more ARGs and virulence genes compared to those isolated from chicken, soil, and humans.

Keywords: multidrug-resistant; rodents; chicken; humans; soil; *E. coli*; PCR; genes

1. Introduction

Escherichia coli (*E. coli*) is a versatile bacterial pathogen that has the ability to cause various infections, most of which are difficult to treat [1,2]. In fact, this bacterium is listed by the World Health Organization (WHO) as one of the critical antimicrobial-resistant bacteria that can cause severe and often deadly infections such as bloodstream infections and pneumonia [3]. The pathogenicity of *E. coli* strains is enhanced by a variety of virulence and resistance genes [4,5]. *E. coli* strains producing extended-spectrum β-lactamases (ESBLs) and carbapenemases are potentially recognized pathogens that can resist most β-lactam antibiotics [6,7]. ESBLs are plasmid-mediated enzymes that hydrolyse β-lactam containing antimicrobial agents including penicillins, cephalosporins, and aztreonam. ESBLs are grouped into three main types: TEM, SHV, and CTX-M [8,9]. Carbapenemases are a major group of β-lactamases capable of hydrolysing penicillins, cephalosporins, monobactams, and carbapenems. They include β-lactamases of classes B (IMP and VIM), D (OXA-23 to -27), and A (IMI, KPC, NMC, and SME) [10,11]. Tetracycline resistance genes (*tetA* and *tetB*) coded for efflux pumps have been frequently detected in human and animal *E. coli* isolates [12]. The genes *qnrA* and *qnrB* are known to confer quinolone resistance in *E. coli* strains and spread horizontally through plasmids [13].

Important virulence factors of *E. coli* are encoded by several genes including: locus enterocyte effacement (LEE), intimin, bundle forming (*bae*, *bfpA*)) [14,15], Shiga toxins, adhesins (*stx1*, *stx2*, *eaeA*, *ehxA, and bfpA*) [15,16], heat-labile, heat stable, and colonization factors (*elt*, *est)* [14,16]. *E. coli* is a typical One Health pathogen, with the potential of resistomes spreading between humans, animals, and the environment, where such interactions exist [17]. In Tanzania, studies conducted in the Karatu ecosystem have revealed intense interactions between humans, rodents, and chicken, leading to frequent occurrence and recurrence of zoonotic infections [18–20]. Previous studies have suggested that the role of rodents in the transmission of multidrug-resistant (MDR) bacterial infections to humans and environmental contamination [21–24]. In a recent phenotypic study conducted in Karatu, we isolated *E. coli* strains from chickens, humans, rodents, and soils which showed high levels of resistance to cefotaxime (79.7%), imipenem (79.8%), and tetracycline (73.7%); 512 out of 650 (78.8%) were MDR [25].

We hypothesize that the intense interactions between chickens, humans, rodents, and soils may lead to the transfer of ARGs and VRGs among them. However, molecular characterization of ARGs and VGs was not conducted in the phenotypic study [25]. Knowledge of ARGs and VGs is important in understanding the pathogenicity and virulence of *E. coli* [26]. This study was conducted in Karatu, Northern Tanzania to provide insights of molecular epidemiology of ARGs and VGs occurring in *E. coli* isolated among chickens, humans, rodents, and soils in households. To our knowledge, this is the first study in Tanzania that has investigated the genotypic diversity of *E. coli* isolated among chicken, humans, rodents, and soils in households. Multiplex PCR [27] was used for detection of genes encoding for tetracycline resistance (*tetA*, *tetB*), ESBL (*blaCTX-M*, *blaSHV*, and *blaTEM*), metallo beta-lactamases (*blaVIM*, *blaIMP*, and *blaNDM*), and virulence genes *bfp*, *east*, *hlyA*, *traT*, *eaeA*, *ompA*, *cnf*, and *stx-1*. The working assumption is that MDR *E. coli* strains circulating in Karatu carry a variety of virulence genes capable of causing life-threatening infections that are difficult to treat.

2. Materials and Methods

2.1. Study Area

This study was conducted between June 2020 and March 2021 in the Karatu district in the northern zone of Tanzania, located between latitudes 3°10′ and 4°00′ S, and longitude 34°47′ and 35°56′ E. The district has a population of 230,166 people comprised of 117,769 men and 112,397 women with an average of five people per household [28]. Karatu has an altitude range from 1000 to 1900 m above sea level with two wet seasons annually (short rains between October and December and long rains from March to June).

2.2. Bacterial Isolates

A total of 50 MDR *E. coli* isolates from chicken cloaca swabs (12), human nasal swabs (14), rodents' deep pharyngeal swabs (10), and household soil (14) samples, particularly those with higher phenotypic resistance to tetracycline, imipenem, and cefotaxime, were selected for genomic DNA extraction and further genomic analyses. All selected isolates were preserved in nutrient broth (TSB) with 50% glycerol (*v*/*v*) at −80 °C until DNA extraction. Isolates that were resistant to at least three different classes of antibiotics were considered as multidrug-resistant (MDR) [29].

2.3. DNA Extraction

The genomic DNA of all phenotypically MDR *E. coli* strains were extracted by using Zymo Research Fungal and Bacterial Genomic DNA MiniPrep™ kit (Zymo Research, Irvine, CA, USA), following the manufacturer's instructions. The purity, quality, and quantity of DNA were determined using a nanodrop spectrophotometer (NanoDrop, Thermo Scientific, Ramsey, NJ, USA) and agarose gel electrophoresis. The extracted DNA samples were stored at −80 °C until when PCR analyses were performed.

2.4. Detection of Antimicrobial Resistance and Virulence Genes

Multiplex PCR [27] was used to detect the tetracycline (*tetA* and *tetB*), ESBL (*blaCTX-M*, *blaSHV*, and *blaTEM*), and Metallo beta-lactamases (*blaVIM*, *blaIMP*, and *blaNDM*) resistance and virulence (*bfp*, *east*, *hlyA*, *traT*, *eae*, *ompA*, *cnf*, and *stx1*) genes. Briefly, lyophilized primers (Macrogen, Amsterdam, The Netherlands) for target genes (in supplementary materials) were reconstituted using nuclease-free water to obtain 100 µM stock and 10 µM working solutions before storage at −20 °C. PCR was carried out in a total volume of 25 µL containing 12.5 µL of 1 X *Taq* PCR Master Mix (Bio Basic, Canada), 1 µL of the forward primer and 1 µL of the reverse primer, 3 µL of DNA template, and 7.5 µL nuclease-free water. Multiplex PCRs were conducted using amplification conditions indicated in Table 1. PCR products were separated by electrophoresis on 1.5% (*w*/*v*) agarose gel pre-stained with Gel Red (Merck, Darmstadt, Germany) at 120 Volts for 1 h, and visualized under UV light using a BioDoc-it™ imaging system (Ultra-Violet Products, Cambridge, UK). PCR product size was determined by conducting electrophoresis along with a GeneRuler 100 bp Plus DNA Ladder (Bioneer, Daedeok-gu, Republic of Korea). DNA from *E. coli* American Type Culture Collection (ATCC) 29522 strain was used for quality assurance.

Table 1. Detection of virulence genes of MDR *E. coli* isolates from different sample sources.

Genes	Different Sample Sources *n* (%)				
	Humans (*n* = 14)	**Chickens (*n* = 12)**	**Rodents (*n* = 10)**	**Soil (*n* = 14)**	**Total (*n* = 50)**
Bfp	0 (0.0)	0 (0.0)	3 (30.0)	2 (14.3)	5 (10.0)
East	0 (0.0)	4 (33.3)	3 (30.0)	2 (14.3)	9 (18.0)
hlyA	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
traT	4 (28.6)	4 (33.3)	4 (40.0)	1 (7.1)	13 (26.0)
eae	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	1 (2.0)
ompA	10 (71.4)	12 (100.0)	7 (70.0)	7 (50.0)	36 (72.0)
cnf	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
stx-1	0 (0.0)	1 (8.3)	1 (10.0)	0 (0.0)	2 (4.0)
Total	2 (14.3)	4 (33.3)	6 (60.0)	4 (33.3)	16 (32.0)
χ^2-square	52.29	46.43	2.00	26.67	
p-value	0.001	0.001	0.0188	0.0004	

2.5. Statistical Analysis

The data obtained were entered into an Excel spreadsheet (Microsoft® Office Excel 2010) and analysed. The differences in occurrence of the genes (%) between categories were compared by chi-square test using R-software, version 4.0.2 (R Foundation for Statistical

computing, Vienna, Austria) [30]. Principal component analysis (PCA) was used to investigate the distribution and relationships of antimicrobial resistance and virulence genes of MDR *E. coli* isolates with respect to their different sample sources. Any *p*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Carbapenems, ESBL, Tetracycline, and Quinolones Resistance Genes in MDR E. coli Isolates from Different Sample Sources

Overall, the resistance genes *blaTEM* (46%), *blaCTX-M* (26%), *tetA* (46%), *tetB* (14%), *qnrA* (24%), *qnrB* (8%), *blaOXA-48* (12%), and *blaKPC* (6%) were detected (Figure 1) and distributed in isolates from human 8/14 (57.1%), chicken 9/12 (75.0%), rodent 8/10 (80.0%), and soil 7/14 (50.0%) samples, as shown in Table 2. For human isolates the most common ARGs were *tetA* 8/14 (57.1%) and *blaSHV* 5/14 (35.7%), while for chicken the most common ones were *tetA* 5/12 (41.7%) and *qnrA* 6/12 (50%), for rodents they were *blaTEM* 6/12 (50%), and *tetA* 6/12 (50%), and for soil they were *blaTEM* 7/14 (50%) and *tetA* 4/14 (28.6%).

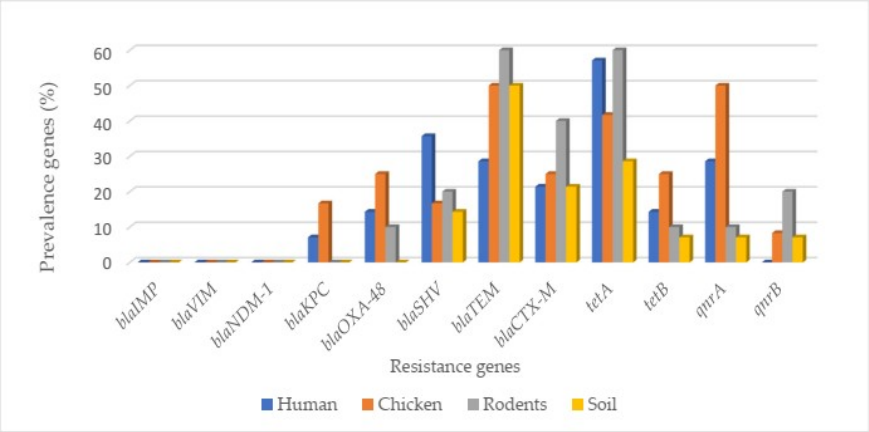

Figure 1. Occurrence of resistance genes in MDR *E. coli* isolates from different sample types.

Table 2. Prevalence of antimicrobial resistance genes in MDR *E. coli* isolates from different sample types.

Genes	Different Types of Sample Sources *n* (%)				
	Human (*n* = 14)	**Chicken (*n* = 12)**	**Rodents (*n* = 10)**	**Soil (*n* = 14)**	**Total Isolates (*n* = 50)**
blaIMP	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
blaVIM	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
blaNDM-1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
blaKPC	1 (7.1)	2 (16.7)	0 (0.0)	0 (0.0)	3 (6.0)
blaOXA-48	2 (14.3)	3 (25.0)	1 (10.0)	0 (0.0)	6 (12.0)
blaSHV	5 (35.7)	2 (16.7)	2 (20.0)	2 (14.3)	11 (22.0)
blaTEM	4 (28.6)	6 (50.0)	6 (60.0)	7 (50.0)	23 (46.0)
blaCTX-M	3 (21.4)	3 (25.0)	4 (40.0)	3 (21.4)	13 (26.0)
tetA	8 (57.1)	5 (41.7)	6 (60.0)	4 (28.6)	23 (46.0)
tetB	2 (14.3)	3 (25.0)	1 (10.0)	1 (7.1)	7 (14.0)
qnrA	4 (28.6)	6 (50.0)	1 (10.0)	1 (7.1)	12 (24.0)
qnrB	0 (0.0)	1 (8.3)	2 (20.0)	1 (7.1)	4 (8.0)
Total	8 (57.1)	9 (75.0)	8 (80.0)	7 (50.0%)	32 (64.0)
χ^2-square	52.29	46.43	2.00	26.67	
p-value	0.001	0.001	0.0188	0.0004	

3.2. Detection of Virulence Genes in MDR E. coli Isolates from Different Sample Sources

Overall, the virulence genes were: *ompA* (72%), *traT* (26%), *east* (18%), *bfp* (10%), *eae* (2%), and *stx-1* (4%), while *hlyA* and *cnf* were not detected (Table 1). For humans the most common VRs were *traT* 4/14 (28.6%) and *ompA* 10/14 (71.4%), while for chicken they were *traT* 4/12 (33.3%) and *ompA* 12/12 (100%), for rodents they were *traT* 4/10 (40%) and *ompA* 7/10 (70%), and for soil isolates they were predominated by *ompA* 7/14 (50%) and *east* 2/14 (14.3) (Figure 2).

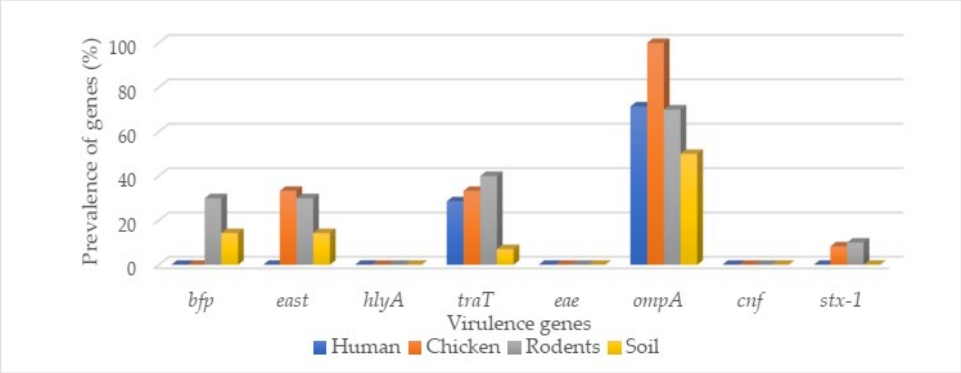

Figure 2. Prevalence of virulence genes in different types of the sample source.

3.3. Comparison between Phenotypic and Genotypic Antibiotic Resistance

We found positive correlations between tetracycline resistance and *tetA* (0.94), *tetB* (=0.90), carbapenem resistance and *blaKPC* (0.90) and *blaOXA-48* (0.89), and quinolone resistance and *qnrA* (0.96). We also found correlation between tetracycline resistance and genotypes for carbapenem (*blaKPC* = 0.90, *blaOXA-48* = 0.91), cefotaxime and *qnrA* (0.96), and quinolone resistance and *qnrA* (0.94). Cefotaxime resistance was correlated with genotypes for carbapenem (*blaKPC* = 0.93, *blaOXA-48* = 0.90) and quinolone (*qnrA* = 0.96) resistance (Table 3). However, we found weak and negative correlation between phenotypes and genotypes for ESBL resistance (*CTX-M* = 0.60, *blaTEM* = −0.63 and *blaSHV* = 0.33) (Table 3).

Table 3. Correlation between phenotypes and genotypes of MDR *E. coli* isolates.

Genotypes of Isolates	Phenotypic Resistance of Isolates			
	Correlation Coefficients (r)			
	Tetracycline	Imipenem	Ciprofloxacin	Cefotaxime
tetA	0.53	0.51	0.62	0.43
tetB	0.90	0.90	0.86	0.93
blaKPC	0.90	0.90	0.86	0.93
blaOXA-48	0.91	0.89	0.90	0.90
qnrA	0.94	0.94	0.90	0.96
qnrB	−0.69	−0.71	−0.67	−0.69
blaCTX-M	−0.54	−0.58	−0.45	−0.61
blaTEM	−0.71	−0.69	−0.78	−0.63
blaSHV	0.43	0.41	0.51	0.33

As shown in Table 4, we found correlations between *qnrB* and *bfp* genes (r = 0.63) and with *blaTEM* and *traT* genes (r = 0.51) and the remaining displayed weak and negative correlations.

Table 4. Correlation between resistance and virulence genes of MDR *E. coli* isolates.

ABR Genes	Virulence Genes					
	Correlation Coefficients (r)					
	bfp	***east***	***traT***	***eae***	***ompA***	***stx-1***
blaKPC	−0.11	0.30	0.41	−0.05	0.11	−0.07
blaOXA-48	−0.16	−0.06	0.15	0.38	−0.05	0.22
blaSHV	−0.24	−0.34	−0.44	−0.10	0.24	−0.15
blaTEM	0.39	0.44	0.51	0.17	−0.39	0.01
blaCTX-M	0.05	0.39	0.31	0.23	−0.22	0.08
tetA	0.36	−0.10	0.11	0.15	0.26	0.22
tetB	−0.18	0.06	−0.05	−0.08	0.18	−0.11
qnrA	−0.26	0.03	0.00	−0.11	0.09	0.10
qnrB	0.63	−0.19	0.12	−0.05	0.13	0.30

3.4. Co-Occurrence between Resistance and Virulence Genes

We observed that 38 out of 50 (76%) MDR *E. coli* isolates had at least one virulence gene. A co-existence of up to six resistance genes and at least one virulence gene was noted. In some cases, four resistance genes co-existed with four virulence genes (Figure 3). The combination consisting of *blaTEM*, *blaCTX-M*, *tetA*, *ompA*, and *traT* genes was common with 55% co-occurrence (Figures 4 and 5).

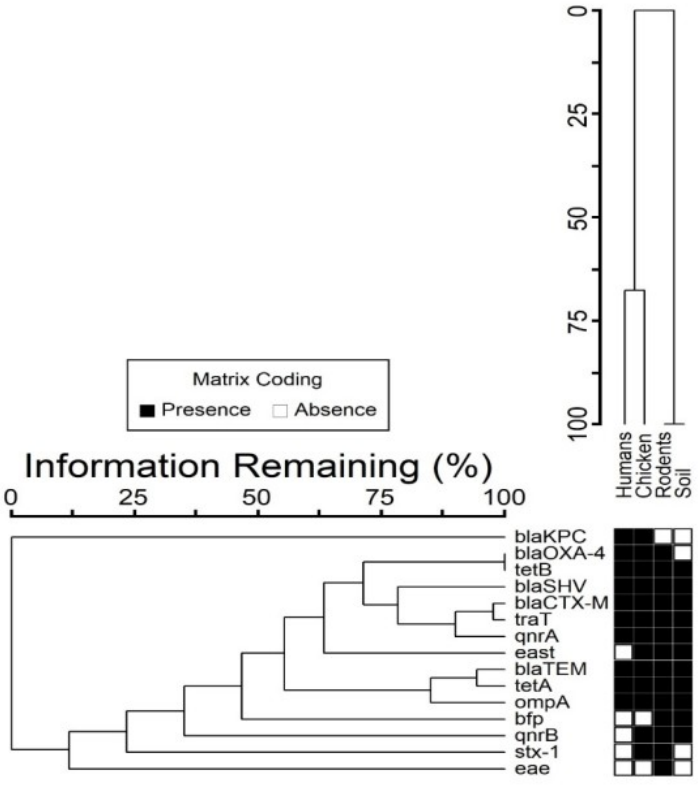

Figure 3. Co-occurrence of resistance and virulence genes in isolates from different sample sources.

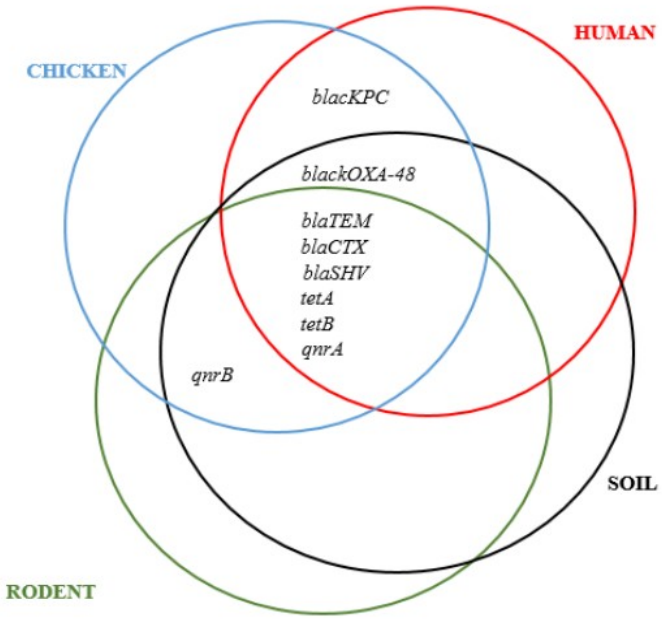

Figure 4. Distribution of resistance genes in various sample sources.

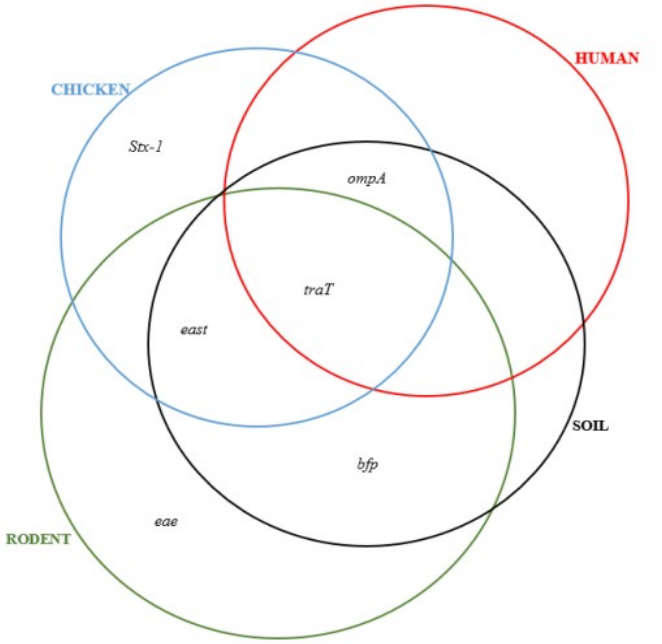

Figure 5. Distribution of virulence genes in various sample sources.

3.5. Principal Component Analysis Results

According to Figure 6 below, the arrows (vectors) for *tetA*, *qnrA*, and *tetB* genes aligned closer to each other in principal component 1 (PC1) indicating greater and positive correlations among them. The lengths of arrows show that *tetB* gene contributed more to the resistance of isolates followed by *qnrB* and *tetA* genes. The vectors for *blaTEM*, *blaCTX-M*, and *qnrB* genes are close to each other and to PC2 showing their influence on resistance. These genes had greater and positive correlations between them, but all were negatively correlated to the *blaSHV* gene. The lengths of the vectors indicate that the *blaTEM* gene had a higher influence on resistance of isolates (PC2), followed by the *blaCTX-M* while *qnrB* had the lowest. According to PCA plane, rodent and chicken ellipses are extended in the upper quadrants indicating higher proportions of ARGs, followed by those from human and soil.

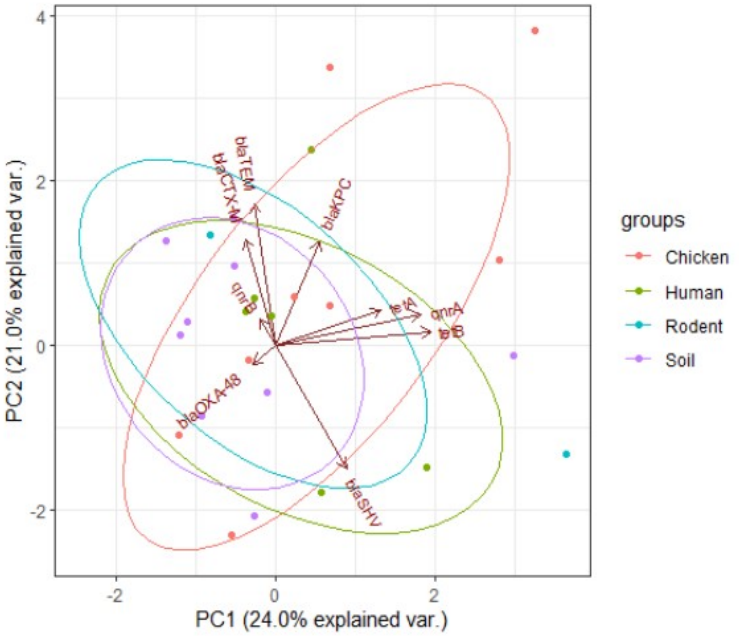

Figure 6. Principal component analysis of resistance genes of *E. coli* isolates. The dots represent isolates from different sources of samples, arrows indicate the original variables (resistance genes of the isolates), and ellipses indicate a region that contains 95% of all samples of a particular source.

The smaller angle between *traT* gene vector and PC1 indicates a greater and positive correlation between them (Figure 7). The same behaviour was displayed by *east* and *eae* genes which show a greater and positive correlation between them. Along PC2, *stx-1*, *bfp* and *ompA* genes had greater and positive correlations with PC2 indicating higher influence on virulence of isolates. The different sizes of loadings indicated higher and positive correlations between them. Different sizes of ellipses indicate variation in the prevalence of virulence genes across different sources of isolates. Rodent isolates had more virulence genes followed by chicken and soil isolates, while those from humans had the lowest gene prevalence.

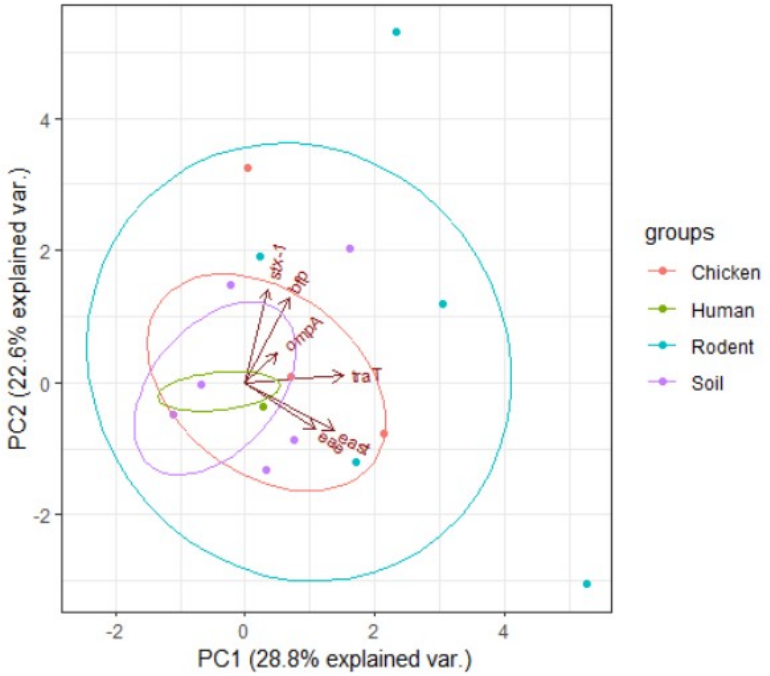

Figure 7. Principal component analysis for virulence genes of *E. coli* isolates. The dots represent isolates from different sources of samples, arrows indicate the original variables (virulence genes of the isolates), and ellipses indicate a region that contains 95% of all samples of a particular source.

4. Discussion

The study found 32/50 (64%) of MDR *E. coli* isolates carrying at least one AMR gene, with 10/50 (20%) having more than three. At the same time, 38 out of 50 (76%) MDR *E. coli* isolates had at least one virulence gene and 8/50 (16%) had more than three. PCA results showed that most of the resistance and virulence genes were found in isolates from rodents and chicken samples compared with human and soil isolates (Figures 6 and 7). The most detected AMR genes included: *tetA* (46%), *blaTEM* (46%), *blaCTX-M* (26%), *qnrA* (24%), *blaSHV* (22%), *tetB* (8%), and *blaOXA-48* (12%). This finding is in agreement with the results of our previous study in Karatu that reported higher resistance of *E. coli* to tetracycline (73.7%), imipenem (79.8%), and cefotaxime (79.7%) where 512 out of 650 (78.8%) isolates were multidrug-resistant [25]. Interestingly, the highest prevalence of AMR genes was observed in isolates from rodent (80.0%) followed by those from chicken (75.0%), human (57.1%), and lastly soil (50.0%) samples. Our findings imply that rodents that invade households have a potential to spread MDR *E. coli* infections with ARGs to other hosts, as observed by others [31–33]. The increased prevalence of resistance genes in isolates from chicken can be associated with frequent use and misuse of antibiotics in the prevention and treatment of poultry diseases, which is a common practice in the area as reported by previous studies [26,34,35]. The high prevalence of ESBL genes; *blaSHV* (20%), *blaCTX-M* (40%), *blaTEM* (60%), tetracycline; *tetA* (60%), and quinolone; *qnrB* (20%) resistance genes indicate the widespread of MDR *E. coli* infections in the Karatu district. This keeps with findings of a study conducted in nearby Arusha that found *blaTEM*, *blaCTX-M*, *tetA*, *tetB*, and *qnrs* [34–36]. This pattern can be explained by the frequent use and misuse of these antibiotics in veterinary and human medicines in the area [35], rendering these groups of antibiotics to be less effective. We found strong and positive correlations between tetracycline resistance and *tetA* (r = 0.94) and *tetB* (r = 0.90), carbapenem resistance and

blaKPC (r = 0.94), as well as *blaOXA-48* (r = 0.89) and quinolone resistance with *qnrA* (r = 0.96), highlighting the dominant role of genes in causing resistance [37–39]. Similarly, we found strong and positive correlation between tetracycline resistance phenotypes and genotypes for carbapenem (*blaKPC* = 0.90, *blaOXA-48* = 0.91), quinolone (*qnrA* = 0.94), as well as ESBL and carbapenem (*blaKPC* = 0.93, *blaOXA-48* = 0.90) and quinolone (*qnrA* = 0.96) resistance genotypes. Such associations have been reported in previous studies [40,41] and can be explained by the fact that most of these genes are carried on similar transferrable plasmids [42,43].

Overall, the detected virulence genes were: *bfp* 5/50 (10%), *east* 9/50 (18%), *traT* 13/50 (26%), *eae* 1/50 (2%), *ompA* 36/50 (72%), and *stx-1* 2/50 (4%). For isolates obtained from human samples, the most common virulence genes were: *traT* (28.6%) and *ompA* (71.4%), for chickens *ompA* (100%), *traT* (33.3%), *east* (33.3%), and *stx1* (8.3%) for rodents *ompA* (70%), *eae* (10%), *traT* (40%), *east* (30%), *bfp* (30%), and *stx1* (10%). Isolates from soil samples contained *bfp* (14.3%), *east* (14.3%), *traT* (7.1%), and *ompA* (50%). The bundle forming pilus (*bfp*) gene codes for adherence of *E. coli* strains to intestinal epithelial cells of the host [44], while *eae* gene promotes secretion of intimin protein for bacterial adherence to enterocytes [45]. The gene stx-1 encodes production of the Shiga toxin (stx) protein in some *E. coli* strains responsible for haemolytic uremic syndrome (HUS) and bloody diarrhoea in humans [45,46]. The gene *east* codes for production of heat-stable enterotoxin 1 in Enteroaggregative *E. coli* (EAST1) which induces diarrhoea in humans and livestock [47]. The gene *ompA* codes for outer membrane protein A, which enables intracellular survival of *E. coli* strains and protects them against host defence mechanism [48]. Meanwhile the *traT* gene codes for outer membrane protein, an important factor during urethral tract infections in humans [49]. The presence of wide-ranging virulence factors indicates that the MDR *E. coli* isolates circulating in Karatu have the ability to cause life-threatening infections that can be difficult to treat, given the fact that they occur in antibiotic-resistant isolates. We noted some significant differences with other studies. In this study, the prevalence of *ompA* in rodent isolates (70%) was lower than 93.5% reported in China by Guan et al. [50]. The 50% occurrence of *ompA* in *E. coli* from soil samples was greater than 42% documented in Indiana, USA [51]. However, we did not detect *stx1*, *eae*, and *hlyA* genes contrary to Cooley et al. [52] who reported *stx1* (100%), *eae* (100%), and *hlyA* (40%) in soil, livestock, wild birds, and water samples, respectively. Interestingly, we found a higher prevalence of virulence genes (60%) among *E. coli* isolates from rodent samples compared to previous studies in Berlin (0%) [21], in Hanoi (1.7%) [53], and in Vancouver (3.8%) [54]. These geographical related differences can be attributed to variations in levels of antibiotics use as well as environmental factors [55]. In this study, we found co-occurrence of resistance and virulence genes in 38/50 (76%) of the isolates. The most common combinations were: *blaSHV*, *tetA*, and *ompA* in humans; *blaTEM*, *tetA*, *tetB*, *qnrA*, and *ompA* in chicken; *blaTEM*, *blaCTX-M*, *tetA*, and *ompA* in rodents; and *blaTEM*, *tetA*, and *ompA* in soil isolates. Importantly, we found varying correlation between ARGs and VGs among the isolates. We found positive correlation between *blaTEM* and *traT* genes (r = 0.51) and *qnrB* and *bfp* genes (r = 0.63), while negative correlations were revealed between *blaOXA-48* and *ompA* (r = −0.05), *blaSHV* and *traT* (r = −0.44), and *tetA* and *east* (r = −0.10). This finding is keeping with those of other studies, showing that acquisition of resistance to certain antimicrobial agents may be associated with an increase or decrease in the virulence levels of a microorganism. This result seems to indicate that acquisition of resistance to certain antibiotics may be associated with an increase or decrease in the virulence levels depending on location and mechanism of transfer of specific genes [27,56,57].

5. Conclusions

Our study revealed that MDR *E. coli* isolates from humans, chicken, rodents, and household soils harbour different ARGs (*blaTEM*, *blaCTX-M*, *blaSHV*, *tetA*, *tetB*, *qnrA*, and *qnrB*) and VGs (*bfp*, *east*, *traT*, *ompA* and *stx-1*). The PCA results show that *traT*, *stx-1*, *bfp*, *ompA*, *east*, and *eae* genes influenced the virulence of MDR *E. coli* isolates. Resistance

(*blaTEM*, *blaCTX-M*, *blaSHV*, *tetA*, *tetB*, and *qnrA*) and virulence (*traT*) genes were detected in isolates from all sample sources, while *stx-1* and *eae* genes were specific to chicken and rodent isolates only. Interestingly, rodents had the highest percentage of both ARGs and VGs, indicating their potential in carriage and transmission of infections to other hosts in the environment. This situation urgently calls for One Health-based interventions including improving hygiene and control of rodents in households.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijerph19095388/s1, Table S1. List of primers used for amplification of selected antimicrobial resistance and virulence genes of *E. coli* isolates. Table S2. Multiplex PCR conditions used during amplification of antibiotic resistance and virulence genes of MDR *E. coli* isolates.

Author Contributions: Collection of samples, laboratory processing, and data analysis V.S.S., supervision and verification of the analytical methods M.I.M. and G.M. Manuscript developed by V.S.S. with input from M.I.M., G.M. and A.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The ethical clearance for the study was issued by the National Institute for Medical Research (NIMR) of Tanzania (NIMR/HQ/R.8a/Vol.IX/3386). NIMR is the national institutional review board that ensures all health research follows the national health research ethics requirements for research involving human subjects. Sokoine University of Agriculture Institutional Animal Care and Use Committee (IACUC) approved the use of animals in this study. The permission to work in the study area was sought from the Regional Administrative Office (Arusha).

Informed Consent Statement: Informed verbal consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Song, Y.; Yu, L.; Zhang, Y.; Dai, Y.; Wang, P.; Feng, C.; Liu, M.; Sun, S.; Xie, Z.; Wang, F. Prevalence and characteristics of multidrug-resistant mcr-1-positive *Escherichia coli* isolates from broiler chickens in Tai'an, China. *Poult. Sci.* **2020**, *99*, 1117–1123. [CrossRef] [PubMed]
2. Kim, Y.B.; Yoon, M.Y.; Ha, J.S.; Seo, K.W.; Noh, E.B.; Son, S.H.; Lee, Y.J. Molecular characterization of avian pathogenic *Escherichia coli* from broiler chickens with colibacillosis. *Poult. Sci.* **2020**, *99*, 1088–1095. [CrossRef] [PubMed]
3. Shrivastava, S.R.; Shrivastava, P.S.; Ramasamy, J. World health organization releases global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. *J. Med. Soc.* **2018**, *32*, 76–77. [CrossRef]
4. Sarowska, J.; Futoma-Koloch, B.; Jama-Kmiecik, A.; Frej-Madrzak, M.; Ksiazczyk, M.; Bugla-Ploskonska, G.; Choroszy-Krol, I. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: Recent reports. *Gut Pathog.* **2019**, *11*, 10. [CrossRef] [PubMed]
5. Abd El-Baky, R.M.; Ibrahim, R.A.; Mohamed, D.S.; Ahmed, E.F.; Hashem, Z.S. Prevalence of virulence genes and their association with antimicrobial resistance among pathogenic E. coli isolated from Egyptian patients with different clinical infections. *Infect. Drug. Resist.* **2020**, *13*, 1221–1236. [CrossRef]
6. Tooke, C.L.; Hinchliffe, P.; Bragginton, E.C.; Colenso, C.K.; Hirvonen, V.H.; Takebayashi, Y.; Spencer, J. β-Lactamases and β-Lactamase Inhibitors in the 21st Century. *J. Mol. Biol.* **2019**, *431*, 3472–3500. [CrossRef]
7. Masoud, S.M.; El-Baky, A.; Mahmoud, R.; Aly, S.A.; Ibrahem, R.A. Co-Existence of Certain ESBLs, MBLs and Plasmid Mediated Quinolone Resistance Genes among MDR E. coli Isolated from Different Clinical Specimens in Egypt. *Antibiotics* **2021**, *10*, 835. [CrossRef]

8. Ibrahim, M.E.; Bilal, N.E.; Magzoub, M.A.; Hamid, M.E. Prevalence of extended-spectrum β-lactamases-producing *Escherichia coli* from Hospitals in Khartoum State, Sudan. *Oman Med. J.* **2013**, *28*, 116–120. [CrossRef]
9. Yano, H.; Uemura, M.; Endo, S.; Kanamori, H.; Inomata, S.; Kakuta, R.; Ichimura, S.; Ogawa, M.; Shimojima, M.; Ishibashi, N. Molecular characteristics of extended-spectrum β-lactamases in clinical isolates from *Escherichia coli* at a Japanese tertiary hospital. *PLoS ONE* **2013**, *8*, e64359. [CrossRef]
10. Queenan, A.M.; Bush, K. Carbapenemases: The versatile β-lactamases. *Clin. Microbiol. Rev.* **2007**, *20*, 440–458. [CrossRef]
11. Livermore, D.M. The impact of carbapenemases on antimicrobial development and therapy. *Curr. Opin. Investig. Drugs (Lond. Engl. 2000)* **2002**, *3*, 218–224.
12. Bryan, A.; Shapir, N.; Sadowsky, M.J. Frequency and distribution of tetracycline resistance genes in genetically diverse, nonselected, and nonclinical *Escherichia coli* strains isolated from diverse human and animal sources. *Appl. Environ. Microbiol.* **2004**, *70*, 2503–2507. [CrossRef] [PubMed]
13. Poirel, L.; Cattoir, V.; Nordmann, P. Plasmid-mediated quinolone resistance; interactions between human, animal, and environmental ecologies. *Front. Microbiol.* **2012**, *3*, 24. [CrossRef] [PubMed]
14. Allocati, N.; Masulli, M.; Alexeyev, M.F.; Di Ilio, C. *Escherichia coli* in Europe: An overview. *Int. J. Environ. Res. Public Health* **2013**, *10*, 6235–6254. [CrossRef]
15. Robins-Browne, R.M.; Holt, K.E.; Ingle, D.J.; Hocking, D.M.; Yang, J.; Tauschek, M. Are *Escherichia coli* pathotypes still relevant in the era of whole-genome sequencing? *Front. Cell. Infect. Microbiol.* **2016**, *6*, 141. [CrossRef]
16. Gomes, T.A.; Elias, W.P.; Scaletsky, I.C.; Guth, B.E.; Rodrigues, J.F.; Piazza, R.M.; Ferreira, L.; Martinez, M.B. Diarrheagenic *Escherichia coli*. *Braz. J. Microbiol.* **2016**, *47*, 3–30. [CrossRef]
17. White, A.; Hughes, J.M. Critical importance of a one health approach to antimicrobial resistance. *EcoHealth* **2019**, *16*, 404–409. [CrossRef] [PubMed]
18. Kilonzo, B.; Mbise, T.; Mwalimu, D.; Kindamba, L. Observations on the endemicity of plague in Karatu and Ngorongoro, northern Tanzania. *Tanzan. J. Health Res.* **2006**, *8*, 1–6. [CrossRef]
19. Makundi, R.H.; Massawe, A.W.; Mulungu, L.S.; Katakweba, A.; Mbise, T.J.; Mgode, G. Potential mammalian reservoirs in a bubonic plague outbreak focus in Mbulu District, northern Tanzania, in 2007. Available online: https://www.degruyter.com/document/doi/10.1515/MAMM.2008.038/pdf (accessed on 2 February 2022).
20. Ziwa, M.H.; Matee, M.I.; Hangombe, B.M.; Lyamuya, E.F.; Kilonzo, B.S. Plague in Tanzania: An overview. *Tanzan. J. Health Res.* **2013**, *15*, 4. [CrossRef]
21. Guenther, S.; Bethe, A.; Fruth, A.; Semmler, T.; Ulrich, R.G.; Wieler, L.H.; Ewers, C. Frequent combination of antimicrobial multiresistance and extraintestinal pathogenicity in *Escherichia coli* isolates from urban rats (*Rattus norvegicus*) in Berlin, Germany. *PLoS ONE* **2012**, *7*, e50331. [CrossRef]
22. Feng, A.Y.; Himsworth, C.G. The secret life of the city rat: A review of the ecology of urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). *Urban Ecosyst.* **2014**, *17*, 149–162. [CrossRef]
23. Hassell, J.M.; Ward, M.J.; Muloi, D.; Bettridge, J.M.; Robinson, T.P.; Kariuki, S.; Ogendo, A.; Kiiru, J.; Imboma, T.; Kangethe, E.K. Clinically relevant antimicrobial resistance at the wildlife–livestock–human interface in Nairobi: An epidemiological study. *Lancet Planet. Health* **2019**, *3*, e259–e269. [CrossRef]
24. Desvars-Larrive, A.; Ruppitsch, W.; Lepuschitz, S.; Szostak, M.P.; Spergser, J.; Feßler, A.T.; Schwarz, S.; Monecke, S.; Ehricht, R.; Walzer, C. Urban brown rats (*Rattus norvegicus*) as possible source of multidrug-resistant Enterobacteriaceae and meticillin-resistant *Staphylococcus* spp., Vienna, Austria, 2016 and 2017. *Eurosurveillance* **2019**, *24*, 1900149. [CrossRef]
25. Sonola, V.S.; Katakweba, A.S.; Misinzo, G.; Matee, M.I. Occurrence of Multi-Drug-Resistant *Escherichia coli* in Chickens, Humans, Rodents and Household Soil in Karatu, Northern Tanzania. *Antibiotics* **2021**, *10*, 1137. [CrossRef] [PubMed]
26. Cepas, V.; Soto, S.M. Relationship between Virulence and Resistance among Gram-Negative Bacteria. *Antibiotics* **2020**, *9*, 719. [CrossRef] [PubMed]
27. Yao, M.; Zhang, X.; Gao, Y.; Song, S.; Xu, D.; Yan, L. Development and application of multiplex PCR method for simultaneous detection of seven viruses in ducks. *BMC Vet. Res.* **2019**, *15*, 103. [CrossRef]
28. United Republic of Tanzania, 2012 Population and Housing Census-Population Distribution by Administrative Areas. Available online: http://tanzania.countrystat.org/fileadmin/user_upload/countrystat_fenix/congo/docs/Census%20General%20Report-2012PHC.pdf (accessed on 2 February 2022).
29. Magiorakos, A.-P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.; Giske, C.; Harbarth, S.; Hindler, J.; Kahlmeter, G.; Olsson-Liljequist, B. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* **2012**, *18*, 268–281. [CrossRef]
30. R: A Language and Environment for Statistical Computing. Available online: https://www.eea.europa.eu/data-and-maps/indicators/oxygen-consuming-substances-in-rivers/r-development-core-team-2006 (accessed on 2 February 2022).
31. Gakuya, F.; Kyule, M.; Gathura, P.; Kariuki, S. Antimicrobial susceptibility and plasmids from *Escherichia coli* isolated from rats. *East Afr. Med. J.* **2001**, *78*, 518–522. [CrossRef]
32. Wang, H.; Sangwan, N.; Li, H.Y.; Su, J.Q.; Oyang, W.Y.; Zhang, Z.J.; Gilbert, J.A.; Zhu, Y.G.; Ping, F.; Zhang, H.L. The antibiotic resistome of swine manure is significantly altered by association with the *Musca domestica* larvae gut microbiome. *ISME J.* **2017**, *11*, 100–111. [CrossRef]

33. Poirel, L.; Madec, J.-Y.; Lupo, A.; Schink, A.-K.; Kieffer, N.; Nordmann, P.; Schwarz, S. Antimicrobial resistance in *Escherichia coli*. *Microbiol. Spectr.* **2018**, *6*, 4. [CrossRef]
34. Comparison of Antibiotic Resistant *Escherichia coli* Obtained from Drinking Water Sources in Northern Tanzania: A Cross-Sectional Study. Available online: https://www.researchgate.net/publication/309765672_Comparison_of_antibiotic_resistant_Escherichia_coli_obtained_from_drinking_water_sources_in_northern_Tanzania_a_cross-sectional_study (accessed on 2 February 2022).
35. Antimicrobial Resistance Profiles of *Escherichia coli* Isolated from Broiler and Layer Chickens in Arusha and Mwanza, Tanzania. Available online: https://www.hindawi.com/journals/ijmicro/2021/6759046/ (accessed on 2 February 2022).
36. Sale of Fluoroquinolones in Northern Tanzania: A Potential Threat for Fluoroquinolone Use in Tuberculosis Treatment. Available online: https://academic.oup.com/jac/article/65/1/145/727526?login=false# (accessed on 2 February 2022).
37. Comparison of the Prevalence of Antibiotic-Resistant *Escherichia coli* Isolates from Commercial-Layer and Free-Range Chickens in Arusha District, Tanzania. Available online: https://www.researchgate.net/publication/308594993_Comparison_of_the_prevalence_of_antibiotic-resistant_Escherichia_coli_isolates_from_commercial-layer_and_free-range_chickens_in_Arusha_district_Tanzania (accessed on 2 February 2022).
38. Subbiah, M.; Caudell, M.A.; Mair, C.; Davis, M.A.; Matthews, L.; Quinlan, R.J.; Quinlan, M.B.; Lyimo, B.; Buza, J.; Keyyu, J. Antimicrobial resistant enteric bacteria are widely distributed amongst people, animals and the environment in Tanzania. *Nat. Commun.* **2020**, *11*, 228. [CrossRef] [PubMed]
39. Genetic Relatedness of Multidrug Resistant *Escherichia coli* Isolated from Humans, Chickens and Poultry Environments. Available online: https://aricjournal.biomedcentral.com/articles/10.1186/s13756-021-00930-x (accessed on 2 February 2022).
40. Mahmoud, N.E.; Altayb, H.N.; Gurashi, R.M. Detection of Carbapenem-Resistant Genes in *Escherichia coli* Isolated from Drinking Water in Khartoum, Sudan. Available online: https://pubmed.ncbi.nlm.nih.gov/32612664/ (accessed on 2 February 2022).
41. Schages, L.; Wichern, F.; Kalscheuer, R.; Bockmühl, D. Winter is coming–Impact of temperature on the variation of beta-lactamase and mcr genes in a wastewater treatment plant. *Sci. Total Environ.* **2020**, *712*, 136499. [CrossRef] [PubMed]
42. Gutkind, G.O.; Di Conza, J.; Power, P.; Radice, M. β-Lactamase-mediated resistance: A biochemical, epidemiological and genetic overview. *Curr. Pharm. Des.* **2013**, *19*, 164–208. [CrossRef] [PubMed]
43. Evans, B.A.; Amyes, S.G. OXA β-lactamases. *Clin. Microbiol. Rev.* **2014**, *27*, 241–263. [CrossRef]
44. Hamilton, M.J.; Hadi, A.Z.; Griffith, J.F.; Ishii, S.; Sadowsky, M.J. Large scale analysis of virulence genes in *Escherichia coli* strains isolated from Avalon Bay, CA. *Water Res.* **2010**, *44*, 5463–5473. [CrossRef] [PubMed]
45. Patel, J.; Yin, H.B.; Bauchan, G.; Mowery, J. Inhibition of *Escherichia coli* O157: H7 and Salmonella enterica virulence factors by benzyl isothiocyanate. *Food Microbiol.* **2020**, *86*, 103303. [CrossRef]
46. Pan, Z.; Chen, Y.; McAllister, T.A.; Gänzle, M.; Plastow, G.; Guan, L.L. Abundance and Expression of Shiga Toxin Genes in Escherichia coli at the Recto-Anal Junction Relates to Host Immune Genes. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 148. [CrossRef]
47. Ménard, L.-P.; Dubreuil, J.D. Enteroaggregative Escherichia coli heat-stable enterotoxin 1 (EAST1): A new toxin with an old twist. *Crit. Rev. Microbiol.* **2002**, *28*, 43–60. [CrossRef]
48. Wang, Y.; Kim, K.S. Role of OmpA and IbeB in Escherichia coli K1 invasion of brain microvascular endothelial cells in vitro and in vivo. *Pediatr. Res.* **2002**, *51*, 559–563. [CrossRef]
49. Firoozeh, F.; Saffari, M.; Neamati, F.; Zibaei, M. Detection of virulence genes in *Escherichia coli* isolated from patients with cystitis and pyelonephritis. *Int. J. Infect. Dis.* **2014**, *29*, 219–222. [CrossRef]
50. Guan, J.; Liu, S.; Lin, Z.; Li, W.; Liu, X.; Chen, D. Severe sepsis facilitates intestinal colonization by extended-spectrum-β-lactamase-producing Klebsiella pneumoniae and transfer of the SHV-18 resistance gene to *Escherichia coli* during antimicrobial treatment. *Antimicrob. Agents Chemother.* **2014**, *58*, 1039–1046. [CrossRef] [PubMed]
51. Duffitt, A.D.; Reber, R.T.; Whipple, A.; Chauret, C. Gene expression during survival of *Escherichia coli* O157: H7 in soil and water. *Int. J. Microbiol.* **2011**, *2011*, 340506. [CrossRef] [PubMed]
52. Cooley, M.B.; Jay-Russell, M.; Atwill, E.R.; Carychao, D.; Nguyen, K.; Quiñones, B.; Patel, R.; Walker, S.; Swimley, M.; Pierre-Jerome, E. Development of a robust method for isolation of Shiga toxin-positive *Escherichia coli* (STEC) from fecal, plant, soil and water samples from a leafy greens production region in California. *PLoS ONE* **2013**, *8*, e65716. [CrossRef]
53. Le Huy, H.; Koizumi, N.; Ung, T.T.H.; Le, T.T.; Nguyen, H.L.K.; Hoang, P.V.M.; Nguyen, C.N.; Khong, T.M.; Hasebe, F.; Haga, T.; et al. Antibiotic-resistant *Escherichia coli* isolated from urban rodents in Hanoi, Vietnam. *J. Vet. Med. Sci.* **2020**, *82*, 653–660. [CrossRef] [PubMed]
54. Himsworth, C.G.; Zabek, E.; Desruisseau, A.; Parmley, E.J.; Reid-Smith, R.; Jardine, C.M.; Tang, P.; Patrick, D.M. Prevalence and characteristics of Escherichia coli and Salmonella spp. in the feces of wild urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*) from an inner-city neighborhood of Vancouver, Canada. *J. Wildl. Dis.* **2015**, *51*, 589–600. [CrossRef]
55. Bengtsson-Palme, J.; Hammaren, R.; Pal, C.; Östman, M.; Björlenius, B.; Flach, C.-F.; Fick, J.; Kristiansson, E.; Tysklind, M.; Larsson, D.J. Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics. *Sci. Total Environ.* **2016**, *572*, 697–712. [CrossRef] [PubMed]
56. Soto, S.; Zuniga, S.; Ulleryd, P.; Vila, J. Acquisition of a pathogenicity island in an *Escherichia coli* clinical isolate causing febrile urinary tract infection. *Eur. J. Clin. Microbiol. Infect. Dis.* **2011**, *30*, 1543–1550. [CrossRef] [PubMed]
57. Hacker, J.; Blum-Oehler, G.; Mühldorfer, I.; Tschäpe, H. Pathogenicity islands of virulent bacteria: Structure, function and impact on microbial evolution. *Mol. Microbiol.* **1997**, *23*, 1089–1097. [CrossRef] [PubMed]

CHAPTER FIVE

MANUSCRIPT IV

Multiplex PCR detection of antibiotic resistance and virulence genes in multidrug-resistant *Staphylococcus aureus* isolated from chickens, humans, rodents and soil in Karatu, Northern Tanzania

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Abstract:

Staphylococcus aureus is a zoonotic pathogen with public health and veterinary importance. We investigated the presence of antibiotic resistance genes (ARGs) and virulence genes (VGs) in 57 Multidrug-resistant (MDR) *S. aureus* isolated from humans (17), chickens (14), rodents (13) and soil (13) using multiplex PCR. Overall, the distribution of ARGs was; *tetK* 18/57 (31.6%), *mecA* 16/57 (28.1%), *tetL* 5/57 (8.9%), and *ermC* 1/57 (1.8%), while *ermA* and *tetM* were not detected. For VGs the distribution was; *clfB* 6/57 (10.5 %), *coa* 8/57 (14.0%), *clfA* 3/57 (5.3%), *hlg* 1/57 (1.8%), *ebpS* 2/57 (3.5%), *fnbB* 2/57 (3.5%), *luk-PV* 6/57 (10.5%) and *tst* 1/57 (1.8%). Resistance genes (*tetK* and *mecA)* and virulence determinants (*clfB, coa* and *luk-PV*) were common in all sample sources, while *tst*, *hlg* and *fnbB* were specific to human, chicken and rodent isolates, respectively. Erythromycin phenotypic resistance results correlated with presence of *ermC* (r=0.42), *tetL* (r=0.98) and *mecA* (r=0.51), while tetracycline resistance correlated with *tetL* (r=1.00) and *mecA* (r=0.57) genes and methicillin resistance (MRSA) correlated with *mecA* (r=0.55) and *tetL* (r=0.98) genes. Positive correlations were noted between ARG (*ermC*) and VGs; *clfA* (r=0.57), *hlg* (r=1.00) and *clfB* (r=0.43) and between *tetK* and *clfB* (r=0.39); *tetK* and *coa* (r=0.36) genes. Principal component analysis (PCA) shows that *tetL, ermC* and *mecA* contributed to tetracycline, erythromycin and methicillin resistance, respectively. The wide spread presence of resistance and virulence genes, often in combination, among MDR *S. aureus* in isolates from humans, chicken, rodents and soil samples require comprehensive One Health interventions.

1. Introduction

Staphylococcus aureus is a globally recognized opportunistic bacteria that colonizes humans, animals and is found in the environment (Katakweba *et al.*, 2016; Wang *et al.*, 2017). In humans, *S. aureus* causes a wide spectrum of infections ranging from skin and

soft tissue to life-threatening infections such as pneumonia, osteomyelitis, endocarditis and bacteraemia (Kayili and Sanlibaba, 2020; Cheung *et al.*, 2021). *S. aureus* is the leading cause of mastitis in dairy animals (Kashoma *et al.*, 2015) and causes several diseases in chickens including omphalitis, dermatitis and arthritis (Abou-Zahr *et al.*, 2018; Gornatti-Churria *et al.*, 2018; Benrabia *et al.*, 2020). Drug resistant strains such as methicillin-resistant *S. aureus* (MRSA) strains, causes infections that are difficult to treat and have been associated with higher mortality and morbidity rates (Watkins *et al.*, 2012) and is on the WHO list of high priority antibiotic-resistant bacteria (Savoldi *et al.*, 2019). Carriage of virulence factors confers some evolutionary benefit to bacteria, which favours the resistant strains (Derakhshan *et al.*, 2021). *S. aureus* infections have been associated with several virulence factors including; haemolysins (*hla, hlb, hld* genes), staphylococcal enterotoxins (*sea, seb, sec, sed, see* genes), toxic shock syndrome toxin-1 (TSST-1) and Panton-Valentine leucocidin (PVL) (lukFand lukS genes) (Li *et al.*, 2015; Zhao *et al.*, 2016; Li *et al.*, 2021;). Molecular techniques like Multiplex PCR are important in detection of resistance genes including *erm(A), erm(B), tet(A), tet(D)* (Adwan *et al.*, 2013), as well as virulence factors such as *hla, hlb, hld, sea, seb, sec, lukF* and *lukS* (Ote *et al.*, 2011; Zhao *et al.*, 2016; Hait *et al.*, 2021).

Antibiotic resistance is recognized as a quintessentially One Health issue, involving cross-transmission of resistant bacteria or their resistance genes across between humans, animals, acquire culture, and environment through their interactions (Ampaire *et al.*, 2016; Mouiche *et al.*, 2019). Genetic determinants of antimicrobial resistance, often located on mobile genetic elements, can be easily transmitted among different hosts including humans, animals and the environment (Baquero *et al.*, 2019). Indeed, antimicrobial resistance studies using a one health approach have found similarities in multidrug-resistance genes in many important and common pathogens such as *Escherichia coli*, *Klebsiella*

pneumoniae and *Staphylococcus aureus* suggesting their potential transmission between humans, animals, aquaculture and the environment (Van Duin and Paterson, 2016). The interaction between these compartments, governed largely by human anthropogenic activities, are key in the spread AMR genes that can be depicted by molecular studies (Nathan and Cars, 2014). In Tanzania, several studies conducted in Karatu in Northern part of the country have revealed intense interactions between rodents, humans and livestock, with occurrence of outbreaks of zoonoses, including plague (Kilonzo *et al.*, 2006; Makundi *et al.*, 2008; Ziwa *et al.*, 2013; Makundi *et al.*, 2015).

In a recent study conducted in the area, *S. aureus* isolated from humans, rodents and environment showed high level of phenotypic resistance against a number antibiotics especially clindamycin (51.0%), erythromycin (50.9%) and tetracycline (62.5%), with 30.2%, of them being multi-drug resistant (MDR) (Sonola *et al.*, 2021). However, molecular studies specifically assessing occurrence and distribution of genes encoding for antimicrobial resistance and virulence factors have not been done. Knowledge regarding genetic diversity antimicrobial resistance and virulence factors is needed for effective control of the spread of antimicrobial resistance given the ability of *S. aureus* to acquire antimicrobial resistance determinants and extensive number of virulence factors (Sonola *et al.*, 2021). We therefore undertook this study to determine and compare profiles of antimicrobial resistance and virulence genes among MDR *S aureus* isolated from chickens, humans, rodents and soil in Karatu, Northern Tanzania, where such interactions are intense. Multiplex PCR was used to amplify the resistance genes (*tetK, tetL, tetM, ermA, ermC* and *mecA*), which encodes tetracycline, erythromycin and clindamycin resistance, respectively (Hait *et al.*, 2021). Virulence factors that were investigated included; clumping factor B (*clfB*), collagen adhesisn gene (*cna*), coagulase protein (*coa*), clumping factor A protein (*clfA*), gamma-hemolysin gene (*hlg*), elastin binding protein gene of *S.*

aureus (*ebpS*), fibronectin-binding protein B (*fbpB*), production of leukocydal toxins (*luk-PV*) and *tst* gene for production of toxic shock syndrome toxin 1 (*TSST-1*). These are important virulence factors which facilitate bacterial adherence and invasion to host epithelial cells, production of toxins and evasion of host defence systems which are necessary for pathogen colonization and initiation of infections in the host's body. The sequences for the genes are conserved among various *Staphylococcus* strains and are often used to screen virulence in this genus (Cotar *et al.*, 2010; Wang *et al.*, 2019).

Bacterial isolates

A total of 57 MDR *S. aureus* isolates from chicken cloaca swabs (14), human nasal swabs (17), rodents' deep pharyngeal swabs (13) and household soil (13) samples were preserved in tryptic soy broth (TSB) with 50% glycerol (v/v) at -80^{0}C, pending DNA extraction. These isolates exhibited phenotypic resistance to at least three different classes of antibiotics.

Genomic DNA extraction

Isolates were sub-cultured on nutrient broth media (NB, Merck, Germany) and incubated at 37 °C for 24 h. Genomic DNA were extracted using Zymo Research Fungal and Bacterial Genomic DNA MiniPrepTM kit (Zymo Research, Irvine, USA), according to manufacturer's recommendations. The purity, quality and quantity of extracted DNA were determined by using a Nanodrop device (NanoDrop, Thermo Scientific, USA), gel electrophoresis, and spectrophotometer. The extracted genomic DNA were stored at -80 °C pending PCR analyses. DNA from *S. aureus* strain ATCC 25923 was used for quality control.

Detection of antibiotic resistance and virulence genes

Multiplex PCR was used to amplify the resistance (*tetK, tetL, tetM, ermA, ermC* and *mecA*) and virulence (*clfB, cna, coa, clfA, hlg, ebpS, fnbB, luk-PV and tst)* genes according to the previously described protocol (Ote *et al.*, 2011; Zhao *et al.*, 2016). The primers used for PCR amplification of different genes are listed in Table 1. Lyophilized primers (Macrogen, Amsterdam, The Netherlands) for targeted genes were reconstituted using DNase/RNase-free sterile water to obtain 100 µM stock solutions which were stored at −20 °C and finally diluted to a working concentration of 10 µM. Amplification was carried out in a total volume of 25 µL containing 12.5 µL of 1X *Taq* PCR Master Mix (Bio Basic, Canada), 1 µL of the forward primer and 1 µL of the reverse primer, 3 µL of DNA template, and 7.5 µL sterile nuclease-free water for every set of PCR. The cycling conditions for all reactions are as shown in Table 2. PCR products were run on 1.5% (w/v) agarose gel using electrophoresis, stained with gel red (Merck, Darmstadt, Germany) at 120 Volts for 1 h, and visualized under UV light using a BioDoc-it™ imaging system (Ultra-Violet Products, Cambridge, UK) by using GeneRuler 100 bp Plus DNA Ladder (Bioneer, Republic of Korea).

Table 1: List of primers that were used for detection of resistance and virulence genes among the MDR *S. aureus* isolates

Targeted Gene	Primer name	Primer sequence (5-3)	Amplicon size (bp)	Annealing temperature (^{0}C)	Reference
tet(K)	*TetK-1*	TTAGGTGAAGGGTTAGGTCC	360	55	Abdolmaleki *et al.*, 2019
	TetK-2	GCAAACTCATTCCAGAAGCA			
tet(L)	*TetL2-2*	ATAAATTGTTTCGGGTCGGTAAT	1077	55	Trzcinski *et al.*, 2000
	TetL2-1	AACCAGCCAACTAATGACAAGAT			
tet(M)	TetM-1	GTCCGTCTGAACTTTGCGGA	158	55	Trzcinski *et al.*, 2000
	TetM-2	GCGGCACTTCGATGTGAATG			
ermA	Tn554-1(ermA)	AAGCGGTAAACCCCTCTGA	139	55	Sidhu et al., 2002
	Tn554-2 (ermA)	TTCGCAAATCCCTTCTCAAC			
ermC	ermC-2	AATCGGCTCAGGAAAAGG	562	55	Pérez-Serrano *et al.*, 2020
	ermC-1	ATCGTCAATTCCTGCATG			
mecA	MecA1-F	AACTCTGTTATTAGGGAAGAACA	293	55	Abdomaleki et al., 2019
	MecA1-R	CCACCTTCCTCCGGTTTGTCACC			
ebpS	EBP-1	CATCCAGAACCAATCGAAGAC	186	55	Peacock *et al.*, 200
	EBP-2	CTTAACAGTTACATCATCATGTTTATCTTTG			
can	CNA-1	AGTGGTTACTAATACTG	560	55	Peacock *et al.*, 2002
	CNA-2	CAGGATAGATTGGTTTA			
clfA	CLFA-1	ATTGGCGTGGCTTCAGTGCT	292	55	Tristan *et al.*, 2003
	CLFA-2	CGTTTCTTCCGTAGTTGCATTTG			
clfB	CLFB-1	ACATCAGTAATAGTAGGGGGCAAC	203	55	Tristan *et al.*, 2003
	CLFB-2	TTCGCACTGTTTGTGTTTGCAC			
coag	COAG-1	ACCACAAGGTACTGAATCAACG	812	55	Mullarky *et al.*, 2001
	COAG-2	TGCTTTCGATTGTTCGATGC			
luk-PV	*LUK-PV-1*	ATCATTAGGTAAAATGTCTGGACATGATCCA	433	55	Jarraud *et al.*, 2002
	LUK-PV-2	GCATCAASTGTATTGGATAGCAAAAGC			
hlg	HLG-1	GCCAATCCGTTATTAGAAAATGC	938	55	Jarraud *et al.*, 2002
	HLG-2	CCATAGACGTAGCAACGGAT			
tst	TST-1	CATCTACAAACGATAATATAAAGG	476	55	Vannuffel *et al.*, 1995
	TST-2	CATTGTTATTTTCCAATAACCACCCG			
fnbB	FNBB-1	GTAACAGCTAATGGTCGAATTGATACT	523	55	Tristan *et al.*, 2003
	FNBB-2	CAAGTTCGATAGGAGTACTATGTTC			

Program	Amplified Genes	Initial denaturation	Denaturation	Annealing	Primer extension	Final extension	Amplification
PCR 1	*ermA*	94ºC 1 min	94ºC, 1 min	55ºC, 1 min	72ºC, 1 min	72ºC 10 min	25 cycles
	ermC	94ºC 1 min	94ºC, 1 min	55ºC, 1 min	72ºC, 1 min	72ºC 10 min	
	tetK	94ºC 1 min	94ºC, 1 min	55ºC, 1 min	72ºC, 1 min	72ºC 10 min	
PCR 2	*mecA*	94ºC 3 min	94ºC, 30 sec	58ºC, 30 sec	72ºC, 30 sec	72ºC 3 min	30 cycles
	tetL	94ºC 3 min	94ºC, 30 sec	58ºC, 30 sec	72ºC, 30 sec	72ºC 3 min	
	tetM	94ºC 3 min	94ºC, 30 sec	55ºC, 30 sec	72ºC, 30 sec	72ºC 3 min	
PCR 3	*fnbB*	94ºC 5 min	94ºC, 1 min	55ºC 1min	72ºC, 1 min	72ºC 10min	25 cycles
	clfA	94ºC 5 min	94ºC, 1 min	55ºC 1min	72ºC, 1 min	72ºC 10min	
	clfB	94ºC 5 min	94ºC, 1 min	55ºC 1min	72ºC, 1 min	72ºC 10min	
	cna	94ºC 5 min	94ºC, 1 min	55ºC 1min	72ºC, 1 min	72ºC 10min	
	ebpS	94ºC 5 min	94ºC , 1 min	55ºC 1min	72ºC, 1 min	72ºC 10min	
PCR 4	*coa*	94ºC 5 min	94ºC, 30 sec	55ºC 1 min	72ºC, 1 min	72ºC 10min	30 cycles
	tst	94ºC 5 min	94ºC , 30 sec	55ºC 1 min	72ºC, 1 min	72ºC 10min	
	luk-PV	94ºC 5 min	94ºC , 30 sec	55ºC 1 min	72ºC, 1 min	72ºC 10min	
	hlg	94ºC 5 min	94ºC , 30 sec	55ºC 1 min	72ºC, 1 min	72ºC 10min	

Statistical analysis

The data obtained were arranged and entered into an Excel spreadsheet (Microsoft® Office Excels 2010) and analysed. The differences in prevalence of the genes (%) between categories were compared by chi-square test while distributions and relationships among the genes in their respective sources of isolates were managed by principal component analysis (PCA). All statistical analyses were performed by using R-software and any *p*-value less than 0.05 was considered significant.

2. Results

Distribution of resistance genes among MDR *S. aureus* isolates from different sample sources

As shown in Table 3, the overall distributions of resistance genes were; *tetK* (31.6%), *mecA* (28.1%), *tetL* (8.9%), and *ermC* (1.8%). These genes were detected in isolates from human 12/17 (70.6%), chicken 14/14 (100.0%), rodent 6/13 (61.5%) and soil 6/13 (46.2%) samples.

Table 3: Prevalence of antibiotic resistance genes in MDR *S. aureus* isolates from different samples

Genes	Different types of sample sources n (%)				
	Human (n=17)	**Chicken (n=14)**	**Rodent (n=13)**	**Soil (n=13)**	**Total isolates (n=57)**
ermC	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	1 (1.8)
tetK	5 (29.4)	5 (35.7)	6 (46.2)	2 (15.4)	18 (31.6)
tetL	3 (17.6)	2 (14.3)	0 (0.0)	0 (0.0)	5 (8.9)
mecA	4 (23.5)	6 (42.9)	2 (15.4)	4 (30.8)	16 (28.1)
Total	12 (70.6)	14 (100.0)	8 (61.5)	6 (46.2)	40 (70.2)
Chi-square	32.6	37.5	29.5	20.67	
p-value	0.001	0.001	0.001	0.002	

PCR Amplification of virulence genes

The PCR results show that, *clfB* (10.5%), *coa* (14.0%), *clfA* (5.3%), *hlg* (1.8%), *ebpS* (3.5%), *fnbB* (3.5%), *luk-PV* (10.5%) and *tst* (1.8%) genes were detected (Table 4). These genes were distributed in isolates from human 7/17 (41.2%), chicken 6/14 (42.9%), rodent 5/13 (38.5%) and soil 11/13 (84.6%) samples.

Table 4: Detection of virulence genes in MDR *S. aureus* isolates from different samples

Genes	Number of samples from different sources n (%)				Total isolates (n=57)
	Human (n=17)	Chicken (n=14)	Rodents (n=13)	Soil (n=13)	
clfB	2 (11.8)	1 (7.1)	1 (7.1)	2 (15.4)	6 (10.5)
coa	2 (11.8)	1 (7.1)	2 (15.4)	3 (23.1)	8 (14.0)
clfA	0 (0.0)	2 (14.3)	0 (0.0)	1 (7.7)	3 (5.3)
hlg	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	1 (1.8)
ebpS	1 (5.9)	0 (0.0)	0 (0.0)	1 (7.7)	2 (3.5)
fnbB	0 (0.0)	0 (0.0)	0 (0.0)	2 (15.4)	2 (3.5)
luk-PV	1 (5.9)	1 (7.1)	2 (15.4)	2 (15.4)	6 (10.5)
tst	1 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)
Total	7 (41.2)	6 (42.9)	5 (38.5)	11 (84.6)	29 (50.9)
Chi-square	4.8	6.0	11.2	7.82	
p-value	0.5697	0.6472	0.1906	0.4514	

We observed multiple occurrences of up to three genes among isolates from human, chicken and soil samples while isolates from rodents were having a maximum of two genes as shown in Table 5. For virulence determinants, combinations of up to four genes were common in chicken-isolates, while co-occurrence of three genes was common in human and soil isolates (Table 5).

Table 5: Co-occurrence between resistance and virulence genes of MDR *S. aureus* from different sample sources

Sample ID	**Source**	**Resistance genes**	**Virulence genes**
EDM 2H	Human	*tetK, tetL, mecA*	
EEG 23H	Human	*tetK, mecA*	*clfB, coa,ebpS*
EEG 16H	Human	*tetL, mecA*	
KS 3H	Human	*tetK, tetL*	*coa, luk-PV*
BSA 2P	Chicken	*tetK, tetL, mecA*	
BSG 6P	Chicken	*ermC, tetK*	*clfB, clfA, coa, hlg*
SL 1P	Chicken	*tetK, mecA*	
Ert 13P	Chicken	*tetL, mecA*	
Ert 12P	Chicken	*mecA*	*clfA, luk-PV*
BSG 14Ra	Rodent	*tetK, mecA*	
KM 3R2	Rodent	*tetK*	*luk-PV*
Ert 10R2b	Rodent	*tetK*	*luk-PV*
Ert 10Ra	Rodent	*tetK*	*clfB, coa*
KS 4S	Soil	*tetK*	*clfB, coa, clfa*
Ert 9S	Soil	*mecA*	*luk-PV*
KS 14S	Soil	*mecA*	*fnbB*
KS 3S	Soil	*tetK, mecA*	*fnbB*
Ert 12S	Soil	*tetK, tetL*	*luk-PV*
Ert 13S	Soil	*tetK, tetL*	*clfB, coa, ebpS*
BSG 2S	Soil	*tetK, tetL, mecA*	

As shown in in Figures 1 and 2, resistance genes (*tetK* and *mecA*) and virulence determinants (*clfB, coa* and *luk-PV*) were common in isolates from all sample sources. However, virulence genes *tst, hlg* and *fnbB* were specific to human, chicken and rodent isolates, respectively.

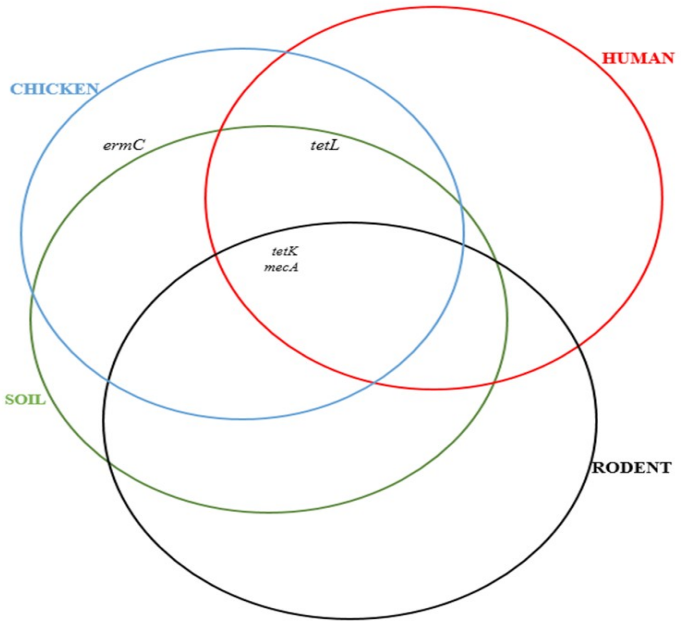

Figure 1: Comparative occurrence of resistance genes among MDR *S. aureus* isolates from all sources

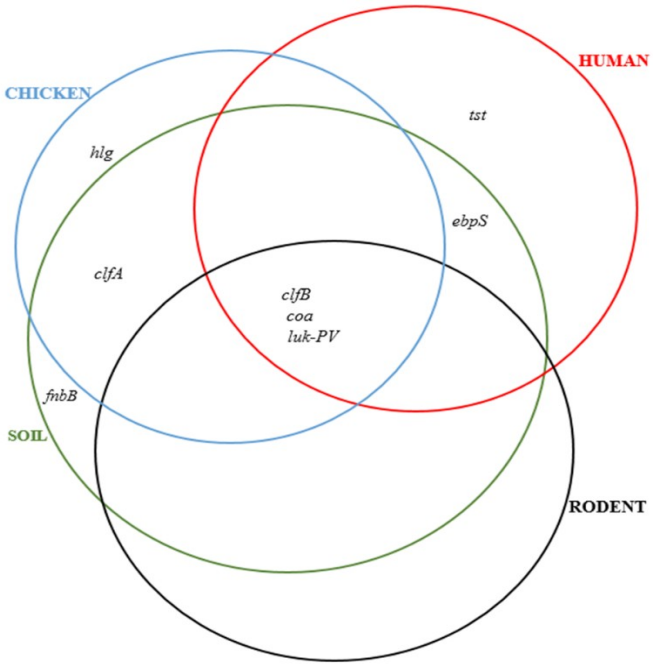

Figure 2: Comparative occurrence of virulence genes among MDR *S. aureus* from all samples

Correlation between phenotypes and genotypes AMR results

We found positive correlations between erythromycin resistance and ermC (r=0.42), tetL (r=0.98) and *mecA* (r=0.51), tetracycline with *tetL* (r=1.00) and *mecA* (r=0.57) and methicillin with *mecA* (r=0.55) and *tetL* (r=0.98) (Table 6).

Table 6: Correlation between resistance phenotypes and genotypes of MDR *S. aureus*

	Correlation coefficients (r)						
	Resistance genotypes				Resistance phenotypes		
	ermC	***tetK***	***tetL***	***mecA***	**Erythromycin**	**Tetracycline**	**Methicillin**
ermC	1	0.19	0.33	0.82	0.42	0.39	0.45
tetK	0.19	1	0.32	-0.24	0.49	0.36	0.46
tetL	0.33	0.32	1	0.54	0.98	1.00	0.98
mecA	0.82	-0.24	0.54	1	0.51	0.57	0.55
Erythromycin	0.42	0.49	0.98	0.51	1	0.99	1.00
Tetracycline	0.39	0.36	1.00	0.57	0.99	1	0.99
Methicillin	0.45	0.46	0.98	0.55	1.00	0.99	1

As shown in Table 7, we found positive correlations between resistance (*ermC*) and virulence genes; *clfA* (r=0.57), *hlg* (r=1.00) and *clfB* (r=0.43), *tetK* and *clfB* (r=0.39); *tetK* and *coa* (r=0.36), while other correlations were weak and negative (Table 7).

Table 7: Correlation coefficients (r) between virulence and resistance genes

Virulence genes	**Correlation coefficients (r) between virulence and resistance genes**			
	Antibiotic resistance genes			
	ermC	***tetK***	***tetL***	***mecA***
clfB	0.43	0.39	0.05	-0.08
coa	0.36	0.36	0.16	-0.14
clfA	0.57	0.14	-0.10	0.01
hlg	1.00	0.17	-0.05	-0.09
ebpS	-0.03	0.24	0.20	0.08
fnbB	-0.03	0.04	-0.08	0.28
luk-PV	-0.05	0.20	0.19	0.01
tst	-0.02	-0.11	-0.05	-0.09

Principal component analysis results for resistance genes

Along PC1 (37.3% explained variance), the vectors for *tetL* and *mecA* genes, each forms a small angle with the x-axis indicating that the genes had higher contribution to tetracycline and methicillin resistance of MDR *S. aureus* isolates. The small angle between these vectors indicates greater and positive correlations between the genes. Considering PC2 (28.2% explained variance), the vector for *ermC* shows greater and positive correlation with PC2 implying higher contribution of *ermC* gene to erythromycin-resistance of the isolates. The ellipses indicate that, most of the resistance genes were found in isolates from chicken samples. The overlapping ellipses for rodents, humans and soil indicate that, the prevalence of resistance genes did not vary much across these sample sources.

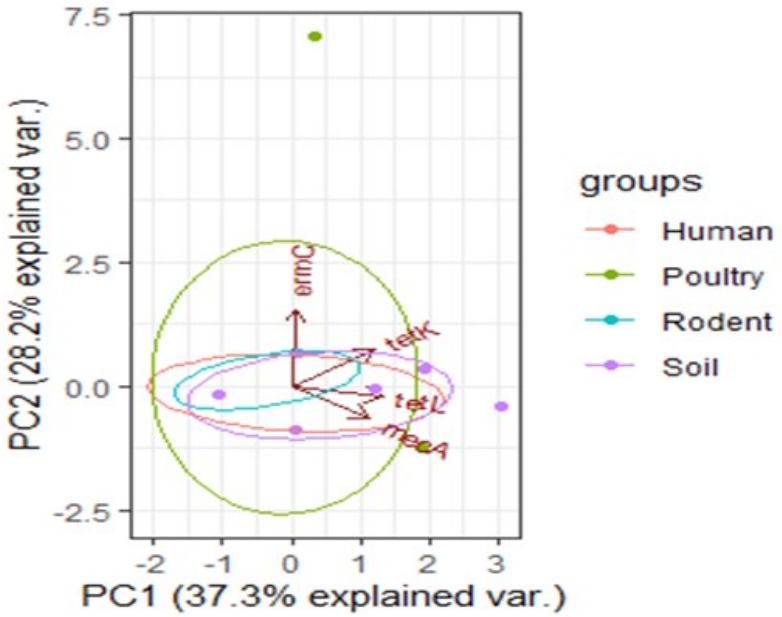

Figure 3: Principal component analysis of resistance genes of MDR *S. aureus* isolates

The dots stand for isolates with their respective sample sources, arrows indicate the vectors for resistance genes of the isolates and ellipses indicate a 95% confidence interval for all samples of a particular source.

Principal Component Analysis (PCA) results for virulence genes

According to Figure 2, *clfB, coa, hlg* and *clfA* genes each forms a small angle with PC1 (34.5% variance), indicating their higher influence to virulence of the MDR *S. aureus* isolates. The genes displayed greater and positive correlations particularly between *clfB* and *coa,* and *hlg* and *clfA.* Like wisely, *fnbB* gene had higher contribution to virulence of isolates but were negatively correlated with *hlg* and *clfA* genes. Along PC2 (17.4% variance), *luk-PV* and *ebpS* were close to the y-axis showing their higher contribution to virulence of the isolates. However, the vectors of these genes were tending to opposite directions indicating that, *luk-PV* and *ebpS* genes are negatively correlated. The biplot shows larger size of soil-ellipse followed by that of humans and rodents both extending in the positive quadrant indicating that most of the virulence genes were found in soil-isolates, followed by those from humans and rodents. The shrunk chicken ellipse extends downward in the negative quadrant showing that MDR isolates from chicken samples had comparatively lower prevalence of virulence genes.

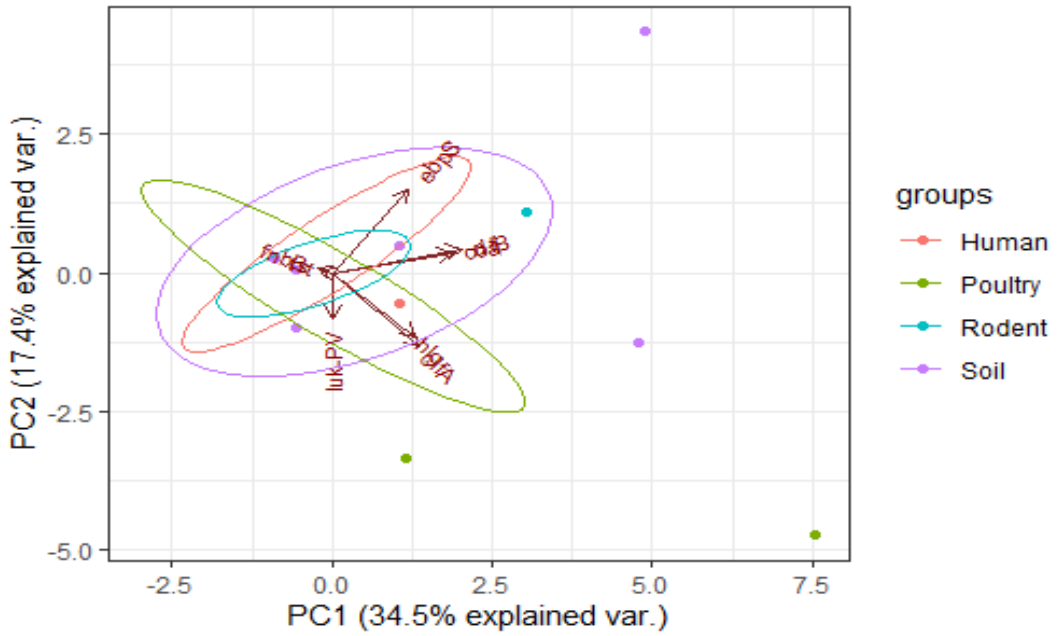

Figure 4: Principal component analysis of virulence genes of MDR *S. aureus* isolates

The dots stand for isolates with their respective sample sources, arrows indicate the vectors for virulence genes of the isolates and ellipses indicate a 95% confidence interval for all samples of a particular source.

Discussion

According to literature review, there is a limited amount of data regarding genetic profile of AMR and virulence genes involving MDR *S. aureus* isolated from humans, poultry, rodents and house-hold soil in Tanzania or surrounding countries. This study was conducted in Karatu in Northern Tanzania where interactions between them is very intense with likelihood for the spread of resistomes and virulence genes (Haule *et al.*, 2013; Ziwa *et al.*, 2013; Makundi *et al.*, 2015; Sonola *et al.*, 2021). A phenotypic study conducted in Karatu indicated high levels of resistance to tetracycline, erythromycin and clindamycin in samples collected from humans, poultry, rodents and house-hold soil (Sonola *et al.*, 2021).

This study, therefore was focused on determining genetic basis of the observed phenotypic resistance and went further to find out the type of virulence genes factors in the isolates. The resistance genes detected in our study were *ermC* (1.8%), *tetK* (31.6%), *tetL* (8.9%) and *mecA* (28.1%). These genes were found in all isolates from chicken (100%) followed by human (70.6%), rodent (61.5%) and soil (46.2%) samples. This finding is plausible with reports of extensive clindamycin, tetracycline, erythromycin and amoxicillin-clavulanate resistance in poultry and humans in Karatu district and other areas in northern Tanzania (Hassell *et al.,* 2019; Gwenzi *et al.,* 2021). Tetracyclines still remain the first-line treatment for a number of human and veterinary infections in many parts of the world, including Tanzania (Sangeda *et al.,* 2021). Our findings show that tetracycline resistance was predominated by *tetK* genes (31.6%) compared with *tetL* genes (8.9%), which is in agreement with previous studies (Dehkordi *et al.*, 2017; Jamali *et al.,* 2015). The *tetK* and *tetL* encode efflux pumping mechanism during *S. aureus* resistance to tetracycline (Lim *et al.,* 2012). Overall, we found that 40.0% of MDR *S. aureus* isolates had *mecA* gene which is greater than 29% reported by Silva *et al.* (2021), in their study on quails slaughtered. In our study, the *ermC* gene was less frequently detected (1.8%), which is unlike other studies reporting prevalence rates ranging from 27.02% to 90.1% (Jamali *et al.*, 2015; Dehkordi *et al.*, 2017; Li *et al.*, 2019; Silva *et al.*, 2021).

The lower prevalence of *ermC* genes in our study, indicates that other mechanisms of resistance such as drug efflux (mediated by *msrA* gene), rather than methylation of ribosomal sites of *S. aureus* which is usually mediated by *ermC,* might have influenced resistance of isolates to erythromycin and clindamycin (Vandendriessche *et al.*, 2011). We noted a co-existence of resistance genes, where the combination of *tetK, tetL* and *mecA* genes was the most common in most isolates. The *mecA* gene, which is carried on Staphylococcal Cassette Chromosome mec (SCC*mec*), is associated with genes encoding

resistance to several non-beta-lactam antibiotic classes, such as tetracyclines and aminoglycosides (Fatholahzadeh *et al.*, 2008).

With regard to virulence genes, the most frequent were *clfB* (10.5%), *coa* (14.0%), *luk-PV* (10.5%) and *clfA* (1.8%). We found lower prevalence of *hlg* (1.8%), *ebpS* (3.5%), *fnbB* (3.5%) and *tst* (1.8%). Most of the genes were found in isolates from soil (84.6%), followed by those from chicken (42.9%), human (41.2%) and rodent (38.5%) samples. Our findings have two implications i) most of these isolates displayed an ability to cause infections that are difficult to treat (Mullarky *et al.*, 2001; Peacock *et al.*, 2002; Tristan *et al.*, 2007) and ii) both rodents and soil environment are potential reservoirs (Lupindu *et al.*, 2015; Hassell *et al.*, 2019; Gwenzi *et al.*, 2021). The virulence genes; *ebpS, clfA, clfB and fnbB* encode for binding proteins that facilitate bacterial adherence to host epithelial cells during invasive infections (Ionescu *et al.*, 2015; Wang *et al.*, 2018). The *luk-PV* and *hlg* genes encode production of toxins that disrupt host immunity resulting into skin lesions and severe pneumonia while coagulase gene (*coa*) is responsible for protection of *S. aureus* cells against phagocytosis and host immunity (Cotar *et al.*, 2010). The *tst* gene codes for production of toxic shock syndrome toxin-1 (TSST-1) protein associated with skin rashes and kidney failure (Bertelloni *et al.*, 2015). According to PCA results, *clfB, coa, ebpS, fnbB, luk-PV, fnbA* and *tst* genes had higher influence to virulence of the MDR *S. aureus* isolates, and there were positive correlations particularly between *clfB* and *coa,* and *hlg* and *clfA,* which comprehends with the results reported by other related studies (Ionescu *et al.*, 2015; Preda *et al.*, 2021). We noted a co-occurrence between resistance and virulence genes in MDR *S. aureus* isolates from humans (*tetK, mecA, clfB, coa* and *ebpS*), chicken (*ermC, tetK, clfB, clfA, coa* and *hlg*), rodents (*tetK, clfB* and *coa*) and soil (*tetK, tetL, clfB, coa and ebpS*). Indeed, PCA confirmed positive correlations between resistance and virulence genes of MDR *S. aureus* highlighting the possibility of co-transmission of

plasmid mediated genes through horizontal gene transfer (Sidhu *et al.,* 2002). Notably, resistance genes (*tetK* and *mecA)* and virulence determinants (*clfB, coa* and *luk-PV*) were common in all sample sources, while *tst, hlg and fnbB* were only specific to human, chicken and rodent isolates, respectively. Our results are in agreement with the findings from different studies on prevalence of virulence and antibiotic resistance genes in *S. aureus* (Tristan *et al.,* 2007; Preda *et al.*, 2021; Silva *et al.,* 2021).

In summary, our results show occurrence and co-occurrence of AMR and virulence genes in most of the isolates implying that the circulating MDR *S aureus* strains are capable of causing infections that are difficult to treat (Ionescu *et al.*, 2015; Wang *et al.*, 2018). Certainly, the predominance of *tetK, tetL* and *mecA* in all sample sources is a reflection of the reported pattern of antibiotic usage in the area (Sonola *et al.*, 2021). The carriage of virulence genes was high even in isolates from soil samples. We are recommending the following; 1) progressive stewardship of antibiotics usage in human and veterinary medicine. 2) improving One Health (OH) interventions to minimize the risks of spreading infections among humans, chickens and soil environment and 3) rodent control practices in households. This will require a multisectoral and multidisciplinary collaborative effort of human health, animal health and environmental sector to attain optimal health for people and animals and protect the environment.

Lastly, we acknowledge that although our study provides important insight regarding the profile of AMR and virulence genes among MDR *S. aureus* strains circulating in Karatu, sequence typing is needed to explore genetic diversity and relatedness, which have consequences in managing the spread and control of these strains between reservoirs.

Conclusion

This study has shown that the *S. aureus* isolates recovered from humans, poultry, rodents and household soil contain a variety of resistance genes, mainly *tetK*, *tetL* and *mecA* and virulence genes mostly; clumping factor B (*clfB*), coagulase protein (*coa*), leukocydal toxins (*LUK-PV*) and clumping factor A protein (*clfA*). PCA revealed that *clfB, coa, hlg* and *clfA* genes had higher influence to virulence of the MDR *S. aureus* isolates. Resistance genes (*tetK* and *mecA)* and virulence determinants (*clfB, coa* and *luk-PV*) were common in all sample sources, while *tst, hlg and fnbB* were only specific to human, chicken and rodent isolates, respectively. AMR and VR genes were found in rodents and soil environment implying that both are potential reservoirs. The large battery of AMR and VR genes among MDR *S. aureus* strains circulating in the area, indicate their ability to cause infections that are difficult to treat endangering public and animal health.

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Author Contributions: Collection of samples, laboratory processing and data analysis (V.S.S.), supervision and verification of the analytical methods (M.I.N.M. and G.M.). Manuscript developed by V.S.S. with input from M.I.N.M., G.M. and A.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The ethical clearance for the study was issued by the National Institute for Medical Research (NIMR) of Tanzania (NIMR/HQ/R.8a/Vol.IX/3386). NIMR is the national health research coordinating body that ensures all health research follows the national health ethics requirements. Sokoine University of Agriculture (SUA) Institutional Animal Care and Use Committee (IACUC) approved the use of animals in this study. The permission to work in the study area was sought from the Regional Administrative Office (Arusha).

Informed Consent Statement: Informed verbal consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

Conflicts of Interest: The authors declare no conflict of interest

References

Abdolmaleki, Z., Mashak, Z. and Dehkordi, F. S. (2019). Phenotypic and genotypic characterization of antibiotic resistance in the methicillin-resistant *Staphylococcus aureus* strains isolated from hospital cockroaches. *Antimicrobial Resistance and Infection Control* 8(1): 1-14.

Abou-Zahr, T., Carrasco, D. C., Shimizu, N., Forbes, N. A., Dutton, T. A., Froehlich, F. and De Bellis, F. (2018). Superficial chronic ulcerative dermatitis (SCUD) in psittacine birds: Review of 11 cases (2008 - 2016). *Journal of Avian Medicine and Surgery* 32(1): 25 - 33.

Adwan, G., Adwan, K., Jarrar, N., Salama, Y. and Barakat, A. (2013). Prevalence of seg, seh and sei Genes among Clinical and Nasal *Staphylococcus aureus* Isolates in Palestine. *Microbiology Research Journal International* 139 -149.

Ampaire, L., Muhindo, A., Orikiriza, P., Mwanga-Amumpaire, J., Boum, Y. and Bebell, L. (2016). A review of antimicrobial resistance in East Africa. *African Journal of Laboratory Medicine* 5(1): 1 - 6.

Baquero, F., Coque, T. M., Martínez, J.L., Aracil-Gisbert, S. and Lanza, V. F. (2019). Gene transmission in the one health microbiosphere and the channels of antimicrobial resistance. *Frontiers in Microbiology* 10: 28 - 92.

Benrabia, I., Hamdi, T. M., Shehata, A. A., Neubauer, H. and Wareth, G. (2020). Methicillin-resistant *Staphylococcus aureus* (MRSA) in poultry species in Algeria: long-term study on prevalence and antimicrobial resistance. *Veterinary Sciences* 7(2): 54.

Bertelloni, F., Fratini, F., Ebani, V. V., Galiero, A., Turchi, B. and Cerri, D. (2015). Detection of genes encoding for enterotoxins, TSST-1 and biofilm production in coagulase-negative staphylococci from bovine bulk tank milk. *Dairy Science and Technology* 95(3): 341 - 352.

Cheung, G. Y., Bae, J. S. and Otto, M. (2021). Pathogenicity and virulence of *Staphylococcus aureus. Virulence* 12(1): 547 - 569.

Cotar, A. I., Chifiriuc, M. C., Dinu, S., Pelinescu, D., Banu, O. and Lazãr, V. (2010). Quantitative real-time PCR study of the influence of probiotic culture soluble fraction on the expression of *Pseudomonas aeruginosa* quorum sensing genes. *Romanian archives of Microbiology and Immunology* 69(4): 213 - 223.

Dehkordi, F. S., Gandomi, H., Basti, A. A., Misaghi, A. and Rahimi, E. (2017). Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant *Staphylococcus aureus* isolated from hospital food. *Antimicrobial Resistance and Infection Control* 6(1): 1-11.

Derakhshan, S., Navidinia, M. and Haghi, F. (2021). Antibiotic susceptibility of human-associated *Staphylococcus aureus* and its relation to agr typing, virulence genes, and biofilm formation. *BMC Infectious Diseases* 21(1): 1-10.

Fatholahzadeh, B., Emaneini, M., Gilbert, G., Udo, E., Aligholi, M., Modarressi, M. H. and Feizabadi, M. M. (2008). Staphylococcal cassette chromosome mec (SCC mec) analysis and antimicrobial susceptibility patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Tehran, Iran. *Microbial Drug Resistance* 14(3): 217-220.

Gornatti-Churria, C. D., Crispo, M., Shivaprasad, H. and Uzal, F. A. (2018). Gangrenous dermatitis in chickens and turkeys. *Journal of Veterinary Diagnostic Investigation* 30(2): 188-196.

Gwenzi, W., Chaukura, N., Muisa-Zikali, N., Teta, C., Musvuugwa, T., Rzymski, P. and Abia, A. L. K. (2021). Insects, Rodents and Pets as Reservoirs, Vectors, and Sentinels of Antimicrobial Resistance. *Antibiotics* 10(1): 68 -76.

Hait, J. M., Cao, G., Kastanis, G., Yin, L., Pettengill, J. B. and Tallent, S. M. (2021). Evaluation of virulence determinants using whole-genome sequencing and phenotypic biofilm analysis of outbreak-linked *Staphylococcus aureus* Isolates. *Frontiers in Microbiology* 12: 67 - 79.

Hassell, J. M., Ward, M. J., Muloi, D., Bettridge, J. M., Robinson, T. P., Kariuki, S. and Kang'ethe, E. K. (2019). Clinically relevant antimicrobial resistance at the wildlife–livestock–human interface in Nairobi: an epidemiological study. *The Lancet Planetary Health* 3(6): e259 - e269.

Haule, M., Lyamuya, E. E., Hang'ombe, B. M. and Matee, M. I. (2013). Investigation of fleas as vectors in the transmission of plague during a quiescent period in North-Eastern, Tanzania. 119pp.

Ionescu, B., Ionescu, D., Gheorghe, I., Curuțiu, C., Banu, O., Bleotu, C. and Bezirtzoglou, E. (2015). Virulence patterns of *Staphylococcus aureus* hospital strains isolated in Bucharest, Romania. *Romanian Biotechnological Letters* 20(3): 10536 - 10546.

Jamali, H., Paydar, M., Radmehr, B., Ismail, S. and Dadrasnia, A. (2015). Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk and dairy products. *Food Control* 54: 383-388.

Jarraud, S., Mougel, C., Thioulouse, J., Lina, G., Meugnier, H., Forey, F. and Vandenesch, F. (2002). Relationships between *Staphylococcus aureus* genetic background, virulence factors, agr groups (alleles) and human disease. *Infection and immunity* 70(2): 631 - 641.

Kashoma, I., Lalata, E., Maiga, C., Mtemela, B. and Medardus, J. (2015). Prevalence and antimicrobial susceptibility profiles of *Staphylococcus aureus* from cow's milk, nasal and environmental swabs in selected dairy farms in Morogoro, Tanzania. *Tanzania Veterinary Journal* 30(2): 61 -75.

Katakweba, A. S., Muhairwa, A. P., Espinosa-Gongora, C., Guardabassi, L., Mtambo, M. M. and Olsen, J. E. (2016). spa typing and antimicrobial resistance of *Staphylococcus aureus* from healthy humans, pigs and dogs in Tanzania. *The Journal of Infection in Developing Countries* 10(02): 143-148.

Kayili, E. and Sanlibaba, P. (2020). Prevalence, characterization and antibiotic resistance of *Staphylococcus aureus* isolated from traditional cheeses in Turkey. *International Journal of Food Properties* 23(1): 1441-1451.

Kilonzo, B., Mbise, T., Mwalimu, D. and Kindamba, L. (2006). Observations on the endemicity of plague in Karatu and Ngorongoro, northern Tanzania. *Tanzania Journal of Health Research* 8(1): 1-6.

Li, H., Tang, T., Stegger, M., Dalsgaard, A., Liu, T. and Leisner, J. J. (2021). Characterization of antimicrobial-resistant *Staphylococcus aureus* from retail foods in Beijing, China. *Food microbiology* 93: 103 - 603.

Li, X., Huang, T., Xu, K., Li, C. and Li, Y. (2019). Molecular characteristics and virulence gene profiles of *Staphylococcus aureus* isolates in Hainan, China. *BMC infectious diseases* 19(1): 1-12.

Lim, K., Hanifah, Y., Yusof, M. and Thong, K. L. (2012). ermA, ermC, tetM and tetK are essential for erythromycin and tetracycline resistance among methicillin-resistant *Staphylococcus aureus* strains isolated from a tertiary hospital in Malaysia. *Indian Journal of Medical Microbiology* 30(2): 203 - 207.

Liu, C., Chen, Z.J., Sun, Z., Feng, X., Zou, M., Cao, W. and Sun, M. (2015). Molecular characteristics and virulence factors in methicillin-susceptible, resistant and heterogeneous vancomycin-intermediate *Staphylococcus aureus* from central-southern China. *Journal of Microbiology, Immunology and Infection* 48(5): 490 - 496.

Lupindu, A. M., Dalsgaard, A., Msoffe, P. L., Ngowi, H. A., Mtambo, M. M. and Olsen, J. E. (2015). Transmission of antibiotic-resistant *Escherichia coli* between cattle, humans and the environment in peri-urban livestock keeping communities in Morogoro, Tanzania. *Preventive Veterinary Medicine* 118(4): 477 - 482.

Makundi, R. H., Massawe, A. W., Mulungu, L. S., Katakweba, A., Mbise, T. J. and Mgode, G. (2008). Potential mammalian reservoirs in a bubonic plague outbreak focus in Mbulu District, Northern Tanzania, in 2007. 87pp.

Makundi, R. H., Massawe, A. W., Borremans, B., Laudisoit, A. and Katakweba, A. (2015). We are connected: flea–host association networks in the plague outbreak focus in the Rift Valley, northern Tanzania. *Wildlife Research* 42(2): 196 - 206.

Mouiche, M. M. M., Moffo, F., Akoachere, J. F. T. K., Okah-Nnane, N. H., Mapiefou, N. P., Ndze, V. N. and Zambou, H. R. (2019). Antimicrobial resistance from a one health perspective in Cameroon: a systematic review and meta-analysis. *BMC Public Health* 19(1): 1 - 20.

Mullarky, I., Su, C., Frieze, N., Park, Y. and Sordillo, L. (2001). *Staphylococcus aureus* agr genotypes with enterotoxin production capabilities can resist neutrophil bactericidal activity. *Infection and immunity* 69(1): 45 - 51.

Nathan, C. and Cars, O. (2014). Antibiotic resistance - problems, progress and prospects. *New England Journal of Medicine* 371(19): 1761 - 1763.

Ote, I., Taminiau, B., Duprez, J.N., Dizier, I. and Mainil, J. G. (2011). Genotypic characterization by polymerase chain reaction of *Staphylococcus aureus* isolates associated with bovine mastitis. *Veterinary microbiology* 153(3-4): 285 - 292.

Peacock, S. J., Moore, C. E., Justice, A., Kantzanou, M., Story, L., Mackie, K. and Day, N. P. (2002). Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*. *Infection and immunity* 70(9): 4987 - 4996.

Pérez-Serrano, R. M., Domínguez-Pérez, R. A., Ayala-Herrera, J. L., Luna-Jaramillo, A. E., de Larrea, G. Z. L., Solís-Sainz, J. C. and Loyola-Rodríguez, J. P. (2020).

Dental plaque microbiota of pet owners and their dogs as a shared source and reservoir of antimicrobial resistance genes. *Journal of Global Antimicrobial Resistance* 21: 285 - 290.

Preda, M., Mihai, M. M., Popa, L. I., Dițu, L. M., Holban, A. M., Manolescu, L. S. C. and Chifiriuc, C. M. (2021). Phenotypic and genotypic virulence features of staphylococcal strains isolated from difficult-to-treat skin and soft tissue infections. *PLoS One* 16(2): e0246478.

Sangeda, R. Z., Saburi, H. A., Masatu, F. C., Aiko, B. G., Mboya, E. A., Mkumbwa, S. and Chambuso, M. (2021). National Antibiotics Utilization Trends for Human Use in Tanzania from 2010 to 2016 Inferred from Tanzania Medicines and Medical Devices Authority Importation Data. *Antibiotics* 10(10): 1249.

Savoldi, A., Carrara, E., Gladstone, B. P., Azzini, A. M., Göpel, S. and Tacconelli, E. (2019). Gross national income and antibiotic resistance in invasive isolates: analysis of the top-ranked antibiotic-resistant bacteria on the 2017 WHO priority list. *Journal of Antimicrobial Chemotherapy* 74(12): 3619 - 3625.

Sidhu, M. S., Heir, E., Leegaard, T., Wiger, K. and Holck, A. (2002). Frequency of disinfectant resistance genes and genetic linkage with β-lactamase transposon Tn 552 among clinical staphylococci. *Antimicrobial Agents and Chemotherapy* 46(9): 2797 - 2803.

Silva, V., Gabriel, S. I., Borrego, S. B., Tejedor-Junco, M. T., Manageiro, V., Ferreira, E. and Igrejas, G. (2021). Antimicrobial Resistance and Genetic Lineages of

Staphylococcus aureus from Wild Rodents: First Report of mecC-Positive Methicillin-Resistant S. aureus (MRSA) in Portugal. *Animals* 11(6): 1537.

Sonola, V. S., Misinzo, G. and Matee, M. I. (2021). Occurrence of Multidrug-Resistant *Staphylococcus aureus* among Humans, Rodents, Chickens and Household Soils in Karatu, Northern Tanzania. *International Journal of Environmental Research and Public Health* 18(16): 84 - 96.

Theonest, N. O., Carter, R. W., Amani, N., Doherty, S. L., Hugho, E., Keyyu, J. D. and Thomas, K. M. (2019). Molecular detection and genetic characterization of Bartonella species from rodents and their associated ectoparasites from northern Tanzania. *PLoS One* 14(10): e0223667.

Tristan, A., Ferry, T., Durand, G., Dauwalder, O., Bes, M., Lina, G. and Etienne, J. (2007). Virulence determinants in community and hospital meticillin-resistant *Staphylococcus aureus*. *Journal of Hospital Infection* 65: 105 - 109.

Tristan, A., Ying, L., Bes, M., Etienne, J., Vandenesch, F. and Lina, G. (2003). Use of multiplex PCR to identify *Staphylococcus aureus* adhesins involved in human hematogenous infections. *Journal of clinical microbiology* 41(9): 4465 - 4467.

Trzcinski, K., Cooper, B. S., Hryniewicz, W. and Dowson, C. G. (2000). Expression of resistance to tetracyclines in strains of methicillin-resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* 45(6): 763 - 770.

Van Duin, D. and Paterson, D. L. (2016). Multidrug-resistant bacteria in the community: trends and lessons learned. *Infectious Disease Clinics* 30(2): 377 - 390.

Vandendriessche, S., Kadlec, K., Schwarz, S. and Denis, O. (2011). Methicillin-susceptible *Staphylococcus aureus* ST398-t571 harbouring the macrolide–lincosamide–streptogramin B resistance gene erm (T) in Belgian hospitals. *Journal of Antimicrobial Chemotherapy* 66(11): 2455 - 2459.

Vannuffel, P., Gigi, J., Ezzedine, H., Vandercam, B., Delmée, M., Wauters, G. and Gala, J. L. (1995). Specific detection of methicillin-resistant Staphylococcus species by multiplex PCR. *Journal of Clinical Microbiology* 33(11): 2864 - 2867.

Wang, F. D., Wu, P. F. and Chen, S. J. (2019). Distribution of virulence genes in bacteremic methicillin-resistant *Staphylococcus aureus* isolates from various sources. *Journal of Microbiology, Immunology and Infection* 52(3): 426 - 432.

Wang, W., Baloch, Z., Jiang, T., Zhang, C., Peng, Z., Li, F. and Xu, J. (2017). Enterotoxigenicity and antimicrobial resistance of *Staphylococcus aureus* isolated from retail food in China. *Frontiers in Microbiology* 8: 22 - 56.

Wang, W., Lin, X., Jiang, T., Peng, Z., Xu, J., Yi, L. and Baloch, Z. (2018). Prevalence and characterization of *Staphylococcus aureus* cultured from raw milk taken from dairy cows with mastitis in Beijing, China. *Frontiers in Microbiology* 9: 11 - 23.

Watkins, R. R., David, M. Z. and Salata, R. A. (2012). Current concepts on the virulence mechanisms of meticillin-resistant *Staphylococcus aureus*. *Journal of Medical Microbiology* 61(Pt 9): 11 - 79.

Zhao, X., Wei, C., Zhong, J. and Jin, S. (2016). Research advance in rapid detection of foodborne *Staphylococcus aureus*. *Biotechnology & Biotechnological Equipment* 30(5): 827 - 833.

Ziwa, M. H., Matee, M. I., Hang'ombe, B. M., Lyamuya, E. F. and Kilonzo, B. S. (2013). Plague in Tanzania: an overview. *Tanzania Journal of Health Research* 15(4): 76 – 88.

CHAPTER SIX

6.0 GENERAL DISCUSSION

This study was conducted in Karatu district, an area reported to have high interactions of rodents, chicken, humans and soil in households that have been associated with spread of zoonotic infections (Kilonzo and Mtoi, 1983; Kilonzo *et al.*, 2006; Makundi *et al.*, 2008; Ziwa *et al.*, 2013; Makundi *et al.*, 2015). The main objective was to determine isolation frequencies, phenotypic and genotypic antimicrobial resistance and virulence profiles of MDR *S. aureus* and *E. coli*. These two bacterial species are One Health bacterial species used for tracking the spread of resistomes and virulence genes across human-animal-environment sources (Aslam *et al.*, 2021).

Results of this investigation revealed high frequencies of isolation for *S. aureus* and *E. coli* in rodents, humans and chicken and soil samples. For *S. aureus,* the isolation frequencies were 52.1%, 66.5%, 74.3% and 24.5% in samples from chicken, human, rodent and soil, respectively. The percentage of occurrence of *S. aureus* in humans comprehends with the findings of other studies in Tanzania which reported a wide range from 59.3% to 71.4% (Kinabo *et al.*, 2013; Silago *et al.*, 2015; Kazimoto *et al.*, 2018). The isolation frequencies of *E. coli* from chicken, humans, rodents and soil were 81.6 %, 86.5 %, 79.2 % and 31.0 %, respectively. This wide spread occurrence of *S. aureus* and *E. coli* highlights the possibility of their spread across human-animal-environment sources (Aworh *et al.*, 2021), with consequential flow of resistomes and virulence genes (Ogundipe *et al.*, 2020).

Antibiotic susceptibility testing results revealed that *S. aureus* isolates exhibited resistance to clindamycin (51%), erythromycin (50.9%) and tetracycline (62.5%) while, *E. coli* isolates showed high resistance against tetracycline (73.7%), imipenem (79.8%) and

cefotaxime (79.8%). This highlights that the drugs could be frequently used and misused during treatment and prevention of bacterial infections in chicken and humans. A recent review reported that the most commonly used classes of antimicrobial agents in animals in Tanzania and Africa in general are tetracycline, sulphonamides, penicillin, macrolides and others including antiprotozoal agents (Kimera *et al.*, 2020; Mdegela *et al.*, 2021).

These antibiotics have also been found in studies investigating residues in foods of animal of animal origin in this country at levels exceeding acceptable regulatory levels (Bilashoboka *et al.*, 2019; Kimera *et al.*, 2020; Mdegela *et al.*, 2021) posing danger to human health. The primary reasons why farmers use antimicrobials are for sickness prevention (60%), growth promotion (26%) and treatment (14%). Farmers usually use antimicrobials (61%) and they sometimes use other disease prevention techniques such as biosecurity and vaccination (39%) (FAO, 2019; Nonga *et al.*, 2009). In most cases, similar antibiotics are shared between the public health, animal health and the food sectors (FAO, 2019; Mboera *et al.*, 2018). Unfortunately, a National Sample Survey indicated that only 20% of Tanzanian farmers utilise extension services (GOT, 2017; Michael *et al.*, 2018).

Ranges of resistance shown by *S. aureus* to all tested antibiotics were between 57.3% (erythromycin) and 65.9% (tetracycline) in isolates from chicken, 51.7% (clindamycin) to 74.4% (tetracycline) from humans, 40% (clindamycin) to 53.3% (tetracycline) from rodents and 31.1% (erythromycin) to 56.3% (tetracycline) from soil samples. For *E. coli*, the resistance ranged from 76.7% (imipenem) to 83.1% (cefotaxime) for chickens, 62.1% to 75.7% for human, 77.5% (cefotaxime) to 83.8% (imipenem) for rodents and 60.0% (tetracycline) to 81.1% (imipenem) for soil-isolates. The occurrence of antimicrobial resistance (AMR) isolates in rodents and soil indicate their significance as vectors that can disseminate AMR to humans via direct contact, human food contamination, and horizontal

gene transfer (Gwenzi *et al.*, 2021). Rodents as wild animals can spread resistant bacterial infections to other rodents or wild animals in the forests or parks close to Karatu district and increase the spectrum of AMR spread.

Specific resistance rates of isolates from chicken, human, rodent and soil samples showed variations against the tested antibiotics. For *S. aureus*, resistance against tetracycline varied from 53.3% to 74.4%, for erythromycin ranged from 31.3% to 62.8% and for clindamycin from 40.0% to 62.2%. For *E. coli* resistance against tetracycline varied from 60.0% to 77.5%for imipenem ranged from 74.4% to 83.8%% and for cefotaxime from 75.7% to 83.1%. The available data obtained from a number of studies in food animals in Tanzania show high proportion of multidrug-resistant *S. aureus* and *E. coli* isolates, including methicillin- resistant *Staph. aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) producers (Kivaria *et al.*, 2006; Kurwijila *et al.*, 2006; Mdegela *et al.*, 2004; Mdegela *et al.*, 2009) Mgaya *et al.*, 2021) at the level found in this study, with minor variations. Similar resistance pattern has also been shown in clinical isolates from humans with various infectious disease conditions (Mshana *et al.*, 2013).

A number of MDR isolates exhibited various phenotypic resistance patterns. The most common combination was CD-E-TE (clindamycin, erythromycin and tetracycline) for *S. aureus* and TE-IMP-CTX (tetracycline, imipenem and cefotaxime) for *E. coli.* MDR isolates were in all sample sources (humans, poultry, rodents and soil), indicating and supporting common use of these antibiotics observed previously in this area (Rugumisa *et al.*, 2016; Caudell *et al.*, 2017). Surprisingly, *S. aureus* isolates from chickens had exhibited high resistance to clindamycin (62.2%) and *E. coli* isolates had high resistance to imipenem (83.1%), which are not commonly used in poultry farming in Tanzania. It has been observed that frequent use of macrolides such as erythromycin trigger production of

erythromycin ribosomal methylase (erm) enzymes in *S. aureus*, with subsequent induction of resistance to clindamycin (Levin *et al.*, 2005). The rate of resistance to imipenem found in this study is alarming since carbapenems are often reserved as the last-line antibiotics against MDR Enterobacteriaceae since they are stable even in the presence of extended-spectrum β-lactamases (ESBLs) and AmpC enzymes (Tsakris *et al.*, 2010). The high level of resistance against imipenem found in this study might be due to co-existence of ESBL and MBL resistance genes on plasmids and hence co-transmission (Masoud *et al.*, 2021).

Antibiotic resistance and virulence genes of MDR *E. coli* and *S. aureus* isolates were detected using multiplex PCR technique (Yao *et al.*, 2019). Various genes that encode for beta-lactamases, tetracycline, quinolone and carbapenem resistance and virulence factors were amplified. Resistance genes detected in MDR *E. coli* were; *blaTEM* (46%), *blaCTX-M* (26%), *blaSHV* (22%), *tetA* (46%), *tetB* (14%), *qnrA* (24%), *qnrB* (8%), *blaOXA-48* (12%) and *blaKPC* (6%). In a recent study in poultry in Dar es Salaam found CTX-M and quinolones resistant gene (*qnrS),* while TEM, SHV, *qnrA, qnrB* and *aac (6')-lb-cr* were not detected (Mgaya *et al.*, 2021) and another study found pork and poultry in Dar es Salaam harbouring *bla*CTX-M, 15% *aac* (*6*)-*lb-cr*, 10% *qnrB*, and 5% *qepA* and none harboured TEM, SHV, *qnrA*, *qnrS*, *qnrC*, or *qnrD* (Kimera *et al.*, 2020). For MDR *S. aureus*, resistance genes detected were; *tetK* (31.6%), *tetL* (8.9%), *ermC* (1.8%) and *mecA* (28.1%). The prevalence rates of *tetK* and *tetL* genes in this study are significantly lower compared to *tetK* (78.8%) and *tetl* (81.4%) reported by Zhou *et al.* (2020) in a related study in China. However, Monecke *et al.* (2016) reported 4.8% rate for both *tetK* and *tetL* genes in *S. aureus* isolated from rodents, which is much, lower than the findings of this study. The gene with lowest frequency in this study was *ermC* (1.8%) which compares with 1.9% reported elsewhere (Zhou *et al.*, 2020), but much lower than 96.6% documented by a South African study on *S. aureus* in pigs and environmental samples (Sineke *et al.*, 2021).

A previous study conducted in Tanzania by Katakweba *et al.* (2016) on *S. aureus* from humans, pigs and dogs did not detect *ermC* (0%) gene. The observed *mecA* (28.1%) in the present study compare with 22.6% findings reported by Okorie-Kanu *et al.* (2020) in Nigeria, but is lower than 63.75% reported by Sineke *et al.* (2021) in South Africa.

Interestingly, positive correlations were observed between *E. coli* resistance phenotypes and genotypes as reported by others (Evans and Amyes, 2014). These correlations included; tetracycline and *tetA* (r=0.53); tetracycline and *tetB* (r=0.90); tetracycline and *blaKPC* (r=0.90); imipenem and *blaKPC* (0.90); imipenem and *blaOXA-48* (0.89); ciprofloxacin and *qnrA* (0.90); cefotaxime and *blaKPC* (r=0.93); cefotaxime and *blaOXA-48* (0.90); cefotaxime and *qnrA* (r=0.96). The lower correlation coefficient between tetracycline resistance phenotype and *tetA* gene (r=0.53) implies that other genes such as *tetC, tetD* and *tetG* could have influenced the phenotypic tetracycline resistance (Skočková *et al.*, 2021). Positive correlations were observed between resistance phenotypes and genotypes for *S. aureus* isolates; erythromycin with *ermC* (r=0.42); *tetL* (r=0.98); *mecA* (r=0.51), tetracycline with *tetL* (r=1.00); *mecA* (r=0.57) and methicillin with *mecA* (r=0.55) and *tetL* (r=0.98). The lowest correlation coefficient was found between erythromycin resistance and its genotype *ermC* (r=0.41) indicating that, different resistance mechanisms such as efflux pumping (encoded by *msrA* gene) or drug inactivation (encoded by *lnu* genes), could have influenced resistance of *S. aureus* to erythromycin and not target site modification by ribosomal methylation (encoded by *ermA* and *ermC* genes) (Saderi *et al.*, 2009).

In this study, virulence genes detected among MDR *E. coli* isolates included; *ompA* (72%), *traT* (26%), *east* (18%), *bfp* (10%), *eae* (2%) and *stx-1* (4%). A number of virulence genes were also found among MDR *S. aureus* isolates, including; *clfB* (10.5%), *coa* (14.0%), *clfA*

*(*1.8%), *hlg (*1.8%), *ebpS (*3.5%), *fnbB (*3.5%), *luk-PV (*10.5%) and *tst* (1.8%). These genes are responsible for various disease causing mechanisms (Firoozeh *et al.*, 2014; Said *et al.*, 2015; Algammal *et al.*, 2020; Pan *et al.*, 2021; Bessaiah *et al.*, 2022; Salamandane *et al.*, 2022).

Most of the AMR genes were found in isolates from rodents (80.0%), followed by chicken (75.0%), human (57.1%), and soil (50.0%), while virulence genes mostly detected in isolates from rodents (80.0%), followed by chicken (75.0%), humans (57.1%), and soil (50.0%) samples. This pattern of predominance of AMR and virulence genes in isolates from rodents and poultry underlines their potential of causing drug resistance infections (Benavides *et al.*, 2021). As a matter of fact, principal component analysis (PCA) revealed that MDR *E. coli* and *S. aureus* isolates from rodents and chicken had more resistance and virulence genes compared to those isolated from soil and humans. Regarding virulence factors, PCA also showed that MDR *E. coli* isolates harboured *traT, east, eae, stx-1, bfp* and *ompA* genes capable of causing wide ranging infections (Firoozeh *et al.*, 2014; Said *et al.*, 2015; Algammal *et al.*, 2020; Pan *et al.*, 2021; Bessaiah *et al.*, 2022; Salamandane *et al.*, 2022). For *S. aureus*, ARGs were found in chicken (100%), human (70.6%) and rodents (61.5%); while VGs were found more commonly in soil (84.6%), chicken (42.9%) and human (41.2%). PCA results showed resistance genes (*tetK* and *mecA)* and virulence determinants (*clfB, coa* and *luk-PV*) were common in all sample sources, while *tst, hlg and fnbB* were only specific to human, chicken and rodent isolates, respectively.

In this study both positive and negative correlations between resistance and virulence genes of *S. aureus* were noted. Positive correlations were found between *blaTEM* and *traT* genes (r=0.51) and *qnrB* and *bfp* genes (r=0.63), while negative correlations were revealed between *blaOXA-48* and *ompA* (r=-0.05), *blaSHV* and *traT* (r=-0.44) and *tetA* and *east*

(r=-0.10). For *S. aureus*, positive correlations were found between resistance (*ermC*) and *clfA* (r=0.57), *hlg* (r=1.00) and *clfB* (r=0.43), *tetK* and *clfB* (r=0.39); *tetK* and *coa* (r=0.36). It has been shown that acquisition of resistance to certain antibiotics may be associated with an increase or decrease in the virulence levels depending on location and mechanism of transfer of specific genes (Boerlin *et al.*, 2005; Diarrassouba *et al.*, 2007). In summary this study has provided useful insight regarding isolation frequencies, phenotypic and genotypic antimicrobial resistance and virulence profiles of MDR *S. aureus* and *E. coli* isolated from human, chicken, soil and rodents in Karatu district.

Limitation of the study

However, the study has some limitations that need to be pointed out including; i) it focused on few AMR and virulence genes of *E. coli* and *S. aureus*, which may underestimate their actual magnitude and co-occurrence ii) the use of conventional PCR, instead of advanced genomic techniques such as whole genome sequencing limited the understanding of AMR transmission between human, chicken, soil and rodents in the area and iii) human, animal and environmental contributors to AMR were not investigated. Lastly, the intermediate isolates were not independently investigated which may undermine their potential in carriage and transmission of resistomes among the microbes.

In summary the findings of these study reveal the following key issues:

i. *E. coli* and *S. aureus* exhibit a very high resistance level of resistance against commonly used antibiotics including tetracycline, cefotaxime, erythromycin and clindamycin.
ii. AMR genes found in *E. coli* were *blaTEM*, *blaCTX-M*, *tetA*, *tetB*, *qnrA*, *qnrB*, *blaOXA-48* and *blaKPC*, while for *S. aurues* were *tetK*, *tetL*, *ermC* and *mecA*

iii. Virulence genes found in *E. coli* were *ompA*, *traT*, *east*, *bfp*, *eae* and *stx-1*, while for *S. aurues* were *clfB*, *coa*, *clfA*, *hlg*, *ebpS*, *fnbB*, *luk-PV* and *tst*. AMR and virulence genes were found in isolates from rodents, chicken, soil and humans.

iv. There were correlations between AMR and virulence genes in both *E. coli* and *S. aureus.*

v. Infections caused by *E. coli* and *S. aureus* will be difficult to treat by the currently used antibiotics.

References

AbuOun, M., O'Connor, H. M., Stubberfield, E. J., Nunez-Garcia, J., Sayers, E., Crook, D. W. and Anjum, M. F. (2020). Characterizing antimicrobial resistant *Escherichia coli* and associated risk factors in a cross-sectional study of pig farms in Great Britain. *Frontiers in Microbiology* 11: 861 -873.

Algammal, A. M., El-Kholy, A. W., Riad, E. M., Mohamed, H. E., Elhaig, M. M., Yousef, S. A. A. and Ghobashy, M. O. (2020). Genes encoding the virulence and the antimicrobial resistance in enterotoxigenic and shiga-toxigenic *E. coli* isolated from diarrheic calves. *Toxins* 12(6): 383 - 398.

Aslam, B., Khurshid, M., Arshad, M. I., Muzammil, S., Rasool, M., Yasmeen, N. and Shahid, A. (2021). Antibiotic Resistance: One Health One World Outlook. *Frontiers in Cellular and Infection Microbiology.* pp.1153.

Aworh, M. K., Kwaga, J. K., Hendriksen, R. S., Okolocha, E. C. and Thakur, S. (2021). Genetic relatedness of multidrug resistant *Escherichia coli* isolated from humans, chickens and poultry environments. *Antimicrobial Resistance and Infection Control* 10(1): 1 - 13.

Azabo, R., Mshana, S., Matee, M. and Kimera, S. I. (2022). Antimicrobial usage in cattle and poultry production in Dar es Salaam, Tanzania: pattern and quantity. *BMC veterinary research* 18(1): 1 - 12.

Baniga, Z., Hounmanou, Y. M. G., Kudirkiene, E., Kusiluka, L. J., Mdegela, R. H. and Dalsgaard, A. (2020). Genome-based analysis of extended-spectrum β-lactamase-producing *Escherichia coli* in the aquatic environment and Nile perch (*Lates niloticus*) of Lake Victoria, Tanzania. *Frontiers in Microbiology* 11: 108 - 117.

Benavides, J. A., Salgado-Caxito, M., Opazo-Capurro, A., González Muñoz, P., Piñeiro, A., Otto Medina, M. and Millán, J. (2021). ESBL-producing *Escherichia coli* carrying CTX-M genes circulating among livestock, dogs and wild mammals in small-scale farms of Central Chile. *Antibiotics* 10(5): 510 - 535.

Bessaiah, H., Anamalé, C., Sung, J. and Dozois, C. M. (2022). What Flips the Switch? Signals and Stress Regulating Extraintestinal Pathogenic *Escherichia coli* Type 1 Fimbriae (Pompilio *et al.*). *Microorganisms* 10(1): 15 - 29.

Bhardwaj, D. K., Taneja, N. K., Shivaprasad, D., Chakotiya, A., Patel, P., Taneja, P. and Sanal, M. G. (2021). Phenotypic and genotypic characterization of biofilm forming, antimicrobial resistant, pathogenic *Escherichia coli* isolated from Indian dairy and meat products. *International Journal of Food Microbiology* 336: 108 - 899.

Bilashoboka, E., Mudenda, B., Munyinda, N., Moshi, F. V. and Kambarage, D. M. (2019). Determination of oxytetracycline residue levels in edible tissues of slaughtered cattle. *Food and Environment Safety Journal* 17(4): 67 – 98.

Boerlin, P., Travis, R., Gyles, C. L., Reid-Smith, R., Heather Lim, N. J., Nicholson, V. and Archambault, M. (2005). Antimicrobial resistance and virulence genes of *Escherichia coli* isolates from swine in Ontario. *Applied and environmental microbiology* 71(11): 753 - 6761.

Caudell, M. A., Quinlan, M. B., Subbiah, M., Call, D. R., Roulette, C. J., Roulette, J. W. and Quinlan, R. J. (2017). Antimicrobial use and veterinary care among agro-pastoralists in Northern Tanzania. *PLoS One 12*(1): e0170328.

Chantziaras, I. (2017). Epidemiology of Antimicrobial Resistance in Commensal *E. coli*: Focus on Selection and Spread of Fluoroquinolone Resistance in Broilers. PhD. Thesis, Ghent University, Ghent, Belgium. 167pp.

Diarrassouba, F., Diarra, M. S., Bach, S., Delaquis, P., Pritchard, J., Topp, E. and Skura, B.J. (2007). Antibiotic resistance and virulence genes in commensal *Escherichia coli* and Salmonella isolates from commercial broiler chicken farms. *Journal of Food Protection* 70(6): 1316 - 1327.

Evans, B. A. and Amyes, S. G. (2014). OXA β-lactamases. *Clinical microbiology reviews* 27(2): 241-263.

FAO (2019). Food and Agriculture Organization of the United Nations. Situation Analysis of Antimicrobial Use and Resistance in the Food and Agriculture Sectors in Tanzania; Internal report of the FAO Representation: Dar es Salaam, Tanzania. 33pp.

Firozeh, F., Saffari, M., Neamati, F. and Zibaei, M. (2014). Detection of virulence genes in *Escherichia coli* isolated from patients with cystitis and pyelonephritis. *International Journal of Infectious Diseases* 29: 219 - 222.

Gakuya, F., Kyule, M., Gathura, P. and Kariuki, S. (2001). Antimicrobial susceptibility and plasmids from *Escherichia coli* isolated from rats. *East African Medical Journal* 78(10): 518 - 522.

Geofrey, A., Abade, A. and Aboud, S. (2015). Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization among Intensive Care Unit (ICU) patients and health care workers at Muhimbili national hospital, Dar Es Salaam, Tanzania, 2012. *The Pan African Medical Journal* 21: 36 - 48.

Ghasemian, A., Peerayeh, S. N., Bakhshi, B. and Mirzaee, M. (2015). The microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) genes among clinical isolates of *Staphylococcus aureus* from hospitalized children. *Iranian journal of pathology* 10(4): 258 - 273.

Gijón, M., Bellusci, M., Petraitiene, B., Noguera-Julian, A., Zilinskaite, V., Moreno, P. S. and Kaiser-Labusch, P. (2016). Factors associated with severity in invasive community-acquired *Staphylococcus aureus* infections in children: A prospective European multicentre study. *Clinical microbiology and infection* 22(7): 643. e641 - 643. e646.

GOT (2017). Government of Tanzania. Tanzania Livestock Master Plan (2017/2018–2021/2022).

Government of Tanzania (Zurfluh). Tanzania Livestock Master Plan (2017/2018–2021/2022). 2017 [https://www.mifugouvuvi.go.tz/uploads/projects /155360 1793- tanzania%20 livestock%20master%20plan.pdf]. Site visited on 6/8/ 2019.

Guenther, S., Bethe, A., Fruth, A., Semmler, T., Ulrich, R. G., Wieler, L. H. and Ewers, C. (2012). Frequent combination of antimicrobial multiresistance and extraintestinal pathogenicity in *Escherichia coli* isolates from urban rats (Rattus norvegicus) in Berlin, Germany. *PLoS One* 7(11): e50331.

Gwenzi, W., Chaukura, N., Muisa-Zikali, N., Teta, C., Musvuugwa, T., Rzymski, P. and Abia, A. L. K. (2021). Insects, rodents and pets as reservoirs, vectors, and sentinels of antimicrobial resistance. *Antibiotics* 10(1): 68 - 98.

Himsworth, C. G., Zabek, E., Desruisseau, A., Parmley, E. J., Reid-Smith, R., Jardine, C. M. and Patrick, D. M. (2015). Prevalence and characteristics of *Escherichia coli* and Salmonella spp. in the feces of wild urban Norway and black rats (Rattus norvegicus and Rattus rattus) from an inner-city neighborhood of Vancouver, Canada. *Journal of Wildlife Diseases* 51(3): 589 - 600.

Jahantigh, M., Samadi, K., Dizaji, R. E. and Salari, S. (2020). Antimicrobial resistance and prevalence of tetracycline resistance genes in *Escherichia coli* isolated from lesions of colibacillosis in broiler chickens in Sistan, Iran. *BMC Veterinary Research* 16(1): 1- 6.

Kajeguka, D. C., Nambunga, P. P., Kabissi, F., Kamugisha, B., Kassam, N., Nyombi, B. and Chilongola, J. O. (2015). Antimicrobial resistance patterns of phenotype Extended. 223pp.

Kalee, N. E., Gahamanyi, N. and Hoza, A. S. (2021). Prevalence and antimicrobial susceptibility profiles of *Staphylococcus aureus* from raw bovine milk in dairy and pastoral farms in Morogoro region, Tanzania. 54pp.

Katakweba, A. S., Muhairwa, A. P., Espinosa-Gongora, C., Guardabassi, L., Mtambo, M. M. and Olsen, J. E. (2016). spa typing and antimicrobial resistance of *Staphylococcus aureus* from healthy humans, pigs and dogs in Tanzania. *The Journal of Infection in Developing Countries* 10(02): 143 - 148.

Katakweba, A.A.S., Møller, K.S., Muumba, J., Muhairwa, A. P., Damborg, P., Rosenkrantz, J. T., Minga, U. M., Mtambo, M. M.A. and Olsen, J. E. (2014). Antimicrobial resistance in fecal samples from buffalo, wildebeest and zebra grazing together with and without cattle in Tanzania. *Journal of Applied Microbiology* 118: 966 – 975.

Kiiti, R. W., Komba, E. V., Msoffe, P. L., Mshana, S. E., Rweyemamu, M. and Matee, M. I. N. (2021). Antimicrobial Resistance Profiles of Escherichia coli Isolated from Broiler and Layer Chickens in Arusha and Mwanza, Tanzania. *International journal of microbiology* 6(7): 59 - 66.

Kilonzo, B. and Mtoi, R. (1983). Entomological, bacteriological and serological observations after the 1977 plague outbreak in Mbulu District, Tanzania. *East African Medical Journal* 60(2): 91 - 97.

Kilonzo, B., Mbise, T., Mwalimu, D. and Kindamba, L. (2006). Observations on the endemicity of plague in Karatu and Ngorongoro, northern Tanzania. *Tanzania Journal of Health Research* 8(1): 1 - 6.

Kimera, Z.I., Frumence, G., Mboera, L.E.G., Rweyemamu, M., Mshana, S.E. and Matee, M.I.N. (2020). Assessment of Drivers of Antimicrobial Use and Resistance in Poultry and Domestic Pig Farming in the Msimbazi River Basin in Tanzania. *Antibiotics* 9: 838 - 984.

Kimera, Z. I., Mgaya, F. X., Misinzo, G., Mshana, S. E., Moremi, N. and Matee, M. I. (2021). Multidrug-resistant, including extended-spectrum beta lactamase-producing and quinolone-resistant, *Escherichia coli* isolated from poultry and domestic pigs in dar es salaam, Tanzania. *Antibiotics* 10(4): 406.

Kivaria, F., Noordhuizen, J. and Kapaga, A. (2006). Evaluation of the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar es Salaam region, Tanzania. *Tropical Animal Health and Production* 38(3): 185 - 194.

Kurwijila, L. R., Omore, A., Staal, S. and Mdoe, N. (2006). Investigation of the risk of exposure to antimicrobial residues present in marketed milk in Tanzania. *Journal of food protection* 69(10): 2487 - 2492.

Le Huy, H., Koizumi, N., Ung, T. T. H., Le, T. T., Nguyen, H. L. K., Hoang, P. V. M. and Haga, T. (2020). Antibiotic-resistant *Escherichia coli* isolated from urban rodents in Hanoi, Vietnam. *Journal of Veterinary Medical Science* 82(5): 653 - 660.

Levin, T. P., Suh, B., Axelrod, P., Truant, A. L. and Fekete, T. (2005). Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant

Staphylococcus aureus: report of a clinical failure. *Antimicrobial Agents and Chemotherapy* 49(3): 1222 - 1224.

Maganga, R. (2019). Antimicrobial resistance in commensal *Escherichia coli* isolated from poultry along a gradient of intensification of poultry production in the Northern Zone of Tanzania. University of Glasgow. 78 pp.

Makundi, R. H., Massawe, A. W., Mulungu, L. S., Katakweba, A., Mbise, T. J. and Mgode, G. (2008). Potential mammalian reservoirs in a bubonic plague outbreak focus in Mbulu District, Northern Tanzania 2007. 90pp.

Makundi, R. H., Massawe, A. W., Borremans, B., Laudisoit, A. and Katakweba, A. (2015). We are connected: flea–host association networks in the plague outbreak focus in the Rift Valley, northern Tanzania. *Wildlife Research* 42(2): 196 - 206.

Manyi-Loh, C., Mamphweli, S., Meyer, E. and Okoh, A. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules* 23(4): 795.

Masoud, S. M., El-Baky, A., Mahmoud, R., Aly, S. A. and Ibrahem, R. A. (2021). Co-existence of certain ESBLs, MBLs and plasmid mediated quinolone resistance genes among MDR E. coli isolated from different clinical specimens in Egypt. *Antibiotics* 10(7): 835 - 897.

Masoud, S.M., Abd El-Baky, R.M., Aly, S.A., Ibrahem, R.A. (2021). Co-Existence of Certain ESBLs, MBLs and Plasmid Mediated Quinolone Resistance Genes

among MDR *E. coli* Isolated from Different Clinical Specimens in Egypt. *Antibiotics* 10: 835.

Massawe, H., Mdegela, R. and Kurwijila, L. (2019). Antibiotic resistance of *Staphylococcus aureus* isolates from milk produced by smallholder dairy farmers in Mbeya Region, Tanzania. *International Journal of One Health* 5: 31 - 37.

Mboera, L. E., Rumisha, S. F., Lyimo, E. P., Chiduo, M. G., Mangu, C. D., Mremi, I. R. and Massawe, I. S. (2018). Cause-specific mortality patterns among hospital deaths in Tanzania, 2006-2015. *PLoS One* 13(10): e0205833.

Mdegela, R., Kusiluka, L., Kapaga, A., Karimuribo, E., Turuka, F., Bundala, A. and Loken, T. (2004). Prevalence and determinants of mastitis and milk-borne zoonoses in smallholder dairy farming sector in Kibaha and Morogoro districts in Eastern Tanzania. *Journal of Veterinary Medicine, Series B* 51(3): 123 -128.

Mdegela, R., Ryoba, R., Karimuribo, E., Phiri, E., Løken, T., Reksen, O. and Urio, N. (2009). Prevalence of clinical and subclinical mastitis and quality of milk on smallholder dairy farms in Tanzania. *Journal of the South African Veterinary Association* 80(3): 163 - 168.

Mdegela, R. H., Mwakapeje, E. R., Rubegwa, B., Gebeyehu, D. T., Niyigena, S., Msambichaka, V. and Fasina, F. O. (2021). Antimicrobial Use, Residues, Resistance and Governance in the food and agriculture sectors, Tanzania. *Antibiotics* 10(4): 454- 497.

Mdegela, R.H., Kusiluka, L.J.M., Kapaga, A.M., Karimuribo, E., Turuka, F.M., Bundala, A., Kivaria, F., Kabula, B., Manjurano, A., Loken, T. (2004). Prevalence and determinants of mastitis and milk borne zoonoses in smallholder dairy farming sector in Kibaha and Morogoro districts, Eastern Tanzania. *Journal of Veterinary Medicine* 51: 123 –128.

Mekonnen, S., Lam, T., Hoekstra, J., Rutten, V. P., Tessema, T., Broens, E. and Koop, G. (2018). Characterization of *Staphylococcus aureus* isolated from milk samples of dairy cows in small holder farms of North-Western Ethiopia. *BMC Veterinary Research* 14(1): 1 - 8.

Mgaya, F. X., Matee, M. I., Muhairwa, A. P. and Hoza, A. S. (2021). Occurrence of multidrug resistant *Escherichia coli* in raw meat and cloaca swabs in poultry processed in slaughter slabs in Dar es Salaam, Tanzania. *Antibiotics* 10(4): 343.

Mgonja, F., Mosha, R., Mabiki, F. and Choongo, K. (2016). A simple and sensitive method for the detection of “Oxytetracycine”xlevels in ready-to-eat beef by liquid chromatography-mass spectrometry. *African Journal of Pharmacology* 10: 571–578.

Mgonja, F., Mosha, R., Mabiki, F. and Choongo, K. (2017). Oxytetracycline residue levels in beef in Dodoma region, Tanzania. *African Journal and Food Sciences* 11: 40 – 43.

Michael, S., Mbwambo, N., Mruttu, H., Dotto, M., Ndomba, C., da Silva, M. and Shapiro, I. (2018). Tanzania Livestock Master Plan (2017/2018-2021/2022). *United Republic of Tanzania, Ministry of Livestock and Fisheries*. 24pp.

Moffo, F., Mouiche, M. M. M., Djomgang, H. K., Tombe, P., Wade, A., Kochivi, F. L. and Ngogang, M. P. (2021). Poultry litter contamination by *Escherichia coli* resistant to critically important antimicrobials for human and animal use and risk for public health in cameroon. *Antibiotics* 10(4): 402 - 564.

Monecke, S., Gavier-Widén, D., Hotzel, H., Peters, M., Guenther, S., Lazaris, A. and Ruppelt-Lorz, A. (2016). Diversity of *Staphylococcus aureus* isolates in European wildlife. *PLoS One* 11(12): e0168433.

Mshana, S. E., Matee, M. and Rweyemamu, M. (2013). Antimicrobial resistance in human and animal pathogens in Zambia, Democratic Republic of Congo, Mozambique and Tanzania: an urgent need of a sustainable surveillance system. *Annual Clinical Microbiology and Ant microbiology* 12(1): 1 - 10.

Mukherjee, S. K. and Mukherjee, M. (2019). Characterization and bio-typing of multidrug resistance plasmids from Uropathogenic *Escherichia coli* isolated from clinical setting. *Frontiers in Microbiology* 10: 2913.

Muriuki, C. W., Ogonda, L. A., Kyanya, C., Matano, D., Masakhwe, C., Odoyo, E. and Musila, L. (2022). Phenotypic and genotypic characteristics of uropathogenic *Escherichia coli* isolates from Kenya. *Microbial Drug Resistance* 28(1): 31- 38.

Nonga, H. E., Mariki, M., Karimuribo, E. and Mdegela, R. (2009). Assessment of antimicrobial usage and antimicrobial residues in broiler chickens in Morogoro Municipality, Tanzania. 96pp.

Nonga, H. E., Mariki, M., Karimuribo, E. D. and Mdegela, R. H. (2009). Assessment of Antimicrobial Usage and Antimicrobial Residues in Broiler Chickens in Morogoro Municipality, Tanzania. *Pak Journal of Nutrition* 8: 203–207.

Nonga, H.E., Simon, C., Karimuribo, E.D. and Mdegela, R.H. (2010). Assessment of Antimicrobial Usage and Residues in Commercial Chicken Eggs from Smallholder Poultry Keepers in Morogoro Municipality, Tanzania. *Zoonoses Public Health* 57: 339–344.

Ogundipe, F. O., Ojo, O. E., Feßler, A. T., Hanke, D., Awoyomi, O. J., Ojo, D. A. and Maurischat, S. (2020). Antimicrobial resistance and virulence of methicillin-resistant *Staphylococcus aureus* from human, chicken and environmental samples within live bird markets in three Nigerian cities. *Antibiotics* 9(9): 588.

Okorie-Kanu, O. J., Anyanwu, M. U., Ezenduka, E. V., Mgbeahuruike, A. C., Thapaliya, D., Gerbig, G. and Olorunleke, S. (2020). Molecular epidemiology, genetic diversity and antimicrobial resistance of *Staphylococcus aureus* isolated from chicken and pig carcasses and carcass handlers. *PLoS One* 15(5): e0232913.

Pan, Z., Chen, Y., McAllister, T. A., Gänzle, M., Plastow, G. and Guan, L. L. (2021). Abundance and expression of Shiga toxin genes in *Escherichia coli* at the recto-anal junction relates to host immune genes. *Frontiers in Cellular and Infection Microbiology* 11: 148.

Pompilio, A., Crocetta, V., Savini, V., Petrelli, D., Di Nicola, M., Bucco, S. and Di Bonaventura, G. (2018). Phylogenetic relationships, biofilm formation, motility,

antibiotic resistance and extended virulence genotypes among *Escherichia coli* strains from women with community-onset primitive acute pyelonephritis. *PLoS One* 13(5): e0196260.

Pozzi, C., Bagnoli, F. and Rappuoli, R. (2016). *Staphylococcus aureus* coagulase R domain, a new evasion mechanism and vaccine target. *The Journal of Experimental Medicine* 213(3): 292 - 308.

Preda, M., Mihai, M. M., Popa, L. I., Dițu, L.-M., Holban, A. M., Manolescu, L. S. C. and Chifiriuc, C. M. (2021). Phenotypic and genotypic virulence features of staphylococcal strains isolated from difficult-to-treat skin and soft tissue infections. *PLoS One* 16(2): e0246478.

Rojas-Jiménez, J., Jiménez-Pearson, M. A., Duarte-Martínez, F., Brenes-Mora, E., Arguedas, R. and Barquero-Calvo, E. (2022). First Report of a Multidrug-Resistant ST58 *Escherichia coli* Harboring Extended-Spectrum Beta-Lactamase of the CTX-M-1 Class in a Fecal Sample of a Captive Baird's Tapir (Tapirus bairdii) in Costa Rica, Central America. *Microbial Drug Resistance* 28(1): 143 - 148.

Rugumisa, B. T., Call, D. R., Mwanyika, G. O., Mrutu, R. I., Luanda, C. M., Lyimo, B. M. and Buza, J. J. (2016). Prevalence of antibiotic-resistant fecal *Escherichia coli* isolates from penned broiler and scavenging local chickens in arusha, Tanzania. *Journal of Food Protection* 79(8): 1424 - 1429.

Sadat, A., Shata, R. R., Farag, A. M., Ramadan, H., Alkhedaide, A., Soliman, M. M. and Awad, A. (2022). Prevalence and Characterization of PVL - Positive

Staphylococcus aureus Isolated from Raw Cow's Milk. *Toxins* 14(2): 97 - 102.

Saderi, H., OULIA, P. and Eslami, M. (2009). Prevalence of Macrolide-Lincosamide-Streptogramin B (MLSB) resistance in *S. aureus* isolated from patients in Tehran, Iran. 117pp.

Said, L. B., Jouini, A., Klibi, N., Dziri, R., Alonso, C. A., Boudabous, A. and Torres, C. (2015). Detection of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in vegetables, soil and water of the farm environment in Tunisia. *International Journal of Food Microbiology* 203: 86 - 92.

Salah, F. D., Soubeiga, S. T., Ouattara, A. K., Sadji, A. Y., Metuor-Dabire, A., Obiri-Yeboah, D. and Simpore, J. (2019). Distribution of quinolone resistance gene (qnr) in ESBL-producing *Escherichia coli* and Klebsiella spp. in Lomé, Togo. *Antimicrobial Resistance and Infection Control* 8(1): 1 - 8.

Salamandane, A., Alves, S., Chambel, L., Malfeito-Ferreira, M. and Brito, L. (2022). Characterization of *Escherichia coli* from Water and Food Sold on the Streets of Maputo: Molecular Typing, Virulence Genes, and Antibiotic Resistance. *Applied Microbiology* 2(1): 133 - 147.

Sassmannshausen, R., Deurenberg, R. H., Köck, R., Hendrix, R., Jurke, A., Rossen, J. W. and Friedrich, A. W. (2016). MRSA prevalence and associated risk factors among health-care workers in non-outbreak situations in the Dutch-German EUREGIO. *Frontiers in Microbiology* 7: 12 - 73.

Serray, B., Oufrid, S., Hannaoui, I., Bourjilate, F., Soraa, N., Mliji, M. and El Azhari, M. (2016). Genes encoding adhesion factors and biofilm formation in methicillin-resistant *Staphylococcus aureus* in Morocco. *The Journal of Infection in Developing Countries* 10(08): 863 - 869.

Sidhu, M. S., Heir, E., Leegaard, T., Wiger, K. and Holck, A. (2002). Frequency of disinfectant resistance genes and genetic linkage with β-lactamase transposon Tn 552 among clinical staphylococci. *Antimicrobial Agents and Chemotherapy* 46(9): 2797 - 2803.

Silva, V., Gabriel, S. I., Borrego, S. B., Tejedor-Junco, M. T., Manageiro, V., Ferreira, E. and Igrejas, G. (2021). Antimicrobial resistance and genetic lineages of *Staphylococcus aureus* from Wild rodents: First report of mecC-positive methicillin-resistant *S. aureus* (MRSA) in Portugal. *Animals* 11(6): 15 - 37.

Sineke, N., Asante, J., Amoako, D. G., Abia, A. L. K., Perrett, K., Bester, L. A. and Essack, S. Y. (2021). *Staphylococcus aureus* in intensive pig production in South Africa: Antibiotic resistance, virulence determinants and clonality. *Pathogens* 10(3): 317 - 346.

Skočková, A., Cupáková, Š., Karpíšková, R. and Janštová, B. (2021). Detection of tetracycline resistance genes in *Escherichia coli* from raw cow's milk. *Journal of Microbiology, Biotechnology and Food Sciences* 2021: 777 - 784.

Sonola, V. S., Katakweba, A. S., Misinzo, G. and Matee, M. I. (2021). Occurrence of Multi-Drug-Resistant *Escherichia coli* in Chickens, Humans, Rodents and Household Soil in Karatu, Northern Tanzania. *Antibiotics* 10(9): 1137.

Talavera-González, J. M., Acosta-Dibarrat, J., Reyes-Rodríguez, N. E., Salgado-Miranda, C. and Talavera-Rojas, M. (2021). Prevalence of the qnrB, qnrA and bla TEM genes in temperate bacteriophages of *Escherichia coli* isolated from wastewater and sewer water from slaughterhouses in the State of Mexico. *Revista Mexicana De ciencias Pecuarias* 12(1): 298 - 305.

Tsakris, A., Poulou, A., Pournaras, S., Voulgari, E., Vrioni, G., Themeli-Digalaki, K. and Sofianou, D. (2010). A simple phenotypic method for the differentiation of metallo-β-lactamases and class A KPC carbapenemases in Enterobacteriaceae clinical isolates. *Journal of Antimicrobial Chemotherapy* 65(8): 1664 - 1671.

Víquez-Molina, G., Aragón-Sánchez, J., Perez-Corrales, C., Murillo-Vargas, C., López-Valverde, M. E. and Lipsky, B. A. (2018). Virulence factor genes in *Staphylococcus aureus* isolated from diabetic foot soft tissue and bone infections. *The International Journal of Lower Extremity Wounds* 17(1): 36 - 41.

Yao, M., Zhang, X., Gao, Y., Song, S., Xu, D. and Yan, L. (2019). Development and application of multiplex PCR method for simultaneous detection of seven viruses in ducks. *BMC Veterinary Research* 15(1): 1 - 10.

Zamani, S., Mohammadi, A., Hajikhani, B., Abiri, P., Fazeli, M., Nasiri, M. J. and Haghighi, M. (2022). Mupirocin-Resistant *Staphylococcus aureus* in Iran: A Biofilm Production and Genetic Characteristics. *BioMedical Research International Journal* 2022.

Zhou, Y., Li, X. and Yan, H. (2020). Genotypic Characteristics and Correlation of Epidemiology of *Staphylococcus aureus* in Healthy Pigs, Diseased Pigs and Environment. *Antibiotics* 9(12): 839 - 856.

Ziwa, M. H., Matee, M. I., Kilonzo, B. S. and Hang'ombe, B. M. (2013). Evidence of Yersinia pestis DNA in rodents in plague outbreak foci in Mbulu and Karatu Districts, Northern Tanzania. *Tanzania Journal of Health Research* 15(3): 35 - 47.

Zurfluh, K., Abgottspon, H., Hächler, H., Nüesch-Inderbinen, M. and Stephan, R. (2014). Quinolone resistance mechanisms among extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* isolated from rivers and lakes in Switzerland. *PLoS One* 9(4): e95864.

CHAPTER SEVEN

7.0 CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

The occurrence of MDR *S. aureus* isolates in all types of sample sources suggests the possibility of cross-transmission of infections among different hosts in household environment through their interactions, leading to widespread of antimicrobial resistance threat. Due to their multiple drug resistance property, these microbes can cause infections that are difficult to treat following a few types of antibiotics to which the organisms are susceptible in the studied area.

The level of antimicrobial resistance, including MDR *E. coli* in isolates from humans, chicken, rodents and soil indicates the possibility of wide spread transmission of resistant bacteria and their genes, with the possibility of causing infections that are difficult to treat. The antibiotics used in this study have limited success in treating for treating both human and animal infections, implying that they are no longer effective in their intended use.

The MDR *E. coli* isolates from humans, chicken, rodents and household soils harbour different antibiotic resistance (*blaTEM, blaCTX-M, blaSHV, tetA, tetB, qnrA* and *qnrB*) and virulence (*bfp, east, traT, ompA* and *stx-1*) genes. The PCA results show that, *traT, stx-1, bfp, ompA, east* and *eae* genes had higher influence on virulence of MDR *E. coli* isolates. Resistance (*blaTEM, blaCTX-M, blaSHV, tetA, tetB, qnrA*) and virulence (*traT*) genes were detected in isolates from all sample sources, while *stx-1* and *eae* genes were specific to chicken and rodent isolates only, respectively. Interestingly, rodents had the highest percentage of both resistance and virulence genes, indicating their potential in carriage and transmission of infections to other hosts in the environment. This situation

urgently calls for One Health based interventions including improving hygiene and control of rodents in households.

From this study, *S. aureus* isolates isolated from humans, poultry, rodents and household soil contain various resistance genes, mainly *tetK*, *tetL* and *mecA* and virulence genes mostly for; clumping factor B (*clfB*), coagulase protein (*coa*), leukocydal toxins (*LUK-PV*) and clumping factor A protein (*clfA*). The PCA results revealed that *clfB, coa, hlg* and *clfA* genes had higher influence to virulence of MDR *S. aureus* isolates. Resistance genes (*tetK* and *mecA)* and virulence determinants (*clfB, coa* and *luk-PV*) were common in all sample sources, while *tst*, *hlg and fnbB* were only specific to human, chicken and rodent isolates, respectively. AMR and VR genes were found in rodents and soil environment implying that both are potential reservoirs. The large battery of AMR and VR genes among MDR *S. aureus* strains circulating in the area, indicate their ability to cause infections that are difficult to treat endangering public and animal health.

7.2 Recommendations

From the study findings, it is evident that comprehensive One Health interventions are needed to minimize the risks of spreading resistant bacterial infections among humans, chicken and soil environment in households. This will require improving our understanding of the human-livestock-environment with well-designed genomic studies involving WGS and metagenomics, to provide a comprehensive picture on the pattern and magnitude of AMR and virulence genes spread. This will ensure that the key drivers of resistance and virulence transmission between human-livestock-environment are accurately identified and the most appropriate interventions can be adopted. It is important to understand the importance of each component of the human-livestock-environment. A OH approach should be deployed to ensure involvement of relevant multisectoral and

multidisciplinary to attain an optimal public and veterinary health and ensure safe environment.

In the meantime, the following can be done:

i. Strengthen infection control and prevention as well as biosecurity measures to reduce incidence of human and animal infections, and hence reducing need of antibiotics, through public awareness campaings using OH approach. This should involve CSOs, NGOs, community leaders and sector ministries.

ii. Perform futher studies to determine behavioural and psychosocial determinants of AMR spread in the interface of humans, livestock and environment.

iii. Mapping of AMR sources and transmission pathways in food animal production.

iv. Enforcing rodent control measures including, biological (use of cats or cat urine to scare rodents), physical by traping them and chemically by using toxins or a combination of them to ensure that rodents have limited contact with humans.

v. Increase access to medical and veterinary services to ensure judicious use of appropriate antimicrobials.

vi. Establish environmental AMR surveillance using simple technologies that can be employed in the field.

vii. Review of the existing laws, policies, guidelines and regulations governing AMU, AMR and AR such as The Veterinary Act, Tanzania Food, Drugs and Cosmetics Act and the Tanzania Medicines and Medical Devices Act and ensure compliance.

APPENDICES

Appendix 1: Informed Consent Statement (English Version)

SOKOINE UNIVERSITY OF AGRICULTURE (SUA)
College of Forestry, Wildlife and Tourism
P.O Box 3073, Chuo Kikuu, Morogoro, Tanzania
Tel: +255 23 2601376
url: www.sua.net

Occurrence of multidrug-resistant *Escherichia coli* and *Staphylococcus aureus* among humans, rodents, chickens and household soils in Karatu, Northern Tanzania

The following statements will be read to head of household to be visited.

My name is ……, from the Sokoine University of Agriculture. We are carrying out a study to determine the antimicrobial resistance profiles of *Escherichia coli* and *Staphylococcus aureus* isolated from rodents, chickens and humans sharing the same environment in Karatu District of northern Tanzania.

Your house is one of those selected for this survey. If you accept, information and samples collected from your house and several others will be analysed to get the general picture of this problem. Our survey involves collecting human nasal swabs, stool samples, chicken faecal materials and capturing rodents inside and outside your house and sending them to laboratory for further analysis. Sampling of humans (stool and nasal secretions), chickens (cloacal swabs), rodents (intestines and pharyngeal swabs) and soils will be done in households.

Information that will be collected and their findings will be treated with confidentiality. Names of the participants will not be mentioned anywhere. Results of this study will provide key information necessary to address and control the problem of antibiotic resistance which is a great public health challenge in our country.

Do you accept our request?

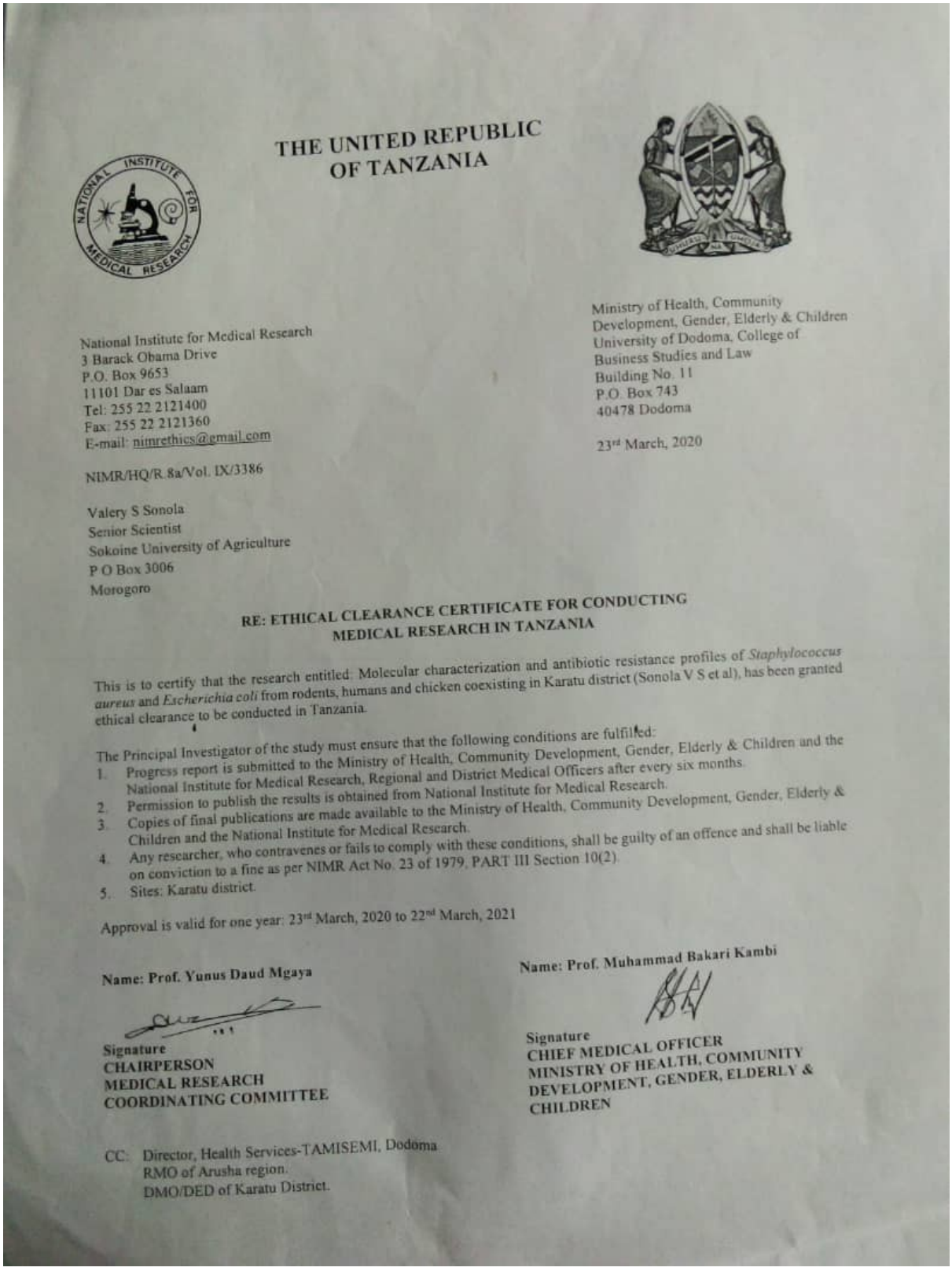
YES ☐

NO ☐

Participant Signature _____ Participant thumbprint ☐

NB: *If you have any questions regarding this research, you may ask the research staff or contact Mr. Valery Sonola, Sokoine University of Agriculture, P.O. Box 3073, Chuo Kikuu, Morogoro, Tanzania; Telephone: +255 763 665 322; E-mail: vssonola@gmail.com*

Appendix 2: Ethical clearance certificate for conducting medical research in Tanzania



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NIMR/HQ/R.8a/Vol. IX/3386

23rd March, 2020

Valery S Sonola
Senior Scientist
Sokoine University of Agriculture
P O Box 3006
Morogoro

RE: ETHICAL CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Molecular characterization and antibiotic resistance profiles of *Staphylococcus aureus* and *Escherichia coli* from rodents, humans and chicken coexisting in Karatu district (Sonola V S et al), has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Sites: Karatu district.

Approval is valid for one year: 23rd March, 2020 to 22nd March, 2021

Name: Prof. Yunus Daud Mgaya

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY & CHILDREN

CC: Director, Health Services-TAMISEMI, Dodoma
RMO of Arusha region.
DMO/DED of Karatu District.

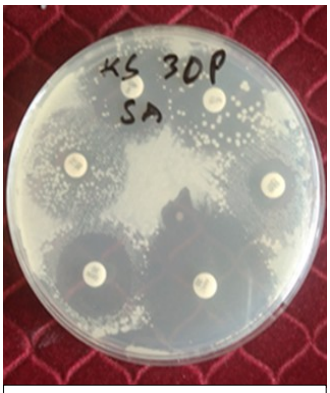
Appendix 3: Some photographs for sample collection in Karatu and laboratory works in Arusha

1. Chicken cloaca swab

2. Rodent samples

3. S. aureus colonies (Mannitol salt agar plate)

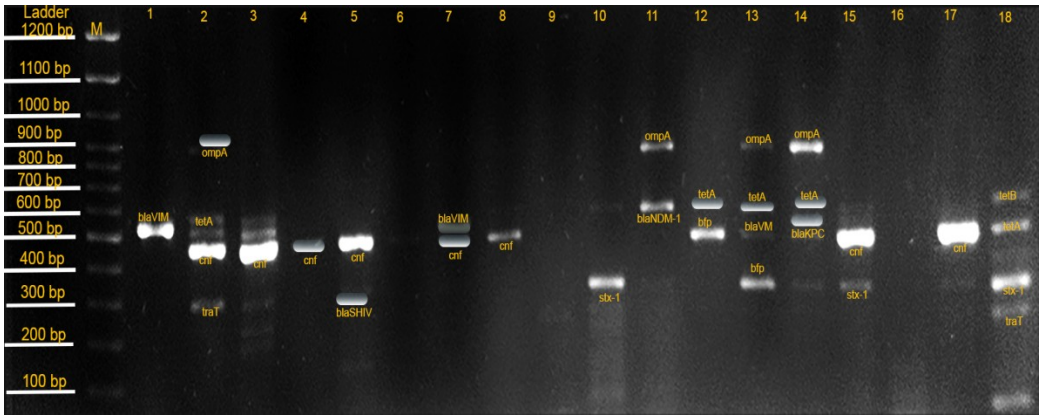
4. *E. coli* colonies (MacConkey agar plate)



5. AST results for S. aureus

6. AST results for E. coli

Appendix 4: Gel electrophoresis of the representative amplification products of MDR *E. coli* isolates



Appendix 5: Map showing the study area (Karatu district) in Arusha and the wards where samples were collected