OCCURRENCE OF MULTIDRUG RESISTANT *ESCHERICHIA COLI* IN RAW CHICKEN MEAT AND CLOACA SWABS COLLECTED FROM SLAUGHTER SLABS IN DAR ES SALAAM, TANZANIA

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ABSTRACT

The current study was carried out to determine the occurrence of multiple drug resistant, resistance profiles and extended spectrum beta lactamase producing *Escherichia coli* in Dar es Salaam, Tanzania. A total of 384 raw chicken meat and cloaca swabs were collected between January and June 2020, a cross sectional study carried out in five Poultry slabs in Dar es Salaam. Out of the 384 samples collected 212 (55.2%) revealed *E. coli*, of which 147(69.3%) were resistant to multiple drugs (MDR). The isolates showed relatively resistant to tetracycline (91.9%), followed by sulfamethoxazole-trimethoprim (80.5%), ampicillin (70.9%), and moderately resistant to ciprofloxacin (40.2%) and 25% cefotaxime. However, they showed low resistance to gentamycin (10.8%) and 8.6% imipenem (95% CI, $P< 0.01$). Ten (10/212) (4.7%) isolates confirmed as ESBL producers and were all MDR. The MDR *E coli* strains were highly resistant to cefotaxime (100%), ampicillin (90%) and moderately resistant to tetracycline, sulfamethoxazole-trimethoprim and ciprofloxacin (40%), low resistant to imipenem (10%) and susceptible to gentamycin. Two isolates detected with cefotaxime munich (CTX-M) and eight isolates revealed *qnrS* genes (Plasmid Mediated Quinolone Resistance) from ESBL producing *E. coli* isolates. The increased levels of antimicrobial resistance revealed in this study pose a major public health danger to human, animals and environment. This calls for an immediate intervention from the public health, veterinary and environmental health authorities to curb the public health threat of antimicrobial resistance. Improved animal husbandry biosecurity and vaccinations in poultry production at the farm level should be implemented to reduce incidence of infections. Equally, urgent action is needed in improving hygiene at poultry slaughter slabs to minimize contamination rate during slaughter and processing of poultry carcasses. It is important to increase public awareness advocacy on proper processing and

waste disposal. Veterinary and environmental authorities should continuously monitor poultry slabs to enforce hygienic standards.

DECLARATION

I, **Fauster Xavery Mgaya**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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DEDICATION

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DEFINITION OF TERMS

1. Antibiotic resistance is the capability of microorganism to resist the killing/inhibition properties of an antibiotic to which it was initially susceptible
2. Multiple drug resistance is the capability of an organism to resist one or more drugs from more than three classes of antibiotics
3. Extended spectrum beta lactamases are enzymes encoded in the chromosomes or on plasmids that are produced by gram-negative bacteria that give resistance to most beta lactam antibiotics such as penicillin, cephalosporin and monobactam.
4. Plasmid mediated quinolone resistance is the capability of gram-negative bacteria to resist against the action of quinolones and fluoroquinolones.

ABBREVIATIONS AND SYMBOLS

%	Percentage
°C	Degree Celsius
µg	Microgram
µl	Microliter
µM	Micromole
AMR	Antimicrobial resistance
AMU	Antimicrobial use
AR	Antibiotic resistance
ATCC	American type culture collection
Br	Broiler
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standard Institute
CN	Gentamycin
CTX	Cefotaxime
CTX- M	Cefotaxime Munich
DNA	Deoxyribonucleotide
dNTP	Deoxyribonucleoside triphosphate
E. coli	*Escherichia coli*
ESBL	Extended spectrum beta lactamase
IMP	Imipenem
MDR	Multiple drug resistance
Mg	Milligram
Mls	Milliter

NC	Negative control
PC	Positive control
PCR	Polymerase chain reaction
P-value	Probability value
Qnr	Quinolone resistance
SIM	Sulfur Indole Motility
SL	Spent Layers
Spps	Species
SXT	Sulfamethoxazole trimethoprim
TE	Tetracycline
TSI	Triple Sugar Iron Agar

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

The increased demand for animal protein, including poultry has led into intensification of farming systems. In Tanzania, the demand of chicken meat was projected to increase from 130 000 tons in 2017 to 465 600 tons in 2020 (URT, 2018). This demand is largely contributed to increase in urbanization and trade liberation of live animals and products (Houmanou *et al.,* 2019). Poultry farming in Dar es Salaam is done urban and peri-urban areas, and is characterized by overcrowding of flocks in unhygienic conditions (Kimera *et al*., 2020). Such conditions are frequently associated with occurrence of diseases and use of excessive antimicrobials as prophylaxis or for treatment of diseases (Mubito *et al*., 2014). The most commonly used antibiotics include tetracycline, sulfamethoxazole-trimethoprim, ampicillin, gentamycin, and ciprofloxacin, which are often obtained over the counter and are administered without advice of veterinary professionals (Founou *et al*., 2018). Knowledge of most poultry keepers on prudent use of antibiotics and their effect is still low, and antimicrobial prescribers and unregistered veterinary drug dealers have inadequate knowledge, which altogether create an environment for development of antimicrobial resistance (Brower *et al.,* 2017, Mubito *et al*., 2014).

Several studies conducted in Tanzania report over usage of antibiotics mainly in promoting growth and prophylactics in poultry production systems, consequently leading to high level of resistant organisms (Houmanou *et al.,* 2019, Rugumisa *et al.,* 2016, Mubito *et al*., 2014). Tanzania has no solid surveillance network in place to monitor veterinary drug use and the existing regulations and guidelines are poorly enforced. This study was done in Dar es Salaam; where demand for poultry meat and products is highest in Tanzania and is

associated with extensive use of antimicrobials by poultry keepers (Lindonne Glasgow *et al.,* 2019). This study aimed at determining the status of multidrug resistant *E. coli* in raw chicken meat and poultry cloaca and their antibiotic resistance pattern as well as detecting the extended spectrum beta lactamase, specifically *CTX-M* and plasmid mediated quinolone resistance genes (*qnrA*, *qnrB*, *qnrS* and *aac (6')-Ib-cr*) amongst *E. coli* isolates.

1.2 Problem Statement and Justification

In Dar es Salaam, bigger demand and consumption of chicken meat has necessitated extensive poultry farming, involving extensive use of antimicrobials for prophylaxis and treatment of disease as well as for promotion of growth which has created antimicrobial resistance (Mubito *et al.,* 2014). In most cases, antibiotics are freely sold in the thoroughfares and circulated all over without prescription by a veterinarian (Houmanou *et al.*, 2019), poultry farmers do not follow withdraw periods as recommended by the drug manufacturers (Houmanou *et al.*, 2019). Due to limited extension services and prohibitive cost farmers resort to treating own livestock without proper diagnosis and dose regulation, and hence metaphylaxis is very common (Haritova *et al.*, 2011, Mubito *et al.*, 2014). Such a scenario has the potential to give rise to multidrug resistant bacteria, such as extended spectrum β-lactamase (ESBL) producer *Enterobacteriaceae* that pose a threat encounter to human and animal health (EFSA, 2015) as well as contamination of the environment (Graham *et al.,* 2019). Multidrug resistant bacteria can cause significant public health problem, food in security and loss of economy (Lilian *et al.,* 2019).

This study will provide an insight on the current extent and antimicrobial patterns of MDR *E. coli* in chicken meat and cloaca, and in the process provide data that can be used by

veterinarians and other public health authorities in tackling the problem of antimicrobial resistance in the poultry industry.

1.3 Objectives

1.3.1 Broad Objective

To determine the magnitude and pattern of multiple drug resistant *Escherichia coli* in raw meat and cloaca of chicken at poultry slaughter slabs in Dar es Salaam, Tanzania.

1.3.2 Specific Objectives

i. To determine the prevalence of MDR *E. coli* in raw meat and cloaca of chicken at poultry slaughter slabs in Dar es Salaam, Tanzania.
ii. To detect the pattern of MDR and ESBL producing, *E. coli* in raw meat and cloaca of chicken at poultry slaughter slabs in Dar es Salaam, Tanzania.
iii. To determine the frequency of occurrence of *CTX-M* and PMQR genes from ESBL producing *E. coli* in raw meat and cloaca of chicken at poultry slaughter slabs in Dar es Salaam, Tanzania.

1.4 Research Questions

i. What is the prevalence of MDR *E. coli* in raw meat and cloaca in chicken at poultry slaughter slabs in Dar es Salaam?
ii. What is the pattern of MDR, including extend of ESBL *E. coli*, in raw meat and cloaca of chicken at poultry slaughter slabs in Dar es Salaam?
iii. What are ESBL and PMQR genes of *E. coli* isolates from in raw meat and cloaca of chicken sold at poultry slaughter slabs in Dar es Salaam?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Poultry Production Systems in Tanzania

Poultry production in Tanzania is primarily conducted in rural and urban areas as source of income, for food security and other social obligations (Kimera *et al.,* 2020, de Bryun *et al.*, 2016). Poultry production in Tanzania comprises commercial and subsistence systems with commercial production involving broilers and Layers. The commercial poultry production in rural and peri-urban settings contribute about 80% of meat and eggs (FAO, 2016) consumed in urban areas. The traditional system involves indigenous breeds raised as free rangers contributes 70% of poultry products (meat and eggs) consumed in countryside areas and 20 % of poultry and products consumed in town areas (FAO, 2019).

In Tanzania chicken meat production does not meet the demands of the market, therefore a significant amount of poultry meat is imported outside the country to meet the demands of consumers (MMA – AMDT, 2016). This situation has caused an intensification of poultry production and use of a wide range of antimicrobials in poultry production in both urban and peri-urban areas (Mubito, 2014) such as in Dar es Salaam.

2.2 Antimicrobial Resistance

Is the capability of a bacterium to resist the killing/inhibitory potential of antibiotics that it was generally susceptible to (Agyrase *et al.*, 2018) and it is a global concern (WHO, 2014; World Health Statistics, 2017). The resistance of some bacteria is not a new phenomenon (Agyrase *et al.,* 2018, Collignon *et al.*, 2015), however, is amplified by excessive usage of

antimicrobials as is the case in poultry husbandly (Kamini *et al.,* 2016, Mubito *et al.*, 2014).

Poultry production is among of sectors that overuse or indiscriminately use of antibiotics for treatment, prophylaxis and improving production by promoting body growth of chickens (Agyrase *et al.,* 2018; Collignon *et al.,* 2015). Poultry production is the highly growing industry worldwide due to the high demand of meat and poultry products (URT, 2018).

The antibiotics used in the poultry industry are those also considered essential drugs in human medicine (Hamis and Shahada, 2014; Agyrase *et al.,* 2018). The indiscriminate use of these essential antimicrobials in animal production is likely to hasten the development of antimicrobial resistance among human pathogenic bacteria, along with commensals (Collignon *et al.,* 2015). The consequences of this is treatment failures, economic losses and transmission of resistance genes to humans (Aniokette *et al.,* 2016). The presence of antimicrobial residues in meat, eggs and other animal products therefore raises concern on human health (Jammoul *et al.* 2019). Resistant bacteria can be transmitted from poultry products to humans through handling and consuming with meat with contaminated pathogens (Odwar *et al.,* 2014). In human system they are able to inhabit the guts where resistant genetic factors can be transmitted to the endogenous colonic flora, and may impair treatments of infection produced by the pathogen in humans (Aniokette *et al.,* 2016).

2.3 Usage of Antimicrobials in Poultry Husbandry

The increased demand of poultry meat as source of protein and income has led to intensive poultry production systems (Odwar *et al.,* 2014, Mubito *et al.,* 2014). This intensification

contributes to usage of wide range antibiotics in poultry farming management and increasing production profits (Kimera *et al.,* 2020; Nonga *et al.,* 2009, Ilea RC., 2009). The excessive usage of antimicrobials for example in chemoprophylaxis, promoting growth and treatment cause emergence of antimicrobials resistance (AMR) in most of bacteria and increases the possibility of developing multiple drug resistance (MDR) among *Enterobacteriaceae,* including *E. coli* (Ilea RC, 2009). Poultry farming in most cities like Dar es Salaam is done in urban and peri-urban districts with improvised shelters and under poor hygienic conditions. These factors contribute to the development of diseases that necessitate treatment with antimicrobials (Kimera *et al.,* 2020).

In Tanzania antimicrobial use accounts between 74% to 100%, use antimicrobials in poultry production, which includes tetracyclines, sulphonamides, penicillin, aminoglycosides macrolides, dihydrofolate and quinolones (Caudell *et al.,* 2017, Nonga *et al.,* 2009) and most of African countries use almost the same antibiotics in poultry production.

2.4 Multiple Drug Resistance *Escherichia coli*

Escherichia coli is a gram-negative bacterium capable of and regularly exchange inherited materials over horizontal genetic factor transfer with another associated bacteria (Agyrase *et al.,* 2018, Jang *et al.,* 2017). *E. coli* is a commensal bacterium living in the gut of humans and animals, although, some strain have been reported to cause gastrointestinal infections (Chishimba *et al.,* 2016). Tetracycline is the most commonly used antibiotic in poultry industry (Mubito *et al.,* 2014). In Tanzania MDR *E. coli* were reported in chicken faeces with emergence of resistance to third generation cephalosporin which is an essential first line drug for treating enteric bacterial infections in humans. The findings showed that

92.6% MDR *E. coli* detected from free range chickens were resistant to tetracycline, penicillin, quinolones, phenols, sulphonamides including third generation cephalosporins (Hamis and Shahada, 2014; Garcia-Migura *et al.,* 2014). Those MDR *E. coli* were detected from non-intensified chicken production not raised with antibiotics, meaning that the environment was highly contaminated with antimicrobial residues. In Nairobi, Kenya, reported contamination of chicken carcases was about 78% with *E. coli* and 98% with other coliforms, the reported *E. coli* were 42.9% MDR, resistant to tetracycline, sulphonamides, penicillin, aminoglycosides, quinolones, phenicols, cephalosporins (Odwar *et al.,* 2014).

In Nigeria, 62% of MDR *Salmonella* spp, from chicken, were reported to be resistant to tetracycline, quinolones and sulphonamides (Fashae *et al.,* 2010). In Indonesia, the prevalence of antimicrobial resistance between associates of *Enterobacteriaceae* in chicken meat sold in traditional markets, the MDR rate of *E. coli* was higher accounting to 86.7% of all *Enterobacteriaceae* tested (Yulistiani *et al.,* 2017), Resistance to were tetracycline was found to be 68.95%, probably due to long and widespread use of this antibiotic in poultry farming as a growth promoter (Beninati *et al.,* 2015, Gelband *et al.,* 2015), chemoprophylaxis and for therapeutic purpose. Several studies report the emergence of MDR bacteria that are mostly compounded by raising birds like chicken, turkeys by using antibiotics which are widely available and are cheapest (Lulijwa *et al*., 2020; Zhao *et al*., 2020; Wongsuvan *et al.,* 2018; Davis *et al.,* 2014).

Beninati *et al.* (2015) reported the highest level of resistant *E. coli* in turkey and chicken retail and imported meat products to ampicillin (100%) and cefotaxime (94%), followed by tetracycline (84%) and chloramphenicol (73%), noted that penicillin and cephalosporin are

drugs of choice used in most European countries in poultry production and, this contributes to the resistance among some bacterial strains to these antibiotics (Beninati *et al.,* 2015).

Multiple drug resistance continues to be a global concern among many bacterial pathogens in human, animal production and the environment (Jammoul *et al.*, 2019), *Salmonella* spp, *Campylobacter* spp, *Klebsiella* spp and *E. coli* as common indicator bacteria for MDR bacteria in poultry and poultry products. These bacterial strains are linked to foodborne diseases increased antimicrobial resistance and treatment failure (Agyrase *et al*., 2018, Sharma *et al.,* 2017). Several studies have reported MDR bacteria in poultry products, remains, clutter and faecal materials which are potentially infectious to handlers and customers (Agyrase *et al.*, 2018, Aniokette *et al.*, 2016, Sharma *et al.*, 2017).

2.5 Control Strategies of Antimicrobial Resistance

In order to combat AMR threats the one health approach is necessary to provide proper education to the society on the danger of over use and misuse of antimicrobials, improved and prevention of infection especially in low income countries. This will reduce unnecessary use of antimicrobials in treating infections, unnecessary reduction in usage of antimicrobials in farming infections and usage of antimicrobials as growth promoters. Introduction of new and rapid diagnostic tools, development of vaccine for disease prevention rather than treatment, training of specialists in all sectors, hospitals, animal health, agriculture and in environmental protection, provision of adequate funds for research on AMR, discovery of new antibiotics and global partnership for rapid action on AMR should be addressed in each country (Global action plan, 2015; WHO, FAO and OIE, 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Dar es Salaam, the most popular commercial city in Tanzania with an area of 1590 square kilometre, Dar es Salaam is located along the East coast of the Indian Ocean at 6^0 48'S latitude and 39^0 17'E longitude and 24 meters above sea level. The city has a population of 4 364 541 million (NBS, 2012) making it the biggest city in the country. Administratively, the city is subdivided into five administrative districts namely, Ubungo, Kinondoni, Ilala, Temeke and Kigamboni. Moreover, it is a major commercial poultry production and consumer of chicken meat and eggs in Tanzania, Approximately 20,000 chicken are slaughtered daily in these five poultry slabs.

3.2 Study Design

A cross sectional study was conducted in five large poultry slabs in the four districts of Ubungo district (Manzese, and Shekilango poultry slabs), Ilala district (Kisutu poultry slab), Kinondoni district (Mtambani poultry slab) and Temeke district (Stereo poultry slab), between January and June 2020, raw chicken meat and cloaca swabs were collected from each of the slabs.

3.3 Inclusion and Exclusion Criteria

Five large poultry slabs were selected since they slaughtered large numbers of chickens from different areas on a daily basis. Broilers and spent layers were the target chicken in the study because they are raised intensively and commonly use antimicrobials for disease

prevention, growth promotion and management of infections, other types of poultry were not included in the study.

3.4 Sample Size Determination

Sample size was estimated using the formula: $n= Z^2 P (1-P) /\Sigma^2$ (Naing *et al*., 2006), where “n" - sample size, "Z"- constant (1.96), P - prevalence and Σ is error margin (0.05). Due to the absence of data on multidrug resistance *E. coli* in chicken meat in Tanzania, an assumed prevalence of 50 percent was used in the calculation, which gave a sample size estimate of n = 384. Overall, two specimens were collected from 192 chickens making total of 384 samples, of which broilers were 96 and spent layers 96.

3.5 Sampling Method

Purposive sampling procedure was used in which cloaca swabs were collected before slaughter at the entry point of the respective poultry slabs and chicken meat swabs were collected after slaughtered.

3.6 Specimen Collection

Chicken meat and cloaca swabs were collected aseptically and placed into a sterile tubes containing Cary Blair transport medium (Oxoid, Basingstoke, UK) (Chishimba *et al.*, 2016). The collected samples were placed in a cooler box at 2 ºC to 8 ºC for transportation and processed within two hours of collection at Muhimbili University of Health and Allied Sciences (MUHAS), Microbiology Teaching Laboratory.

3.7 Media Preparation

Media were prepared following manufacturer instructions, sterilization was done using steam under pressure (autoclave) at 121 ºC for 15 minutes. After sterilization media were poured into the respective sterile petri dishes, allowed to solidify at room temperature, after media solidification at 42 ºC the plates were dried in a hot air oven at temperature of 50 ºC in 10 minutes, and kept at 2 - 8 ºC refrigerator ready for use.

Media quality and Sterility checks

Few prepared uninoculated media plates were kept at 37 ºC incubator for 24 hour for sterility check, and two media plates were inoculated with reference strains (ATCC 25922 *E. coli* or *Klebsiella pneumonia* (ATCC 700603) to ascertain the growth.

3.8 Isolation and Identification of Enterobacteria

The swabs were cultivated onto the MacConkey Agar (Oxoid, Basingstoke, UK) without antibiotic and the inoculated plates incubated at 37ºC incubator aerobically for 24 hour. The grown colonies on MacConkey Agar were identified (Chishimba *et al.,* 2016) by colonial morphology (Fig. 1), lactose fermentation and gram staining. Lactose fermenters were subjected to conventional phenotypical identification using Triple Sugar Iron agar (TSI), Sulphide Indole Motility(SIM) agar and Citrate utilization test, then further identified into species level by API 20E (Biomerieux, Marcy-Etoile, France) tests (Feglo and Opoku, 2014) for *Enterobacteriaceae* differentiations.

Figure 1: *E. coli* colonies on MCA

3.9 API 20E Identification System for *Enterobacteriaceae*

Briefly, a single colony was emulsified into sterile saline and filled into the API 20 E strips chamber covered with dry bacterial media/biochemical components in 20 distinct compartments, then left at 37ºC in an incubator for 18 to 24 hour aerobically in a wet chamber of API 20 E strips (Biomerieux, Marcy-Etoile), France This standardized method permitting only biochemical identifier of an *Enterobacteriaceae* strain (Salah *et al*., 2019) using an isolated colony, with this system *E. coli* was identified to species level and the results were interpreted following API 20 E manufacturer instructions.

The confirmed *E. coli* isolates were then cultivated onto MacConkey agar containing 2mg/l cefotaxime for preliminary screening of ESBL producers (Chishimba *et al.*, 2016). ESBL were confirmed using a disk of cefotaxime 30µg alone and in combination with clavulanic acid 10 µg (Azam *et al.*, 2016) along with ceftazidime 30µg alone and in combination with clavulanic acid 10 µg (Taraghian *et al.*, 2020). The difference size of inhibition zone of more than or equal to 5mm were confirmed as ESBL positive (CLSI, 2018). *Klebsiella. pneumoniae* strain (ATCC 700603) used as an ESBL positive strain) and *E. coli* ATCC

25922 as an ESBL negative strain, results were interpreted following the CLSI 2018 standard.

3.10 Antimicrobial Susceptibility Testing

The antimicrobial sensitivity testing was perfomed using the Kirby-Bauer disc diffusion technique on MH Agar (Oxoid, Basingstoke, UK), following CLSI 2018 guidelines. Seven antibiotics from different classes were used including, ampicillin (10µg), tetracycline (30µg), gentamycin (10µg), ciprofloxacin (5µg), imipenem (10µg), sulfamethoxazole-trimethoprim (1.25/23.5µg), and cefotaxime (30µg).

Five colonies of lactose fermenters identified as *E. coli* were emulsified into 5 mls of sterile physiological saline, and adjusted to achieve turbidity comparable to 0.5 McFarland tube which is equivalent to 10^8 cfu/ml (CLSI, 2014), then spread using a sterile cotton swabs onto MH agar and incubated at 37 °C for 16 to 18 hour. Zones of inhibition for each antibiotic were examined within 16 – 18 hours of incubation (Nahar *et al.*, 2018; CLSI, 2018) as showed in Fig. 2. The inhibition zones were read following the CLSI (2018) guideline. The ATCC 29522 *E. coli* was incorporated as control (CLSI, 2018; Nahar *et al*., 2018). The *E. coli* isolates were considered MDR if they were resistant to a minimum of one antibiotic from three or more antimicrobial classes (Nahar *et al*., 2018; CLSI, 2014).

Figure 2: Inhibition zones in Muller Hinton Agar

3.11 DNA Extraction and Polymerase Chain Reaction (PCR)

Few colonies of confirmed ESBL *E. coli* were emulsified into 2 mls of nuclease free sterile distilled water and DNA was extracted by boiling in a water bath at 100ºC for 10 min, then centrifuged at 1500 revolution per minute for 3 min (Chishimba *et al.*, 2016), supernatant containing DNA was transferred into sterile Eppendorf tube, centrifuged and resuspended and the process were repeated three times. The concentration of DNA contents were determined by Nano drop spectrophotometer at 260/280 and 260/230 wavelength. DNA was stored at -20ºC, then the DNA of suspected *CTX- M* and *PMQR* genes were detected by PCR according to Park *et al*. (2006) and Kim *et al*. (2013).

The Dream Tag DNA polymerase kit were used in finding resistance genes, in a PCR reaction volume of 25µL consisting of 10X dream Tag Buffer 5 µL, 1 µL of forward primer, DNA template 2 µL, reverse primer 1 µL, Dream Tag DNA Polymerase (1.25 U) 1 µL dNTP 5 µL, and nuclease free water 10 µL. The primers used in amplification of respective *E. coli* resistance genes are listed in Appendix 3.

3.12 Molecular Detection of CTX -M Genes

All confirmed as ESBL producers *E. coli* were screened by uniplex PCR based technique (Park *et al.,* 2006). Amplification involved initial denaturation at 98 ºC for 3 min, 35 cycles were employed for denaturation at 98 ºC for 15 s, annealing at 60 ºC for 5 s, at 72 ºC temperature extension for 45 s and ended with extension at 72 ºC for 5 min (Park *et al.*, 2006).

3.13 Detection of PMQR Genes

These Quinolone resistant genes (A, B and S) were amplified by multiplex PCR assay (Yan*g et al.,* 2013), using amplifications condition i.e early denaturation at 94 ºC for 5 min and 32 cycles again for the denaturation at 94 ºC in 45 s, followed by annealing at 53 ºC for 60 s and extension at 72 ºC for 60 s and ended with extension at 72 ºC intended 10 min (Yang *et al.,* 2013).

3.14 Ampilification of *Aac (6')-lb-cr* gene

Aac (6')–lb-cr gene was screened by uniplex PCR based assay (Park *et al.,* 2006), using the amplification condition initial denaturation at 94 ºC for 5 min and 34 cycles of denaturation at 94 ºC for 45 s, annealing at 55 ºC for 45 s, extension at 72 ºC for 45 s and ended with extension at 72 ºC for 10 min (Park *et al.,* 2013). The amplified DNA products were stained with gel red stain and examined by 1.5 % agarose gel at 100 voltages for 35 minutes.

3.15 Analysis of Data

The Microsoft Excel program was used in organizing and cleaning collected data; which was analysed by *Chi-square test*. A paired *t-test* assuming unequal variance was used for comparing overall prevalence and comparing resistance rate among confirmed antibiotics using SPSS 16 version software and probability value of less than or equal to 0.05 was confirmed statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Results

4.1.1 Frequency of *E. coli* in cloaca and chicken meat from broiler and spent layers

Out of the 384 chicken meat and cloaca swabs collected to establish *E. coli* prevalence in five selected poultry slabs in Dar es Salaam, 212 (55.2%) were identified to have *E. coli.*

A total of 147 (69.3%) of 212 isolated *E. coli* were resistant to more than three classes of tested antibiotics. The distribution of MDR isolates was 18/19 (94.7%) at Stereo Poultry slab in Temeke District, 37/43 (86%) at Shekilango Poultry slab in Ubungo district, followed by Manzese in Ubungo district 28/40 (70%), Mtambani Poultry slab in Kinondoni district 14/20 (70%) and Kisutu Poultry slab 50/90 (55.6%) (Table 1).

Table 1: Isolation frequency of *Escherichia coli* in among Poultry slabs in Dar es Salaam region (n= 384)

Poultry Slabs	MDR		Not MDR		Overall	
	n	%	n	%	n	%
Stereo	18	94.7	1	5.3	19	100.0
Manzese	28	70.0	12	30.0	40	100.0
Mtambani	14	70.0	6	30.0	20	100.0
Shekilango	37	86.0	6	14.0	43	100.0
Kisutu	50	55.6	40	44.4	90	100.0
Total	**147**	**69.3**	**65**	**30.7**	**212**	**100.0**

MDR- multidrug resistant

4.1.2 Assessment of antibiotic resistance rates in isolated *E. coli*

The highest resistance was detected against tetracycline (91.9%), followed by trimethoprim-sulfamethoxazole (80.5%), ampicillin (70.9%), ciprofloxacin (40.2%), cefotaxime (22.5%), 10.8% gentamycin (10.8%) and imipenem (3.3%) (Table 2).

Table 2: Antibiotic resistance patterns of *E. coli* isolates (n = 212)

Poultry slabs	**Antibiotics tested**													
	TE (n)	**%**	**CN (n)**	**%**	**CIP (n)**	**%**	**IMP (n)**	**%**	**SXT (n)**	**%**	**CTX (n)**	**%**	**AMP (n)**	**%**
		95.		16.		46.		16.		89.		100.		100.
Stereo	18	0	3	7	9	7	3	7	17	4	19	0	22	0
		95.				63.				84.				
Manzese	38	7	3	7.3	25	7	0	0.0	34	7	0	0.0	24	62.9
		88.				25.				82.				
Mtambani	18	1	0	0.0	7	0	0	0.0	15	1	0	0.0	14	73.8
		97.		22.		36.				86.				
Shekilango	42	5	5	4	16	1	0	0.0	37	3	5	12.5	30	70.1
		83.				29.				59.				
Kisutu	75	1	7	7.4	29	7	0	0.0	56	9	0	0.0	45	47.9
	19	**91.**		**10.**		**40.**		**3.3**		**80.**				
Total	**1**	**9**	**18**	**8**	**86**	**2**	**3**	**4**	**159**	**5**	**24**	**22.5**	**135**	**70.9**

TE=Tetracycline, CN=Gentamycin, CIP=Ciprofloxacin. IMP=Imipenem, SXT=Trimethoprim-sulfamethoxazole, CTX=Cefotaxime, AMP=Ampicillin

Out of 147 MDR *E. coli* isolated in the study, 49% showed resistance to three classes of antibiotics, 33.3% to at least four classes, 14.3% resistance to five classes, 2.7% resistance to six classes and one isolate (0.7%) were resistant to all seven tested antibiotics (Table 3 and Appendix 1).

Table 3: Classes of antimicrobial patterns resisted (%)

MDR *E. coli* isolates	**Classes of antibiotics**				
	3	**4**	**5**	**6**	**7**
147	72 (49)	49 (33.3)	21(14.3)	4(2.7)	1(0.7)

There were differences in isolated MDR *E. coli* by location of poultry slabs, in spent layers' meat and cloaca (CI: 14.6 – 77.7, p-value < 0.001). At Mtambani Poultry slab MDR *E. coli* isolation rate in cloaca of spent layers was 57.1%, Manzese Poultry slab (32.0%), Shekilango poultry slab were (29.7%), Stereo Poultry slab were (27%) and Kisutu slab were 8.0%, while MDR *E. coli* isolated in Spent layers' meat at Shekilango were 27.0%, Stereo Poultry slab were (22.2%), Manzese Poultry slab were (21.4%), Kisutu poultry slab were (16.0%) and at Mtambani (14.3%) (Table 4).

For MDR *E. coli* isolates in broiler cloaca at Kisutu were (44.0%), Stereo (38.9%), Shekilango (27.0%), Manzese were (25.0%) and Mtambani (7.1%), finally MDR *E. coli* isolated in broiler meat at Kisutu were (32.0%), Manzese and Mtambani were (21.0%), Shekilango poultry slab were (16.2%) and Stereo poultry slab were (11.1%) (Table 4). No statistically significant difference existed in MDR *E. coli* isolation frequency between broiler meat and broiler cloaca by poultry slabs (*P> 0.410*).

There were significantly higher MDR *E. coli* isolation rates of chicken types by location of poultry slabs i.e isolates from cloaca and Meat of broilers VS isolates from cloaca and meat of spent layers (*P< 0.001*). Also, no statistically significant difference existed between multiple drug resistant isolated *E. coli* and type of chicken (Broilers and spent layers) (*P> 0.267*).

Table 4: MDR *E. coli* isolates by location of poultry slabs and chicken category

Poultry slabs	Meat Br		Cloaca Br		Meat SL		Cloaca SL		Total
	n	%	n	%	n	%	n	%	n
Stereo	2	11.1	7	38.9	4	22.2	5	27.8	**18**
Manzese	6	21.4	7	25.0	6	21.4	9	32.1	**28**
Mtambani	3	21.4	1	7.1	2	14.3	8	57.1	**14**

Shekilango	6	16.2	10	27.0	10	27.0	11	29.7	**37**
Kisutu	16	32.0	22	44.0	8	16.0	4	8.0	**50**
Total	**33**	**22.4**	**47**	**32.0**	**30**	**20.4**	**37**	**25.2**	**147**

Meat Br =broiler meat, Cloaca Br=broiler cloaca, Meat SL=spent layers' meat, Cloaca SL=Spent layers' cloaca

4.1.3 Extended spectrum beta lactamase producers

Out of 212 identified *E. coli* ten (4.7%) isolates were screened and established as ESBL producers. All 10 isolates were resistant to more than three different antibiotics classes, were 100% resisted cefotaxime and ampicillin, 90% resisted tetracycline and sulfamethoxazole-trimethoprim, 40% resisted ciprofloxacin and 10% were resistant to imipenem. However, all ten (100%) ESBL *E. coli* were susceptible to gentamycin (Table 5). All ESBL producers isolated from Stereo Poultry slab in Temeke district, and mostly obtained from cloaca and raw chicken meat of spent layers.

Table 5: Confirmed ESBL producing *E. coli* isolates and their antibiotic resistance pattern (n=10)

			Chicken category				
Antibiotic	**Isolates (n)**	**%**	**Meat SL**	**cloaca SL**	**Meat Br**	**Cloaca Br**	**Total**
TE	9/10	90	3	4	1	1	**9**
CN	0/10	0	0	0	0	0	**0**
CIP	4/10	40	3	1	0	0	**4**
IMP	1/10	10	1	0	0	0	**1**
SXT	9/10	90	3	4	1	1	**9**
CTX	10/10	100	4	4	1	1	**10**
AMP	10/10	100	4	4	1	1	**10**

Meat SL= spent layers' meat, cloaca SL=spent layers' cloaca, cloaca meat Br =broiler meat, Br= broiler cloaca, TE=Tetracycline, CN=Gentamycin, CIP=Ciprofloxacin, IPM = imipenem, SXT= Trimethoprim-Sulfamethoxazole, CTX=Cefotaxime, AMP= ampicill5n

4.1.4 Detection of CTX- M and *qnrA, qnrB, qnrS* and *aac (6')-lb-cr.*

Detection of PMQR genes in eight out of ten ESBL producers by PCR indicated that the genes either exist singly genes or in combination with *CTX-M*. The *qnrS* were present in eight (80%) of the isolates from four spent layers' meat, two spent layers' cloaca, one broiler meat and one cloaca of broilers (Table 6 and Fig. 3). *CTX-M* were present in two (20%) isolates of which one from spent layers' meat and the other from cloaca of spent layers (Fig. 4), the *CTX-M* was found to be associated with *qnrS*, while, for the PMQR determinants none of *qnrA, qnrB* and *aac (6')-lb-cr* were detected in all 10 *E. coli* isolates tested (Table 6).

Table 6: Detection of specific AMR encoding genes using PCR and agarose gel Electrophoresis (n = 10)

Detected genes	*E. coli* No (%)	Spent layers meat	Spent layers cloaca	Broiler meat	Broiler cloaca
CTX- M	2/10 (20)	1	1	0	0
qnrA	0/10 (0.0)	0	0	0	0
qnrB	0/10 (0.0)	0	0	0	0
qnrS	8/10 (80)	4	2	1	1
Aac (6')-Ib-cr	0 (0.0)	0	0	0	0
Total	**10/10(100)**	**5**	**3**	**1**	**1**

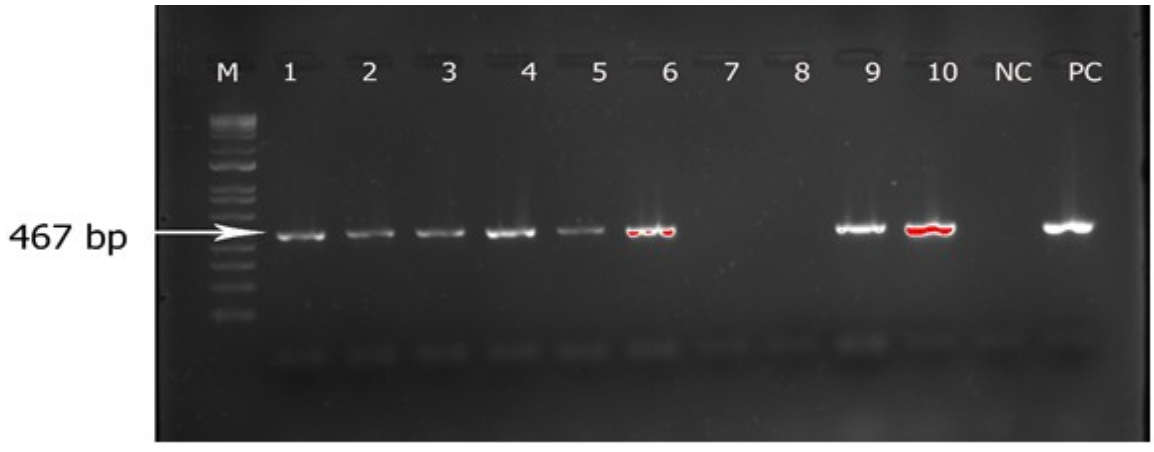

Figure 3: Shows M- 1 kb Ladder, amplified *qnrS* gene in sample 1- 6, 9 and 10, NC- Negative control and PC- Positive control

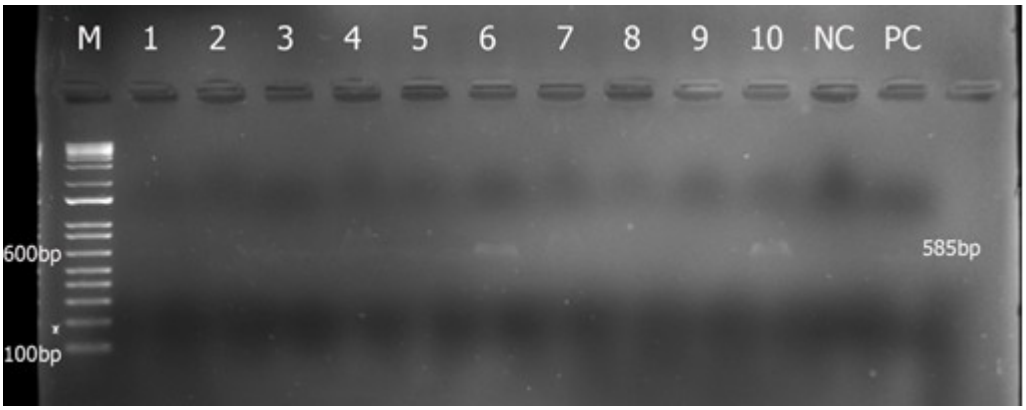

Figure 4: Shows amplified *CTX- M* in sample 6 and 10, M- 1kb Ladder, NC- Negative control and PC- Positive control

CHAPTER FIVE

5.0 DISCUSSION

The findings of the current study indicate that the overall frequency of *E. coli* isolated from both meat and cloaca swabs of broilers and spent layers was 55.2%. Contamination may have occurred during slaughtering and dressing processes and as well as poor hygienic condition of poultry slabs, they have been previously reported elsewhere to be the sources of contamination (Zhao *et al.*, 2012; Odwar *et al.*, 2014).

The findings of the antimicrobial susceptibility testing of *E. coli* isolates in this study showed that the majority of the isolates were resistant to tetracycline (91.9%), sulfamethoxazole-trimethoprim (80.5%), ampicillin (70.9%), ciprofloxacin (40.2%) and cefotaxime (25%). Furthermore, (69.3%, CI: 63.5 – 81.5) of the *E. coli* detected were resistant to three or more different classes of antibiotics namely; tetracycline, Ciprofloxacin ampicillin and sulfamethoxazole-trimethoprim.

The results are similar to findings from other studies conducted in Tanzania, which indicated high level of resistance rates to ampicillin, tetracycline (75.8%) and sulfamethoxazole-trimethoprim (62.3%) (Kissinga *et al.,* 2018; Rugumisa *et al.,* 2016; Hamis and Shahada, 2014). Furthermore, studies have also described high levels of resistance to the same antibiotics in *Campylobacter* species detected from poultry and other animal products (Nonga *et al.,* 2009; Kashoma *et al.,* 2016).

The trend of antibiotic resistance found in this study can be explained by the fact that tetracycline, ampicillin, sulfamethoxazole-trimethoprim and cefotaxime are commonly used antibiotics in poultry production in Tanzania as prophylaxis, growth promoters and in treatment of diseases (Hamis and Shahada, 2014; Rugumisa *et al.,* 2016; Kissinga *et al.,* 2018). Such antibiotics were easily accessible over the counters and sold cheaply to poultry and other livestock keepers (Mubito *et al.,* 2014; Lupindu *et al.,* 2014; Katakweba *et al.,* 2012). Poor dosage adherence by farmers, poor enforcement regulatory measures in use of these antibiotics also compound the problem of antimicrobial resistance in animal production in Tanzania, farmers ignorance of withdraw period (Mubito *et al.,* 2014; Kimera *et al.,* 2020), and the common practice were farmers/peasants themselves in treating their animals (Karimuribo *et al.,* 2005) and arbitrary drug combinations (Mmbando, 2004). Further exacerbate the problem of antibiotic resistance by microbial organisms.

In this study, ESBL were detected in 10 out of 212 (4.7%) isolates. The observed frequency was lower than 20.1% reported by Chishimba *et al*. (2016) in neighbouring Zambia. Among the ESBL producers *E coli* isolates reported in the current study, *CTX-M* was found in 2 of 10(20%) and plasmid mediated quinolone resistant gene (*qnrS*) was found in 8 out of 10 (80%) isolates. Of particular note is that AMR encoded by inherited

elements such as plasmids and transposons can quickly spread along the food chain and in other ecosystems (Salah *et al.,* 2019; Ben Sallem *et al.,* 2014; Sidiabat *et al.,* 2006) therefore, requiring strict monitoring, including avoiding the use of quinolones in poultry production.

During this study, unregulated waste disposal was observed in the five poultry slabs. This practice is likely to spread resistomes through contamination of the environment from the poultry effluence. The wastewater from the poultry slabs spreads in the environment through drainages (Graham *et al.,* 2019; Rousham *et al.,* 2018) and exposes other animals and human to AMR bacteria. Solid wastes eg. Faeces and liquid waste from slaughter poultry slabs like blood and waste water dressing of chicken carcases were disposed into Municipal drainage directly (Rousham *et al.,* 2018). This contributes to the risks of spreading of AMR in the environment (UNICEF and ICDDRB, 2015).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The high levels of antimicrobial resistance to antibiotics revealed in this study pose a major public health threat to humans, animals as well as the contaminating the environment. This situation calls for an immediate intervention from the public health, veterinary and environmental health authorities across the country to curb the public health threat of AMR. Therefore, need for understanding and advocacy of prudent use of antimicrobials use cannot be underscored in poultry production systems, in Tanzania.

6.2 Recommendations

It is recommended to improved animal biosecurity and vaccinations in poultry production at the farm level to lessen the occurrence of infections and the necessity to use excessive antimicrobial agents; furthermore it is being recommended to improve hygiene at poultry slaughter slabs to minimize contamination of poultry carcases during slaughter and processing.

It is important to enhance for public awareness and advocacy on proper processing and disposal of waste not only at farm level but also at the poultry slabs premises as well as strict regulation of animal waste disposal by both local and national public health authorities, particularly in urban and peri-urban settings of Tanzania if the fight against AMR is to be realized.

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APPENDICES

Appendix 1: Antimicrobial resistance pattern of *E. coli*

Antibiotic Combination	Number of resistant Isolates	%	Number of antibiotics resisted
TE, CN, CIP	1	0.7	3
TE, CN, SXT	1	0.7	3
TE, CIP, SXT	17	11.6	3
TE, CN, AMP	1	0.7	3
TE, IMP, SXT	3	2.0	3
TE, CIP, AMP	8	5.4	4
TE, CN, CIP, SXT	3	2.0	4
TE, IMP, AMP	1	0.7	4
TE, SXT, AMP	39	26.5	4
TE, CIP, IMP, SXT	1	0.7	4
CIP, SXT, AMP	1	0.7	3
TE, CN, SXT, AMP	4	2.7	4
TE, CIP, IMP, AMP	1	0.7	4
TE, CIP, SXT, AMP	29	19.7	4
TE, IMP, SXT, AMP	1	0.7	4
TE, CN, CIP, IMP, AMP	1	0.7	5
TE, IMP, CTX, AMP	1	0.7	4
TE, CN, CIP, SXT, AMP	4	2.7	5
TE, SXT, CTX, AMP	9	6.1	4
TE, CIP, IMP, SXT, AMP	3	2.0	5
TE, CN, SXT, CTX, AMP	3	2.0	5
TE, CIP, SXT, CTX, AMP	9	6.1	5
TE, CN, CIP, IMP, SXT, AMP	2	1.4	6
TE, IMP, SXT, CTX, AMP	1	0.7	5
TE, CN, CIP, SXT, CTX, AMP	2	1.4	6
TE, CN, CIP, IMP, SXT, CTX, AMP	1	0.7	7

Appendix 2: Data collection tool

Research title: Occurrence of Multidrug resistant *Escherichia coli* in raw chicken meat and cloaca swabs in slaughter slabs in Dar es Salaam, Tanzania

1. Name of poultry slab……
2. Type of chicken ☐ Broiler ☐ Spent layer
3. Type of specimen collected ☐ Cloaca swab ☐ Meat swab
4. Source of water used in the Slab ☐ Well water ☐ Tape water
5. Original source of chicken, (if known) ……
6. Estimated number of chickens slaughtered per day in each poultry slab……....
7. Number of poultry vendors per slab…….
8. Date of specimen collection……...

Appendix 3: Summary of primers

Gene	Primer set	Amplicon size	Reference
CTX-M	F: SCSATGTGCAGYACCAGTAA R: ACCAGAAYVAGCGGBGC	585 bp	Valat *et al.,* 2016
QnrA	F: TCAGCAAGAGGATTTCTCA R: GGCAGCACTATTACTCCCA	627 bp	Moawad *et al.,* 2017
QnrB	F: GGMATHGAAATTCGCCACTG R: TTTGCYGYYCGCCAGTCGAA	264 bp	Moawad *et al.,* 2017
QnrS	F: ATGGAAACCTACAATCATAC R: AAAAACACCTCGACTTAAGT	467 bp	Moawad *et al.,* 2017
aac (6')-Ib-cr	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGTTT	482 bp	Moawad *et al.,* 2017 Park *et al.*,2006