OCCURRENCE OF SELECTED VIRULENCE GENES IN MULTIDRUG-RESISTANT *ESCHERICHIA COLI* ISOLATES FROM HUMANS, ANIMALS AND ENVIRONMENT IN DAR ES SALAAM, TANZANIA

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By

Edwin M. James

A Dissertation Submitted in (Partial) Fulfilment of the Requirements for the Degree of Master of Science (Biochemistry) of

Muhimbili University of Health and Allied Sciences
October, 2024

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences of the dissertation entitled; **''occurrence of selected virulence genes in multidrug-resistant *Escherichia coli* isolates from humans, animals and environment in Dar es Salaam Tanzania'',** in (partial) fulfilment of the requirements for the degree of Master of Sciences (Biochemistry) of Muhimbili University of Health and Allied Sciences

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DECLARATION AND COPYRIGHT

I, **Edwin M. James,** declare that this **dissertation** is my original work and that it has not been presented and will not be presented at any other university for a similar or any other degree award.

Signature …… **Date**……..

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DEDICATION

This research work is dedicated to my beloved mother, Sarah L. Nyato, whose unwavering support and encouragement have been a constant source of strength throughout my academic journey.

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ABSTRACT

Background: *Escherichia coli* is listed by the World Health Organization as one of the critical pathogen due to its vast array of virulence and antimicrobial resistance genes. The genes are widely spread between humans, animals and the environment through horizontal gene transfer. In humans, the bacterium is capable of causing a broad spectrum of intestinal and extra-intestinal diseases.

Aim of the study: To determine the occurrence of virulence genes *bfp, ompA, traT, eaeA* and *stx1* in multidrug-resistant *E. coli* isolates from humans, animals, and the environment in Dar es Salaam Tanzania.

Methodology: This cross-sectional study involved 50 MDR *E. coli* isolates; 15 from humans, 6 isolates from the environment and 29 isolates from animals, of them 16 were from domestic pigs and 13 from poultry. These isolates were cultured on MacConkey agar (Oxoid Ltd, Hampshire, UK) and Nutrient agar (Oxoid Ltd, Hampshire, UK) aerobically at 37°C for 24 hrs. Multiplex PCR (New England Biolabs, Ipswich, MA, USA) was used to detect virulence genes *ompA, traT, eaeA* and *stx1,* while *bfp* was detected using a uniplex PCR (New England Biolabs, Ipswich, MA, USA). T. Descriptive analysis was used to determine the prevalence of virulence gene(s) among MDR *E. Coli*. Differences in the occurrence of the five genes between sample sources were determined using Chi-square and $p < 0.05$ was considered to be significant. Principal component analysis (PCA) was used to determine distribution and relationships of antimicrobial resistance and virulence genes of MDR *E. coli* isolates.

Results: All 50/50 (100%) MDR *E. coli* isolates were found to carry at least one virulence gene, with 19/50 (38%) carrying four genes, *bfp + traT + eaeA + ompA.* The findings showed high occurrence of virulence genes *bfp* (82%), *traT* (82%), *eaeA* (78%) and *ompA* (72%), whereas *stx1* was not detected in any of the isolates. For humans, the most detected virulence genes were *bfp* and *traT* 14/15 (93.3%), for poultry, was *eaeA* 13/14 (92.9%), for pigs were *bfp* and *traT* 13/15 (86.7%) while for river water the most detected gene was *eaeA* 6/6 (100%). PCA results show the virulence genes *ompA, traT, eaeA* and *bfp* contributed to virulence of the isolates. The

PCA ellipses show that isolates from pigs had more virulence genes compared to those isolated from poultry, river water, and humans. No association was observed between virulence genes and the antimicrobial-resistant genes.

Conclusion: The high frequency of virulence genes in MDR *E. coli* isolates from humans, animals, and the environment indicates these isolates have very high potential to cause diseases which are difficult to treat, emphasizing the need for integrated surveillance and control measures.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistance
ARG	Antimicrobial Resistant Gene
CLSI	Clinical Laboratory Standard Institute
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
EPEC	Enteropathogenic *E. Coli*
IRB	Institutional Review Board
MCA	MacConkey Agar
MDR	Multidrug Resistance
MUHAS	Muhimbili University of Health and Allied Sciences
NA	Nutrient Agar
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PMQR	Plasmid Mediated Quinolone Resistance
TAE	Tris-acetate EDTA
UV	Ultraviolet
VG	Virulence Gene
WHO	World Health Organization

DEFINITION OF KEY TERMS

Antimicrobial resistance:	A state where by microorganisms (bacteria, viruses, fungi, parasites etc.) no longer respond to antimicrobial agents which are design to kill them or inhibit their growth
Multidrug resistance:	Non-susceptibility to at least one agent in three or more different antimicrobial categories
Resistant genes:	Genetic elements that confer resistance to antimicrobial agents
Virulence:	Ability of microorganism to cause damage to host
Virulence factors:	The molecules that assist a pathogen colonize and multiply in the host
Virulence gene:	A genetic element coding for virulence
Multiplex PCR:	A molecular biology technique in which multiple primers are used allowing detection of several genes in a single assay

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

In the recently released 2024 list of World Health Organization (WHO) priority pathogens, Gram-negative bacterial pathogens, including third-generation cephalosporin-resistant *Escherichia coli (E. coli)* and carbapenem-resistant *Klebsiella pneumoniae (K. pneumoniae)*, maintain their critical status (1). Infections caused by these bacteria pose a formidable global challenge, because of their virulence, resistance, and limited treatment options, leading to severe nosocomial infections, especially among intensive care patients, and alarmingly high mortality rates (1). These pathogens pose the highest estimated burden among all multidrug resistant (MDR) Gram-negative bacteria due to their widespread prevalence and resistance(1). These groups of bacteria possess a wide array of antimicrobial resistance genes (ARGs) and virulence genes making them formidable pathogens. The co-occurrence of virulence genes and AMR genes in bacteria can lead to increased severity and extent of infections, posing a greater challenge to public health (2).

ARGs are genetic elements that confer resistance to antimicrobial agents. *E. coli* may carry a variety of ARGs including Extended Spectrum Beta-lactamase (ESBL) genes (*blaSHV, blaCTX-M* and *blaTEM*), Carbapenemase genes (*blaKPC, blaNDM, blaOXA-4*), plasmid mediated quinolone resistance (PMQR) genes (*qnrA, qnrB, qnrS, qnrC, qnrD, qepA,* and *aac(6)-Ib-cr*) and tetracycline resistance genes (*tetA, tetB*). Other genes include macrolide resistant genes (*ermB*), sulfonamide resistant genes (*sul1,sul2,sul3*), trimethroprim resistant genes (*dfrA*), aminoglycoside resistant genes (*aac(3), aph(3), aadA*) and chloramphenicol resistant genes (*catA1*) (3–5).

Mechanisms of virulence of *E. coli* includes adherence to the host cell membrane, invasion into the host cell, competing for iron, toxin production and host immune evasion, among others(3,5–7). The bundle forming pilus (*bfp*) gene and *E. coli* attaching and effacing (*eae*) gene involved in *E. coli* adherence to host cell, the gene *stx-1* encodes production of the Shiga toxin (*stx*) while

the outer membrane protein A (*ompA*) gene and the *traT* gene help *E. coli* to evade host immune system (3,5–9). These virulence genes have been found in *E. coli* isolates from humans, animals and environment with varying distribution from different regions worldwide (2,3,5,10–14).

While AMR and virulence are distinct phenomena, both are essential for pathogenic bacteria to adapt to and survive in competitive microbial environments (2,15). Studies have been conducted to assess distribution of ARGs, virulence genes or both of them together in MDR *E.coli* isolates from humans, animals and environment (4,5,10,13,14,16,17), but fewer studies have been gone further to assess the association between ARGs and virulence genes (3,10,18–23). This study aimed to use the Multiplex PCR technique to assess the presence and distribution of virulence genes amongst the stocked MDR *E. coli* isolates from previous studies (4,24–26).

Multiplex polymerase chain reaction (mPCR) is a molecular biology technique that enables the simultaneous amplification of multiple target DNA sequences in a single reaction. Multiplex PCR has been used in several procedures including pathogen identification and detection of virulence and resistance genes with great success (27). The use of multiplex PCR, as compared to conventional PCR, has been proven to be time-saving and cost-effective, in which two or more primers are mixed in the same tube with the DNA template whose multiple segments need to be amplified (28). In general, several considerations and optimization steps are involved in developing a robust and efficient multiplex PCR (29).

In Tanzania, there is extensive interaction between humans, animals, and the environment. While the presence of antimicrobial resistance *E. coli* isolates from humans, animals, and the environment is well-documented, understanding their virulence potential is equally crucial for effective disease management and treatment. A study conducted in northern Tanzania observed that 76% of MDR *E. coli* isolates, from humans, chicken, rodents, and household soil, had at least one virulence gene, with some isolates exhibiting a co-existence of up to six resistance genes and at least one virulence gene (3). Other studies reported the varying prevalence of virulence genes in *E. coli* from different sources of isolates ranging from 22.9% to 100% (30–34). The presence of MDR *E. coli* has been reported in different studies conducted in Tanzania including humans, environment, and animals (4,24–26).

1.2 Problem Statement

E. coli is a remarkably versatile microorganism and an important member of the normal intestinal microbiota of humans and animals. This harmless commensal organism can acquire a mixture of comprehensive mobile genetic elements that contain genes encoding for virulence factors, becoming an emerging human and animal pathogen capable of causing a broad spectrum intestinal and extra-intestinal diseases (6). In addition to the role of the acquisition of virulence genes and its effect on pathogenicity, the acquisition of resistance genes plays an important role in therapeutic failure and the increase of mortality rate (2). The presence of virulence genes in MDR *E. coli* can increase the severity and extent of infections.

Studies show that there is a co-occurrence of virulence and resistance genes in *E. coli* isolated from animals and humans from different parts of the world (17,22,35–37). In Tanzania, there have been many studies that focused on the antimicrobial resistance of *E. coli*, but there have been fewer studies on the epidemiology of antimicrobial resistance and virulence factors of *E. coli* (38).

The virulence genes *bfp, eaeA, traT, ompA,* and *stx1* were carefully chosen since they represent the different mechanisms of the bacterium – bundle forming pilus, aggregative adherence fimbriae, inhibitors of OmpA-specific phages, Shiga toxin production, and Outer membrane protein formation. Besides their association with ARGs in MDR *E. coli* has never been evaluated in our setting. This study was conducted to determine the distribution of virulence genes *bfp*, *eaeA, traT, ompA* and *stx1* and their association with ARGs in MDR *E. coli* isolated from humans, animals and environment in Dar es Salaam, Tanzania.

1.3 Conceptual framework

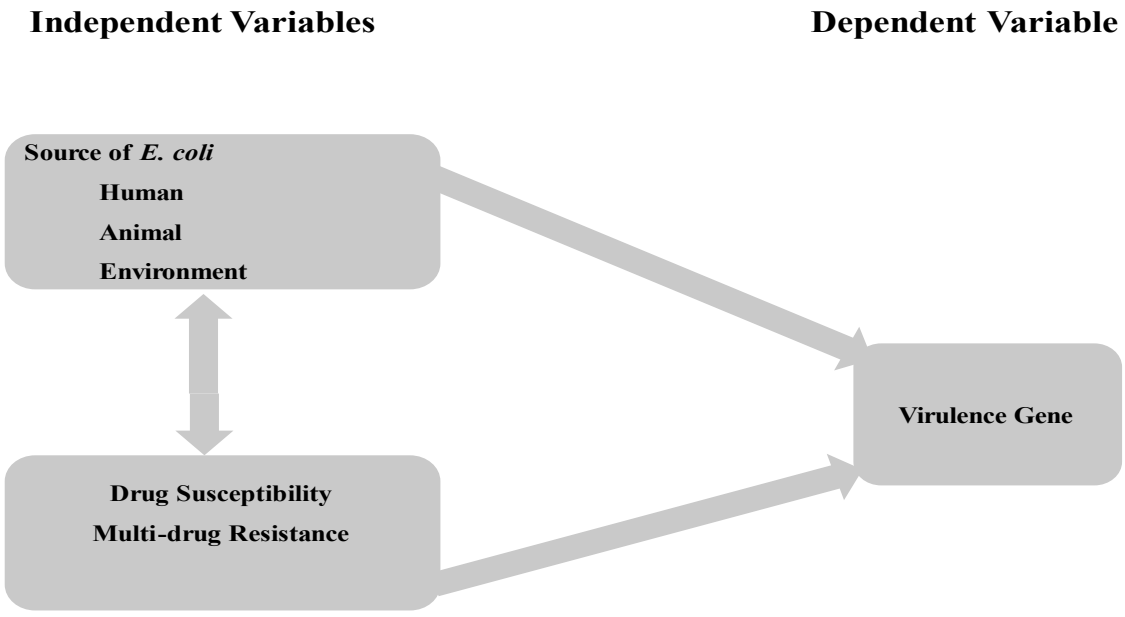


Figure 1: Conceptual Framework

The presence and diversity of virulence genes in *E. coli* are influenced by their sources such as humans, animals, and the environment. *E. coli* from human hosts may carry virulence genes linked to human infections while those from animal sources may harbor genes adapted to animal hosts (6,39,40). Similarly, drug susceptibility patterns among *E. coli* from different sources is different due to differences in drug exposure among sources (41,42).

Drug resistance and virulence help microorganisms to survive in the host against medications and host immunity. The relationship between drug susceptibility patterns and virulence gene distribution in *E. coli* is explained by the relationship in the acquisition of virulence genes and genes responsible for drug resistance (43). Different sources of *E. coli* have different patterns of exposure to antimicrobial agents but due to increased interaction among these sources, there is transmission of virulence genes and genes for drug resistance among *E. coli* from different sources with environment saving as reservoirs for the genes (44,45).

1.4 Rationale

The knowledge of virulence genes distribution in multidrug-resistant *E. coli* isolated from humans, animals and the environment will help to understand the pathogenicity and virulence of *E. coli* as well as possible means of transmission of these genes among humans, animals, and the environment. These will assist in designing proper measures for dealing with infections caused by *E. coli* in both humans and animals by taking into consideration the possible interaction between themselves and the environment.

1.5 Research Questions

1.5.1 Broad Research Question

What is the occurrence and distribution of virulence genes *bfp, traT, eaeA, ompA,* and *stx1* in MDR *E. coli* isolates from humans, animals, and the environment in Dar es Salaam Tanzania? Is the occurrence of these genes associated with the occurrence of multidrug resistance genes?

1.5.2 Specific Research Questions

i. What is the distribution of virulence genes *bfp, traT, eaeA, ompA,* and *stx1* in MDR *E. coli* isolates from humans, animals, and the environment in Dar es Salaam Tanzania?

ii. Is there an association between the occurrence of virulence genes *bfp, traT, eaeA, ompA,* and *stx1* and AMR genes in MDR *E. coli* isolates from humans, animals, and the environment in Dar es Salaam Tanzania?

1.6 Research Objectives

1.6.1 General objective

To determine the occurrence of virulence genes *bfp, traT, eaeA, ompA,* and *stx1* and their association with ARGs in MDR *E. coli* isolates from humans, animals and environment in Dar es Salaam Tanzania

1.6.2 Specific Objectives

i. To determine the distribution of virulence genes *bfp, traT, eaeA, ompA,* and *stx1* in MDR *E. coli* isolates from humans, animals, and environment in Dar es Salaam Tanzania
ii. To determine correlation between virulence genes *bfp, traT, eaeA, ompA,* and *stx1* and ARGs in MDR *E. coli* isolates from humans, animals, and environment in Dar es Salaam Tanzania

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of virulence genes

Virulence genes are genetic elements that carry information on which type of virulence factors to be synthesized by the microbe (18). Virulence genes are responsible for the production of virulence factors which play an important role in the pathogenicity of bacteria through multiple mechanisms such as adhesion, invasion, toxin production, and immune evasion (44). Important virulence factors of *E. coli* are encoded by several genes including the following: (i) The bundle forming pilus (*bfp*) gene codes for adherence and involved in attachment of *E. coli* strains to intestinal epithelial cells of the host, which is the first step in the infection process (6); (ii) *E. coli* attaching and effacing (*eae*) gene promotes secretion of intimin protein which is responsible for the attaching and effacing of enterocytes, which allows *E. coli* to adhere to the host's intestinal epithelium and cause damage (46); (iii) the gene *stx-1* encodes production of the Shiga toxin (*stx*) protein in some *E. coli* strains responsible for haemolytic uremic syndrome (HUS), renal failure and bloody diarrhoea in humans (3,47); (iv) the outer membrane protein A (*ompA*) gene codes for outer membrane protein A, which enables intracellular survival of *E. coli* strains and protects them against host defense mechanism (3); (v) the *traT* gene codes for outer membrane protein, an important factor during urethral tract infections in humans (3); and also is responsible for the translocation of the intimin receptor, which is essential for the attachment and effacing (AE) phenotypes in *E. coli* (46).

2.2 Distribution of virulence genes *bfp, traT, eae, ompA,* and *stx1* among MDR *E. coli* isolates from humans, animals, and environment

Studies indicate a significant presence of these genes in *E. coli* isolates from humans, animals, and the environment. However, the frequency and combination of these genes can differ based on the source and geographic location. For instance, studies conducted in Slovakia and Lithuania reported that *traT* gene was among the virulence genes found in the MDR *E. coli* isolates from bloodstream-infected patients (18,20). In a study on multidrug-resistant *E. coli* strains isolated

from bovine feces and carcasses in Northeast Mexico, *stx1* was detected (19). In other studies, there was the co-occurrence of two or more virulence genes. In China, the virulence-associated genes (VAGs) *ompA* and *traT* were among the *E.coli* virulence genes found in isolates from a dairy farm environment (44). Another study in China reported *E.coli* isolates from calves with diarrhea carry virulence genes *eaeA* and *ompA* at varying distribution (48).

In Iran, the presence of virulence genes *stx1* and *eae* was reported in all *E.coli* isolates from diarrheic calves (49). In Spain, a study by (50) reported the presence of *E.coli* virulence genes *stx1, eae* and *bfp*A with varying distribution. A study in Malaysia detected the presence of *eae*A and *bfp*A genes in MDR *E. coli* isolated from wild birds, chicken, and the environment (51). Virulence genes *stx1* and *eae* were reported in study conducted in Vietnam using *E.coli* isolates from diarrheic calve (52). Virulence genes *stx*1, *eae* and *bfpA* are among the virulence genes found in MDR *E. coli* in the study conducted in Saudi Arabia (53).

In Africa, a systematic review conducted reported the presence of different virulence genes including *stx1, eae,* and *bfp* with varying distribution in isolates from multiple health compartments (13). In Egypt, *E. coli* isolates from Egyptian patients were found to possess the virulence gene *traT* (2), while virulence genes *stx1, eaeA* were found among diarrheic dogs (37) , and *eae*A genes were found among cows, environmental, avian sources, diarrheic lambs and kids (18,36,54). Studies in Tunisia reported the presence of virulence genes *eae* among isolates from diarrheic rabbits (35), *traT* among isolates from chicken (55) and *eaeA, stx1,* and *traT* among isolates from animals and animal food products (22). Virulence genes *traT, eae,* and *stx* were among the *E. coli* virulence gene detected in isolates from poultry and children with diarrhoea in Nigeria (56–58). In South Africa, *eaeA* and *stx1* genes were found in well and borehole water used for domestic purposes in a peri-urban community (59). Virulence genes *eae* and *stx* were reported to be present among *E. coli* from water and food sold on the streets in Mozambique (60).

In Tanzania, the presence of *stx1* gene was detected in healthy cattle and their attendants (30). Virulence genes *eaeA* was detected in *E. coli* isolated from patients (61). The virulence gene *OmpA* was among the virulence genes detected in isolates from Tanzania and Uganda study

(62). In another study conducted under One Health perspective virulence genes *bfp, traT, stx1, ompA,* and *eae* were detected in rodents, humans, chicken, and household soil isolates (3).

The presence of virulence genes *bfp, traT, ompA,* and *eae* increased persistence of *E. coli* in their hosts, animals and environment, resulting into risk of further transmission to humans. Also their presence in humans and animals lead to chronic infections. The presence of *stx1* results into diarrhoea in humans and animals (3,20,46).

2.3 Association between virulence genes *bfp, traT, eae, ompA*, and *stx1* and ARGs in MDR *E. coli* isolates from humans, animals, and environment

Research indicates a potential link between the presence of virulence genes and ARGs in MDR *E. coli*. This association could result from horizontal gene transfer mechanisms facilitated by mobile genetic elements like plasmids, transposons, and integrons (63). A study conducted in Mexico reported that although the prevalence of virulence factors was low, the strains harboring the virulence factors were frequently associated with MDR (19). A study in Slovenia reports a significant association between virulence and resistance genes among isolates from patients (20). In India, there was a significant positive association between virulence genes and drug resistance in a study using uropathogenic isolates (21). A meta-analysis study reported a significant association between virulence and resistant genes among isolates from patient in different countries in the world (10).

In Egypt, studies reported varying correlations between virulence and resistance genes in isolates from cattle, dogs and patients. A study conducted in Tunisia indicated the correlation of virulence and resistance genes among isolates from animals and animal food products (22). Another study from Nigeria reported on the resistant isolates having more than one virulence marker, showing the correlation between virulence and resistance genes (23).

However, there are other studies which reported lack of significant association between virulence genes and ARGs in different parts of the world. In Brazil, the isolates from animals and farms had no statistical significant association between virulence gene *stx1* and antimicrobial resistance (64). In Kilimanjaro, Tanzania no statistically significant correlation

was found between the number of antibiotic resistance and virulence genes in isolates from patients (65). This lack of association between virulence genes and ARGs lead to complexity in predicting pathogenicity and drug sensitivity of isolates resulting to challenges in infection control.

In Dar es Salaam, Tanzania, the occurrence and distribution of these virulence genes in MDR *E. coli* from various sources have not been extensively studied. However, regional studies suggest a high prevalence of MDR *E. coli* in both human and animal populations, with significant public health implications (66). The environmental reservoirs, including water sources, also harbor MDR *E. coli*, contributing to the cycle of resistance. Positive correlation between some of resistant and virulence genes has been reported (3). Other studies in Tanzania reported the co-occurrence of virulence and resistant genes without assessing their correlation (30,62,67).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design

This study was analytical cross-sectional study which was conducted in Dar es Salaam.

3.2 Study Site and Study Population

This study was carried out at the Muhimbili University of Health and Allied Sciences (MUHAS), Department of Microbiology and Immunology at the Microbiology Research Laboratory between April and May 2024, using stocked isolates of MDR *E. coli* isolated from humans, animals, and the environment in Dar es Salaam (4,24–26). The isolates were collected from January 2020 to January 2022 at different districts of Dar es Salaam and were stored under -80^0C at the Microbiology Research Laboratory, MUHAS. There were no stocked isolates from other species than humans, poultry, domestic pigs and river water.

3.3 Identification of the Bacterial Isolates

Isolate identification and drug susceptibility tests were conducted as described before (24–26). Briefly, bacteria were identified by colonial morphology, Gram stain, and a set of conventional biochemical tests (catalase, oxidase, indole, methyl red, Voges–Proskauer and citrate utilization tests, and lactose fermentation) (24–26). Stocked isolates from animals and environment were later confirmed by API 20E following the manufacturer's recommendations (BioMérieux, Marcyl'Etoile, France). *E. coli* was identified to the species level (24,26).

3.4 Phenotypic and Genotypic Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was done using the Kirby–Bauer disc diffusion method on Mueller Hinton agar (Becton, Dickinson and Company, New Jersey, USA) based on the 2022 Clinical Laboratory Standard Institute (CLSI) standards, using most commonly prescribed antibiotics. An isolate was considered to be MDR if it was non-susceptible to three or more drugs from different classes of antimicrobial (24–26). The isolates involved in this study were

highly resistant to ciprofloxacin, trimethoprim/sulfamethoxazole, tetracycline, and ampicillin (24–26).

The ARGs in the MDR *E. coli* isolates from poultry, domestic pigs and environment were screened using PCR as described in previous study (4). Briefly, the fresh cultured MDR *E. coli* isolates were used for DNA extraction by boiling method. After PCR, gel electrophoresis and imaging were conducted to detect the presence of AMR genes. The screened ARGs were ESBL genes (*bla*CTX-M, *bla*TEM, and *bla*SHV) and plasmid-mediated quinolone resistance (PMQR) genes (*qnrA, qnrB, qnrS, qnrC, qnrD, qepA*, and *aac (6)-Ib-cr*) (4). The isolates involved in this study possess ARGs *bla*CTX-M, *bla*TEM, *qnrB, qnrS* at varying distribution.

3.5 Sample Size and Sampling Technique

This study involved a total of 50 MDR *E. coli* isolates, 15 isolates from humans, 6 isolates from environment and 29 isolates from animals, of them 16 were from domestic pigs and 13 from poultry. Only six stocked isolates from environment met the criteria to be included in this study.

To ensure a fair representation of the entire study population, a stratified random sampling technique was used to obtain the required number of isolates from humans and animals. In each compartment, a list of stocked MDR *E. coli* isolates was made, each isolate was assigned a unique number, then using a random number generator a total of 44 MDR *E. Coli* isolates were obtained with each compartment contributing as stated earlier. For isolates from environment, a convenient sampling method was used in which all 6 available MDR *E. coli* isolates were obtained to make a total of 50 MDR *E. coli*.

3.6 Inclusion & Exclusion Criteria

3.6.1 Inclusion Criteria

- Isolates from humans, poultry, domestic pigs, and river water from Dar es Salaam
- Isolates which were MDR *E. coli*

3.6.2 Exclusion Criteria

- Isolates which were not MDR or their drug susceptibility results were unavailable
- Isolates which were not *E. coli*

3.7 Study Variables

3.7.1 Independent variables

- Isolate sources, which can be humans, river water, poultry and domestic pigs
- Drug resistance status, in which MDR isolates were used

3.7.2 Dependent variable

Virulence gene, whereby any isolate with at least one virulent gene upon multiplex PCR was termed positive and those lacking any virulence gene were negative.

3.8 Data collection

3.8.1 DNA Extraction

MDR *E. coli* isolates were inoculated on MacConkey agar (MCA) (Oxoid Ltd, Hampshire, UK) and incubated aerobically at 37°C for 24 hrs. Colonies that were lactose fermenters, appeared pink or red on MCA, were considered to be *E. coli*, were peaked and inoculated on Nutrient agar (NA) (Hi media, Mumbai, India) and incubated aerobically at 37°C for 24 hrs. Colonies on NA were used for DNA extraction by boiling in a water bath at 100°C for 10 min, followed by centrifugation at 13000 rpm for 10 min. The boiling method provides similar results as other methods of DNA extraction and the use of the boiling method in this study was because of its simplicity, cost-effectiveness and short handling time compared to other methods (68,69). The supernatant containing DNA was transferred into a sterile Eppendorf PCR tube (Eppendorf AG, Hamburg, Germany) where centrifugation and separation of supernatant were repeated three times. The concentration and quality of DNA were determined using Nanodrop spectrophotometer (Biochrom LTD, Cambridge, England) at 260/280 wavelength. The extracted DNA was stored at -20°C, before being used for detection of virulence genes.

3.8.2 PCR mixture for Detection of Virulence Genes

The One Tag Master Mix Hot Start DNA polymerase kit (New England Biolabs, Ipswich, MA, USA) was used in the detection of virulence genes *bfp, traT, ompA, stx1 and eaeA* through multiplex PCR. Primers (forward and reverse) for each gene are shown in **Table 1** below. Lyophilized primers for target genes were reconstituted by following manufacturer instructions using nuclease-free water to obtain 100 µM stock and 10 µM working solutions before storage at -20 °C.

3.8.2.1 The *ompA* and *traT* genes

PCR was carried out in a total volume of 25 µL containing 12.5 µL of 2X Taq PCR Master Mix, 0.5µL of the 10µM forward primer for each gene and 0.5 µL of the 10µM reverse primer for each gene, 2 µL of DNA template, and 8.5 µL nuclease-free water.

3.8.2.2 The *stx1* and *eaeA* genes

PCR was carried out in a total volume of 25 µL containing 12.5 µL of 2X Taq PCR Master Mix, 0.5µL of the 10µM forward primer for each gene and 0.5 µL of the 10µM reverse primer for each gene, 2 µL of DNA template, and 8.5 µL nuclease-free water.

3.8.2.3 The *bfp* gene

PCR was carried out in a total volume of 25 µL containing 12.5 µL of 2X Taq PCR Master Mix, 0.5µL of the 10µM forward primer and 0.5 µL of the 10µM reverse primer, 2 µL of DNA template, and 9.5 µL nuclease-free water.

Virulence gene *bfp* was amplified with uniplex PCR although it has the same PCR conditions as virulence genes *stx1* and *eaeA*. This was due to the close proximity of amplicon size of *bfp* and *stx1* gene which could provide challenges during visualization after gel electrophoresis.

PCR optimization and conditioning were as indicated in **Table 2**.

Table 1: Primers for virulence genes *bfp, traT, eaeA, ompA,* and *stx1*

Target gene	Primers	Primer Sequences (5'----3')	Annealing temp (°C)	Amplicon size (bp)	References
ompA	*ompA-F*	AGCTATCGCGATTGCAGTG	52	919	(3)
	ompA-R	GGTGTTGCCAGTAACCGG			
stx1	*stx1-F*	TCTCAGTGGGCGTTCTTATG	58	388	(3)
	stx1-R	TACCCCCTCAACTGCTAATA			
Bfp	*bfp-F*	AATGGTGCTTGCGCTTGCTGC	56	324	(3)
	bfp-R	GCCGCTTTATCCAACCTGGTA			
traT	*traT-F*	GGTGTGGTGCGATGAGCACAG	63	290	(3)
	traT-R	CACGGTTCAGCCATCCCTGAG			
eaeA	*eaeA-F*	ATGCTTAGTGCTGGTTTAGG	58	248	(3)
	eaeA-R	GCCTTCATCATTTCGCTTTC			

Table 2: Multiplex PCR conditions for amplification of virulence genes in MDR *E. coli* isolates.

Program	Targeted Genes	Amplification conditions for virulence genes					
		Initial Denaturation	**Denaturation**	**Annealing**	**Primer extension**	**Final extension**	**Number of cycles**
PCR 1	*ompA, traT*	94°C for 5min	94°C for 1min	58°C for 30sec	68°C for 3min	72°C for 10min	x30
PCR 2	*stx1, eae*	95°C for 5min	95°C for 30sec	55°C for 30sec	72°C for 3min	72°C for 7min	x35
PCR 3	*Bfp*	95°C for 5min	95°C for 30sec	55°C for 30sec	72°C for 3min	72°C for 7min	x35

The PCR product size was determined by conducting electrophoresis. The 1X Tris-acetate – EDTA (TAE) working buffer was prepared by measuring 980 mL of the distilled water and mixed with 20 mL of 50X TAE buffer stock solution in a conical flask. Thereafter, 1.5% of the Agarose gel was prepared by measuring 1.5 gram of agarose (Merck, SA) which was then dissolved in 100 mL of the working 1X TAE buffer solution and mixed by heating in a microwave until completely dissolved to form homogenous. To facilitate visualization of DNA 5µL gel red stain (Sigma-Aldrich, USA) was added to the agarose gel. After cooling the solution to about 60°C it was then poured to the casting tray containing sample comb and allowed to solidify at room temperature. The amplicons of each sample were mixed with 2µL loading dye and a 100-base pair marker (New England Biolabs, Ipswich, MA, USA) was included for DNA band size estimation. All gels were run in 1X TAE buffer at 120V for 60 minutes, and visualized by UV trans-illumination. Those that produced bands were termed positive, whereas those that did not show bands were termed as negative.

3.9 Data Management and Analysis

The data obtained were entered into an Excel spreadsheet (Microsoft® Office Excel 2016) and analyzed. Descriptive analysis was used to determine the frequency of virulence gene(s) among MDR *E. Coli*. Differences in the occurrence of the five genes between sample sources were determined using Chi-square and $P< 0.05$ was considered to be significant. The Spearman rank correlation test was used to elucidate the association that exists between virulence genes and ARGs. Principal component analysis (PCA) was performed by using the R-statistical software of version 4.1.3 (2022-03-10) to assess the relationships antimicrobial resistance and virulence genes in the different sample sources.

3.10 Quality Control

To exclude the possibility of using the contaminated isolates, the stocked isolates were cultured in MacConkey agar where lactose-fermenting colonies, which appeared pink or red, were peaked and cultured again on Nutrients agar for DNA extraction. Two samples from each compartment were used to optimize multiplex PCR before using it. The negative control was a DNA-free template (nuclease-free water). Isolates culture, DNA extraction, and mPCR were done in the laboratory with experienced technicians.

3.11 Ethical Consideration

For stocked isolates from humans, this study used stored isolates from an already ethically cleared study with reference number NIMR/HQ/R.8a/Vol.IX/3580 (25). For stocked isolates from animals and environment, this study used stored isolates from already cleared studies with reference numbers NIMR/HQ/R.8a/Vol. IX/3133 and DA.282/298/01.C (24,26).

CHAPTER FOUR

4.0 RESULTS

4.1 Distribution of virulence genes in MDR *E. coli* isolates from different sample sources

Overall, each of the MDR *E. coli* isolates 50/50 (100%) was found to carry at least one of the virulence genes with 19/50 (38%) carrying four types of virulence genes, *eaeA* + *bfp* + *ompA* + *traT*, **Table 4**. The virulence genes were; *bfp* (82%), *traT* (82%), *eaeA* (78%) and *ompA* (72%) while *stx1* was not detected (**Table 3**). For humans, the most detected virulence genes were *bfp* and *traT* 14/15 (93.3%), for poultry, was *eaeA* 13/14 (92.9%), for pigs were *bfp* and *traT* 13/15 (86.7%) while for river water the most detected gene was *eaeA* 6/6 (100%) (**Figure 1**).

As shown in **Table 3**, the difference in the distribution of virulence gene *eaeA* in different sample sources was statistically significance, while the distribution of other virulence genes had no statistical significance.

Table 3: Distribution of virulence genes in MDR *E. coli* isolates from different sample sources.

Genes	Different Sample Sources n (%)				Total (n=50)	p-value
	Human (n=15)	Poultry (n= 14)	Pigs (n=15)	River water (n=6)		
ompA	12 (80%)	10 (71.4%)	10 (66.7%)	4 (66.7%)	36 (72%)	0.855
traT	14 (93.3%)	11 (78.6%)	13 (86.7%)	3 (50%)	41 (82%)	0.122
eaeA	13 (86.7%)	13 (92.9%)	7 (46.7%)	6 (100%)	39 (78%)	0.005
bfp	14 (93.3%)	9 (64.3%)	13 (86.7%)	5 (83.3%)	41 (82%)	0.062

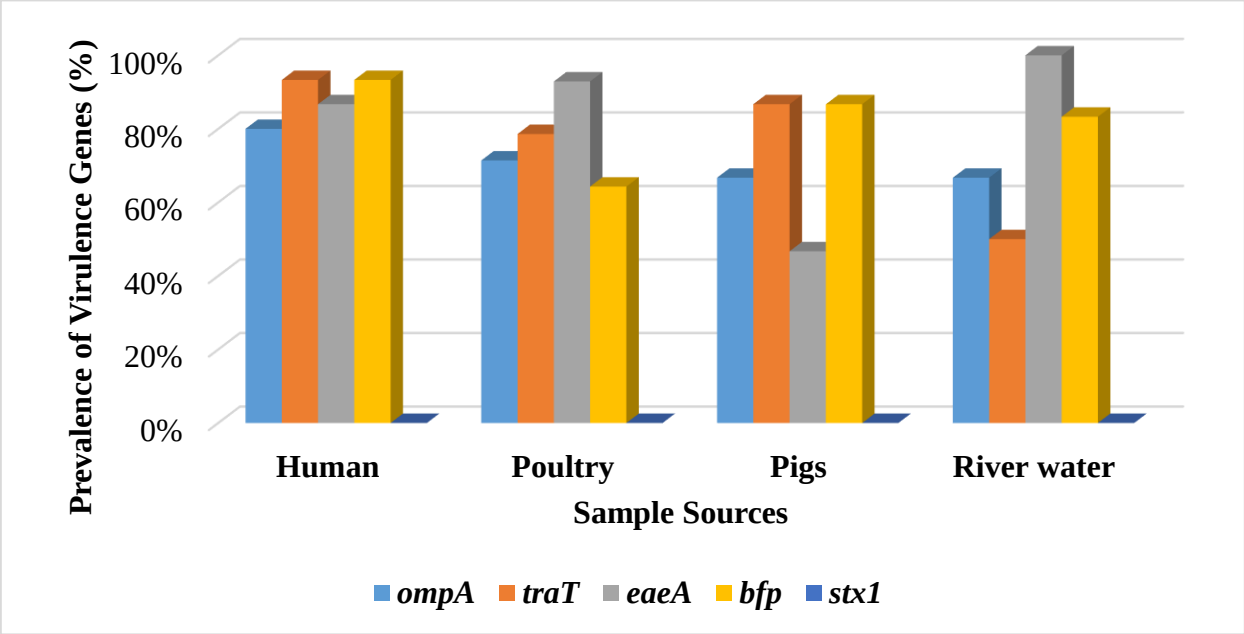


Figure 2: Frequencies of virulence genes in MDR *E.coli* isolates from different sample sources.

4.2 Co-occurrence of virulence genes in MDR *E. coli* isolates from different sample sources

As shown in **Table 4** below, 38% isolates found to carry four different types of virulence genes, as mention before. Most of these isolates were from humans, in which 66% of all isolates from humans carried four virulence genes. Majority of the isolates (42%) carried three different types of virulence genes with varying combinations in which *bfp+traT+eaeA* was the most frequently (16%) observed combination of three genes, followed by *ompA+bfp+eaeA* (14%). The co-occurrence of two genes was observed in 16% of the isolates where *traT+eaeA* and *traT+bfp* were most frequently observed (6% each). Only two isolates were found to carry only one type of virulence gene *ompA* and *traT* and both isolates were from pigs.

Table 4: Co-occurrence of virulence genes in MDR *E. coli* isolates from different sample sources

Virulence Genes	Different Sample Sources n (%)				Total (n=50)
	Humans (n=15)	Poultry (n=14)	Pig (n=15)	River Water (n=6)	
ompA	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)	1 (2%)
traT	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)	1 (2%)
ompA+bfp	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)	1 (2%)
traT+bfp	2 (13.3%)	0 (0%)	1 (6.7%)	0 (0%)	3 (6%)
traT+eaeA	0 (0%)	1 (7%)	2 (13.3%)	0 (0%)	3 (6%)
ompA+eaeA	0 (0%)	0 (0%)	0 (0%)	1 (16.7%)	1 (2%)
bfp+traT+eaeA	1 (6.7%)	3 (21%)	2 (13.3%)	2 (33.3%)	8 (16%)
ompA+bfp+eaeA	1 (6.7%)	4 (29%)	0 (0%)	2 (33.3%)	7 (14%)
ompA+traT+bfp	0 (0%)	0 (0%)	4 (26.6%)	0 (0%)	4 (8%)
ompA+traT+eaeA	1 (6.7%)	0 (0%)	1 (6.7%)	0 (0%)	2 (4%)
ompA+traT+bfp+eaeA	10 (66.6%)	6 (43%)	2 (13.3%)	1 (16.7%)	19 (38%)

4.3 Association between virulence genes and ARGs

As indicated in **Table 4**, there was no association between virulence genes and ARGs.

Table 5: Association between resistance and virulence genes of MDR *E. coli* isolates.

AMR Genes	Virulence Genes			
	Correlation Coefficients (r)			
	ompA	***traT***	***eaeA***	***bfp***
bla-CTX-M	-0.08	-0.21	0.23	-0.3
bla-TEM	0.39	-0.21	0.23	0.27
qnrB	-0.11	0.18	-0.33	-0.26
qnrS	-0.35	0.25	0.24	0.12

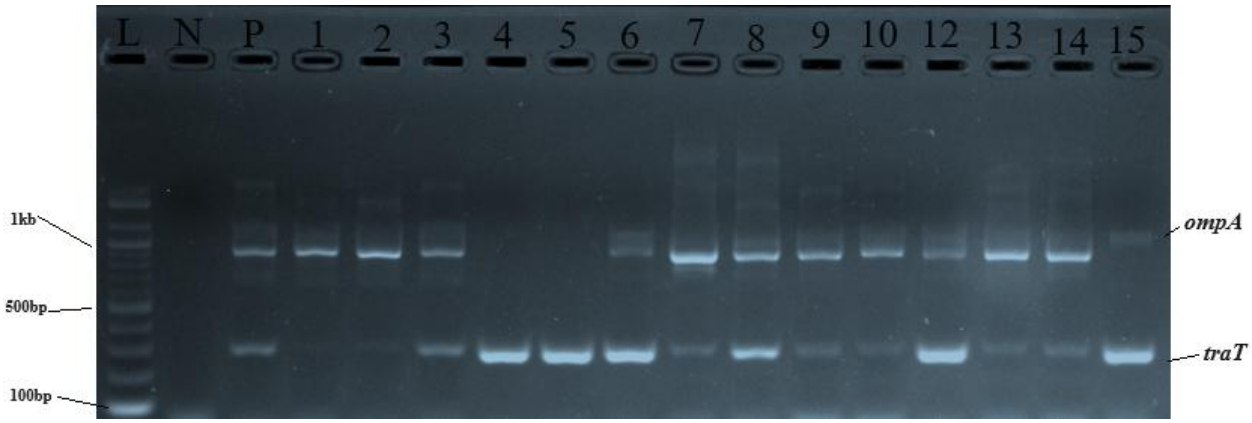


Figure 3: Gel electrophoretic bands of virulence genes (*ompA* and *traT)*.

Letters L–DNA ladder, N–negative control and P–positive control. Numbers 3,6,7,8,9,10,12,13,14 and 15 are positive samples for both genes. Numbers 1 and 2 are positive samples for *ompA* gene while 4 and 5 are positive samples for *traT* gene.

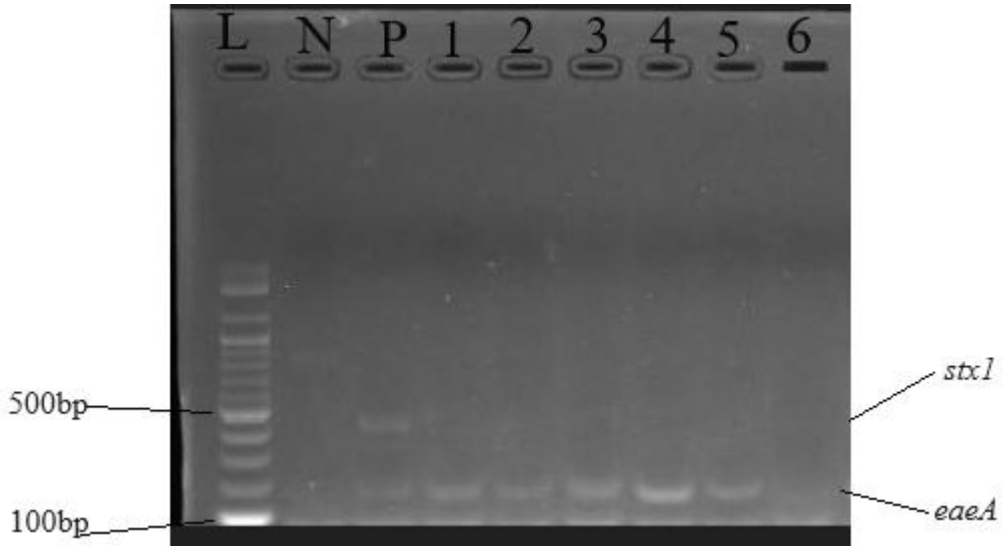


Figure 4: Gel electrophoretic bands of virulence genes *eaeA* and *stx1*.

Letters L–DNA ladder, N–negative control and P–positive control. Numbers 1,2,3,4 and 5 are positive samples for *eaeA* gene. Number 6 is negative sample for both genes.

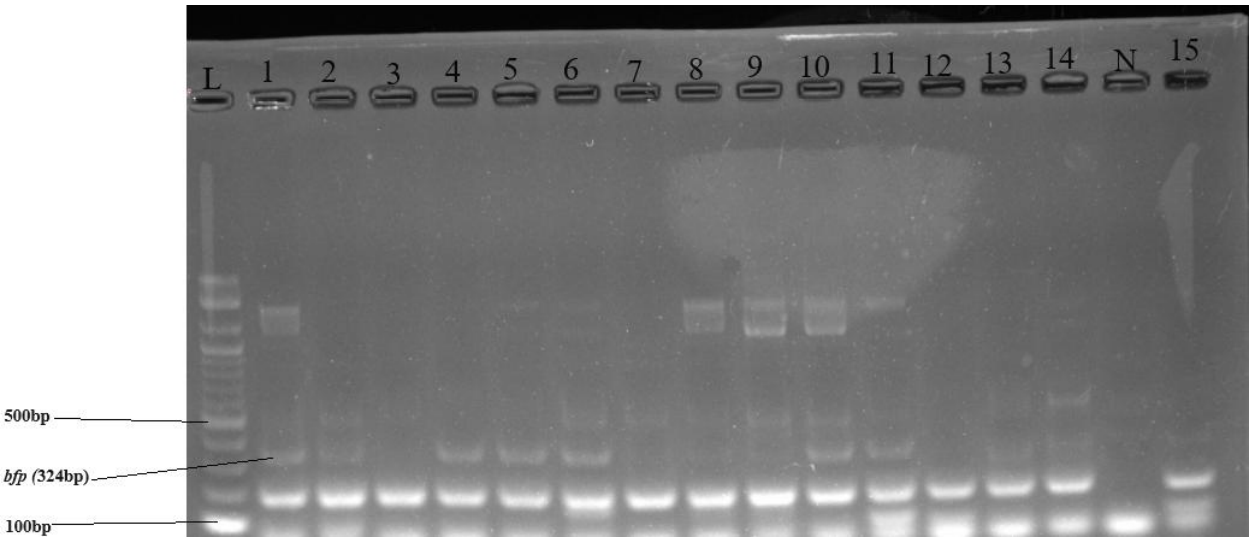


Figure 5: Gel electrophoretic bands of virulence gene *bfp*.

Letters L–DNA ladder, N–negative control. Numbers 1,2,4,5,10,11,13 and 14 are positive samples. Numbers 3,7,8,9,12 and 15 are negative samples.

4.3 Principal Component Analysis Results

According to **Figure 6** below, the arrows (vectors) for *ompA* and *traT* genes align closer to PC1 indicating greater and positive correlation between them and PC1. Vectors for *ompA* and *traT* genes pointing in opposite directions indicate negative correlation between them. The length of arrows shows that *ompA* gene contributed more to the virulence of isolates than *traT*. The vectors for *bfp* and *eaeA* genes are close to each other and to PC2 showing their influence on virulence. The length of vectors indicates that the *eaeA* gene had higher influence on virulence of isolates than *bfp* gene.

The vectors for *blaTEM, blaCTX-M qnrS* genes show that these genes contributed more to the resistance of isolates than other ARGs (**Figure 7**). The sizes of the ellipses show indicate that poultry isolates had highest prevalence of ARGs followed environment isolates, with pig isolates having lowest prevalence.

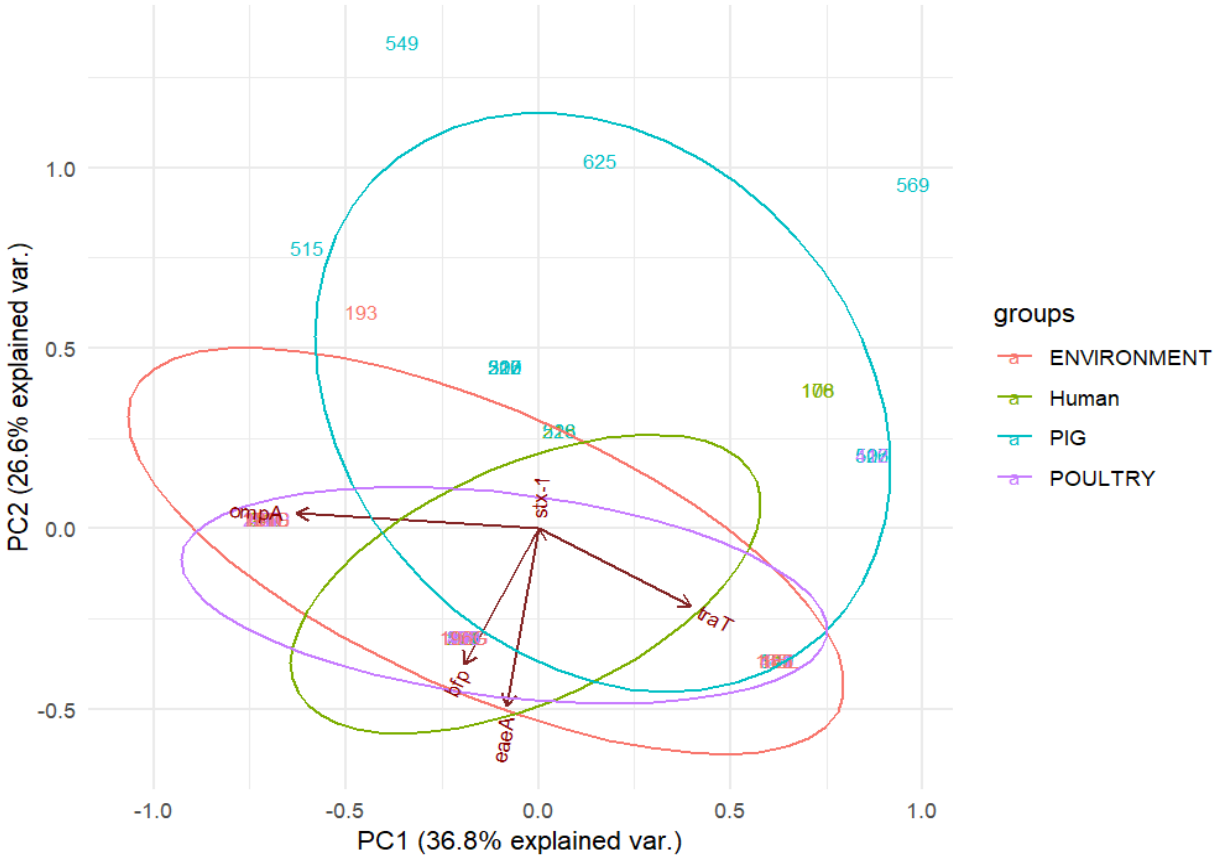


Figure 6: Principal component analysis for virulence genes of *E. coli* isolates.

PC1 indicate genes which have largest difference of occurrence across sample sources. PC2 indicate the other significant difference not covered by PC1. Arrows indicate the original variable (virulence genes of the isolates) and ellipses indicate a region that contains 95% of all samples of a particular source.

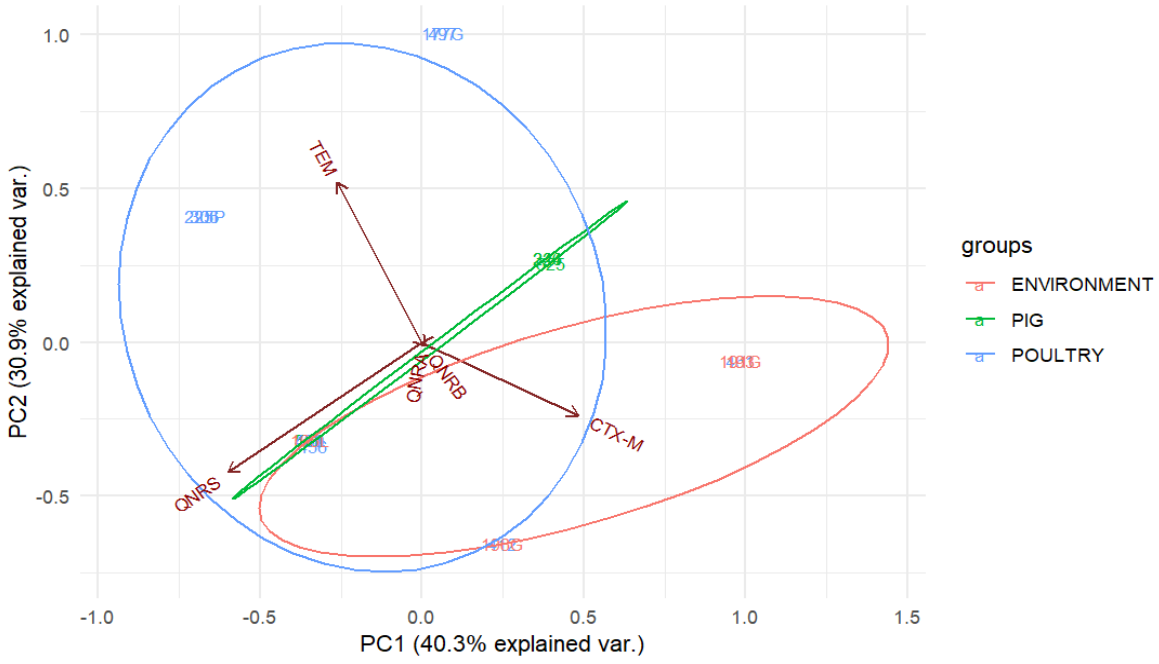


Figure 7: Principal component analysis of resistance genes of MDR *E. coli* isolates.

PC1 indicate genes which have largest difference of occurrence across sample sources. PC2 indicate the other significant difference not covered by PC1. Arrows indicate the original variable (ARGs of the isolates) and ellipses indicate a region that contains 95% of all samples of a particular source.

CHAPTER FIVE

5.0 DISCUSSION

This study determined the occurrence and distribution of virulence genes *bfp*, *traT*, *ompA*, *stx1* and *eaeA* as well as their association with ARGs in MDR *E. coli* isolates from humans, animals and environment in Dar es Salaam, Tanzania. The study found all 50/50 (100%) of the MDR *E. coli* isolates carrying at least one virulence gene, with 19/50 (38%) having four. These results align with a study conducted in Karatu, Tanzania in which high proportion (72%) of the MDR *E. coli* isolates from humans, animals, and environment were found to carry at least one virulence gene (3). In this study, the findings showed a high occurrence of virulence genes *bfp* (82%), *traT* (82%), *eaeA* (78%), and *ompA* (72%) while in Karatu the distribution of virulence gene *ompA* was the same as in this study, 72%, but the virulence genes *traT, bfp* and *eae* had lower frequencies compared to this study 26%, 10%, and 2% respectively (3). The frequency of virulence gene *stx1* in Karatu was higher (4%) than that found in this study which was 0%. However, the absence of virulence gene *stx1* in all isolates aligns with the study conducted in Egypt in which none of the isolates from retail meat and workers were found to carry the *stx1* gene (70).

5.1 Distribution of Virulence Genes *traT, ompA, bfp, stx1,* and *eaeA* in MDR *E. coli* isolates from humans, animals, and environment

For isolates from humans, virulence gene *traT* which encodes for outer membrane protein (71), was among the most frequently detected genes, alongside the virulence gene *bfp* which confer the ability to adhere to host cells using bundle-forming pili (3), with each found in 93.3% of the isolates. This prevalence is higher compared to studies in Karatu, Slovakia and Lithuania, which reported *traT* gene prevalence rates of 28.6%, 59.4% and 81.3% respectively (3,20,72). Similarly, the frequency of the virulence gene *bfp* (93.3%) in this study was higher than that reported in Karatu (0%) and Syria (42%) (3,73). Additionally, the *eaeA* gene, which encodes intimin involved in *E. coli* adherence to the host cell (74), was present in 86.7% of *E. coli* isolates from human samples, compared to 0% in Karatu and 42% in Syria (3,73). The *ompA* gene,

encoding for outer membrane protein A, helps *E. coli* to evade the host immune system (75), was detected in 80% of isolates, which is nearly equal to the 71.4% reported in Karatu (3). These four genes, which occurred in 80% to 93% of the isolates from humans seem to have a high significance in human infections in this area.

In poultry isolates, the *eaeA* gene was the most frequently detected, present in 92.9% of the samples followed by *traT* (78.9%), *ompA* (71.4%) and *bfp* (64.3%). This differs from study in Karatu in which the prevalence of virulence genes was *eaeA* (0%), *traT* (33.3%), *ompA* (100%), *bfp* (0%) and *stx1*(8.3%) (3). In China, 60% of poultry found to carry *traT* gene (76) while in Iraq poultry reported to carry bfp gene by 22% (77). The high prevalence of *eaeA* in poultry, which encodes for the intimin protein, suggests a significant risk of enteropathogenic *E. coli* (EPEC) infections originating from poultry, a common food source.

Isolates from pigs this study found frequencies of virulence genes *traT* (86.7%), *bfp* (86.7%), *ompA* (66.7%), and *eaeA* (46.7%). These findings show higher frequency of *eae* gene compared to that found in rabbit (28.3%) in Tunisia (35). However, these findings are similar to studies conducted in China which reported *ompA* (86.9%) and *traT* (84.9%) were among the most frequently detected virulence genes in *E. coli* isolates from animals (44). In Pakistan, the prevalence of *eae* gene (90%) and *stx1* gene (25%) in animals (78) were higher than the ones reported in this study. The high presence of *traT*, *bfp*, *ompA*, and *eaeA* genes in pig isolates means they can adhere to host cells and evade the host immune system, contributing to their persistence and pathogenicity.

In river water samples, the *eaeA* gene was detected in all samples (100%), underscoring the environmental persistence of EPEC strains. Other virulence genes distribution was *bfp* (83,3%), *ompA* (66.7%), *traT* (50%) and *stx1* (0%). These findings differ with those in Karatu where the distribution of virulence genes in environment isolates were *eaeA* (0%), *traT* (7.1%), *bfp* (14.3%) and *ompA* (50%) while there are similarities the virulence gene *eaeA* which was not detected in isolates from environment (3). In Pakistan, the prevalence of *eaeA* gene (100%) in *E. coli* isolates from the environment (78) was the same as in this study, while the prevalence was low (16.8%) in Thailand of *eaeA* in seawater *E. coli* isolates (74). Collectively, these results

and those of others strongly indicate the virulence potential of *E. coli* in water sources and the consequences for public health.

The study found the significant difference in distribution of the *eaeA* gene across different sample sources (p=.0.005). This indicates a non-random distribution and suggesting specific varied epidemiological patterns and transmission dynamics in the sampled environments (18,35,45,59). In contrast, the difference in distribution of other virulence genes, *bfp, traT* and *ompA*, did not show statistical significance, suggesting a more uniform distribution across different sources. This uniformity might indicate a broader adaptive advantage conferred by these genes, enabling *E. coli* to thrive in diverse environments (2,44,72).

5.1.1 Principal Component Analysis Results

PC1 indicate the genes which show biggest difference in distribution among different sources of isolates while PC2 shows the genes with second biggest difference, i.e. not larger difference than those shown by PC1. From **Figure 6** above, the distribution of virulence gene *ompA* is most important in differentiating samples followed by distribution of virulence genes *traT*, *eaeA* and *bfp*. This is due to the fact that, the vectors for virulence genes *ompA* and *traT* were close to PC1 and those for *eaeA* and *bfp* were close to PC2.Using PC1 and PC2 together it shows that, the virulence genes *ompA, traT, eaeA* and *bfp* contribute to the virulence of these isolates. These findings align with those from Karatu, Tanzania in which the vectors of virulence genes *traT* and *bfp* were in close proximity with PC1 and PC2 respectively (3).

Different sizes of ellipses indicate variation in the proportion of virulence genes across different sources of isolates. Isolates from pigs had a higher proportion of virulence genes compared to isolates from other isolates, as their ellipse is the largest, followed by isolates from the environment while isolates from poultry and humans had the lowest proportion. These results align with those from Karatu, Tanzania in which the isolates from animals and environment were found to have higher prevalence of virulence genes while isolates from humans had lowest gene prevalence (3). This suggests animals and the environment can act as reservoirs for virulence with the potential to transmit to humans.

5.2 Association between Virulence genes *ompA, traT, eaeA, bfp* and *stx1* and ARGs

The Spearman rank correlation tests between specific virulence genes and antimicrobial resistance genes (ARGs) were analyzed to understand their associations. The association between virulence genes and ARGs, although not statistically significant, found to be weak to moderate which were either positive or negative. These findings differ from study conducted in Karatu, Tanzania in which positive correlation was found between *blaTEM* and *traT* genes (r = 0.51) and *qnrB* and *bfp* genes (r = 0.63), while negative correlations were revealed between *blaSHV* and *traT* (r = -0.44) (3). This difference can be due to differences in the geographical location of isolate sources in these studies, which has impact on selective pressures leading to variation in bacterial genetics (79,80). Various studies have shown varying relationships in the occurrence of virulence and antimicrobial resistance genes, implying that the relationship is not straightforward. The interplay between these factors depends on four main factors: the bacterial species involved, virulence and resistance mechanisms, the ecological niche, and the host. Expression of a certain virulence gene may be associated with over or under-expression of a particular AMR gene as a matter of adaptation to the surrounding environment (81). This lead to challenges in infection control as lack of significant association between VGs and ARGs can lead to complexity in predicting pathogenicity and drug sensitivity of isolates.

5.3 Study Limitations and Mitigations

This study involved the use of stocked isolates of MDR *E. coli*, but isolates from humans and some isolates from poultry, pigs, and river water missed information on ARGs. Due to this, the association between virulence genes and ARGs was assessed in only 21 isolates that had ARGs information. For those isolates with ARGs results, few types of ARGs were included leaving large number of ARGs unknown. This might be a contributing factor for the observed lack of association between virulence genes and ARGs.

Limited resources were another limitation. Due to limited resources, more advanced techniques on evaluating the distribution of virulence genes could not be used. The resources available did not allow the use of whole genome sequencing as well as the use of study design which could allow the detection of transmission of these genes across different sample sources.

Multiplex PCR allowed the detection of only those virulence genes selected by the principal investigator, leaving the distribution of other virulent genes unknown. The assessment of virulence genes found to be more prevalent, with serious health outcomes, in other studies conducted in areas with similar geographical conditions as the study area helped minimize the effect of such limitation. The use of whole genome sequencing could have provided the really distribution of all virulence genes in the isolates.

This study was a cross-sectional study in which the outcomes were assessed simultaneously. This provided insight about the distribution of virulence genes but did not show the causal relationship among isolates sources.

Amplification of virulence gene *bfp* showed multiple bands as shown in **Figure 5**. Although the targeted bands were present, the presence of other, unintended bands, which were due to nonspecific binding of the primers needs to be confirmed by another primer set for the same gene.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

This study demonstrates the widespread distribution of virulence genes *bfp*, *traT*, *eaeA*, and *ompA* in MDR *E. coli* isolates from various sources in Dar es Salaam, Tanzania. The high frequency of virulence genes in MDR *E. coli* isolates from humans, animals, and the environment indicates that these isolates have high potential to cause diseases that are difficult to treat. This study emphasizes the need for integrated surveillance and control measures to mitigate the spread of MDR *E. coli* and reduce the associated infection burden. The co-occurrence of multiple virulence genes and ARGs highlights the urgent need for comprehensive public health strategies to address the growing threat of MDR bacterial infections.

Further research is needed to explore the mechanisms underlying the co-occurrence of virulence genes with one another and with ARGs. Longitudinal studies and genomic analyses could provide deeper insights into the evolutionary dynamics and transmission pathways of MDR *E. coli*, informing more effective intervention strategies.

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APPENDICES

Appendix I: Approval for ethical clearance

UNITED REPUBLIC OF TANZANIA

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

OFFICE OF THE DIRECTOR – RESEARCH, PUBLICATIONS AND INNOVATION

Ref. No.DA.282/298/01.C/2116 Date: 25/03/2024

MUHAS-REC-03-2024-2116
EDWIN M. JAMES
MUHAS

RE: APPROVAL FOR ETHICAL CLEARANCE FOR A STUDY TITLED: EVALUATION OF PREDOMINANT VIRULENCE GENES IN MULTIDRUG-RESISTANT ESCHERICHIA COLI ISOLATES FROM HUMAN, ANIMALS AND ENVIRONMENT IN DAR ES SALAAM TANZANIA

Reference is made to the above heading,

I am pleased to inform you that the Chairman has on behalf of the University Senate, approved ethical clearance of the above-mentioned study, on recommendations of the Senate Research and Publications Committee meeting accordance with MUHAS research policy and Tanzania regulations governing human and animal subjects research.

APPROVAL DATE: 25/03/2024
EXPIRATION DATE OF APPROVAL: 25/03/2025

STUDY DESCRIPTION:
Purpose:

The purpose of this observational cross sectional study is to determine the types and predominance of virulence genes associated with multidrug-resistant E. coli isolates from human, animals and environment in Dar es Salaam Tanzania

The approved protocol and procedures for this study is attached and stamped with this letter, and can be found in the link provided: https://irb.muhas.ac.tz/storage/Certificates/Certificate%20-%2014554.pdf and in the MUHAS archives.

The PI is required to:

1. Submit bi-annual progress reports and final report upon completion of the study.
2. Report to the IRB any unanticipated problem involving risks to subjects or others including adverse events where applicable.
3. Apply for renewal of approval of ethical clearance one (1) month prior its expiration if the study is not completed at the end of this ethical approval. You may not continue with any research activity beyond the expiration date without the approval of the IRB. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.
4. Obtain IRB amendment (s) approval for any changes to any aspect of this study before they can be implemented.
5. Data security is ultimately the responsibility of the investigator.
6. Apply for and obtain Data Transfer Agreement (DTA) from NIMR if data will be transferred to a foreign country.
7. Apply for and obtain Material Transfer Agreement (MTA) from NIMR, if research materials (samples) will be shipped to a foreign country.
8. Apply for and obtain permission to publish the results from National Institute for Medical Research. The application should be before submitting to a publisher and not concurrently through the email publications@nimr.or.tz.
9. Any researcher, who contravenes or fail 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)
10. The PI is required to ensure that the findings of the study are disseminated to relevant stake holders.
11. PI is required to be versed with necessary laws and regulatory policies that govern research in Tanzania. Some guidance is available on our website https://drp.muhas.ac.tz/.

Dr. Nahya Salim Masoud,

Chairperson, MUHAS Research and Ethics Committee

