STATUS OF ANTIMICROBIAL RESISTANCE IN BACTERIA ISOLATED FROM KENYAN CHICKENS

TINO AYUL DENG AJAK (BSc. University of Juba)

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE MASTER OF SCIENCE DEGREE OF UNIVERSITY OF NAIROBI IN APPLIED MICROBIOLOGY (BACTERIOLOGY OPTION)

DEPARTMENT OF VETERINARY PATHOLOGY, MICROBIOLOGY AND PARASITOLOGY, FACULTY OF VETERINARY MEDICINE

UNIVERSITY OF NAIROBI

2017

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Sign..... Date..4/12/2017.....

TINO A. DENG (BSc)

This thesis has been submitted for examination with our approval as University supervisors:

Sign..... Date..4/12/17.....

PROF. LILLY C. BEBORA (BVM, MSc, PhD)

Department of Veterinary Pathology, Microbiology and Parasitology

Sign..... Date..7/12/2017.....

PROF. SAMUEL M. KARIUKI (BVM, MSc, PhD)

Center for Microbiology Research, Kenya Medical Research Institute (KEMRI)

Sign..... Date..4/12/2017.....

PROF. PETER K. GATHUMBI (BVM, MSc, PhD)

Department of Veterinary Pathology, Microbiology and Parasitology

Sign..... Date..4/12/2017.....

DR. GERALD M. MUCHEMI (BVM, MSc, PhD)

Department of Public Health, Pharmacology and Toxicology

DEDICATION

I dedicate this work to the soul of my late father Dr. Ayul Deng Ajak

My mother Nyabage Chol Deng

My brothers Oyenj Ayul, Banian Ayul, Amooj Ayul and Kimo Ayul

Thanks for the prayers, patience and Love

ACKNOWLEDGEMENTS

I would like to give thanks to God for His goodness and mercy endures forever; without The Almighty God, I wouldn't have been able to accomplish my studies.

Many thanks to my supervisors: Prof. Lilly C. Bebora, Prof. Samuel M. Kariuki, Prof. Peter K. Gathumbi and Dr. Gerald M. Muchemi for their valuable comments, guidance and support through this study. Special thanks to my lead supervisor Prof. Lilly C. Bebora for everything she has done to make sure this research becomes a success.

Thanks to Dr. Mahacla O. Odongo for his guidance and support. I appreciate all staff in the Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi for supporting me during the studies. I thank Mr. Ezekiel H. Weda, Mrs. Mary N. Mutune, Mrs. Lydia N. Maina, Mrs. Ann K. Munene and Mrs. Charity Gathenya for their assistance with the laboratory work.

Special thanks to my mother for her endless love, encouragement and inspiration throughout my studies. Much appreciation to my auntie Dr. Sarah O. Dak for her love and support. I would like to thanks my friends, George Otieno, Jackson Mushiri and Jasinta Mamur for their moral and technical support.

I would also like to thank the German Academic Exchange Service (DAAD) for awarding me a scholarship, without which this research would not have been possible. Many thanks to Prof. Samuel M. Kariuki, Mr. John N. Kiiru, Mr. Ronald Ngetich and Mr. Samuel Njoroge of the Center for Microbiology Research, Kenya Medical Research Institute (KEMRI) for technical support in molecular analysis.

May God bless you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ACMSF	Advisory Committee on the Microbiological Safety of Food
AFLP	Amplified Fragment Length Polymorphism
AIDS	Acquired Immune Deficiency Syndrome
ABR	Antibiotic Resistance
AMR	Antimicrobial Resistance
ATCC 25922	American Type Culture Collection 25922
CDC	Center for Disease Control and Prevention
CFLP	Cleavase Fragment Length Polymorphism
CLSI	Clinical and Laboratory Standards Institute
CTX-M	Cefotaxime Hydrolyzing Capabilities
dfr	Dihydrofolate Reductase gene
DNA	Deoxyribonucleic Acid
E.coli 0157:H7	Enterohemorrhagic *Escherichia coli*
EAggEC	Entero-aggregative *Escherichia coli*
EDTA	Ethylene Diamine Tetra Acetic Acid
EIEC	Enteroinvansive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ESBL	Extended Spectrum β-Lactamases
ETEC	Enteroxigenic *Escherichia coli*
FAO	Food and Agriculture Organization
GARP	Global Antimicrobial Resistance Partnership
GES	Guiana Extended-Spectrum

GLASS	Global Antimicrobial Resistance Surveillance System
IBC	Integron-prone Cephalosprinase
LSD	Least Significant Difference
MDR	Multidrug Resistance
MHA	Muller Hinton Agar
MIC	Minimum Inhibitory Concentration
MLC	Minimal Lethal Concentration
MRSA	Methicillin Resistant *Staphylococcus aureus*
OIE	Office International des Epizooties (World Organization for Animal Health)
OXA	Oxacillin Hydrolysing Capabilities
PBPs	Penicillin Binding Proteins
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
RAPD	Application of Random Amplified Polymorphic DNA assays
Rep-PCR	Repetitive extragenic palindromic Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
RT-PCR	Reverse Transcriptase PCR
SHV	Sulfhydryl Variable
TEM	Temoneira
TLA	Tlahuicas Indians
TMP	Trimethoprim
UNGA	United Nations General Assembly

VEB	Vietnam Extended-Spectrum β-Lactamase
VTEC	Verototoxin-producing *Escherichia coli*
WHO	World Health Organization

ABSTRACT

The term antimicrobial resistance refers to the ability of microorganisms to grow in the presence of an antimicrobial (drug) at a concentration that would normally kill them or inhibit their growth. Antimicrobial resistance has become a big threat to global health; having risen to dangerously high levels in all parts of the World, making it difficult to treat infectious diseases. This is forcing patients to incur extra expenses as they have to buy more expensive second-generation or third-generation medicines. Also, as a result of medicines not being effective, patients are forced to stay longer in hospitals; this translates to higher hospital bills.

In an effort to establish the antimicrobial resistance status of bacteria isolated from chickens, a cross-sectional study was conducted to demonstrate the antimicrobial resistance profiles of bacteria isolated from three groups of chickens [sick (clinical), farm and slaughter]. The three chicken groups were studied so as to determine whether there are any differences, with respect to antimicrobial resistance, between them. Intestinal swabs were taken from the first 50 chickens brought to the clinic (for post mortem examination) during the study period, while, for farm and market (slaughterhouse) categories, cloacal swabs were randomly taken from a total of 122 birds. Bacteriological isolation and characterization was then carried out, using the conventional methods, and six genera were identified; the most prevalent being organisms of the genus *Streptococcus* (40.7%), followed by *E. coli* (31.4%), then *Staphylococcus* (26.2%), *Bacillus* (9.3), *Proteus* (2.9%). The least isolated were in the genus *Corynebacterium* (2.3%). Due to financial constraints, while all the *E. coli* isolates were tested for antibiotic susceptibility/resistance, only a few of the other bacterial isolates were tested, using the 8 antibiotics supplied by HiMedia (HiMedia Laboratories-INDIA). Overall, the study demonstrated existence of antimicrobial resistance, both single and multiple (some up to 7

antimicrobials), in the tested bacteria. The antimicrobial resistance was mostly towards the commonly-used antibiotics, namely: ampicillin (76.0%), tetracycline (71.1%), sulphamethoxazole (69.5%) and co-trimoxazole (65.5%). They were least resistant to Gentamycin (8.3%). The study also showed that, overall, a higher percentage of *Escherichia coli* isolates demonstrated multi-drug resistance compared to the other isolates. When comparing the three study groups, the general picture indicated higher multidrug resistance prevalence in bacteria isolated from clinical cases, followed by market birds (Table 3.3). It was, however, encouraging that there were some bacterial strains that were still susceptible to the commonly-used antimicrobials

The resistant *E. coli* isolates were further tested for carriage of antimicrobial resistant genes; three Extended-spectrum β-lactamase (ESBL) - coding genes: bla_{TEM}, *dfrA1* and $bla_{\text{CTX-M,}}$ using multiplex Polymerase Chain Reaction. Only 3 (10.7%) of the 28 isolates tested had the *dfrA1*gene; none carried the $bla_{\text{CTX-M}}$ and bla_{TEM}. This showed that 25 (89.3%) of the tested resistant *E. coli* isolates utilised other means to express their antimicrobial resistance. Results from the two studies will thus contribute towards data on current antimicrobial resistance status in bacteria harboured by chickens in Kenya, which will help in informing the policy makers as they embark in their fight towards reduction of antimicrobial resistance.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background information

Antimicrobial resistance (AMR) has become a big threat to global health. It has risen to dangerously high levels in all parts of the World, making it difficult to treat infectious diseases (GEN, 2010; Maron 2016; Parovic and Schultz, 2016). This is forcing patients to incur extra expenses as they have to buy more expensive second-generation or third-generation medicines. Also, as a result of medicines not being effective, patients are forced to stay longer in hospitals; this translates to higher hospital bills (OIE, 2015; WHO, 2015$_{a, b}$). The higher rate of development of antimicrobial resistance (AMR) has attracted the attention of international bodies such as WHO, FAO, OIE, who have now forged a united approach to combat it as a common force (Maron, 2016; Perovic and Schultz, 2016; Teale and Moulin, 2012). The situation seems so dire that it is now estimated that, worldwide, 700,000 patients die annually as a result of resistant infections. If nothing is done to combat AMR, the death rate is estimated to escalate to 10 million annually by the year 2050 (O'Neill, 2016).

Antimicrobial are also used in animals to treat animal diseases and also as growth promoters in an effort to increase productivity. It needs to be appreciated that, though rated second to humans, animals are important to the well-being of the humans; they contribute to their nutrition, wealth, status and also serve as their "banks" – to be sold whenever the owners face financial difficulties. Therefore, as the prevalence of AMR increases, livestock farmers lose sick animals from treatment failure. Thus, they are tempted to use more effective and often more expensive antimicrobial; ending-up infringing upon those that are last line options for use in humans; especially if they can easily be bought over the counter (OIE, 2015). This ends-up in the development of resistance to the few antimicrobials that are relied on.

One health concept was embraced when it was realized that most bacteria that are pathogenic to humans come from animals. In fact, it has been quantified that about 60% of human bacterial pathogens are shared between animals and humans (OIE, 2015). Since the same antibiotics are used to treat diseases in humans and animals (OIE, 2015; GEN, 2010), the war on AMR in humans cannot be won without launching a parallel war in animal health; that is: addressing AMR in animals is just as important as in humans. This also includes use of AMR in agriculture and fisheries. However, in order to tackle AMR, its current situation needs to be known. Thus there is need to carry out routine surveillance in order to monitor reduction of antibiotic use and subsequent reduction of AMR (WHO, 2015_b). In Kenya, as in most developing countries, it is difficult to get a complete picture of the AMR situation as antibiotic susceptibility testing is not done routinely. It is only done on specific requests and specific researches on AMR are minimal and scattered (personal observation); there is therefore need of researching and consolidating the data. As part of data collection, this study was undertaken to establish the extent of antimicrobial resistance in Kenyan chickens.

1.2 Objectives

1.2.1 Overall objective

To determine extent of antimicrobial resistance in bacteria isolated from Kenyan chickens

1.2.2 Specific objectives

1. To establish antimicrobial resistance profiles of bacteria isolated from sick (clinical), farm and market chickens in Nairobi, Kenya.
2. To detect whether the *E. coli* isolates carried bla_{TEM}, *dfrA1* and $bla_{\text{CTX-M}}$ antimicrobial resistant genes

1.3 Hypothesis

1. Bacteria isolated from Kenyan chickens are resistant to commonly-used antimicrobials
2. *Escherichia coli* isolates from the chickens carry bla_{TEM}, *dfrA1* and $bla_{\text{CTX-M}}$ antimicrobial resistant genes

1.4 Justification of study

Much as a number of studies on antimicrobial resistance (AMR), which is mostly antibacterial resistance (ABR), have been carried out in Kenya and a number of them have been published, including reviews by Mitema *et al.* (2004) and Kariuki *et al.* (2010; 2016), it is believed that the consolidated situation analysis is not exhaustive, especially with respect to animals. Compounding the situation is the fact that, despite all the studies carried out on antimicrobial resistance, the scourge is still on, either at the same level or higher (GEN, 2010; Maron, 2016; Parovic and Schultz, 2016). There is, therefore, need for more data generation so as to have a broad baseline picture of the current situation of AMR in bacteria isolated from animals. This study focused on chickens because they are kept by many Kenyans; there is also a high tendency of using antimicrobials when the chickens are kept under intensive farming system. Due to the close relationship between man and chicken, there is possibility of resistant bacteria in chickens finding their way to humans, thus pass the resistance traits to the human bacteria; not to mention that some of the chicken bacteria can cause severe illness in man. The results of this study will contribute towards establishment of the AMR status in Kenya and formulation of intervention criteria for reduction of antimicrobial resistance locally and, by extension, internationally.

CHAPTER 2: LITERATURE REVIEW

2.1 Antimicrobials and antimicrobial resistance

The term "antimicrobial" refers to drugs/medicines used to treat all types of microorganisms such as bacteria, viruses, parasites, or fungi while the term "antibiotic/antibacterial" refers to drugs/medicines used to treat bacteria (www.reactgroup.org cited 2017 Jan 28). The term "antimicrobial resistance" refers to the ability of microorganisms to grow in the presence of an antimicrobial (drug) at a concentration that would normally kill them or inhibit their growth (www.reactgroup.org cited 2017 Jan 28). However, since antibacterials form a major fraction of antimicrobials, the two terms are mostly used interchangeably, which will be the case in this study. Effective antimicrobial drugs are essential for both preventive and curative measures, protecting patients from potentially fatal diseases and ensuring that complex procedures, such as surgery, can be provided at low risk (www.reactgroup.org cited 2017 Jan 28).

Antimicrobials are essential for human and animal health, but need to be used cautiously. Food animals (including poultry and fish) are important to human welfare. Thus, animal health is important in two ways: (1) to improve animal welfare, which translates to improved productivity and economic status for the farmer, thus contribute towards food security and (2) to ensure food safety, since it is estimated that over 60% of bacteria that are pathogenic to humans are from animals/animal products (OIE, 2015), The major problem, with respect to development of antimicrobial resistance, is based on the fact that same drugs/medicines are used in both humans and animals (de Souza and Hidalgo, 1997; GEN, 2010; OIE, 2015), for treatment and prophylaxis, and a large percentage of bacteria are shared between the two groups. Prudent use of antimicrobials in animals is therefore, important as it will control the transfer of bacterial antimicrobial resistance between animals and humans (Mitema *et al.*, 2001). This means that when resistance occurs in animals, there is a high chance that it will

get to the humans; either indirectly, via the food chain, or directly from the animal (Helmuth and Hensel, 2004) – the *vice versa* is also possible, leading to a cycle of transmission - human to animals and back to humans (WHO, 2015_b). Indiscriminate usage of antimicrobials, for example, as growth promoters in veterinary medicine (Hart *et al*., 2004) should be discouraged. Antimicrobials should not be used to offset the shortcomings of poor management or insufficient hygiene standards in farms – i.e. antimicrobials should not be a substitute for efficient management or good husbandry – when good management or good husbandry is implemented all the time, there will be no need to give untargeted antimicrobial cover (OIE. 2010; O'Neill, 2016). In cases of antimicrobial resistance, the resultant food-borne or animal-acquired illness in humans will be less responsive to treatment with respect to antimicrobial drugs (Fair and Tor, 2014).

2.2 Global approaches/concerns to antimicrobial resistance

Antimicrobial resistance is a major global challenge and it is of particular concern in developing countries. It is rising to dangerously high levels in all parts of the world (GEN, 2010; Maron, 2016; Parovic and Schultz, 2016), compromising the ability to treat infectious diseases and undermining many advances in health and medicine. When the common antimicrobials are no longer effective, patients are forced to use newer antimicrobials which are more expensive. This becomes worse in hospitalization cases, where patients end-up staying longer in hospitals due to the antimicrobial(s) not working or having less effect. In such circumstances, the disease burden may be tremendously increased (OIE 2015; WHO $2015_{a,\ b}$). Management of infectious diseases, e.g. gastrointestinal, respiratory, sexually transmitted bacterial diseases, and hospital - acquired infections has been compromised to some extent by the appearance and spread of antimicrobial resistance. The Global Antimicrobial Resistance Partnership (GARP) is currently involved in campaigns to try and

slow-down the spread of resistance without impairing access to antimicrobials , when required (Kariuki, 2011).

Public health significance of the transmission of resistant bacteria from animals to humans has been addressed in several international meetings (de Souza and Hidalgo, 1997). At one meeting it was recommended, *inter alia,* that the use of antimicrobials as growth promoters in production animals should be discontinued. This is especially important if the same antimicrobial or class of antimicrobials are used for human therapeutics or known to select for cross-resistance to antimicrobials used in human medicine (de Souza and Hidalgo, 1997; OIE, 2015; GEN, 2010). At another meeting, it was agreed that there was an urgent need to develop prudent guidelines for antimicrobial use in food-producing animals and that the indiscriminate use of fluoroquinolones must be discouraged (Tovey *et al.,* 2010). A European Scientific Conference entitled 'The use of antibiotics in animals, ensuring the protection of public health' focused on implementing strategies and actions to control and reduce the possibility of antibiotic resistance occurring subsequent to use of antibiotics in animals (Vuuren, 2001).

World Health Organization (WHO) is leading a global campaign towards reduction of antimicrobial use, which will consequently result in a reduction in antimicrobial resistance. In its report of year 2014 (WHO, 2014) on global surveillance of antimicrobial resistance, it revealed that "antimicrobial resistance is no longer a prediction for the future; it is happening right now across the world, and that it is putting at risk the ability to treat common infections in the community and hospitals". WHO warned that, without urgent co-ordinated action, the world is heading towards a post-antibiotic era in which common infections and minor injuries, which have been treatable for decades, can once again kill. Currently, WHO is working closely with other world bodies – Food and Agriculture Organization (FAO) and

World organization for Animal Health (OIE) to address antimicrobial resistance. In fact, in year 2015, a tripartite agreement between WHO, FAO and OIE was signed campaign to achieve the following objectives were: (1) To make antimicrobial resistance a globally-recognized health issue, (2) To raise awareness of the need to preserve the power of antimicrobials through appropriate use, (3) To increase the recognition that individuals, health and agriculture professionals, and governments must all play a role in tackling antimicrobial resistance, and (4) To encourage behavior change and convey the message that simple actions can make a difference (WHO, 2015_a; WHO, 2015_b).

On 21st September 2016 United Nations General Assembly (UNGA) reported that all the 193 members of the United Nations signed a landmark agreement promising to tackle drug resistant infections (the super bugs); recognizing that antimicrobial resistance is one of the biggest threats to global health (UNGA, 2016). The WHO action plan (WHO, 2015_a) underscores the need for effective "One health" approach involving coordination among numerous international sectors and actors, including human and veterinary medicine, agriculture and fisheries. The Global Antimicrobial Resistance Surveillance System (GLASS) manual (WHO, 2015_b) emphasizes on the need for continuous surveillance of AMR so as to establish the current status and to follow-up the trends for improvement. The current study is based on this and is geared towards establishing the current status of AMR in Kenyan chicken.

One of the Global action plans of the Objective One is to "improve awareness and understanding of antimicrobial resistance through effective communication, education and training". As part of respective activities two AMR awareness weeks were set aside – the first on 16-22nd November 2015; the second on 14-20th November 2016. Many activities marked the weeks, including launching of the week's activities, media outreach, engagement with the

public through social media and local-awareness – raising events around the World. Kenya was fully engaged in both occasions. Partners such as UN agencies; Ministry of Health and Ministry of Agriculture, Livestock and Fisheries; non-governmental organizations; human and animal health professional groups; and others were involved. Talks to various professional groups – doctors, pharmacists, veterinarians, animal health assistants took place. Various posters tailor-made for various groups – doctors, veterinarians, farmers have been produced. All these were geared towards sparking mind changes to ensure antimicrobials are used only when necessary and as prescribed by a health professional.

In line with the global agreement, and as a collaboration between the Ministry of Health, Ministry of Agriculture, livestock and Fisheries; facilitated by international bodies (WHO, FAO, CDC, OIE), Kenya has completed preparation of the National AMR Policy, AMR Action Plan and AMR Surveillance Plan. All this would not have been possible without the support of the Kenyan Government.

2.3 Status of antimicrobial resistance in Kenya

Literature review on AMR in Kenya has identified four study categories: **Category one** includes AMR demonstrated in bacteria isolated from animals. It included studies by Bebora *et al*. (1989) who studied *Salmonella* Gallinarum isolated from chicken; Bebora *et al*. (1994) who studied *E. coli* isolated from chicken; Ombui *et al*. (2000) who studied *Staphylococcus aureus* isolated from milk and meat; Njagi (2003) who studied *Listeria* isolated from chicken; Mapeney *et al*. (2002) who studied *E. coli* isolated from pigs, chickens and cattle; Gakuya *et al*. (2007) who studied bacteria isolated from rats; Kikuvi *et al*. (2007a) who studied *E. coli* isolated from cattle, pigs and chicken; Kikuvi *et al*. (2007b) who studied *Salmonella* isolates from slaughtered pigs; Allorechtova *et al*. (2012) who studied *E. coli* from dogs. About 67% of these studies were based on phenotypic profiling, using diffusion

technique; other techniques used were: plasmid finger-printing and Pulsed Field Gel Electrophoresis (FPGE) banding patterns. The antimicrobials variably studied included: nitrofurantoin, gentamycin, chloramphenicol, tetracycline, ampicillin, furazolidone, neomycin, co-trimoxazole, erythromycin, nalidixic acid, streptomycin, sulfamethoxazole, ampicillin, trimethoprim, kanamycin, penicillin, augmentin, sulphonamides, doxacillin, lincomycin, minocycline, methicillin, cefuroxime, apramycin, cefotaxime, cephradine, co-amoxyclav, ciprofloxacins. The test bacteria showed varying degrees of AMR; all studies recording aspects of multiple drug resistances; some to over six antimicrobials (Ombui *et al*. 2000; Gakuya *et al*. 2007; Kikuvi *et al*. 2007a).

Category two includes AMR demonstrated by bacteria isolated from the environment and othe sources. It included studies by Wambugu *et al* (2015) who studied on *E. coli* isolated from Athi river in Machakos County, and Kutto (2012) who worked on *Salmonella* isolated from kale leaves. Both used diffusion technique. The antimicrobials variably studied included: ampicillin, amoxicillin, cefoxin, sulfamethoxazole, tetracycline, cefpodoxim, aztreonam, nalidixic acid, ceftazidime, ciprofloxacin. chloramphenicol, cetepime, gentamycin, cefriazone, cefuroxime, ampicillin-cloxacillin, trimoxazole, erythromycin, penicillin. The test bacteria demonstrated varying degrees of AMR; they also demonstrated aspects of multi-drug resistance – some bacteria resistant to up-to seven antimicrobials.

Category three includes AMR demonstrated by bacteria isolated from humans. It includes studies by: Kariuki *et al* (1996) who studied non-typhi salmonellae isolated from patients in Kenya; Bururia (2005) who worked on *Klebsiella* isolated from urinary and non-urinary isolates from patients at Kenyatta National Hospital; Kariuki *et al* (2006) who studied non-typhoidal salmonellae from children presenting with fever; Kariuki *et al* (2007) who studied *E. coli* from community-acquired urinary tract infections; and Oundo *et al* (2008) who studied entero-aggregative *E. coli* isolated from food handlers. The antimicrobials used in

these studies were similar to those used under Category two. The researchers also used diffusion technique and demonstrated presence of AMR in the study bacteria – some of which manifested multi-drug resistance.

Reviews on situation of AMR in bacteria in Kenya have also been published by Mitema, 2010 and Kariuki, 2011; they gave an overview of AMR in both human and animal bacteria.

2.4 Scope of antimicrobial resistance in bacteria

Microbial resistance to antimicrobials emerged soon after the first use of these agents in the treatment of infectious diseases; the problem seems to continue to date (GEN, 2010; Maron, 2016; Parovic and Schultz, 2016), posing a challenge in the health sector. Resistance, which was once primarily associated with health care institutions, is now widely distributed within communities (Wright, 2011). The dynamic profiles of risk factors associated with antimicrobial resistance have greatly contributed to the worsening of condition in the World (OIE, 2015). Since the fight against antimicrobial resistance is of the global significance (Maron, 2016; Perovic and Schultz, 2016; Teale and Moulin, 2012), it is important for each country to establish its current status, and also carry out continuous surveillance to follow-up the trend, as the fight continues. It is difficult to assess the extent of antimicrobial resistance in Kenya because antimicrobial susceptibility tests are not run routinely, and where done, it has been mainly at the national levels (e.g., referral and private hospitals/laboratories), with limited sharing of information and data analysis (personal observation). The changing status of antimicrobial resistance should, therefore, be strategically and continuously monitored to update the prevailing situation and inform the mitigation measures (WHO, 2015_b). The current study endeavored to establish the status of AMR in bacteria isolated from chickens.

2.5 Modes of action of antimicrobials

Based on the fact that antimicrobials act on bacteria by inhibiting bacterial cell metabolic pathways, antimicrobials can be divided into five classes, with respect to their modes of action: (1) cell wall inhibitors, such as beta-lactams (cephalosporins, penicillin), carbapenems (imipenem), and glycopeptides (vancomycin); (2) protein synthesis inhibitors, such as aminoglycosides (streptomycin, gentamicin), tetracyclines and chloramphenicol; (3) nucleic acid synthesis inhibitors, such as fluoroquinolones, which inhibit nucleic acid (DNA) synthesis, and rifampin, which inhibits RNA synthesis; (4) anti-metabolites, such as the sulfonamides (trimethoprim, methoxazole); and (5) cell membrane inhibitors, such as polymyxin B, gramicidin and daptomycin (Woodin and Morrison, 1994).

2.6 Mechanisms of antimicrobial resistance in bacteria

Antimicrobial resistance is the ability of bacteria to grow in presence of an antimicrobial to which it was previously susceptible; this antimicrobial resistance may be by intrinsic resistance (an inherent possession of resistance to the antibiotic(s) or acquired resistance (one which is a consequence of mutational events or gene acquisition via horizontal gene transfer, namely: transformation, conjugation, transposition and transduction). Four general mechanisms leading to acquired antimicrobial resistance have been described: (1) decreased uptake of the antimicrobial into the bacterial cell; (2) increased extrusion of the antimicrobial by bacterial efflux pump; (3) mutational modification of the antimicrobial's target and; (4) production of antimicrobial-inactivating enzymes (Georgios *et al.*, 2014) Acquisition of antimicrobial resistance can be as a result of mutation in chromosomal genes or acquisition of plasmid and mobile genetic elements such as transposons and integrons, which carry the antimicrobial resistance genes (Barabra *et al.*, 2006).

2.7 Predisposing factors for development of antimicrobial resistance

The main drivers of antimicrobial resistance are: (1) over the counter medication/access to medicines, (2) counterfeit drugs, (3) under-dosing [due to (a) lack of resources, (b) lack of knowledge)], (4) indiscriminate use [in (a) humans, (b) animals)], (5) not observing the recommended withdrawal period, (6) quacks and (7) high cost of genuine drugs (Laxminarayan and Chaudury, 2016). Antimicrobial resistance in animals can be transferred to humans, while humans can also be a source of antimicrobial resistance for animals; hospital setting is also conducive for development of antimicrobial resistance (WHO, 2012).

2.8 Detection of antimicrobial resistance/susceptibility in bacteria

Antimicrobial resistance can be detected using either phenotypic or molecular methods as given below.

2.8.1 Phenotypic methods

Phenotypic methods are techniques used to demonstrate metabolic, physiological and biochemical characteristics of the respective microorganism e.g. disc diffusion technique, double disc synergy technique, Imipenem-EDTA synergy technique, boronic acid technique, Hodge technique, combination meropenem disc technique, which is mainly used in biological researches (Weatherall, 2001). Phenotypic methods are used in the daily laboratory practice in order to identify the antimicrobial susceptibility/resistance status among frequently isolated nosocomial pathogens (Georgios *et al.*, 2014). Reproducibility is especially important for the construction of reliable information containing all strains within a species to which unknown organisms can be compared for classification. Variable expression of phenotypic characteristics, such as sporadic expression of resistance genes, can contribute to problems with reproducibility (Arbeit, 1995).

2.8.1.1 Diffusion technique

The Disc Diffusion Technique is one of the methods of antimicrobial susceptibility testing, manifested by inhibition of growth of the bacterium in Mueller Hinton Agar; it is commonly used for *E. coli* and *Staphylococcus aureus.* It is also known as a Kirby Bauer method (Bonev *et al*., 2008). Determination of bacterial sensitivity to antimicrobials is essential for the accurate management of bacterial infections and for comparative analysis of antimicrobial resistant agents.

Disc diffusion technique, using Mueller Hinton Agar (MHA), is commonly used for determination of antimicrobial resistance. The study bacterium is streaked onto the medium to produce confluent growth. Antimicrobial-impregnated discs are placed onto the streaked agar and incubated overnight at 37^{o} C. Antimicrobial diffuses from these discs into the medium, inhibiting growth of susceptible bacteria; this manifests as clear 'zones' within the bacterial lawn of growth. The size/diameter of respective inhibition zone is directly proportional to concentration of the tested antimicrobial (Bonev *et al*., 2008). The zone diameter can be measured using a ruler, and interpretation of the results done according to Clinical and Laboratory Standards Institute (CLSI, 2008) [formerly known as the National Committee for Clinical Laboratory Standards (NCCLS)], where 10 mm or less is taken as resistance, 11-18 mm is taken as intermediate and over 19 mm is taken as susceptible.

2.8.1.2 Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest antimicrobial concentration which inhibits the growth of bacteria. In broth dilution test, the MIC is determined by adding various dilutions of the test antimicrobial into respective tubes containing broth culture of the same bacterial type and concentration; growth (in form of turbidity) read after overnight incubation. The highest dilution (which contains the minimum antimicrobial concentration) indicating no growth is taken as the MIC for the particular antimicrobial, with respect to the

particular bacterium (Carson *et al*., 2002). Antimicrobial minimum inhibitory concentration can also be determined using other techniques, which include: the disc diffusion method (when dilutions of the same antimicrobial are used) and the E-test. Also, in addition to testing for effectiveness of an antimicrobial through assessment of MIC, which may measure the ability of an antimicrobial to inhibit bacterial growth, one can do it through minimal lethal concentration (MLC), which measures the antimicrobial's ability to kill the bacterium. Disc diffusion and E-test are usually done on solid media (Mueller Hinton Agar), whereas broth dilution assays can be carried-out using any of the methods described by different researchers (Mishra *et al*., 2006; Macias *et al*., 1994; Lang and García, 2004).

Determination of MIC includes a semi-quantitative test procedure which gives an approximation to the minimum concentration of an antimicrobial needed to inhibit microbial growth. Using a semi-automated microtitre method, where the turbidity of the test compound interferes with the test, indicators can be used for the determination of the endpoint or minimal concentration of antimicrobials (Lambert and Pearson, 2000).

2.8.2 Genotypic methods

2.8.2.1 Definition and Examples

Genotypic methods are techniques used to identify the genetic make-up of resistant strains of a microorganism (Weatherall, 2001) for example Pulsed-field gel electrophoresis of whole chromosomal DNA, Southern blotting and Restriction fragment length polymorphism (RFLP), PCR-based locus-specific Restriction fragment length polymorphism (RFLP), Application of random amplified polymorphic DNA(RAPD) assays, Repetitive sequence-Based PCR (Rep-PCR), Cleavase fragment length polymorphism method (CFLP), Amplified fragment length polymorphism (AFLP) assays and DNA sequencing (Olive and Bean, 1999). Many of the researchers using genotypic techniques for typing rely on electrophoretic

separation of DNA fragments of different molecular lengths; the fragments appearing as precipitin bars within the gel. Since these patterns may be extremely complex, the ease with which the fragments are interpreted and related is a factor in evaluating the utility of a particular typing method (Arbeit, 1995).

2.8.2.2 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is a molecular biology technique used to amplify a single or a few copies of genetic information (DNA) to generate thousands of millions of copies of the particular DNA sequence (Joshi, 2010). The development of PCR was by the American biochemist, Kary Mullis in 1984. However, the basic principle of replicating a piece of DNA using two primers had already been described by Gobind Khorana in 1971(Bartlett and Stirling, 2003). Polymerase Chain Reaction is a common technique used in medical and biological research laboratories for a variety of applications, including antimicrobial sensitivity testing (Kleppe *et al*., 1971).

Polymerase Chain Reaction is closely designed after the natural nucleic acid (DNA) replication process (Saiki *et al*., 1985). To start DNA amplification by using two primers of DNA molecules, these primers hybridize and exchange to opposite strands of the DNA to serve as initiation sites for the synthesis of new DNA strands. An enzyme, Taq DNA polymerase, is used as a catalyst for this synthesis. In PCR technique, there are three major steps involved: denaturation of primer at 94-96 ºC, annealing of primer at 45-60 ºC, and extension of primer usually at 72 ºC (Joshi, 2010). The resultant fragments can then be separated and visualized by gel electrophoresis. Polymerase Chain Reaction assays are used for the detection of genes for ampicillin resistance (bla_{TEM} and bla_{PSE}), tetracycline resistance (*tet(A), tet(B), tet(C),* and *tet(H)*), chloramphenicol resistance (*catA1*, *catA3*, and *cmlA*), and streptomycin resistance (*strA* and *aadA1*) using specific primers. The plasmids and PCR products are detected by electrophoresis in 0.8 % and 1.5% agarose gels, respectively (Kikuvi

et al., 2010). There are different types of PCR that can be used, namely: Nested PCR, Real Time PCR, Reverse Transcriptase PCR (RT-PCR), Multiplex-PCR, and Semi-quantitative PCR (Rodriguez and Ramirez, 2012).

2.9 Extended – spectrum beta-lactamases

Extended-spectrum β-lactamases (ESBLs) break down third and fourth-generation cephalosporins and monobactams, as well as the earlier generation cephalosporins and penicillins. Extended-spectrum β-lactamases have been discovered in many different genera of *Enterobacteriaceae* and *Pseudomonas aeruginosa*. However, they are mostly present in *Escherichia coli* and *Klebsiella pneumoniae*. Extended-spectrum β-lactamases are plasmid mediated and are inhibited by β-lactamase inhibitors such as clavulanic acid. There are four main types of ESBLs: bla_{SHV}, bla_{TEM}, bla_{OXA} and $bla_{CTX\text{-}M}$ (http://www.lahey.org/studies/webt.asp cited 2017 Jan 30).

The bla_{SHV} and bla_{TEM} derived enzymes, first secluded in Western Europe in the mid-1980s, are mainly in *Klebsiella* spp., followed by *E. coli*. These enzymes are capable of hydrolysing broad spectrum cephalosporins and monobactams but are inactive against cephamycins and imipenem. Enzyme TEM-1 is the most commonly expressed β-lactamase in Gram-negative bacteria. Up to 90% of ampicillin resistance in *E. coli* is due to the production of $bla_{TEM\text{-}1}$. This enzyme is also accountable for the ampicillin and penicillin resistance that is seen in *Hemophilus influenzae* and *Neisseria gonorrhoeae* in increasing numbers. Enzyme TEM-1 is also capable of hydrolysing penicillins and first generation cephalosporins such as cephalothion and cephaloridine. Enzyme TEM-2, the first derivative of $bla_{TEM\text{-}1}$, had a single amino acid replacement from original β-lactamase (Chaudhary and Aggarwal, 2004). Enzyme SHV-1 is most commonly found in *Klebsiella pneumoniae* and it accounts for up to 20% of the plasmid mediated ampicillin resistance in this species (Bradford, 2001). Analysis of

bla$_{SHV-2}$ gene showed that it was the outcome of a point mutation in the SHV-1 gene, which resulted in an amino acid modification from glycine to serine at position 238 (Sougakoff *et al.,* 1988). Enzyme CTX-M is fundamentally found in strains of *Salmonella enterica*, subspecies 1 *serovar typhimurium* and *E. coli*, but has also been described in other species of *Enterobacteriaceae*. They contain CTXM-type enzymes: *bla*$_{CTX-M-1}$ (formerly called MEN-1), *bla*$_{CTX-M-2}$ through to *bla*$_{CTX-M-10}$. These enzymes are not closely related to *bla*$_{TEM}$ or *bla*$_{SHV}$ in that they show only approximately 40% identity with these two generally isolated β-lactamases (Tzouvelekis *et al.,* 2000). The OXA-type enzymes are another rising family of ESBLs. These β-lactamases vary from the *bla*$_{TEM}$ and *bla*$_{SHV}$ enzymes in that they belong to molecular class D and functional group 2d. The OXA-type β-lactamases mediate resistance to ampicillin and cephalothion and are characterized by their high hydrolytic activity against oxacillin and the fact that they are poorly prevented by clavulanic acid (Bradford, 2001).

2.10 Bacteria that inhabit the gastrointestinal tract (GI) of chickens

The gastrointestinal tract of chickens contains several bacteria, both aerobic and anaerobic; the aerobic ones including: *Staphylococcus* spp, *Streptococcus* spp, *Campylobacter* spp, *Salmonella* serotypes, *Listeria* and coliforms (*E. coli, Klebsiella, Enterobacter*) (Chopra and Roberts, 2001). These bacteria tend to occur as commensals but some of them, for example: *Escherichia coli, Campylobacter, Listeria* and *Salmonella* spp, are of public health importance – they can cause disease in humans, depending on their pathogenicity and the number and concentration of bacteria/dose (FAO, cited Oct 12, 2017). They are normally associated with gastro-intestinal upsets, causing diarrhoea, but sometimes they can become septicaemic (Abbas and Newsholme, 2009)

Most antimicrobial susceptibility studies are done using *Escherichia coli* because they are the most prevalent commensal enteric bacteria in both animals and humans and are also important zoonotic agents that can be implicated in both animal and human infectious

diseases (Costa *et al*., 2010). They can be taken as a good microbial indicator of the potential presence of disease caused by bacteria and also show the general sanitary quality of the food since they are closely associated with fecal contamination (Costa *et al*., 2010); they are also easy to grow. *Escherichia coli* would, therefore, easily serve as a representative for the other bacteria within the same environment.

2.11 Usage of chicken as the study animal

Chickens are preferred as study animals by many researchers because they are small and easy to handle. They are cheap to acquire and also kept by many Kenyans, including the resource-poor ones in villages; thus, if antimicrobial resistance develops in chicken's bacteria, the chances of the resistant bacteria getting to humans are high. This is more so considering the way humans handle and intermingle with chickens, especially in the rural areas. There is also a lot of abuse of antimicrobials in chicken farms as growth promoters for increasing egg-production; popular growth formulae on the market are: Egg formula, chick formula, growth formula; which contain vitamins and other substances, e.g. antibiotics; not to mention indiscriminate uses by unprofessional persons, including respective farmers, when chickens fall sick (Landers *et al*., 2012). It will, therefore, be interesting to determine the antimicrobial resistance patterns of bacteria carried by chickens. This study used chicken as its study animal

CHAPTER 3: DETERMINATION OF ANTIMICROBIAL RESISTANCE PROFILES OF BACTERIA ISOLATED FROM CHICKEN

3.1 INTRODUCTION

In order to establish the current status of AMR in animal bacteria, it is necessary to carry out several surveys on bacteria isolated from various animals, including chickens and fish. This, together with continuous surveillance exercises, as efforts are made to reduce the level of AMR in bacteria, is the only way that will enable gauging of any improvements (reduction of AMR in bacteria) over time. This study has determined the antimicrobial resistance profiles of bacteria isolated from chickens in Kenya. Three groups of chickens [sick (clinical), farm and slaughter] were studied so as to determine whether there are any differences, with respect to antimicrobial resistance, between them. Chickens were chosen for this study because they are kept by many Kenyans; there is also a high tendency of using antimicrobials when the chickens are kept under intensive farming system. Also, while most of the bacteria that inhabit the intestinal tract of chickens are commensals, some are pathogenic to the chickens and some are zoonotic. These bacteria can acquire resistance to antimicrobials and can be a source of resistance genes to human pathogens. Thus, they are a threat to humans that consume the chickens.

3.2 MATERIALS AND METHODS

3.2.1 Study Area and study animals

The study was carried out in Nairobi County. Samples were obtained from three types of chickens: (1) those that were brought to the Poultry clinic of the department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, for disease diagnosis (post-mortem examination) (50 samples) - these chickens came from different areas of Kenya, and were of different ages and breeds, (2) chickens from a commercial farm in Nairobi (University poultry farm) (50 samples), and (3) chickens from a slaughterhouse in

Nairobi (Kariokor slaughterhouse) (72 samples). This slaughterhouse handles chickens from various parts of Kenya.

3.2.2 Study design

This was a convenience sampling approach. Selection of chicken from the farm and slaughterhouses was based on ease of availability and access while sampling the sick ones depended on what was brought to the clinic.

3.2.3 Sample Size Calculation

Since this was a non-probability sampling approach, it was not possible to obtain a representative sample. However in order to guide the investigation on the adequate number of samples needed a Probability sampling calculation was used. Sample size was calculated using the Fisher formula (Charan and Biswas, 2013), taking prevalence rate of 12.8%, as established for *E. coli* in a study carried-out by Sang *et al.* (2012), as follows:

$$n = \frac{Z^2 \propto_2 PQ}{L^2}$$

Where;

n = the sample size

Z = the standard deviation at 95% confidence level, giving Z-statistic of 1.96

P = the proportion in the study population.

Q= 1-P

L = the statistical significance level at 0.05.

$$n = \frac{(1.96^2)(0.128)(0.872)}{(0.05^2)} = 172$$

Therefore, a total of 172 chickens were sampled.

3.2.4 Sampling method and data collection

Intestinal swabs were taken from the first 50 chickens brought to the poultry clinic (for post mortem examination) during the study period, while, for farm and market (slaughterhouse) categories, cloacal swabs were randomly taken from a total of 122 birds.

3.2.5 Sample collection, handling and processing

Intestinal/cloacal swabs were aseptically collected from the study chickens, placed in separate bottles containing sterile Stuart's transport medium and transported to the bacteriology laboratory of the department of Veterinary Pathology, Microbiology and Parasitology in a cool box. Samples that were not processed immediately were refrigerated at 4° C.

3.2.6 Disposal of carcasses

For clinical cases, disposal of carcasses was done carefully, to minimize environmental contamination. The carcasses were disinfected with 1% hypochlorite and buried in a specially-prepared disposal pit, covered with lime. The disposal pit is normally manned by trained technical staff of the department of Veterinary Pathology, Microbiology and Parasitology. The area where a post-mortem examination was carried-out was cleaned and disinfected using 1% hypochlorite post examination and sampling.

3.2.7 Isolation and identification of bacteria

MacConkey agar and Blood agar were used for isolation; pre-enrichment in Selenite broth (Oxoid, Basingstoke, United Kingdom) was used to pick any possible *Salmonella* bacteria present, while sorbitol MacConkey was used to detect the possible presence of *E. coli* 0157:H7 - for this, a colony of *E. coli* was picked, streaked onto sorbitol MacConkey and incubated at 37º C overnight. Isolated colonies were then identified using the criteria given in Bergey's Manual of systemic bacteriology (Holt *et al* 1994). Breakdown of various

characteristics used for identification of the various bacteria isolated is given in Appendix 1 and Figure 3.1 shows the researcher culturing the samples. All the media used were manufactured by Oxoid, Basingstoke United Kingdom.

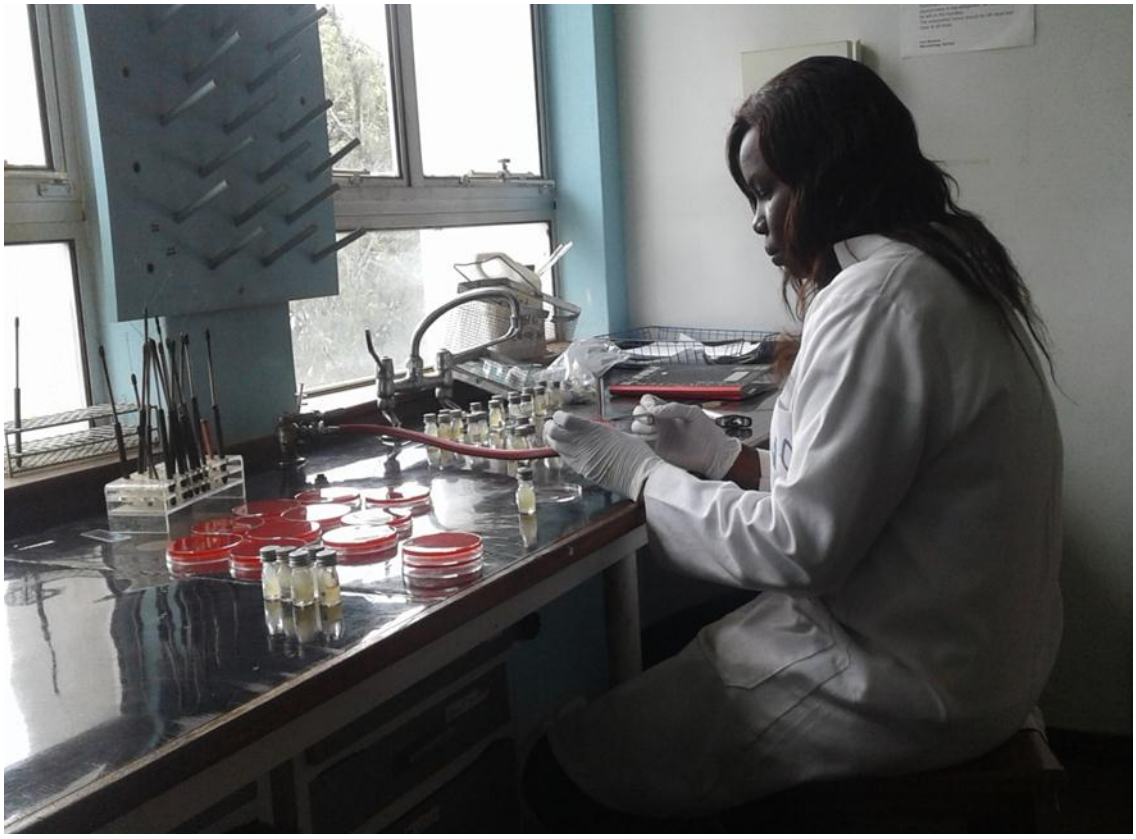


Figure 3.1: Researcher working in the Bacteriology laboratory

3.2.8 Phenotypic antimicrobial sensitivity testing of the bacterial isolates

Antimicrobial sensitivity testing was done by disc diffusion on Mueller-Hinton (MH) agar (Oxoid, Basingstoke, United Kingdom) according to the method given by the Clinical and Laboratory Standards Institute (CLSI; 2008). Bacterial suspensions of turbidity matching 0.5 MacFarland turbidity tube (1.5×10^8 CFU/µl) were prepared in saline. Sterile cotton swabs were separately dipped into the suspensions, then, on removal, pressed firmly to the inside of the tube wall, to remove excess liquid. Each swab was then streaked on the surface of Mueller Hinton agar (Oxoid, Basingstoke, United Kingdom) three times while rotating the plate 60 degrees, to produce confluent growth (Kutto, 2012). Each bacterial isolate was spread-plated in triplicate.

Eight antimicrobials, obtained from HiMedia (HiMedia Laboratories-INDIA) were used for this testing; they included ampicillin (25µg), tetracycline (100µg), nitrofurantoin (200µg), nalidixic acid (30µg), streptomycin (25µg), sulphamethoxazole (200µg), co-trimoxazole (25µg) and gentamycin (10µg). After streaking, the antimicrobial discs were placed on the agar using sterile forceps; the agar plate was then incubated aerobically at 37ºC for 24 hours. After incubation, the diameters of the growth-inhibition zones around the discs were measured using a ruler. The reference strain, *E. coli* - ATCC 25922 (CD and WHO, 2003**),** obtained from Department of Public Health Pharmacology and Toxicology (PHPT), University of Nairobi, was used as the standard control organism. The results of the inhibitory zone diameters were interpreted according to the guidelines provided by the CLSI (2008). In this study, by design, the diameters measuring up to 10 mm were taken as being resistant, while diameters measuring beyond 10 mm were taken as being susceptible to the respective antimicrobial (this includes the intermediate ranges); the size of the inhibition zone being directly proportional to the susceptibility of the organism to the particular antimicrobial (Coyle, 2005).

3.2.9 Statistical analysis

Statistical analysis was done using the R statistical program. Descriptive statistics and appropriate hypothesis tests were carried out to establish the associations and correlations between antimicrobial resistance and the selected variables.

3.3 RESULTS

3.3.1 Aerobic bacteria isolated from the study chickens

Figure 3.2 shows the genera of aerobic bacteria that were isolated from the study chickens. Of the six genera isolated, the most prevalent were organisms of the genus *Streptococcus* (40.7%), followed by *E. coli* (31.4%), then *Staphylococcus* (26.2%). The least isolated were in the genus *Corynebacterium* (2.3%); some specimens yielding organisms from more than one genus. Analysing them per study group (Table 3.1): for clinical cases: *E. coli* was the most prevalent (72%), followed by *Streptococcus* (42%), *Bacillus* (18%), then *Proteus* (10%) - *Staphylococcus* and *Corynebacterium* were not isolated from clinical cases. For farm chickens: *Streptococcus* and *Staphylococcus* had highest prevalence rates (each at 40%), followed by *E. coli* (22%), *Bacillus* (14%), and then *Corynebacterium* (8%) - *Proteus* was not isolated from farm birds. For market chickens: *Streptococcus* had the highest prevalence rate (40.3%), followed by *Staphylococcus* (34.7%), *E. coli* (9.7%), *Corynebacterium* (7%); and then *Bacillus* (1.4%) - *Proteus* was not isolated from market birds. *Salmonella* and *E. coli 0157:H7* were not isolated from the study chickens.

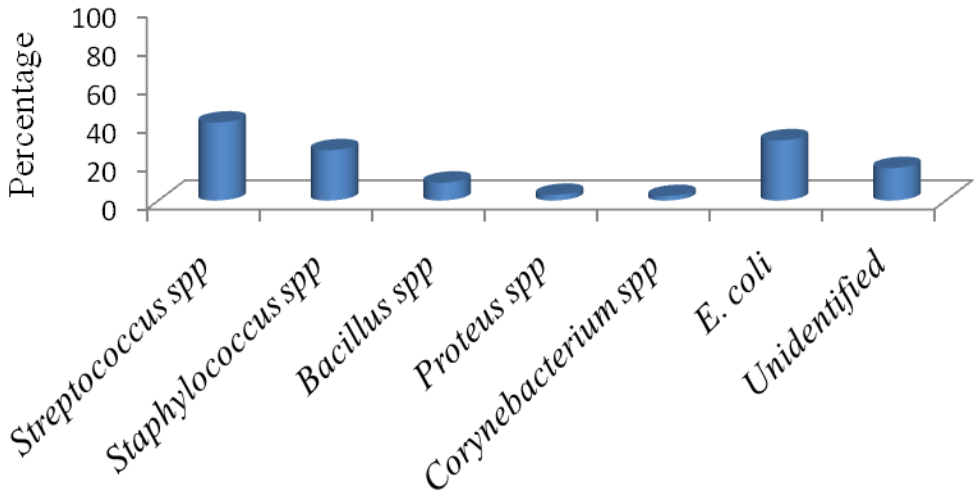


Figure 3.2: Overall proportion of bacteria isolated from chickens

Table 3.1: Prevalence of bacteria isolated from chickens per study group

Study group	***Strept***		***Staph***		***Bacillus***		***Proteus***		***Coryne***		***E. coli***	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Clinical cases N = 50	21	42	0	0	9	18	5	10	0	0	36	72
Farm chickens N = 50	20	40	20	40	7	14	0	0	4	8	11	22
Market chickens N= 72	29	40.3	25	34.7	1	1.4	0	0	0	0	7	9.7

Key: *Strept - Streptococcus, Staph - Staphylococcus, Coryne - Corynebacterium*

E. coli - Escherichia coli, n – number, and % - percent

3.3.2 Antibiotic resistance test results of the bacterial isolates

Due to financial constraints, while all the *E. coli* isolates were tested for antimicrobial susceptibility/resistance, only a few of the other bacterial isolates were tested, using the 8 antimicrobials supplied by HiMedia (HiMedia Laboratories-INDIA). Overall, the respective resistance patterns of the studied bacteria were as given in Table 3.2. *Escherichia coli* showed higher resistance to ampicillin (83%), followed by *Proteus* (33.3%), *Bacillus* (20%), *Staphylococcus* (8.3%) and then *Streptococcus* (5.9%). *Staphylococcus* showed higher resistance percent to gentamycin (8.3%) compared to *E. coli* (3.7%). *Proteus* was fully (100%) resistant to co-trimoxazole, while other bacteria showed moderate resistance (55.6%, 40%, 16.7%, for *E. coli*, *Bacillus* and *Staphylococcus,* respectively). *Proteus* was 100% resistant to sulphamoxazole followed by *E. coli* (63%), *Bacillus* (40%), and *Staphylococcus* (16.7%). Resistance to streptomycin was higher in *Proteus* (66.7%) compared to other bacteria: *Streptococcus* (23.5%), *E. coli* (18.5%) and *Staphylococcus* (16.7%). *Proteus* was fully (100%) resistant to nalidixic acid, while *Staphylococcus* and *Streptococcus* showed moderate resistance (50% and 47.1%, respectively) and *E. coli* showed low resistance (18.5%). Resistance to nitrofurantoin was high for *Proteus* (66.7%) compared to other organisms: *E. coli* (20.4%), *Staphylococcus* (8.3%) and *Streptococcus* (5.9%). *Proteus* was fully (100%) resistant to tetracycline while *E. coli* showed moderate resistance (55.6%) compared to lower resistance which was observed in both *Staphylococcus* and *Streptococcus* (8.3% and 5.9%, respectively). *Corynebacterium* was not subjected to antimicrobial sensitivity testing. Figure 3.3 illustrates the susceptibility profile of one of the bacterial isolates. Bacteria isolated from clinical chickens showed higher prevalence of AMR to most of the tested antimicrobials than those isolated from farm and market birds, However, the difference was not statistically significant except for Sulphamethoxazole ($P = 0.005$),

Nitrofurantoin (P = 0.008) and Co-trimoxazole (P = 0.002). Details of the Chi-square values are given in Appendix 2.

Table 3.2: Overall antimicrobial resistance patterns demonstrated by the isolated bacteria

Tested antibiotics	***Streptococcus*** **n = 17**		***Staphylococcus*** **n = 12**		***Bacillus*** **n = 5**		***Proteus*** **n = 3**		***E. coli*** **n = 54**	
	N resistant	**% resistant**	**N resistant**	**% resistant**	**N resistant**	**% resistant**	**N resistant**	**% resistant**	**N resistant**	**% resistant**
Ampicillin 25 µg	1	5.9	1	8.3	1	20	1	33.3	45	83.3
Gentamycin 10 µg	0	0	1	8.3	0	0	0	0	2	3.7
Co-trimoxazole 25 µg	0	0	2	16.7	2	40	3	100	30	55.6
Sulphamoxazole 200 µg	0	0	2	16.7	2	40	3	100	34	63
Streptomycin 25 µg	4	23.5	2	16.7	0	0	2	66.7	10	18.5
Nalidixic Acid 30 µg	8	47.1	6	50	0	0	3	100	10	18.5
Nitrofurantoin 200 µg	1	5.9	1	8.3	0	0	2	66.7	11	20.4
Tetracycline 100 µg	1	5.9	1	8.3	0	0	3	100	30	55.6

Key: *E. coli - Escherichia coli, µg - microgram, n – number, and % - percent*

Figure 3.3: Antimicrobial susceptibility profiles (clear zones) for one *E. coli* isolates to various antimicrobial discs on Mueller Hinton agar

Clear zones (arrow) indicate susceptibility (no growth)

3.3.3 Multidrug resistance demonstrated by the bacterial isolates

Even though the number tested was small, the results were interesting, as shown in Tables 3.3 and 3.4, taking multi-drug resistance (MDR) to include resistance to two or more antimicrobials. Overall, more *Escherichia coli* isolates demonstrated MDR than the other isolates, while comparing the three study groups, the general picture indicated a higher MDR prevalence in bacteria isolated from clinical cases, followed by market chickens (Table 3.3). *Escherichia coli, Streptococcus, Staphylococcus* and *Proteus* had isolates showing resistances to more than three antibiotics: *Streptococcus* had one isolate resistant to 4

antibiotics, *Staphylococcus* had one isolate resistant to 4, while *Proteus* had 2 isolates resistant to 6 and one isolate resistant to 5 antimicrobials.

Overall, antimicrobials that were mostly included in the multi-drug resistance blocks were: nalidixic acid (40.9%), sulphamethoxazole and streptomycin (each at 36.4%), then co-trimoxazole (31.8%). With respect to the three study groups: for clinical cases, the mostly included antimicrobials were: cotrimoxazole (75%), followed by sulphamethoxazole (62.5%), then nalidixic acid and streptomycin (each at 50%); for farm chickens, all the seven tested antimicrobials occurred at the same rate (1/6 = 16.7%); for market chickens, the mostly included antimicrobials were: nalidixic acid and streptomycin (each at 50%), followed by sulphamethoxazole and nitrofurantoin (each at 12.5%) (Table 3.4). Gentamycin was included in the multi-drug resistant blocks for *E. coli* only; not for other bacterial isolates. Detailed data were given in Appendices 3, 4, 5, 6, and 7.

Of the 9 isolates which were resistant to only one antimicrobial [5 (22.7%) overall; 4 (46.7%) from farm chickens; one (12.5%) from market chickens] were resistant to nalidixic acid, two [(9.1% overall; both (25%) from market chickens] were resistant to streptomycin, while 2 [(9.1% overall; one (12.5%) from market chickens and one (16.7%) from farm chickens] were resistant to gentamycin and ampicillin.

Table 3.3: Multidrug resistance patterns demonstrated by isolates from chickens in the three study groups

Organism tested	**Clinical cases**		**Farm chickens**		**Market chickens**	
	Number showing some resistance (%)	Multi-resistant isolates	Number showing some resistance (%)	Multi-resistant isolates	Number showing some resistance (%)	Multi-resistant isolates
Strept 17 tested	2/2 (100%)	*One resistant to two antimicrobials: S and NA *One showing resistance to 4 antimicrobials: AMP, S, NA and TE	2/3 (66.7%)	No MDR - Both resistant to one antimicrobial – NA	3/12 (25%)	Two MDR: both resistant to 2 antimicrobials: *one resistant to NA and NIT; *One resistant to NA and S. The third one was resistant to one antimicrobial - NA
Staph 12 tested	1/1 (100)	Resistant to 4 antimicrobials: AMP, COT, SXT and TE	3/3 (100%)	One MDR – resistant to 2antimicrobials: SXT and COT The other 2 were resistant to one (same) antimicrobial - NA	5/8 (62.5%)	Two were MDR – both resistant to 2 antimicrobials: *one resistant to SX and S *one resistant to S and NA The other 3 were resistant to one

						antimicrobial each: two to S; one to GEN
Bacillus 5 tested	2/3 (66.7%)	Both MDR – resistant to 2 antimicrobials: SXT and COT	1/2 (50%)	No MDR -resistant to one antimicrobial - AMP	-	-
Proteus 3 tested	3/3 (100%)	Two were resistant to 6 antimicrobials: *one resistant to: NA, NIT, TE, COT, SXT and S *one resistant to: NA, TE, AMP, COT, SXT, S The third one was resistant to 5 antimicrobials: NA, TE, AMP, COT and SXT.	-	-	-	-
E. coli 54 tested	33/36 (94.4%)	32 (88.9%) were MDR: *three were resistant to 6 antimicrobials – two had combination of AMP, TE, NA, S, SXT and COT; one had combination of AMP, TE, NA, SXT, COT and GEN *four were resistant to 5 antimicrobials – three had combination of AMP, TE, S, SX and COT; one had combination of AMP, TE, NA, SXT and COT *10 had resistance to 4 antimicrobials	5/11 (45.5%)	Four (36.4%) were MDR: *2 resistant to 4 antimicrobials – one combination being: TE, S, SX and COT; the other combination being: AMP, NIT, SXT and COT *one resistant to 3 antimicrobials: AMP. NIT and SXT *two resistant to 2	5/7 (71.4%)	Three (42.9%) were MDR: *2 resistant to 4 antimicrobials – one combination being: AMP. S, SXT and COT; the other combination being: AMP, NA, SXT and COT *one resistant to 2 antimicrobials: AMP and TE The other two were

		– five had combination of AMP, TE, SXT and COT; two had combination of AMP, S, SXT and COT; two had combination of AMP. NA. SXT and COT; one had combination of AMP, NIT, SXT and COT *9 had resistance to 3 antimicrobials – five had combination of TE, SXT and COT; two had combination of AMP, SXT and COT; one had combination of AMP, TE and SXT; and one had combination of NA, SXT and COT *six had resistance to 2 antimicrobials – two had combination of AMP, TE; while the other 4 had varying combinations of SXT, COT; TE, S; AMP, NA and AMP, SXT; respectively The other 3 had resistance to one antimicrobial – NA; AMO and TE, respectively		antimicrobials – one combination being: AMP and NIT; the other combination being: AMP and SXT The fifth one was resistant to one antimicrobial – TE		resistant to one antimicrobial: AMP and TE, respectively

Key: *Strept - Streptococcus, Staph - Staphylococcus, Coryne - Corynebacterium, E. coli - Escherishia coli, AMP - ampicillin, SXT - sulphamethoxazole, NA - Nalidixic acid, S - streptomycin, GEN - gentamycin, TE - tetracycline, COT - co-trimoxazole, NIT - nitrofurantoin and MDR - multidrug resistance.*

Table 3.4: Antimicrobial inclusion rates in the multi-drug resistance blocks: overall and per study group

Antimicrobial	**Clinical isolates n = 8**		**Farm isolates n = 6**		**Market isolates n = 8**		**Combined isolates n = 22**	
	Number	**%**	**Number**	**%**	**Number**	**%**	**Number**	**%**
AMP	3	37.5	1	16.7	0	0	4	18.2
TE	4	50	1	16.7	0	0	5	22.7
NA	4	50	1	16.7	4	50	9	40.9
SXT	5	62.5	1	16.7	1	12.5	8	36.4
COT	6	75	1	16.7	0	0	7	31.8
S	4	50	0	0	4	50	8	36.4
NIT	1	12.5	0	0	1	12.5	2	9.1
GEN	0	0	0	0	0	0	0	0

Key: *AMP - ampicillin, TE - tetracycline, NA - Nalidixic acid, SXT - sulphamethoxazole, COT - cotrimoxazole, S - streptomycin, NIT - nitrofurantoin, GEN - gentamycin, n – number and % - percent.*

3.4 DISCUSSION

Overall, in this study, *E. coli* was isolated at a rate of 31.4%, However, it was interesting to note a low *E. coli* prevalence of 9.7% (7/72) in market chickens (Table 3.1), since *E. coli* is normally the most commonly-found bacteria (coprobacteria) in the faeces of both humans and animals (Buxton and Frazer, 1977). Thus, even though not excreted all the time, low occurrence of 9.7% in market chickens, compared to occurrence of 72% in clinical cases, can only be explained by the fact that cloacal swabbing cannot be the same as intestinal-content swabbing – the amount of fecal material in cloacal swabbing is much less. Prevalence from farm birds was also at lower level (22%). Other researchers (Njagi, 2003) reported an *E. coli* prevalence of 40.2% from cloacal swabs of apparently healthy indigenous chickens in Kenya. This low prevalence in market birds cannot be blamed on the medium used, since the same medium (from the same source) was used to isolate *E. coli* from the three tested groups.

Other bacteria isolated from chickens were *Streptococcus* spp (40.7%), *Staphylococcus* spp (26.2%), *Bacillus* spp (9.3%), *Proteus* spp (2.9%), and *Corynebacterium* spp (2.3%). Similar bacteria have been isolated from chickens, for example: Bebora (1979), working on chickens from farms and slaughterhouse, isolated the following bacteria, respectively: from farms *Salmonella* spp (0.37%), *E. coli* (53.6%), *Proteus* spp (18.3%), *Aerobacter* spp (3.6%), *Streptococcus* spp (5.3%), *Staphylococcus* spp (4%), *Citrobacter* spp (3.8%) and *Pseudomonas* spp (1.5%); from slaughterhouse *Salmonella* spp (0.5%), *E. coli* (81.5%), *Proteus* spp (17.7%), *Aerobacter* spp (2.1%), *Streptococcus* spp (4.2%) and *Staphylococcus* spp (4.8%). Njagi (2003) isolated the following bacteria from market and trading centers: *E. coli* (33.9%), *Staphylococcus aureus* (20%), and *Streptococcus* (14.3%); from farm: *E. coli* (48.1%), *Staphylococcus aureus*

(23.1%), *Streptococcus* (9.7%), and *Erysipelothrix* spp (1.8%); from slaughter houses: *E. coli* (40.1%), *Staphylococcus aureus* (28.4%), *Streptococcus* spp (22.5) and *Erysipelothrix* spp (4.9%). Also, when diagnostic records for years 2015 and 2016, from departmental bacteriology laboratory, University of Nairobi, were perused, it was found that 125 bacterial isolates were recovered from chickens that were brought to the departmental poultry clinic. These included *E. coli* (75.2%), *Staphylococcus aureus* (17.6%), *Streptococcus* spp (16.8%), *Pasteruella* spp (5.6%), *Salmonella gallinarum* (4%), *Pseudomonas* spp (3.2%), *Proteus* spp (3.2%), *Corynebacterium* spp (1.6%), *Klebsiella pneumoniae* (1.6%), *Clostridium* spp (1.6%), *Bacillus* spp (0.8%), *Citrobacter* spp (0.8%), *Rhodococcus equi* (0.8%) and *Enterobacter* spp (0.8%).

This study recorded *Proteus* prevalence of 2.9% which is lower than that found by Bebora (1979) and Mutsami (2011) who recorded *Proteus* prevalences of 18.2% and 66.7%, respectively. The prevalence is, however, similar to that found by Wandili (2013; 6%). Different species of *Proteus*, which commonly occur as saprophytes are known to cause septic infections in humans (Wilson and Miles, 1975) and animals (Murdoch and Baker 1977; O'Driscoll, 1977; Pine *et al* 1973) under certain conditions. *Proteus* has been implicated in the persistent yolk sac infection, omphalitis and embryonic death in chickens, ducks and turkeys (Bhatia *et al*., 1972; Baruah *et al*., 2001).

This study recorded 9.3% prevalence of *Bacillus* spp and 2.3% *Corynebacterium* spp in chickens. From the bacteriology laboratory diagnostic records (2015-2016) prevalence of 0.8% and 1.6% for *Bacillus* and *Corynebacterium*, respectively, was recorded. Osman and Elsanousi (2013) reported (2.83%) *Bacillus* and (2.36%) *Corynebacterium* in Sudan.

In this study, the overall prevalence of *Staphylococcus* was found to be 26.2%. These results are in agreement with that of Njagi (2003), who reported an overall prevalence rate of 20.1%. In contrast, Bebora (1979) reported an overall prevalence of 4.3% and Igbinosa *et al.* (2016) found a prevalence of 100% *Staphylococcus* spp isolated from chicken carcasses in Nigeria. *Staphylococcal* infections are widespread in poultry and these infections are caused by *Staphylococcus aureus* (Kibenge *et al*., 1982). *Staphylococcus aureus* are an important cause of avian disease and may thus pollute food as a result of processed carcasses (Mead and Dodd, 1990).

The prevalence of *Streptococcus* organisms in this study was 22.5%, which was almost close to the prevalence reported by Njagi, (2003) at 21.2%. In contrast, Bebora (1979) reported an overall prevalence of 4.9% in Kenya and Kolar *et al*, (2002) reported 14.8% prevalence of *Streptococcus* isolated from poultry in Czech Republic. *Streptococcus* occurs globally in chickens; it is associated with both chronic and acute septicemic infections, causing mortality rates of between 0.5% and 50% (Verma *et al.*, 2013).

The various bacteria isolated in this study demonstrated high levels of resistance to commonly used antimicrobials. Considering individual bacterial types, *E. coli* demonstrated highest resistances to commonly-used antimicrobials: tetracycline, sulphamethoxazole, ampicillin, streptomycin and co-trimoxazole (Tables 3.2). This has also been reported by other researchers (Bebora, 1987; Ombui *et al*. 2000; Mapeney *et al*. 2006; Gakuya *et al*. 2007; Kikuvi *et al*. 2007_b and Allorechtova *et al*. 2012). The bacteria showed resistance to amoxicillin at rate of 1.9%, it

was encouraging to find that there were some bacterial strains that were still susceptible to the commonly-used antimicrobials, for example: two *E. coli* isolates from farm and two isolates from market chickens were susceptible to all the tested antimicrobials.

Considering the 3 study groups, overall, isolates from farm chickens showed higher antimicrobial susceptibility than those from the other two groups (prevalence of 90.3%; Table 3.3); isolates from market birds being more susceptible than those from clinical cases (prevalence of 78%; Table 3.3). This shows that although this particular farm was practicing intensive farming system, it was not misusing antimicrobials. Market chickens were from a slaughterhouse which received chickens from various parts of Kenya. These were mainly indigenous chickens and, as is generally practiced, these birds are normally raised free-range and are hardly given antimicrobials. In agreement with this Naliaka (2011), working with *Salmonella* and *E. coli* isolated from indigenous chicken and broilers, reported high antimicrobial susceptibility due to low usage of antimicrobials by the respective farmers. However, Odwar *et al*, (2014) found high level of antimicrobial resistance in bacteria from market chickens.

Multi-drug resistance was recorded in *E. coli* in this study. The combinations were variable, but four antimicrobials were mostly included in the resistance blocs; these were: ampicillin, sulphamethoxazole (74.4%), and co-trimoxazole (69.8%). With respect to the three study groups: for clinical cases, the mostly included antimicrobials were: ampicillin (79.4%), tetracycline, co-trimoxazole (each at 76.5%) and sulphamoxazole (73.5%); for farm chickens, the mostly included antimicrobials were: ampicillin (88.3%), tetracycline, sulphamethoxazole, nitrofurantoin (each at 66.7%); for market birds, the mostly included antimicrobials were:

ampicillin, tetracycline, sulphamethoxazole (each at 100%) and nalidixic acid, cotrimoxazole (each at 66.7%) (Table 3.4). Multi-drug resistance is a worrying trend world-wide. It has been reported by a number of researchers; the Kenyan ones being: in animals – Bebora (1987), Ombui *et al*. (2000), Mapeney *et al*. (2006), Gakuya *et al*. (2007), Kikuvi *et al*. (2007b), Allorechtova *et al*. (2012); in an environment – Wambugu *et al*. (2015), Kutto (2012); in humans – Kariuki *et al*. (1996; 2006), Bururia (2005), Oundo *et al*. (2008). Many more researchers outside Kenya have reported on MDR: Van den Bogaard *et al*. (2001), Ryu *et al*. (2012), Adzikey *et al*. (2012). This emphasizes the need to join the fight against further development of MDR, by advocating for prudent use of antimicrobials.

Proteus spp showed high prevalence of resistance to most of the antimicrobials tested; being fully (100%) resistant to cotrimoxazole 25µg, sulphamoxazole 200µg, tetracycline 100µg and nalidixic acid 30µg. This Multiple drug resistance (MDR) was also reported by Amare *et al*. (2013) in Ethiopia; they recorded resistance at prevalences of 100% to bacitracin 10µg, 84.7% to penicillin G 10µg, 69.2% to tetracycline 30µg, 43.6% to ampicillin 10µg and 23.1% to erythromycin 15µg, and Nemati, (2013) from Iran; they recorded resistance of 100% to gentamycin, 93% to nalidixic acid, 91% to doxycycline, 89% to oxytetracycline and 22% to ampicillin. Nahar *et al*., (2014) in Bangladesh recorded resistance levels of 94%, 88.9%, 66.7%, 52.8%, to tetracycline, nalidixic acid, ampicillin and trimethoprim, gentamycin, respectively, and 16.7% to ciprofloxacin.

In this study, *Staphylococcus* showed resistance of 50 % to nalidixic acid 30µg and resistance of 16.7% to the other antimicrobials tested (co-trimoxazole 25µg, sulphamethoxazole 200µg and

streptomycin 25µg). This was in contrast with another study done in Kenya by Shitandi and Mwangi (2004); they found 72% resistance to penicillin G 10µg, 59% trimethoprim/sulphamethoxazole 35µg, 57.9% tetracycline 30µg, and 46.8% chloramphenicol 30µg. Amare *et al*. (2013) in Ethiopia, found *Staphylococcus aureus* that was fully resistant (100%) to bacitracin 10µg, 97.5% penicillin G 10µg, 92.3% tetracycline 30µg, 47.5% ampicillin 10µg, and less resistant 12.5% to streptomycin 10µg. Kolar *et al*, (2002) in Czech Republic recorded resistances in *Staphylococcus* organisms to erythromycin (39%), clindamycin (19%), tetracycline (14%), and ofloxacin (13%).

Streptococcus isolated in this study was resistant to nalidixic acid at 47.1%, streptomycin 25µg at 23.5% and ampicillin 25µg, nitrofurantoin 200µg and tetracycline 100µg at 5.9%, each. These findings are in agreement with those of Imohl and Van der Linden (2015) of Germany; between years 2003 and 2013, they recorded resistance levels of *Streptococcus pyogenes* as: 9.7% to tetracycline, 3.9% to macrolides, and 0.7% to sulphamethoxazole. Camara *et al*. (2013) in Senegal reported on *Streptococcus pyogenes* that were fully (100%) resistant to tetracycline, 37.5% to spiramycine (Macrolides) and 2.5% to pristinamycin (Streptogramins).

Multi-drug resistances have been reported in this study – to 2, 3, 4, up to 6 antibiotics – this is with respect to the number of antimicrobials tested – they could have been more if more antimicrobials were tested. There is a high chance that these resistances are coded-for by plasmids which can be transmitted horizontally and vertically between various bacterial species and genera. The American Infectious Disease Society has identified extended-spectrum β-lactamase producing *Enterobacteriaceae*, methicillin-resistant *Staphylococcus aureus* (MRSA),

vancomycin-resistant *Enterococcus faecium*, MDR *Acinetobacter baumannii*, *Pseudomonas aerouginosa*, *Clostridium difficile* and extensively- drug resistant *Tuberculosis* (XDR-TB) and the list is growing (Sebaihia *et al*., 2006; Talbot *et al*., 2006; Dorman and Chaisson, 2006; Wright and Sutherland, 2007).

Chances are also that all these resistant traits are on one plasmid, hence transferred as a block, as is the case of methicillin-resistance in *Staphylococcus* spp. [Ombui *et al*., (2000) in Kenya; Waters *et al*., (2011) in USA; Al-haddad *et al*., (2014) in Libya]. Zarfet *et al*. (2014) in Austria demonstrated the presence of ESBL genes in *E. coli*, *mecA* harbouring staphylococci and vancomycin-resistant enterococci (VER). Various cases of multidrug-resistance in bacteria have been reported by other researchers for example:, Margaret *et al*. (1999) in Hong Kong; Moyo *et al*. (2012) in Tanzania; and Kobayashi *et al*. (2017) in Kenya reported multidrug resistance in *Streptococcus*. Bebora and Nyaga (1989) reported multidrug resistance in *Salmonella* Gallinarum in Kenya. Zhang *et al*., (2011), detected multidrug resistance gene *cfr* in pBs-02 *Bacillus* in China. Bhatt *et al*. (2014) reported MDR for both Gram-negative and Gram-positive bacteria; *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *E. coli* and *Staphylococcus aureus* in Nepal.

This study has confirmed existence of resistance, both single and multiple, in the various bacteria, including *E. coli*, isolated from the study chickens. It thus contributes towards data on current AMR status in bacteria harboured by chickens/animals in Kenya. The results will help in informing the policy makers as they embark in their fight towards reduction of AMR.

CHAPTER 4: DETECTION OF ANTIMICROBIAL RESISTANT GENES IN *E. COLI* ISOLATES USING POLYMERASE CHAIN REACTION

4.1 INTRODUCTION

One way that bacteria become resistant to a particular antimicrobial is through production of enzyme(s) capable of breaking down the particular antimicrobial (Fair and Tor, 2014). There are many different types of enzymes produced by different bacteria. One group of enzymes that is often produced by resistant Gram-negative bacteria are the Extended-spectrum β-lactamases (ESBLs), which have the ability to break down third and fourth-generation cephalosporins and monobactams, as well as the earlier generation cephalosporins and penicillins. They consist of four main types, coded by genes: bla_{SHV}, bla_{OXA}, bla_{TEM} and bla_{CTX-M} (http://www.lahey.org/studies/webt.asp cited Feb 2 2017). It was interesting to find out if the resistant *E. coli* isolated from chickens carried any of these ESBL genes. This study, therefore, sought to determine the presence of the respective genes in the resistant *E. coli* isolates, with the understanding that, being proteins, ESBLs, which are responsible for antimicrobial resistance, are coded-for by specific genes. Polymerase Chain Reaction (PCR) and specific primers were used to detect presence of the specific genes. Absence of these genes in these isolates would then mean that the antimicrobial resistance, portrayed by the isolates, was coded-for by some other gene or other characteristic.

4.2 MATERIALS AND METHODS

4.2.1 Study organisms

The study used the 28 *E. coli isolates* that were obtained from chickens as described in Chapter 3 section 3.2.7.

4.2.2 Isolation of DNA by boiling method

A single colony of each isolate was suspended in 0.5 ml of extraction buffer (100 µl of 1ml buffer Tris Borate and 2µl of 0.5 EDTA). Then, 400 µl buffer suspension (herein-after referred-to as reaction mixer), in Eppendrof tube, was boiled for 10 minutes at 100ºC. After boiling, centrifugation was done at 14,000 rpm for 5 minutes at 4°C. The DNA-containing supernatant was stored in -20°C and later used as the source of DNA template for further PCR amplification experiments (Solberg *et al*., 2006).

4.2.3 Polymerase Chain Reaction procedure

This was done according to the method described by Brody and Kern (2004). The DNA extract of each sample was used as a template in the specific Polymerase Chain Reaction (PCR) amplifications for detection of bla_{TEM}, *dfrA1* and $bla_{\text{CTX-M}}$ genes. For each sample a total volume of 23.07µl of reaction mixture per gene was mixed in the Eppendrof tube as follows: 11µl Taq PCR master mix, 11µl DNAs free water, 0.5µl primer forward, 0.5µl primer Reverse, and lastly 0.07µl DNA. Primers, as given in Table 4.1, were used according to the manufacturer's instructions. The PCR reaction cycle for $bla_{\text{CTX-M}}$ was carried out using Thermocycler at a denaturation temperature of 95ºC for 5 minutes followed by annealing at 55ºC for 1 minute, then extension at 72ºC for 30 minutes. The cycles for bla_{TEM} and *dfrA1* were run in a Thermocycler at a denaturation temperature of 95ºC for 5 minutes followed by annealing at 60ºC for 1 minute and then extension at 75ºC for 30 minutes.

Table 4.1: Primers and annealing temperatures used in the PCR

Primer Name	5'-3' Sequence	Product size	Annealing TM	Accession Number
bla$_{CTX-M}$ (consensus)-F	ATGTGCAGACCAGTAAGTATGGC			
bla$_{CTX-M}$ (consensus)-R	TGGGTAATAGTACCAGAACAGCGG	593bp	60	Y10278
bla$_{TEM}$ (consensus)-F	ATGAGTATTCAACATTTCCG			
bla$_{TEM}$ (consensus)-R	CCAATGCTTAATCAGTGAGG	840bp	55	EF125012
dfrA1-F	CATCTGACAATGAGAACGTAT			
dfrA1-R	ACCCTTTTGCCAGATTTGGTA	269	60	KX242350

Key: *CTX-M-Cefotaxime hydrolyzing capabilities, dfr A1-Dihydrofolate reductase, TEM-Temoneira TM- Temperature, bla- β-lactam,* F – forward, and R – reverse

4.2.4 Gel preparation, Electrophoresis and photography

Gel preparation, electrophoresis and photography of the PCR products were done as described by Ausubel *et al*. (2003).

4.2.4.1 Gel preparation

The buffer was prepared by diluting 0.5µl 10X TBE buffer into 1000µl distilled water. The gel was prepared by accurately weighing 2.75g of agarose and dissolving it in 250µl X TBE (TBE = Tris/borate). The agarose was melted in a microwave oven and swirled to ensure even mixing and then cooled to 55°C in a water bath. Ethidium bromide (0.5µg/µl, w/v) was then added to the agarose.

4.2.4.2 Electrophoresis and photography

The electrophoresis gel casting platform was sealed at the end, the agarose was poured into the gel tank. The gel comb was inserted making sure that there were no bubbles before the gel sets.

After the agarose gel had solidified, the tape from the open ends of the electrophoresis gel platform and the comb were removed carefully. The electrophoresis gel casting platform containing the set gel was placed in the electrophoresis tank and electrophoresis buffer was then added to a depth of 1mm. Carefully, the DNA ladder, *E. coli* DNA samples and the negative control were loaded into the specified wells after adding the loading buffer containing the tracking dye. To begin the electrophoresis the electrodes were connected to the power supply and the voltage set to 1 to 10 v/cm of gel. The power supply was turned off after 40 minutes when the tracking dye had migrated and reached near the end of the gel. DNA visualization and photography was done after placing the gel with migrated DNA samples on a UV transilluminator (>2500 $\mu W/cm^2$).

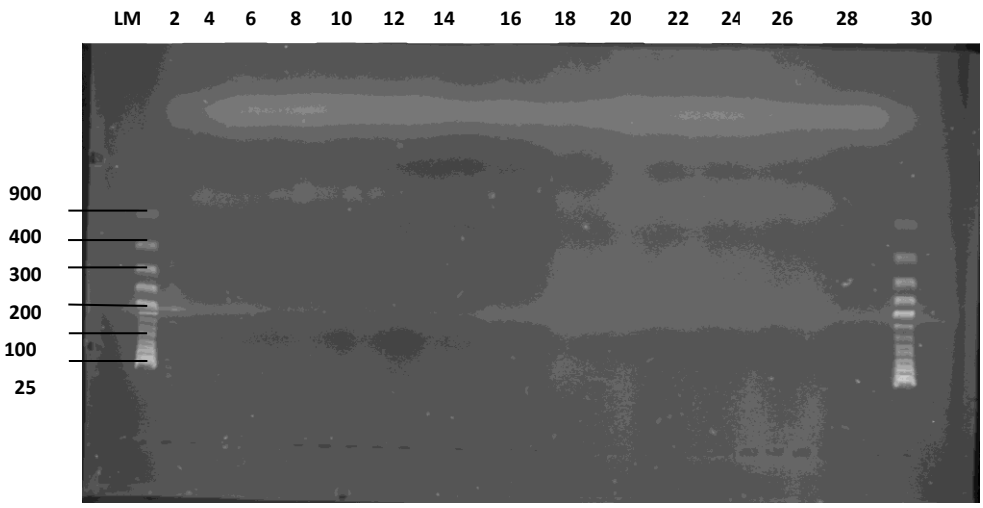
4.3 RESULTS

A total of 28 *E. coli* isolates were subjected to PCR to test for the presence of bla_{TEM}, $bla_{CTX\text{-}M}$, and *dfrA1* genes. Genes $bla_{CTX\text{-}M}$ and bla_{TEM} were not detected; while 3 isolates (10.7%) had *dfrA1* (Table 4.2). Figures 4.1, 4.2 and 4.3 show the electrophoretic/amplification reactions, with respect to the 3 genes.

Table 4.2: Proportion of isolated genes in *E. coli* strains

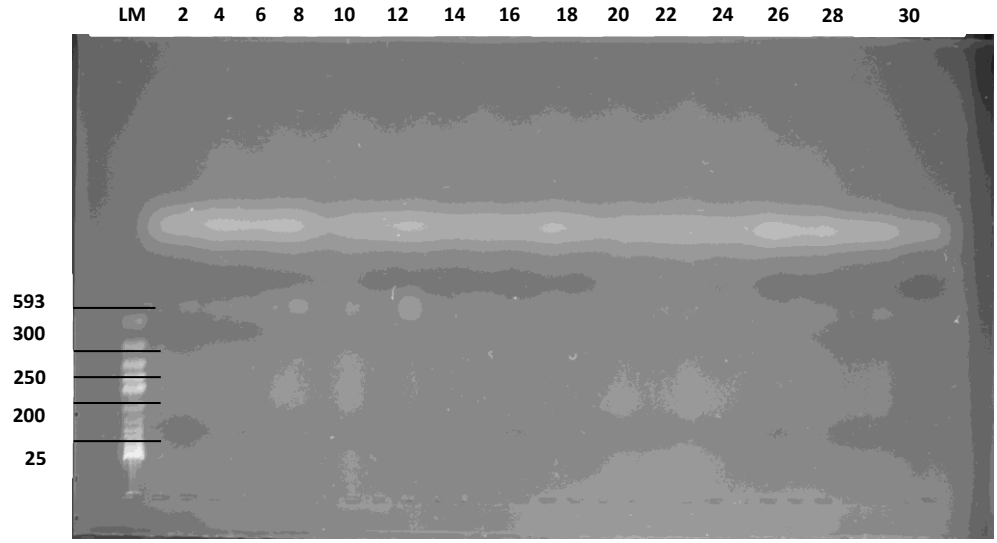
Genes n = 28	**Proportion (%)**
bla_{TEM}	0% (0/28)
$bla_{CTX\text{-}M}$	0% (0/28)
dfrA1	10.7% (3/28)

Key: *CTX-M-Cefotaxime hydrolyzing capabilities, dfr A1-Dihydrofolate reductase, TEM-Temoneira and bla- β-Lactam*



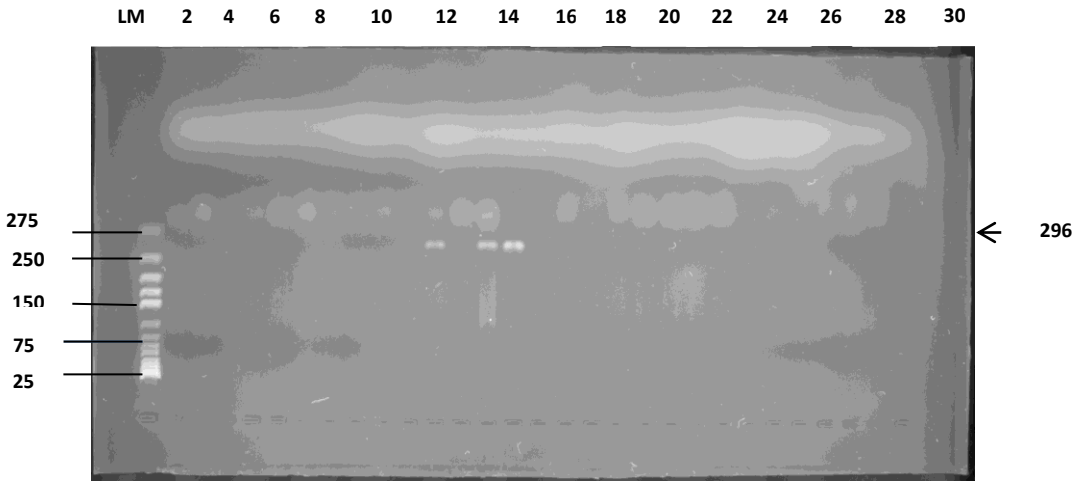
Key: *LM-DNA Ladder, from numbers 1- 28 are E. coli samples, 29 negative control (Distilled water)*

Figure 4.1: PCR amplification reaction for *E. coli bla*$_{TEM}$ gene – negative reaction



Key: *LM-DNA ladder, from 1-28 are E. coli samples, 29 is negative control (Distilled water)*

Figure 4.2: PCR amplification reaction for *E. coli bla*$_{\text{CTX-M}}$ gene – negative reaction



Key: *LM-DNA ladder, from 1-28 are E. coli samples 11, 13, and 14 are positive for dfr A1, 29 is negative control (Distilled water)*

Figure 4.3: PCR amplification reaction for *E. coli dfrA1* gene – positive reaction for 3 samples

4.4 DISCUSSION

Only three of the 28 screened resistant *E. coli* demonstrated presence of *dfrA1* gene, giving a prevalence of 10.7%; none of isolates had bla_{TEM} nor $bla_{CTX\text{-}M}$ genes; while the resistance rate of the *E. coli* isolates from chickens was 92.6%. This shows that 25 of the resistant *E. coli* (89.3%) utilised other means than Extended-spectrum β-lactamases to express their resistance. This is not surprising as bacteria have various ways (not necessarily enzymes) which they use to make themselves resistant to particular antimicrobials. These ways include: (1) gaining ability to eject the antimicrobial once it enters the bacterium; (2) if the antimicrobial blocks an enzyme which produces a vital product, the bacterium may produce more of this enzyme so that there is excess of the enzyme. (3) if the antimicrobial blocks a certain pathway, the bacterium may produce the same product through a different pathway, and (4) if the antimicrobial breaks down a vital nutrient/product, the bacterium may evolve not to rely on that particular nutrient/product (Merchant and Parker 1967; Buxton and Fraser 1977; Forbes *et al*., 2002). These changes are facilitated by either mutation or trans-bacterial genetic transfer (Merchant and Parker 1967; Buxton and Fraser 1977).

The observations of this study, therefore, show that phenotypic antimicrobial susceptibility/resistance studies may be better than genotypic ones, where one is looking for the presence of one or two resistance genes (for example: ESBL or Methicillin resistant genes); the bacterium may be expressing resistance through other enzymes/genes or systems. This is supported by observations made by other researchers (Ombui *et al.*, 2000; Bururia, 2005), who used both typing methods and found that isolated plasmids did not correspond to any resistance

patterns shown. Thus genotypic typing is only helpful if one is interested in finding out the presence of a particular gene, as was the case in this particular study.

Just like ESBLs, these other traits may also be carried by plasmids, making it easy for them to be transferred widely among bacteria, as this horizontal transfer has been demonstrated by other researchers (Bebora *et al.*, 1994; Kikuvi *et al.,* 2007a; Bururia, 2005, Van den Bagaard *et al.*, 2001; Stokes and Gillings, 2011; O'Leary, 2015).

Presence of ESBL- positive *E. coli* in animals is showing a general propensity to increase in some countries, among the bacteria from the poultry intestinal tract (Liebana *et al*., 2004; Hasman *et al*., 2005; Liebana *et al*., 2006; Roest *et al*., 2007; Hunter *et al*., 2010; and Leverstein-van Hall *et al*., 2011). This ESBL-group has the capability to break down and cause resistance to third-generation β-lactam antimicrobials (Pitout and Laupland, 2008); enzyme bla_{TEM} being responsible for 90% of ampicillin resistance in *E. coli* isolates (Livermore, 1995).

In spite of the rise in proportion of ESBL producing *E. coli* in some countries (AitMhand *et al*., 2002), there are very few research papers from Africa and especially Kenya on this (Blomberg *et al*., 2005; Kariuki *et al*., 2007). This is because, in developing countries, including Kenya, the identification of ESBL producing *E. coli* is not often carried out in many microbiology research institutes because of lack of facilities and resources for conducting ESBL detection, and also due to the fact that many clinicians have not fully appreciated the massive significance of ESBL (Thiong´o, 2012). In other related studies, *E. coli* producing $bla_{\text{CTX-M}}$ β-Lactamase with decreased susceptibility to cephalosporins, for example ceftiofur and penicillin, has been reported in poultry in Japan (Shiraki *et al.,* 2004). Another study in Japan by Ogutu *et al.* (2015),

reported *bla*$_{TEM}$ prevalence of 77.6% in clinical *E. coli* strains. This is in contrast with the finding of this study (zero prevalence). The results of this study highlight the use of multiplex-PCR system in detecting specific β-lactamase group genes and the resistance of the *E. coli* to multiple β-lactam antimicrobials. These multi-resistance genes may also be in form of large and easily transmissible plasmids, consisting β-lactamase and other genes (Pitout and Laupland, 2008). The presence of multiple resistance genes in one strain thus increases the probability of spreading the genes to other bacteria. Another study in Iran, reported prevalences of 60.3% and 37.7% for the *bla*$_{CTX-M}$ and *bla*$_{TEM}$ genes among ESBL-producing *E. coli* isolates, respectively (Khoshbakht *et al*., 2016).

The *dfrA1* is a gene which is found in *E. coli* resistant to trimethoprim and, most commonly in combination with sulfonamides (sulfamethoxazole), which is the antimicrobial of choice for the treatment of gastrointestinal tract infections and urinary tract infections (Ŝeputiene *et al*., 2010). The existence of *dfrA1* gene cassettes within integrons indicates a possibility of horizontal gene transfer of trimethoprim resistance among bacteria existing in different environments, including livestock and poultry, where trimethoprim is used for antimicrobial treatment and prophylaxis of food-producing animals (Mathew *et al*., 2007; Prescott, 2008). Using PCR-restriction fragment length polymorphism analysis, Saenz *et al.* (2004) found fifteen *E. coli* isolates resistant to trimethoprim; *dfrA1* gene being detected in seven (46.7%) of them. In the current study, *dfrA1* was found in three out of twenty eight (10.7%) *E. coli* isolates using multiplex-PCR method. The difference in the methods used for the two researches could be the reason behind the big difference in the recorded prevalences of *dfrA1* in respective *E. coli* strains. In another study in Lithuania by Ŝeputiene *et al.* (2010) in which a comparison between diseased and healthy-looking animals was made, frequencies of trimethoprim resistance among diseased animal

isolates were 23, 33, and 40% for swine, poultry and cattle, respectively. For the healthy-looking animals, 9% of isolates from poultry and 20% of isolates from cattle were trimethoprim resistant. The reason of variation in resistance averages was thought to be partly due to the sampling methodology, and partly due to previous unsuccessful treatment, which would favour the emergence of resistant bacteria; it was possible that some of the sick animals were recuperating. Studies on the ESBL genes presence in other bacteria, especially the rising cases in *Klebsiella pneumoniae*, have been documented (Ndiba, 2013; Magwenzi *et al.*, 2017; Ulstad *et al.*, 2016).

This study has shown that some of the *E coli* isolated from the study chickens carried one of the ESBL genes, which contributes towards antimicrobial resistance. Being easily transferable between bacteria, these genes play a role in dissemination of AMR among bacteria. This study has thus contributed data to the situation of AMR in chickens/animals in Kenya. The results will help in informing the policy makers as they embark in their fight towards reduction of AMR.

CHAPTER 5: OVERALL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 OVERALL DISCUSSION

This study demonstrated that antimicrobial resistant bacteria are circulating in chickens in Kenya. These include multi-drug resistant (MDR) bacterial ones – some to 6, up to 7 antimicrobials. The combinations of drug resistance were variable, but four antimicrobials were mostly included in the resistance blocks. These were: ampicillin (76.0%), tetracycline (71.1%), sulphamethoxazole (69.5%) and co-trimoxazole (65.5%). With respect to the three study groups: from clinical cases, resistance was mostly detected to ampicillin (75.0%), tetracycline, co-trimoxazole and sulphamethoxazole (each at 72.2%). From farm chickens, the mostly included antimicrobials were: ampicillin (71.4%), tetracycline, sulphamethoxazole, nitrofurantoin (each at 57.1%). However, from market chickens, the mostly included antimicrobials were: ampicillin, tetracycline (each at 100%), sulphamethoxazole (83.4%) and; nalidixic acid and cotrimoxazole (each at 66.7%). Evidence of correlation between antimicrobial usage and development of antibiotic resistance was demonstrated in this study when bacteria from 3 different study groups (clinical, farm and market chickens) were tested for antimicrobial susceptibility/resistance. Clinical isolates demonstrated higher resistance prevalence than farm and market chickens, while market chickens showed the least resistance prevalence.

Review of literature revealed that AMR is normally higher in cases where there is high antimicrobial usage, and that is, AMR development is directly associated with overuse of antimicrobials (Van den Bogaard *et al.*, 2001; Nys *et al.*, 2004). In a study carried out by Van den Bogaard *et al.* (2001), the proportion of samples containing resistant *E. coli* and percentages of resistant *E. coli* were significantly higher in turkeys and broilers (which were commonly given antimicrobials) than in laying productions (infrequently given antimicrobials). Working on

antimicrobial resistance of fecal *E. coli* from 8 developing countries, Nys *et al* (2004) documented higher antimicrobial prevalences in isolates from urban areas (where antimicrobials are used more frequently) compared to isolates from non-urban areas.

A report by Coghan (2016) that gut bacteria inside a 1000-year-old mummies from the Inca Empire are resistant to most of the currently-used antimicrobials (antibiotics were only discovered within the last 100 years) confirms what is already known that, for some of the bacteria, antimicrobial resistance is an inherent property. This gives them an advantage of survival, against the particular antimicrobial. Development of the antimicrobial resistance in currently susceptible bacterial populations suggests the continuous possibility that some (may be a very small percentage) of the bacteria mutate back to the original resistant state. In cases where the resistance is not manifested, chances are that these resistant mutants are kept under check, through bacterial competitiveness, by the many susceptible ones. Overuse of antimicrobials removes the susceptible population, through killing, thus giving a chance for the resistant few to multiply and flourish. This theory is supported by the observation that there has been high level of resistance towards commonly-used antimicrobials like tetracycline and less to the newer ones that are used sparingly, like ciprofloxacin and gentamycin. However, what is worrying is the latest development of resistance to the remaining few effective antimicrobials, even to the reserve drugs such as colistin (Kennedy and Collignon, 2010; Ulstad *et al.,* 2016; Anon, 2016; Liu *et al.,* 2016). This means that soon there may be no effective antimicrobial, since there are no indications of new antimicrobials being developed, except for the very few cases (Torome, 2015); meaning that the situation may get back to the pre-antibiotic *era* where treatable diseases/surgical contaminations killed patients – a genuine case for concern globally.

Demonstration of antimicrobial resistance in bacteria isolated from wild birds (Borges *et al.*, 2016; Simoes *et al.,* 2010) and colistin resistance plasmids in *E. coli* isolated from seagulls (McKenna, 2016) is worrying since the birds migrate widely across borders, so they can easily disseminate these hard-to-treat bacteria and their resistance genes across the world. While noting that wildlife migration is not the only way that bacteria can cross borders (there is plenty of opportunity through global trade and travel) seagull discovery, which translates to the role of wild birds in general, illustrates just how hard it is to control antimicrobial resistance once it takes root. It is, therefore, important to press-on and support the World bodies in their efforts to reduce the indiscriminate use of antimicrobials, which include: taking antimicrobials when sick, even if the sickness is not caused by a bacterium; underdosing; not completing the prescribed dosage; misuse of antimicrobials in animals – as growth factors or in order to increase production – this is notorious for poultry, fish and pig farmers (pressure of producing more to earn more). This is demonstrated in Van den Bogaard *et al*. (2001)'s publication where they reported higher resistance in turkeys and broilers (where antimicrobials were used a lot) and less in layers (where antimicrobials were less used). Also in the report of McKenna, (2016) which mentions that colistin resistance was first reported in an intensive pig farm in China (in November 2015), where colistin was used widely to make pigs grow faster. Reduction of this indiscriminate use of antimicrobials can only be effected through attitudinal change of the people and farmers.

In this study, when the resistant *E. coli* isolates were tested for the presence of ESBL genes (*bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$ and *dfrA1*), none of them had *bla*$_{\text{TEM}}$ and *bla*$_{\text{CTX-M}}$ genes and only three (10.7%) had the *dfrA1* gene. This shows that most of the resistance, demonstrated in bacteria in this study, was through mechanisms other than the three tested genes/enzymes. Noting that there

are chances of these resistant traits being carried by a plasmid, the resistance can easily be transmitted to other bacteria, as has been demonstrated by other researchers (Bebora *et al.,* 1994; Kikuvi *et al*., 2007$_a$; Bururia, 2005; Van de Bogaard *et al*., 2001; Stokes and Gillings, 2011; O'Leary, 2015). This will provides a potential for the resistance gene to spread widely within a short time.

This study has demonstrated existence of antimicrobial resistance, both single and multiple, in bacteria of various genera isolated from chickens in Kenya; most of the AMR being to the commonly-used antimicrobials. The study has also shown that some of the *E coli* isolated from the study chickens carried one of the ESBL genes, *dfrA1* gene, which contributes towards antimicrobial resistance. It thus contributes towards data on current AMR status in bacteria harboured by chickens/animals in Kenya. The results will help in informing the policy makers as they embark in their fight towards reduction of AMR.

5.2 CONCLUSIONS

- The various aerobic bacteria isolated from chickens in this study included genera: *Streptococcus*, *Escherichia*, *Staphylococcus*, *Bacillus*, *Proteus* and *Corynebacterium.*
- This study has demonstrated existence of antimicrobial resistance, both single and multiple (some up to 7 antimicrobials), in bacteria of various genera isolated from chickens in Kenya.
- The AMR was mostly towards the commonly-used antimicrobials, namely: ampicillin (76.0%), tetracycline (71.1%), sulphamethoxazole (69.5%) and co-trimoxazole (65.5%). They were least resistant to gentamycin (8.3%).
- Overall, more *Escherichia coli* isolates demonstrated multi-drug resistance compared to the other isolates.
- When comparing the three study groups, the general picture indicated a higher MDR prevalence in bacteria isolated from clinical cases, followed by market birds (Table 3.3).
- There were some bacterial strains that were still susceptible to the commonly-used antimicrobials.
- The study has also shown that some of the *E coli* isolated from the study chickens carried one of the ESBL genes, *dfrA1* gene, which contributes towards antimicrobial resistance.
- Since only 3 (10.7%) of the 28 resistant *E. coli* isolates studied carried the *dfrA1* gene, it shows that 25 (89.3%) of the studied resistant *E. coli* utilised other means to express their antimicrobial resistance.

5.3 RECOMMENDATIONS

- More studies need to be carried out on other chickens and other animals, so as to get the full picture of the extent of antimicrobial resistance in animals.
- Since antimicrobial resistance status could change with time, a surveillance system needs to be put in place to monitor the trends.
- As a means towards reduction of AMR development in bacteria, awareness campaigns need to be encouraged in order to effect behavioural change towards reduction of indiscriminate usage of antimicrobials.

REFERENCES

Abbas A and Newsholme (2009). Diagnosis and recommended treatment of Helminth infections. www.prescriber.co.uk cited Oct 12, 2017.

AitMhand, R., Soukri, A., Moustaoui, N., Amarouch, H., ElMdaghri, N., Sirot, D., and **Benbachir, M., (2002).** Plasmid-mediated TEM-3 extended spectrum beta-lactamase production in Salmonella Typhimurium in Casablanca. *Journal of Chemotherapy* 49:169-172.

Al-haddad, O. H., Zorgani, A., and **Ghenghesh, K. S., (2014).** Nasal carriage of multi-drug resistant panton-valentine leucocidin-positive methicillin-resistant *Staphylococcus aureus* in children in Tripoli-Libya. *American Journal of Tropical Medicine and Hygiene*, 90: 724-727.

Allorechtova, K., Dolejska, M., Cizek, A., Tausova, D., Klimes, J., Bebora, L. C., and **Literak, I., (2012).** Dogs of nomadic pastoralists in Northern Kenya are reservoirs of plasmid-mediated cephalosporins- and quinolone-resistant *Escherichia coli*, including pandemic clone B2-O25-ST131. *Journal of Antimicrobial agents and chemotherapy* 56 (7): 4013-4017.

Amare, A. Amin, A. M. Shiferaw, A. Nazir, S. and **Negussie, H. (2013).** Yolk sac infection (Omphalitis) in Kombolcha Poultry farm, Ethiopia. *American Eurasian Journal of Scientific Research* 8 (1): 10-14.

Angulo, F. J., Nargund, V. N. and **Chiller, T. C., (2004)**. Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. *Journal of Veterinary Medicine*. B, *Infectious diseases and veterinary public health*, 51:8-9.

Anon, (2016): Antibiotic resistance grows as last-line drugs fail. GEN News Highlights

November 21. *www.genengnews.com/gen-news-highlights (cited Mar 12 2017)*

Arbeit, R., (1995). Laboratory procedures for the epidemiologic analysis of microorganisms. *Manual of Clinical Microbiology http://ci.nii.ac.jp/naid/10018346571/ (cited Jan 22 2017)*

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and **Struhl, K., (2003).** Current Protocols in Molecular Biology, ringbou editions, Copyright © 2003 John Wiley & Sons, Inc.).

Bacteriology Laboratory Diagnostic Records, (2015-2016). University of Nairobi, College of Agriculture and Veterinary Sciences-Faculty of Veterinary Medicine, Department of Veterinary pathology, Microbiology and Parasitology (Bacteriology laboratory).

Barabra, B., and **Jane, J., (2006).** Infection: Microbiology and Management: Barbara Bannister, Stephen Gillespie, Jane Jones: 9781405126656*: Amazon.com:* Books. 4:64-68.

Bartlett, J. M. S. and **Stirling, D., (2003).** A short history of the polymerase chain reaction. Methods in molecular biology (Clifton, N. J.), 226: 3–6.

Baruah, K. K., Sharama, P. K., and **Bora, N. N., (2001).** Fertility, hatchability and embryonic mortality in ducks. *Indian veterinary journal* 78: 529-530.

Bebora, L. C., (1979). A study of the occurrence of avian Salmonellosis in some farms and a slaughterhouse in Kenya. MSc. Thesis, University of Nairobi.

Bebora, L. C., (1987). Fowl typhoid The disease, immunity and control. PhD thesis, University of Nairobi.

Bebora, L. C., and **Nyaga P. N., (1989).** Antibiotic resistance in *Salmonella gallinarum* isolates recovered from Kenya. Paper presented at 1st Annual Scientific Conference of the Faculty of Vet. Medicine, University of Nairobi.

Bebora, L. C., and **Nyaga, P. N., (1989).** Antibiotic Resistance In *Salmonella Gallinarum*

Isolates Recovered From Kenya. *Bulletin of Animal Health and Production in Africa,* (Special issue) 115–118.

Bebora, L. C., Oundo, J. O. and **Yamamoto, H., (1994).** Resistance of *E. coli* strains, recovered from chickens to antibiotics with particular reference to trimethoprim-sulfamethoxazole (Septrin*). East African Medical Journal,* 71 (10): 624–627.

Bebora, L. C., Oundo, J. O., Khamala. J., Saidi, S., Sang, W. K., Yamamoto, H., and **Mukundi, P.W. (1993).** Some *E. coli* strains causing septicaemia in chicks in Kenya the Kenyan Veterinarian, volume 17:1-2.

Bebora, L. C., Yamamoto, H., Sang, W. K., Oundo, J. O., Khamala, A., Mwangudza, A., and **Mukundi, P. W., (1994).** Plasmid profiles of *Escherichia coli*, *Salmonella* and *Shigella* organisms isolated in Kenya. *East African Medical Journal* 71**:**619–623.

Bhatia, K. C., Sharama, U. K., and **Singh, N., (1972).** Studies of persistent yolk sac condition in chicks. *Indian journal of animal health* 11:173-176.

Bhatt, C. P., Baidya, R., Karki, P., Shah, R. K., Miya, R., Mahashate, P., and **Mishra, K., (2014).** Multi Drug Resistance, Bacterial Isolates of Surgical Site Infection. *Open Journal of Medical Microbiology* 4: 203-209.

Blomberg, B., Jureen, R., Manji, K., Tamim, B., Mwakagile, D., Urassa, W., Fataki, M., Msangi, V., tellevik, M., Maselle, S., and **Langland, N., (2005).** High rate of fatal cases of pediatric septicemia caused by gram negative bacterial with extended-spectrum beta-lactamases in Dar es Salam, Tanzania. *Journal of clinical Microbiology* 43:745-749.

Bonev, B., Hooper, J., and **Parisot, J., (2008).** Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *The Journal of antimicrobial chemotherapy*, 61(6): 1295–301.

Borges, C. A., Beraldo, L. G., Maluta, R. P., Cardozo, M. V., Barboza, K. B., Guastalli, E. A. L., S. Kariyawasam, S., DebRoy, C., and **Ávila, F. A., (2016)**. Multidrug-resistant pathogenic *Escherichia coli* isolated from wild birds in a veterinary Hospital. *Avian Pathology* http://dx.doi.org/10.1080/03079457.2016.1209298.

Bradford, P. A., (2001). Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clinical Microbiology Reviews* (4): 933-51.

Brien, T. F., (2002). Emergence Spread and Environmental Effect of Antimicrobial Resistance: How Use of an Antimicrobial Anywhere, can Increase Resistance to Any Antimicrobial Anywhere Else resistance genes and the emergence of resistance, 02115 (Suppl 3). *https://www.ncbi.nlm.nih.gov/pubmed/11988877*

Brody, J., and **Kern, S., (2004):** History and principles of conductive media for standard DNA electrophoresis. *Annals Biochemistry* 333:1-13.

Bururia, J. M., (2005). Characterisation of multiple drug resistant Klebsiella strains isolated in Kenya. MSc thesis, University of Nairobi.

Buxton, A., and **Fraser, G., (1977).** Animal Microbiology Black well Scientific Publication volume 1. London, Oxford, UK.

Camara, M., Dieng, M., and **Boye, C. H. B., (2013).** Antibiotic Susceptibility of *Streptococcus Pyogenes* Isolated from Respiratory Tract Infections in Dakar, Senegal. *Microbiology Insights* 6: 71–75.

Carson, C. F., Mee, B. J., and **Riley, T. V., (2002).** Mechanism of Action of Melaleuca alternifolia (Tea Tree) Oil on *Staphylococcus aureus* Determined by Time-Kill, Lysis, Leakage, and Salt Tolerance Assays and Electron Microscopy 46 (6): 1914–1920.

Chakraborty, R., and **Coates, J. D., (2005).** Hydroxylation and carboxylation--two crucial steps of anaerobic benzene degradation by Dechloromonas strain RCB. *Applied and Environmental microbiology*, 71 (9): 5427–32.

Charan, J., and **Biswas, T., (2013).** How to calculate sample size for different study designs in medical research? Indian Journal of Psychological Medicine 35(2): 121-126.

Chopra I and Roberts M (2001). Tetracycline Antibiotics: Mode of Action, Application molecular biology and Epidemiology of Bacterial Resistance. Microbiology and Molecular Biology 65(2): 232-260

CLSI-Clinical and Laboratory Standards Institute (2008). Performance standards for antimicrobial susceptibility testing; Eighteenth informational supplement vol. 28 No.1 M100-s18, ISBN1-56238-653-0.

Coghan, A. (2016): Antibiotic resistance discovered in the guts of ancient mummies. New Scientist, 8 July 2016; Museum of Applied Arts and Sciences in Sydney Australia.

Costa, M. M., Drecher, G., Maboni, F., Webar, S. S., Schrank, A., Vainstein, M. H., Schrank, I. S., and **Vargas, A. C., (2010).** Virulence factors, antimicrobial resistance and plasmid content of *Escherichia coli* isolated in swine commercial farms. *Veterinary Microbiology* 62 (1): 30–36.

Coyle, M. B., (2005). Manual of antimicrobial susceptibility test, American Society for Microbiology. *https://www.researchgate.net/file.PostFileLoader.html?id...assetKey.*

de Souza, C. M. and **Hidalgo, M. P. L., (1997).** The Medical Impact of Antimicrobial Use in Food Animals. Report of a WHO Meeting. Berlin, Germany, 13-17 October 1997. World Health Organization, (October): 13–17.

Dorman, S. E., and **Chaisson, R. E., (2007).** From magic bullets back to the Magic Mountain:

the rise of extensively drug-resistant tuberculosis. *Nature Medicine* 13: 295–298.

Fair, R. J., and **Tor, Y.,** (2014). Antibiotic and bacterial resistance in the 21st Century, *Perspectives in Medicinal Chemistry* 6: 25-64.

FAO, OIE and **WHO (2014)**. Collaboration: sharing responsibilities and coordinating global activities to address health risks at the animal-human-ecosystems interfaces – a tripartite concept note, 2010.

(FAO) poultry and poultry products risk for human health (slaughtering and processing (MariaVenterura de silva cited Oct 12 2017) *www.fao.org/docrep/013/al742e/al742e00.pdf*

Forbes, B. A., Sahm, D., and Weissfield, A. S., (2002). Baiely and Scotts Diagnostic Microbiology, 11th Edition. Mosby. Westline Industrial Drive, St Louis-Missouri 63146.

Fratamico, P., and **Smith, J., (2006).** *Escherichia coli* infections. Foodborne infections and intoxications. (3rd Edn.) Florida, Academic Press, an imprint of Elsevier. PP: 205-208.

Gakuya, F. M., Kyule, M. N., Gathura, P. B., and **Kariuki, S. M., (2007):** Antimicrobial resistance of bacterial organisms isolated from rats. *East African Medical Journal* 78 (2): 646-649.

Georgios, M., and **Egki, T., (2014)** Phenotypic and Molecular Methods for the Detection of Antibiotic Resistance Mechanisms in Gram Negative Nosocomial Pathogens. *https://www.intechopen.com/.../phenotypic-and-molecular-methods-for-the-detection-of.*

Hart, W. S., Heuzenroeder, M. W., and **Barton, M. D., (2004).** Antimicrobial resistance in *Campylobacter* spp., *Escherichia coli* and Enterococci associated with pigs in Australia. *Journal of Veterinary Medicine* Series B: *Infectious Diseases and Veterinary Public Health*, 51(5): 216–221.

Hasman, H., Mevius, D., Veldman, K., Olesen, I., and **Aarestrup, F. M., (2005)**. β-lactamases

among extended-spectrum-b-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and human patients in the Netherlands. *Journal of Antimicrobial Chemotherapy*. 56:115–21.

Helmuth, R., and **Hensel, A., (2004).** Towards the Rational Use of Antibiotics: Results of the First International Symposium on the Risk Analysis of Antibiotic Resistance. *Journal of Veterinary Medicine*, 51:357–360.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, S. T., (1994). Bergey's Manual of Determinative Bacteriology, ninth edition. Williams and Wilkins 428 East Preston Street-Baltimore, Maryland 21202, USA.

Hunter, P. A., Dawson S., French G. L., Goossens H., and **Hawkey P. M., (2010)**. Antimicrobial-resistant pathogens in animals and man: prescribing, practices and policies. *Journal of Antimicrobial Chemotherapy* 65 Suppl 1:3-17.

Igbinosa, E. O., Beshiru, A., Akporehe, L. U., Oviasogie, F. E., and **Igbinosa, O. O., (2016).** Prevalence of Methicillin-Resistant *Staphylococcus aureus* and Other *Staphylococcus* species in raw meat samples intended for human consumption in Benin City, Nigeria: implication for public health. *International Journal of Environmental Research and Public Health* 13 (10): 949-960.

Imohl, M., and **Van der Linden, M., (2015).** Antimicrobial Susceptibility of invasive *Streptococcus pyogenes* isolates in Germany during 2003-2013. *PloS ONE* 10 (9): e0137313.

Jenkins, S. G., and **Schuetz, A. N., (2012).** Current concept in laboratory testing to Guide antimicrobial therapy. *Mayo Clinic Proceedings* 87 (3): 290-308.

Joshi, M., (2010). Polymerase Chain Reaction: Methods, Principles and. *International Journal of Biomedical Research.* Available at: www.ssjournals.com *ssjournals.com/index.php/ijbr/article/view/640/636*

Kariuki, S. M., (2010). Antimicrobial Resistance in Enteric Pathogens in Developing Countries. Antimicrobial Resistance in Developing Countries A. Sosa, D. K Byarugaba, C.F. Amabile--Cuevas*et al*, Springer.

Kariuki, S. M., (2011). Antibiotic Use and Resistance in Kenya-GRAP. (August), p.30. *https://cddep.org/.../situation_analysis_and_recommendations_antibiotic_use_and_res.*

Kariuki, S. M., and **Dougan, G., (2014).** Antimicrobial resistance in Sub-Saharan Africa: an underestimated emergency. *Annals of the New York Academy of Science* 1323:43-55.

Kariuki, S. M., and **Revathi, G., (2006).** Invasive multidrug-resistant non-typhoidal *Salmonella* Infections in Africa: zoonotic or anthroponotic transmission? *Journal of Medical Microbiology* 55:585-591.

Kariuki, S. M., Gilks, C., Corkill, J., Benea, A., Waiyaki, P., and **Hart, C. A., (1996):** Multi-drug resistant, non-typhi salmonellae in Kenya. *Journal of Antimicrobial Chemotherapy* 38 (3): 425-434.

Kariuki, S. M., Gilks, C., Kimari, J., Obanda, A., Muyodi, J., Waiyaki, P., and **Hart, C. A. (1999).** Genotype analysis of *Escherichia Coli* strains isolated from children and chickens living in close contact. *Applied and Environmental Microbiology* 65: 472-476.

Kariuki, S. M., Gunturu, R., Francis, G., Victor, Y., and **Muyodi, J., (2002).** Lack of clonal relationship between non-typhi Salmonella strain types from humans and those isolated from animals living in close contact. *FEMS Immunology and Medical Microbiology* 33.165–171.

Kariuki, S. M., Revathi G., Corkill J., Kiiru J., Mwituria J., Mirza, N.. and **Hart, C. A., (2007$_a$).** *Escherichia coli* from community-acquired urinary tract infections resistant to fluoroquinolones and extended-spectrum beta lactams. *Journal of Infection in Developing Countries* (JIDC) 1 (3): 257-262.

Kariuki, S. M., Revathi, G., Corkill, J., Kiiru, J., Mwituria, J., Mirza, N., and **Hart, C. A (2007).** *Escherichia coli* from community-acquired urinary tract infections resistant to fluoroquinolones and extended-spectrum beta-lactams *Journal of Infection in Developing Countries*; 1 (3): 257-262.

Kariuki, S. M., Revathi, G., Kariuki, N., Kiiru J., Mwituria J. and **Hart, C. A., (2006).** Characterization of community-acquired non-typhoidal Salmonella from bacteraemia and diarrhoeal infections in children admitted to hospital in Nairobi, Kenya. *BMC Microbiology* 20066:101. DOI:10.1186/1471-2180-6-101.

Kariuki, S. M., Revathi, G., Kiiru. J., Mengo, M. D., Mwituria, J., Muyodi, J., Munyalo, A., Teo, Y. Y., Holt, K. E., Kingsley, R. A., and **Dougan, G., (2010)**. Typhoid in Kenya is associated with a dominant multidrug-resistant Salmonella enterica serovar Typhi haplotype that is also widespread in Southeast Asia. *Journal of Clinical Microbiology*, 48 (6): 2171–6.

Kariuki, S., Gilks, C., Kimari, J., Muyodi, J., Waiyaki, P., and **Hart, C. A., (1997**). Plasmid diversity of multi-drug-resistant *Escherichia coli* isolated from children with diarrhoea in a chickens farming area in Kenya. *Annual Tropical Medical and Parasitology* 91**:** 87–94.

Kennedy, K., and **Collington, P., (2010):** Colonisation with *Escherichia coli* resistant to "critically important" antibiotics: a high risk for international travellers. *European Journal of Clinical Microbiology and Infectious Diseases* 29 (12): 1501-1506.

Khoshbakhat, R., Seifi, S., and **Raeisi, M., (2016).** Antibiotics susceptibility and high

prevalence of extended spectrum beta-lactamase producing *Escherichia coli* in Iranian broilers. *Revue de Médecine Vétérinaire* 167, 5-6,133-137.

Kibenge, F. S., Wilcox, G. E., and **Perret. (1982).** *Staphylococcus aureus* Isolated from poultry in Australia phage typing and Cultural characteristics. *Veterinary Microbiology* 7: 471-483.

Kikuvi, G. M., Ole-Mapenay I. M., Mitema, E. S., and **Ombui J. N., (2013).** Antimicrobial resistance in *Escherichia coli* isolates from faeces and carcass samples of slaughtered cattle, swine and chickens in Kenya. *Israel Journal of Veterinary Medicine*. 2006; 61(3–4):82–88

Kikuvi, G. M., Ombui J. N., and **Mitema E. S., (2010).** Serotypes and antimicrobial resistance profiles of *Salmonella* isolates from pigs at slaughter in Kenya. *Journal of Infection in Developing Countries* (JIDC) 4 (4): 243-248.

Kikuvi, G. M., Ombui, J. N., Mitema, E. S., and **Schwartz, S., (2007_b).** Antimicrobial resistance in *Salmonella* serotypes isolated from slaughter animals in Kenya. *East African Medical Journal* 84 (5): 233-239.

Kikuvi, G. M., Schwartz, S., Ombui J. N., Mitema, E. S., and **Kehrenberg, C., (2007_a).** Streptomycin and Chloramphenicol resistance genes in *Escherichia coli* isolates from cattle, pigs and chicken in Kenya. *Microbial Drug Resistance* 13 (1): 62-68.

Kleppe, K., and Ohtsuka, E., (1971). Studies on polynucleotides XCVI Repair replications of short synthetic DNA's as catalyzed by DNA polymerases. *Journal of Molecular Biology*, 56 (2): 341–61.

Kobayashi, M., Conklin, L. M., Bigogo, G., Jagero, G., Hampton, L., Fleming-Dutra, K. F., Junghae, M., Carvalho, M., Pimenta, F., Beall, B., Taylor, T., Laserson, K. F., Vulule, J., Beneden, C., Kim, L., Feikin, D. R., Whitney, C. G., and **Breiman, R. F., (2017)**. Pneumococcal carriage and antibiotic susceptibility patterns from two cross-sectional

colonization surveys among children aged <5 years prior to the introduction of 10-valent pneumococcal conjugate vaccine—Kenya, 2009–2010. *BMC Infectious Diseases* 17(1):25. doi: 10.1186/s12879-016-2103-0.

Kolar, M., Pantucek R., Bardon J., Vagnerova I., Typovska H., Valka I., and **Doskar J., (2002).** Occurrence of antibiotic resistant bacterial strains isolated in poultry. *Veterinární medicína* 47: 52-59.

Kutto, E. K., (2012). Bacteriological contamination of farm and market kale in Nairobi and its environs: Pathogenicity and Antibiotic sensitivity of isolated salmonellae. MSc. Thesis, University of Nairobi.

Lalitha, M. K., (2004). Manuel on antimicrobial susceptibility testing (under the auspices of *Indian Association of Medical Microbiologists*).

Lambert, R. J. W., and **Pearson, J., (2000).** Susceptibility testing: accurate and reproducible minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) values *Journal of Applied Microbiology* 88 (5): 784–790.

Landers T F, Cohen B, Wittum T E, Larson, E L (2012) A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential, *Public Health Reports*. 2012 Jan-Feb; 127(1): 4–22.

Lang, L., and **García, F., (2004)**. Comparison of E-test and disk diffusion assay to evaluate resistance of Helicobacter pylori isolates to amoxicillin, clarithromycin, metronidazole and tetracycline in Costa Rica. *International Journal of Antimicrobial Agents* 24 (6): 572–577.

Laxminarayan, R., and **Chaudury, R. R., (2016).** Antibiotic resistance in India; Drivers and opportunities for action. *PLOS Medicine* 13 (3): e1001947.

Leverstein-van hall, M. A., Dierik, C. M., Cohen, S. J., Voets, G. M., and **van den**

Munckhof, M. P., (2011). Dutch patients, retail chicken meat and poultry share the same ESBL genes. Plasmid and strains. *Clinical of Microbiology Infection* 17: 873-880.

Liebana, E., Batchelor, M., Hopkins, K. L., Clifton-Hadley, F. A., and **Teale, C. J., (2006).** Longitudinal farm Study of extended-spectrum β-lactamase-mediated resistance. *Journal of Clinical Microbiology* 44: 1630-1634.

Liebana, E., Gibbs, M., Clouting, C., Baker, L., and **Clifton-Hadley, F. A., (2004).** Characterization of β-lactamases responsible for resistance to extended-spectrum cephalosporins in *Escherichia coli* and *Salmonella* enterica strains from food- producing animals in the United Kingdom. *Microbial drug Resistance* 10: 1-9.

Liu, Y-Y., Wang, Y., Walsh, T. R., Xlan, L., Zhang, R., Spencer, J., Dol, Y., Tian, G., Dong, B., Huang, X., Yu, L. F., Gu, D., Ren, H., Chen, X., LV, L., He, D., Zhou, H., Liang, Z., Liu, J. H., and **Shen, J., (2016).** Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *The Lancet Infectious Diseases* 16:161–8.

Livermore, D. M., (1995). Beta-Lactamases in laboratory and clinical resistance. *Clinical Microbiology Reviews* 8: 557-584.

Macias, E. A., Mason, E. O., Ocera, H. Y., and **Larocco, M. T., (1994)**. Comparison of E test with standard broth microdilution for determining antibiotic susceptibilities of penicillin-resistant strains of Streptococcus pneumoniae. *Journal of Clinical Microbiology* 32 (2): 430–2.

Magwenzi, M. T., Guda-Mugable, M., Mujuru, H. A., Dangarembizi-Bwakura, M., Robertson, V., and Aiken, A. M., (2017). Carriage of Antibiotic-resistant Enterobacteriaceace in hospitalized children in tertiary hospitals in Harare, Zimbabwe.

Antimicrobial Resistance and Infection Control 6:10. https://doi.org/10.1186/s13756-016-0155-y

Mapeney, J. M., Kikuvi, G. M., Mitema, E. S., and **Ombui, J. N., (2006).** Antibiotic resistance of *E. coli* isolated from healthy food animals in Kenya. *Kenya Veterinarian* 30 (1): 23-27.

Margaret, I. P., Lyon, D. J., Yung, R. W. H., Chan, C., and **Chenege, A. F. B., (1999).** Evidence of clonal dissemination of multidrug-resistant streptococcus pneumonia in Hong Kong. *Journal of Clinical Microbiology* 37 (9): 2834-2839.

Maron, D. F., (2016): Superbug explosion triggers UN General Assembly (UNGA) meeting. Scientific America. *https://www.scientificamerican.com/.../superbug-explosion-triggers-u-n-general-assem cited Mar 12 2017*

McKenna, M., (2016). Seagulls are carrying a Dangerous Superbug Through the skies. *marynmckenna.com/by-pub/ Feb 22 2017*

Mead, G. C., and **Dodd, C. E., (1990).** Incidence, origin and significance of staphylococci on processed poultry. *Society for Applied Bacteriology Symposium Series* 19:815-915.

Merchant I. A., and **Packer, R. A (1967).** Veterinary Bacteriology and Virology. Seventh Edition. The Lowa State University press. Ames, USA.

Mathew, A. G., Cissell, R. & Liamthong, S. (2007). Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. *Foodborne Pathogen Disease* 4, 115–133.

Mishra, K., Srivastava, A., and **Garg, A., (2006)**. Antibiotic susceptibility of Helicobacter pylori clinical isolates: comparative evaluation of disk-diffusion and E-test methods. *Current Microbiology*, 53 (4): 329–34.

Mitema, E. S., and **Kikuvi G. M., (2004).** Surveillance of the overall use of antimicrobial drugs

in humans over a 5 year period (1997-2001) in Kenya. *Journal of Antimicrobial Chemotherapy*. 54: 966-967.

Mitema, E. S., Kikuvi G. M, Wegener, H. C., and **Stohr, K., (2001).** An Assessment of antimicrobial consumption in food producing animals in Kenya. *Journal of Veterinary Pharmacology and Therapeutics*, 24: 385–390.

Mitema, E. S., Kikuvi, G. M., Wegener, H. C., and **Stohr, K. (2001).** Antimicrobial susceptibility and plasmid from *Escherichia coli* isolated from rat, *East African Medical Journal* 78: 518-522.

Mitema, E. S. (2010). The role of unregulated sale and dispensing of antimicrobial agents on the development of antimicrobial resistance in developing countries. In: Antimicrobial resistance in developing countries, Antimicrobial Resistance in Developing Countries edit. Springer, NY,

Mølbak, K., (2004). Spread of Resistant Bacteria and Resistance Genes from Animals to Humans – The Public Health Consequences *Journal of Veterinary Medical B Infection Disease Veterinary* 51 (8-9): 364–369.

Moyo, S. J., Steinbakk., M, Aboud., Mkopi, N., Kasubi, M., Blomberg, B., Manji, K., Lyamuya, E. F, Maselle, S. Y., and **Langeland, N., (2012).** Penicillin resistance and serotype distribution of *Streptococcus pneumoniae* in nasopharyngeal carrier children under 5 years of age in Dar es Salaam, Tanzania. *Journal of Medical Microbiology* 61:952-959.

Murdoch, D. B., and **Baker, J. R., (1977).** Bacterial endocarditis in the dog, *Journal of Small Animal Practice*, 18:687-699.

Mustsami N A., (2011). A comparative study of Antibiotic resistance profiles among enteric bacteria in broilers and traditional chicken from selected farms in Kericho, Kenya. MSc

Thesis. Jomo Kenyatta University. *ir-library.ku.ac.ke/bitstream/handle/123456789/.../Mutsami%20Anne%20Naliaka.pdf;...*

Ndiba, P. M., (2013). Phenotypic detection and susceptibility pattern for the detection of extended spectrum beta-lactamase – producing *Klebsiella pneumonia*e isolates in Nairobi, Kenya. *Open Journal of Medical Microbiology* 3:91-94.

Nahar, A., Siddiquee, M., Nahar, S., Anwar, K. S., Ali, S.I and **Islam, S (2014).** Multidrug resistance-*Proteus mirailis* isolated from chicken droppings in commercial poultry farms, Bio-security concern and emerging public Health in Bangladesh. *Journal of Biosafety and Health Education* 2: 120 doi:10.4172/2332-0893.1000120.

Nemati, M., (2013). Antimicrobial resistance of *Proteus* isolates from poultry. *European Journal of Experimental Biology* 3 (6): 499-500.

Njagi, L. W., (2003). Carrier status, Antibiotic and disinfectant sensitivity patterns of *Listeria monocytogenes* and other aerobic bacteria in scavenging chickens and ducks. MSc Thesis, University of Nairobi.

Njagi, L. W., Mbuthia, P. G., Bebora, L. C., Nyaga, P. N., Minga, U. M., and **Olsen, J. E., (2004).** Sensitivity of *Listeria* species recovered from indigenous chickens, to antibiotics and disinfectants. *East African Medical Journal* 81 (10): 534-537.

Nys, S., Okeke, N., Kariuki, S., Dinant, C. J., Driesen, C., and **Stobberingh E. E., (2004).** Antibiotic resistance of fecal *Escherichia coli* from healthy volunteers from eight developing countries. *Journal of Antimicrobial Chemotherapy* 54 (5): 952-955

O'Driscoll, J., (1977). Venereal infection in through breads with bacillus *Proteus mirabilis*. *Veterinary Record* 100 (25): 534.

O'leary, D., (2015). Horizontal Gene Transfer: Sorry, Darwin, It's Not your Evolution Any

More. Evolution News and Views *https://evolutionnews.org/2015/08/horizontal_gene/* (cited April 13 2017).

O'Neill, J., (2016). Tackling drug-resistant infections globally: Final report and recommendations. Review on antimicrobial resistance. Wellcome Trust and UK Government *https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf (cited feb 22, 2017)*

Odwar, J. A., Kikuvi, G., Kariuki, J. N., and **Kariuki, S. M., (2014).** A cross-sectional study on the Microbiological quality and safety of raw chicken meats sold in Nairobi, Kenya. *BMC Research Notes* 7: 627.

Ogutu, J. O., Zhang, Q., Huang, Y., Yan, H., Su, L., Gao, B., Zhang, W., Zhao, J., Gai, W., Li, W., Zhao, H., Chen, Y., Song, W., Chen, X., Fu, Y., and **Zhang, F., (2015)**. Development of multiplex PCR System and it is application in detection of *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-9}$ and *bla*$_{OXA-1}$ group genes in clinical *Klebsiella Pneumonia* and *Escherichia coli* strains. *The Journal of antibiotics* 68:725-733.

OIE (2010). Guidelines for the prudent use of veterinary antimicrobial drugs – with notes for guidance. Revised version. Supplement to the *Germany Veterinary Journal* volume 10. *www.oie.int/fileadmin/Home/eng/Health_standards/tahc/.../chapitre_antibio_use.pdf (cited Feb 28 2017.)*

OIE (2015). Fact sheet on Antimicrobial resistance. *www.oie.int/en/for-the-media/key-documents/fact-sheets/cited (Feb 28 2017)*

Olive, D. M. and **Bean, P., (1999).** MINIREVIEW Principles and Applications of Methods for DNA-Based Typing of Microbial Organisms. *Journal of Clinical Microbiology* 37 (6):

1661–1669.

Ombui, J. N., Kimotho, A. M., and **Nduhiu, G., (2000).** Antimicrobial resistance patterns and plasmid profiles of *Staphylococcus aureus* isolated from milk and meat. *East African Medical Journal* 77 (9): 463-467.

Osman, T. M., and **El Sanousi, S. M., (2013)**. Aerobic Bacteria Isolated from Dead –in-Shell Chick Embryo in Khartoum State. *The Sudan Journal of Veterinary Research* 28: 1-5.

Oundo, J. O., Kariuki, S. M., Boga, H. I., Muli, F. W. and **Iijima, Y. (2008).** High incidence of enteroaggregative *Escherichia coli* among food handlers in three areas in Kenya: A possible transmission route of travellers' diarrhoea. *Journal of Travel Medicine* 15 (1): 31-38.

Perovic, O., and **Schultz, C., (2016).** Stepwise approach for implementation of antimicrobial resistance surveillance in Africa. *African Journal of Laboratory Medicine*. ISSN: (On line), pages 5(3): 482-488.

Pine, J. H., Ritches, W. R., and **Esterly J. R., (1973).** Bacterial Pneumonia: Ultrastructural, auto radiographic and histochemical observations. *American Journal of Pathology* 73:115-124.

Pitout, J. D., and **Laupland, K. B., (2008).** Extended-spectrum beta-lactamase-producing Enterobacteriaceae an emerging-health concern. *Lancet Infectious Disease* 8: 159-166.

Prescott, J. F., (2008). Antimicrobial use in food and companion animals. *Animal health Research Review* 9: 129-133.

Rodriguez, P. H., and **Ramirez, A. G., (2012).** Polymerase chain reaction; Types, utilities and limitation www.intechopen.com published May 30 (8): 157-172.

Roest, H. I., Liebana, E., Wannet, W., van Duynhoven, Y., and **Veldman, K. T., (2007).**

Antibiotic resistance in *Escherichia coli* O157:H7 isolated between 1998 and 2003 in the Netherlands. Tijdschr Diergeneeskd 132: 954-958.

Saenz, Y. Brinas, L. Dominguez, E. Ruiz, J. Zarazaga, M, Vila, J and **Torres, C. (2004).** Mechanisms of resistance in multiple-Antibiotics resistant *Escherichia coli* strains of human, Animal, and food origins. *Antimicrobial Agents and Chemotherapy* 40: 3996-4001.

Saiki, R. K., Scharf, S. Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A and **Arnheim. N., (1985)**. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia. Science (New York, N.Y.), 230 (4732): 1350–4.

Sang, W. K., Boga H. I., Waiyaki, P. G., Schnabel, D., Wamae, N. C., and **Kariuki, S. M., (2012).** Prevalence and genetic characteristics of Shigatoxigenic *Escherichia coli* from patients with diarrhoea in Maasailand, Kenya. *Journal of Infection in Developing Countries*, 6 (2): 102–8.

Sebaihia, M., Wren, B. W., Mullany, P., Fairweather, N. F., Minton, N., Stabler, R., Thomson, N. R., Roberts, A. P., Cerdeño-Tárraga, A. M., Wang, H., Holden, M. TG., Wright, A., Churcher, C., Quail, M. A., Baker, S., Bason, N., Brooks, K., Chillingworth, T., Cronin, A., Davis, P., Dowd, L., Fraser, A., Feltwell, T., Hance, Z., Holroyd, S., Jagels, K., Moule, S., Mungall, K., Price, C., Rabbinowitsch, E., Sharp, S., Simmonds, M., Stevens, K., Unwin, L., Whithead, S., Dupuy, B., Dougan, G., Bart Barrell, B., and **Parkhill, J., (2006).** The multidrug-resistant human pathogen *Clostridium difficile* has a highly mobile, mosaic genome. *Nature Genetics* 38: 779 – 786.

Seputiene, V., Povilonis, J., Ruzauskas, M., Povilonis, A., and **Suziedeliene, E., (2010)**. Prevalence of Trimethoprim resistance genes in *Escherichia coli* isolated of human and

animal origin in Lithuania. *Journal of Medical Microbiology* 59: 315-322.

Shiraki, Y., Shabata, N., Doi, Y., and **Arakawa, Y., (2004**). *Escherichia coli* Producing CTX-M-2 β-Lactamase in cattle, Japan. *Emerging Infectious Diseases*, 10 (1): 69-75.

Shitandi, A., and **Mwangai, M., (2004).** The occurrence of multiple antimicrobial resistances among *Staphylococcus aureus* isolates from milk. *The Journal of Food Technology in Africa.* Vol9 No 1 23-25.

Simoes, R. R., Poirel L., Dausta P. and **Nordmann P. (2010).** Seagulls and Beaches as reservoirs of multi-drug resistant *Escherichia coli. Emerging Infectious Diseases* 16 (1): 110-112.

Solberg, O. D., Ajiboye, R. M., and **Riley, L. W., (2006).** Origin of class 1 and 2 integrons and gene cassettes in a population-based sample of uropathogenic *Escherichia coli*. *Journal of Clinical Microbiology* 44: 1347-1351.

Sougakoff, W., Goussard, S., Gerbaud, G., and **Courvalin, P., (1988).** Plasmid-mediated resistance to third-generation cephalosporins caused by point mutations in TEM-type penicillinase genes. *Reviews of Infectious Diseases* 10 (4): 879-84.

Stokes, H. W., and Gillings, M. R., (2011). Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. FEMS-Microbiology Review 35: 790-819.

Swayne, D. (1998). Laboratory manual for the isolation and identification of avian pathogens. American association of avian pathologist university of Pennsylvania 4th edition 4-6.

Talbot, G. H., Bradley, J., Edwards, J. E. Jr., Gilbert, D., Scheld, M., and **Bartlett, J. G., (2006)**. Bad Bugs Need Drugs: An Update on the Development Pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America.

Clinical Infectious Diseases 42: 657–68.

Teale, C. J., and **Moulin, G., (2012)**. Prudent use guidelines: A review of existing veterinary guidelines. *Revue scientifique et technique (International Office of Epizootics)* 31 (1): 343-354.

Tovey, M. G., Wadhwa, M., and **Thorpe, R., (2010)**. WHO International Cytokine Standards and reference preparations. Cytokine, 52 (3): II–IV.

Thiong´o, L. N., (2012). Molecular characterization multi-drug resistant *Escherichia coli* isolated from urine sample in Mater hospital, Nairobi, Kenya. MSc, Thesis-Jomo Kenyatta University of Agriculture and Technology.

Torome, T. K., (2015): Isolation and characterisation of antibiotic producing *Bacillus* organisms in Lake Bogoria. MSc, Thesis-Jomo Kenyatta University of Agriculture and Technology.

Tudor, K., (2002). Online Textbook of Bacteriology. Todar's Text Book of Bacteriology.

Tzouvelekis, L. S., Tzelepi, E., Tassios, P. T., and **Legakis, N. J., (2000**). CTX-M-type beta-lactamases: an emerging group of extended-spectrum enzymes. *International Journal of Antimicrobial Agents – Elsevier* 14 (2): 137-42.

Ulstad, C. R., Solheim M., Berg, M., Liudbaek, M., Dable, U. R., and **Wester, A. L., (2016).** Carriage of ESBL/Amp C – producing or Ciprofloxacin non-susceptible *Escherichia coli* and *Klebsiella* spp in healthy people in Norway. *Antimicrobial Resistance and Infection Control* 5: 57.

UNGA-United Nation General Assembly (2016). UN meeting tackles the fundamental threat's of antibiotic resistant superbugs. *https://www.theguardian.com › World › United Nations*

Van den Bogaard A. E., London, N., Driessen, C., and **Stobberingh, E. E., (2001).** Antibiotic resistance of fecal *E. coli* in poultry, poultry farmers and poultry slaughterers. *Journal of*

Antimicrobial Chemotherapy 47 (6): 763-771.

Van den Bogaard, A. E., and **Stobberingh, E. E., (2000).** Epidemiology of resistance to antibiotics links between animals and humans. *International Journal of Antimicrobial Agent* 14: 327-335.

Verma, A. A., Saminathan, M., Neha., Tiwari, R., Dhama, K., and **Vir Singh, S. (2013).** Glanders-A Re-emerging Zoonotic Disease; A Review. *Journal of Biological Sciences* 14: 38-51.

Vuuren, M. Van., (2001). Antibiotic Resistance with Special Reference To Poultry Production. Conference OIE: 135–146. *https://www.oie.int/doc/ged/D2955.PDF(cited Mar 16 2017)*

Wambugu, P., Habtu, M., Impwi, P., Matiru, V., and **Kiiru, J., (2015).** Antimicrobial susceptibility profiles among *Escherichia coli* strains isolated from Athi River water in Machakos County, Kenya. *Advances in Microbiology* 5: 711-719.

Wandilim, S. A., Onyango. D. M., and **Waindi. E. N., (2013)**. Prevalence and antimicrobial resistance profile of clinical *Salmonella* isolates from Nandi central of. *International Journal of Innovative Biotechnology and Biochemistry*, 1(1), pp: 1–10.

Weatherall, D. J., (2009). Genotype – Phenotype Relationships. DOI: 10.1002/9780470015902.a0003403.pub2

WHO (2003). Implementation workshop on the WHO global strategy for containment of antimicrobial resistance. Geneva: World Health Organization.

WHO (2012). Research and development to meet health needs in developing countries: strengthening global financing and coordination. Report of the consultative expert working group on research and development: financing and coordination. Geneva: World Health Organization.

WHO (2012). The evolving threat of antimicrobial resistance Options for action WHO Library Cataloguing-in-Publication Data, World Health Organization.

WHO (2014). Antimicrobial resistance: global report on surveillance 2014. Geneva: World Health Organization; 2014.

WHO (2014). Several existing initiatives were reviewed at the WHO's Technical Consultation on Innovative Models for New Antibiotics' Development and Preservation (Geneva, 13 May 2014) (http://www.who.int/phi/implementation/consultation_imnadp/en/, accessed 20 November 2014).

WHO (2015_a). Global Action Plan on Antimicrobial Resistance. *www.wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf (cited May 22 2017)*

WHO (2015_b). Global Antimicrobial Resistance Surveillane Syatem (GLASS) manual for early implementation. *apps.who.int/iris/bitstream/10665/188783/1/9789241549400_eng.pdf Cited May 22 2017*

Wilson, G. S., and **Miles, A., (1975).** Principle of Bacteriology, Virology and immunity. 6th Edition. London, Edward Arnold Ltd., 887-900.

Woodin, K. A. and **Morrison, S. H., (1994).** Antibiotics: mechanisms of action. Pediatrics in review. *American Academy of Pediatrics* 15 (11) 440–7.

Wright, G. D and Sutherland, A. D., (2007). New strategies for combating multidrug-resistant bacteria. TRENDS in *Molecular Medicine* 13 (6): 260-267.

Wright, G. D., (2011). Molecular mechanisms of antibiotic resistance. Chemical communications (Cambridge, England), 47 (14): 4055–4061.

www.lahey.org/studies/webt.asp. ß-Lactamase Classification and Amino Acid Sequences for

TEM, SHV and OXA Extended-Spectrum and Inhibitor Resistant Enzymes. (cited June 10 2017)

www.reactgroup.org. (2015) Action on Antibiotic resistance (The global threat of antibiotic resistance) cited June 1 2017).

Yewale, V. N., (2014). Antimicrobial resistance, 51 (3): 171–172.

Yoneyama, H., and **Katsumata, R., (2006).** Antibiotic Resistance in Bacteria and Its Future for Novel Antibiotic Development. *J-Stage*, 70 (5): 1060–1075.

Zarfet, G., Galler, H., Luxner, J., Petternel, C., Reinthaler, F. F., Haas, D., Kittinger, G., Grisold, A. J., Pless, P., and **Feierl, G., (2014).** Multiresistant Bacteria Isolated from Chicken Meat in Austria. *International Journal Environmental Research in Public Health* 11: 12582-12593.

Zhang, W. J., Wu, C. M., Wang, Y., Shen, Z., Dai, L., and **Han, J., (2011).** The new genetic environment of *cfr* on plasmid pBS-02 in a *Bacillus* strain, *Journal of Antimicrobial Chemotherapy* 66: 1174-1175.

APPENDICES

Appendix 1: Identification criteria of the various bacteria isolated

Sample	Colonies on MacConkey agar	Colonies on Blood agar	Gram stain		Oxi	Cat	Gel	IMVIC					
			MAC	BA				Indole	Cit	Urea	MR	PW	VP
E. coli	Pink color Non lactose ferments Medium in size	White color Non- hemolytic Medium in size	Gram -ve rod	Gram -ve Rod	-ve	+ve	_	+ve	-ve	-ve	+ve	+ve	-ve
Proteus	Pink color Non-lactose ferments Small size	Brown color spread in the media Non-hemolytic Small size	Gram –ve rod	Gram –ve rod	-ve	+ve	_	-ve	+ve	+ve	+ve	+ve	-ve
Strept	Pink color Non-lactose ferments Small size	White color Hemolytic Small size	Gram +ve cocci	Gram +ve cocci	-ve	+ve	_	_	_	_	_	_	_
Staph	-ve	White color Non-hemolytic Medium size	-ve	Gram +ve cocci (Clusters)	-ve	+ve	_	_	_	_	_	_	_
Bacillus	-ve	Creamy color Very hemolytic Big size	-ve	Gram +ve rod	-ve	+ve	+ve	_	_	_	_	_	_
Coryne	-ve	White color Non-hemolytic Medium size	-ve	Gram +ve rod Coccobacilli	-ve	+ve	_	_	_	_	_	_	_

Key: *Strept-Streptococcus, Staph-Stapylococcus, Coryne-Corynebacterium, Oxi-Oxidase test, Cat- Catalase Test, Gel-Gelatin, Indole-Indole test, Cit- Citrate, MR- Methyl Red, PW-Peptone Water, and VP-Voges Proskauer*

Appendix 2: Chi-square for each antimicrobial by group

```
> AMP0.025 <-table(Tino2$AMP0.025, Tino2$Group)
> AMP0.025

            Clinic Market Poultry-unit
  Resistant       33      5           7
  Susceptible      3      2           4
> fisher.test(AMP0.025)

            Fisher's Exact Test for Count Data

data:  AMP0.025
p-value = 0.0411
alternative hypothesis: two.sided


> TE0.1 <-table(Tino2$TE0.1, Tino2$Group)
> TE0.1

            Clinic Market Poultry-unit
  Resistant       24      4           2
  Susceptible     12      3           9
> fisher.test(TE0.1)

            Fisher's Exact Test for Count Data

data:  TE0.1
p-value = 0.01618
alternative hypothesis: two.sided

> SX0.2 <-table(Tino2$SX0.2, Tino2$Group)
> SX0.2

            Clinic Market Poultry-unit
  Resistant       28      2           4
  Susceptible      8      5           7
> fisher.test(SX0.2)

            Fisher's Exact Test for Count Data

data:  SX0.2
p-value = 0.005887
alternative hypothesis: two.sided
```

```
> GN0.01 <-table(Tino2$GN0.01, Tino2$Group)
> GN0.01

            Clinic Market Poultry-unit
  Resistant        2      0           0
  Susceptible     34      7          11


> fisher.test(GN0.01)

            Fisher's Exact Test for Count Data

data:  GN0.01
p-value = 1
alternative hypothesis: two.sided

>
> S0.025 <-table (Tino2$S0.025, Tino2$Group)
> S0.025

            Clinic Market Poultry-unit
  Resistant       16      2           3
  Susceptible     20      5           8
> fisher.test(S0.025)

            Fisher's Exact Test for Count Data

data:  S0.025
p-value = 0.5859
alternative hypothesis: two.sided


> NA0.03 <-table(Tino2$NA0.03, Tino2$Group)
> NA0.03

            Clinic Market Poultry-unit
  Resistant        9      1           0
  Susceptible     27      6          11
> fisher.test(NA0.03)

            Fisher's Exact Test for Count Data

data:  NA0.03
p-value = 0.1767
alternative hypothesis: two.sided
```

```
> NIT0.2 <-table(Tino2$NIT0.2, Tino2$Group)
> NIT0.2

            Clinic Market Poultry-unit
  Resistant        4      0           7
  Susceptible     32      7           4
> fisher.test(NIT0.2)

            Fisher's Exact Test for Count Data

data:  NIT0.2
p-value = 0.0008296
alternative hypothesis: two.sided

> COT0.025 <-table(Tino2$COT0.025, Tino2$Group)
> COT0.025

            Clinic Market Poultry-unit
  Resistant       26      2           2
  Susceptible     10      5           9

> fisher.test(COT0.025)

            Fisher's Exact Test for Count Data

data:  COT0.025
p-value = 0.002359
alternative hypothesis: two.sided
```

Appendix 3: Results of susceptibility profiles of *E. coli* Isolates

Sample No	AMP 25	TE 100	NIT 200	NA 30	S 25	SXT 200	COT 25	GEN 10
ATCC 25922	**24 (S)**	**27 (S)**	**28 (S)**	**30 (S)**	**19 (S)**	**25 (S)**	**25 (S)**	**32 (S)**
DV 60	20 (S)	16 (S)	23 (S)	25 (S)	20 (S)	6 (R)	6 (R)	34 (S)
DV 61	6 (R)	25 (S)	25 (S)	28 (S)	9 (R)	6 (R)	6 (R)	30 (S)
DV 62	11 (S)	8 (R)	20 (S)	24 (S)	18 (S)	6 (R)	6 (R)	30 (S)
DV 63 A	6 (R)	10 (R)	20 (S)	26 (S)	15 (S)	6 (R)	6 (R)	27 (S)
DV 67 A	6 (R)	8 (R)	14 (R)	22 (S)	23 (S)	10 (R)	6 (R)	30 (S)
DV 67 B	6 (R)	10 (R)	20 (S)	28 (S)	6 (R)	6 (R)	6 (R)	27 (S)
DV 67 C	6 (R)	6 (R)	18 (S)	6 (R)	15 (S)	6 (R)	6 (R)	28 (S)
DV 70	15 (S)	9 (R)	22 (S)	25 (S)	25 (S)	6 (R)	6 (R)	26 (S)
DV 71	30 (S)	14 (R)	21 (S)	6 (R)	15 (6)	35 (S)	30 (S)	30 (S)
DV 73	11 (S)	8 (R)	20 (S)	24 (S)	18 (S)	6 (R)	6 (R)	30 (S)
DV75	6 (R)	10 (R)	22 (S)	20 (S)	6 (R)	6 (R)	6 (R)	27 (S)
DV76	6 (R)	8 (R)	18 (S)	25 (S)	15 (S)	6 (R)	6 (R)	27 (S)
DV 77	6 (R)	6 (R)	16 (S)	6 (R)	15 (S)	6 (R)	6 (R)	6 (R)
DV 78	9 (R)	9 (R)	20 (S)	25 (S)	6 (R)	6 (R)	6 (R)	30 (S)
DV 79	6 (R)	10 (R)	20 (S)	28 (S)	16 (S)	35 (S)	35 (S)	25 (S)
DV 81	6 (R)	8 (R)	16 (S)	21 (S)	19 (S)	28 (S)	25 (S)	25 (S)
DV 85	6 (R)	6 (R)	20 (S)	20 (S)	19 (S)	6 (R)	17 (S)	25 (S)
DV 86	20 (S)	6 (R)	22 (S)	25 (S)	6 (R)	35 (S)	30 (S)	29 (S)
DV 86 EX	14 (S)	6 (R)	19 (S)	21 (S)	19 (S)	6 (R)	6 (R)	30 (S)
DV 83	15 (S)	10 (R)	18 (S)	25 (S)	23 (S)	6 (R)	6 (R)	25 (S)
DV 87	6 (R)	6 (R)	14 (S)	6 (R)	13 (R)	6 (R)	6 (R)	10 (R)
DV 89	6 (R)	20 (S)	16 (S)	20 (S)	16 (S)	6 (R)	6 (R)	25 (S)
DV 90	6 (R)	21 (S)	20 (S)	23 (S)	6 (R)	6 (R)	6 (R)	27 (S)
DV 91	6 (R)	15 (S)	22 (S)	20 (S)	19 (S)	6 (R)	6 (R)	25 (S)
DV 92	6 (R)	21 (S)	20 (S)	25 (S)	22 (S)	35 (S)	30 (S)	26 (S)
DV 93 A	6 (R)	21 (S)	20 (S)	6 (R)	19 (S)	31 (S)	30 (S)	25 (S)
DV 93 B	6 (R)	9 (R)	21 (S)	6 (R)	6 (R)	6 (R)	6 (R)	30 (S)
PU 16 A	6 (R)	20 (S)	22 (S)	20 (S)	18 (S)	6 (R)	23 (S)	25 (S)
PU 16 B	15 (S)	12 (S)	16 (S)	6 (R)	23 (S)	6 (R)	6 (R)	29 (S)
DV 94	6 (R)	6 (R)	19 (S)	22 (S)	15 (S)	6 (R)	6 (R)	30 (S)
DV 98	6 (R)	20 (S)	10 (R)	20 (S)	18 (S)	6 (R)	6 (R)	26 (S)
DV 99	6 (R)	10 (S)	18 (S)	25 (S)	16 (S)	6 (R)	6 (R)	25 (S)
DV 103	6 (R)	15 (S)	18 (S)	6 (R)	15 (S)	6 (R)	6 (R)	20 (S)
DV 7	6 (R)	20 (S)	20 (S)	6 (R)	22 (S)	6 (R)	6 (R)	30 (S)
DV 8	11 (S)	22 (S)	18 (S)	20 (S)	16 (S)	33 (S)	30 (S)	25 (S)

DV 9 A	11 (S)	10 (R)	22 (S)	20 (S)	15 (S)	30 (S)	30 (S)	20 (S)
PUS 1	22 (S)	23 (S)	11 (S)	30 (S)	22 (S)	29 (S)	23 (S)	25 (S)
PUS 2	19 (S)	25 (S)	22 (S)	25 (S)	24 (S)	35 (S)	30 (S)	28 (S)
PUS 3	6 (R)	19 (S)	10 (R)	25 (S)	22 (S)	30 (S)	30 (S)	30 (S)
PUS 4	6 (R)	19 (S)	10 (R)	25 (S)	19 (S)	6 (R)	29 (S)	28 (S)
PUS 5	6 (R)	19 (S)	11 (S)	28 (S)	15 (S)	6 (R)	30 (S)	28 (S)
PUS 6	11 (S)	10 (R)	11 (S)	25 (S)	6 (R)	6 (R)	6 (R)	30 (S)
PUS 7	13 (S)	10 (R)	15 (S)	25 (S)	15 (S)	35 (S)	30 (S)	28 (S)
PUS 8	11 (S)	18 (S)	22 (S)	28 (S)	22 (S)	30 (S)	30 (S)	20 (S)
PUS 9	22 (S)	28 (S)	23 (S)	26 (S)	18 (S)	28 (S)	29 (S)	30 (S)
PUS 10	20 (S)	19 (S)	23 (S)	28 (S)	19 (S)	30 (S)	30 (S)	25 (S)
PUS 12	6 (R)	20 (S)	10 (R)	20 (S)	18 (S)	6 (R)	6 (R)	20 (S)
K11	6 (R)	20 (S)	20 (S)	29 (S)	20 (S)	30 (S)	30 (S)	25 (S)
K16	15 (S)	6 (R)	20 (S)	31 (S)	20 (S)	30 (S)	30 (S)	30 (S)
K14	6 (R)	12 (S)	25 (S)	25 (S)	9 (R)	6 (R)	6 (R)	30 (S)
K2	13 (S)	23 (S)	22 (S)	30 (S)	19 (S)	30 (S)	30 (S)	29 (S)
K59	6 (S)	10 (R)	21 (S)	6 (R)	6 (R)	6 (R)	6 (R)	32 (S)
K29	20 (S)	23 (S)	24 (S)	26 (S)	23 (S)	30 (S)	30 (S)	30 (S)
K54	6 (R)	11 (S)	25 (S)	6 (R)	9 (S)	6 (R)	6 (R)	30 (S)

Key: *AMP-ampicillin, SXT-sulphamethoxazole, GEN-gentamycin, TE-tetracycline, NIT-nitrofurantoin, S-streptomycin, NA-nalidixic acid and COT-co-trimoxazole,*

Appendix 4: Results of susceptibility profiles of *Streptococcus* isolates

Sample No	AMP 25	GEN 10	COT 25	SXT 200	S 25	NA 30	NIT 200	TE 100
PU16 B	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	31 (S)	30 (S)	30 (S)
PUS 36	31 (S)	31 (S)	30 (S)	30 (S)	30 (S)	6 (R)	30 (S)	30 (S)
DV 87	6 (R)	30 (S)	30 (R)	30 (S)	6 (R)	6 (R)	25 (S)	6 (R)
PUS 35	30 (S)	30 (S)	30 (S)	31 (S)	25 (S)	6 (R)	30 (S)	30 (S)
DV 61	20 (S)	15 (S)	30 (S)	30 (S)	6 (R)	6 (R)	15 (S)	30 (S)
K 17	30 (S)	30 (S)	31 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)
K 67	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)
K 62	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)
K 61	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)
K 60	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)
K 55	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)
K 50	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	6 (R)	6 (R)	15 (S)
K 79	30 (S)	30 (S)	30 (S)	30 (S)	6 (R)	6 (R)	30 (S)	30 (S)
K 44	20 (S)	20 (S)	30 (S)	30 (S)	6 (R)	6 (R)	23 (S)	30 (S)
K 52	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)
K 59	30 (S)	30 (S)	30 (S)	25 (S)	15 (S)	6 (R)	30 (S)	30 (S)
K 53	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)	30 (S)

Key: *AMP-ampicillin, SXT-sulphamethoxazole, GEN-gentamycin, TE-tetracycline, NIT-nitrofurantoin, S-streptomycin, NA-nalidixic acid and COT-co-trimoxazole,*

Appendix 5: Results of susceptibility profiles of *Staphylococcus* isolates

Sample No	AMP 25	GEN 10	SX 200	S 25	NA 30	NIT 200	TE 100	COT 10
PUS 28	30 (S)	30 (S)	28 (S)	20 (S)	6 (R)	25 (S)	35 (S)	25 (S)
PUS 52	30 (S)	31 (S)	6 (R)	29 (S)	19 (S)	25 (S)	30 (S)	6 (R)
PUS 19	28 (S)	30 (S)	31 (S)	31 (S)	6 (R)	33 (S)	24 (S)	30 (S)
DV 67 B	6 (R)	30 (S)	6 (R)	6 (R)	28 (S)	25 (S)	11 (R)	6 (R)
K 52	25 (S)	6 (R)	30 (S)	30 (S)	30 (S)	20 (S)	25 (S)	30 (S)
K 81	30 (S)	35 (S)	25 (S)	6 (R)	6 (R)	20 (S)	25 (S)	26 (S)
K 43	19 (S)	25 (S)	28 (S)	25 (S)	6 (R)	6 (R)	28 (S)	25 (S)
K 70	30 (S)	30 (S)	25 (S)	25 (S)	6 (R)	25 (S)	30 (S)	25 (S)
K 53	30 (S)	35 (S)	25 (S)	25 (S)	25 (S)	20 (S)	30 (S)	30 (S)
K 58	30 (S)	28 (S)	28 (S)	20 (S)	20 (S)	30 (S)	30 (S)	25 (S)
K 80	28 (S)	25 (S)	25 (S)	20 (S)	20 (S)	30 (S)	30 (S)	25 (S)
K 55	30 (S)	33 (S)	25 (S)	29 (S)	6 (R)	25 (S)	25 (S)	30 (S)

Key: *AMP-ampicillin, SXT-sulphamethoxazole, GEN-gentamycin, TE-tetracycline, NIT-nitrofurantoin, S-streptomycin, NA-nalidixic acid and COT-co-trimoxazole.*

Appendix 6: Results of susceptibility profiles of *Bacillus* isolates

Sample No	AMP 25	GEN 10	SX 200	S 25	NA 30	NIT 200	TE 100	COT 10
DV 68 A	18 (S)	30 (S)	18 (S)	22 (S)	24 (S)	23 (S)	25 (S)	20 (S)
PUS 31	6 (R)	30 (S)	28 (S)	22 (S)	24 (S)	23 (S)	25 (S)	23 (S)
PUS 28	32 (S)	32 (S)	32 (S)	32 (S)	20 (S)	32 (S)	32 (S)	32 (S)
DV 77	20 (S)	30 (S)	6 (R)	24 (S)	25 (S)	23 (S)	25 (S)	6 (R)
DV 65	16 (S)	35 (S)	6 (R)	32 (S)	26 (S)	23 (S)	33 (S)	6 (R)

Key: *AMP-ampicillin, SXT-sulphamethoxazole, GEN-gentamycin, TE-tetracycline, NIT-nitrofurantoin, S-streptomycin, NA-nalidixic acid and COT-co-trimoxazole.*

Appendix 7: Results of susceptibility profiles of *Proteus* isolates

\Sample No	AMP 25	GEN 10	SX 200	S 25	NA 30	NIT 200	TE 100	COT 25
DV 100	25 (S)	30 (S)	6 (R)	6 (R)	6 (R)	6 (R)	6 (R)	6 (R)
DV 66	10 (R)	28 (S)	6 (R)	18 (S)	6 (R)	17 (S)	11 (R)	6 (R)
DV 103	6 (R)	27 (S)	6 (R)	6 (R)	6 (R)	13 (R)	11 (R)	6 (R)

Key: *AMP-ampicillin, SXT-sulphamethoxazole, GEN-gentamycin, TE-tetracycline, NIT-nitrofurantoin, S-streptomycin, NA-nalidixic acid and COT-co-trimoxazole.*