BACTERIAL CARRIAGE AND RESPECTIVE ANTIMICROBIAL SUSCEPTIBILITY PROFILES, WITH RESPECT TO INDIGENOUS CHICKENS MARKETED IN NAIROBI, KENYA; AFTER HEAVY RAINS.

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

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

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DEDICATION

I dedicate this work to my late father Muzirikana Jason; though I have never seen your face, I hope that we will meet in heaven.

I am dedicating this work to my lovely Mother Nyirabaganizi Damarce and to all family members, especially my brother Masengesho Amouram.

I dedicate also this work to my friends, who are always supportive toward my work. May the almighty God bless you.

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°C:	Degree centigrade
µg:	Microgram
µl:	Microliter
AMC:	Amoxycillin
AMP:	Ampicillin
ANOVA:	Analysis of variance
ATCC	American Type Culture Collection
ATP:	Adenosine triphosphate
BA:	Blood agar
C:	Chloramphenicol
***Camp*.:**	*Campylobacter*
CAMP:	Christie, Atkins and Munch Peterson
CIP:	Ciprofloxacin
CLSI:	Clinical and Laboratory Standards Institute
CN:	Gentamycin
CO_2:	Carbon dioxide
D.C:	District of Colombia
DA:	Clindamycin

DGHM:	German Society for Hygiene and Microbiology.
***E. coli*:**	*Escherichia coli*
E:	Erythromycin
EMB:	Eosin Methylene Blue
G+ve:	Gram positive
G-ve:	Gram Negative
H_2S:	Hydrogen Sulphide
HCl:	Hydrogen Chloride
I:	Intermediate
IMVIC:	Indole, methyl red, Voges-Proskauer and citrate
Ltd:	Limited
m/v:	Mass per volume
MDR:	Multi-drug resistance
MH:	Mueller-Hinton
Mm:	Millimeter
MSA:	Mannitol salt agar
NS:	No significant
OIE:	World Organisation for Animal Health
QAC:	Coco-benzyl-dimethyl-Ammonium Chloride
R- plasmid:	Resistance plasmid
R:	Resistance

RL:	Sulphamethoxazole
S:	Susceptible
SIM:	Sulfide indole motility
Spp:	Species
SPSS:	Statistical Package for the Social Sciences
SSA:	Salmonella Shigella agar
***Staph*:**	*Staphylococcus*
***Strep*:**	*Streptococcus*
TCBS:	Thiosulfate Citrate Bile Salts Sucrose Agar
TE:	Tetracycline
TSI:	Triple Sugar Iron agar
v/v:	Volume per Volume
w/v:	Weight per Volume
β:	Beta

ABSTRACT

Indigenous chickens are mostly raised free-range, where they scavenge around, picking their food from the environment with little or no supplementation. During periods of heavy rains, the flooding water carries different materials and wastes, like bacteria, antibiotics and disinfectants among others, from one place to another. These may end up being picked by the feeding free-range chickens. If the picked bacteria are antibiotic/disinfectant resistant, the respective resistance may be transferred to other bacteria in chicken and humans who consume the chicken products. If transferred to pathogenic ones, this will complicate control of the resultant disease(s). Antimicrobial resistance is currently a worldwide problem that is attracting a lot of attention to scientists. This study was carried out to determine bacterial carriage and respective antimicrobial susceptibility profiles in slaughtered indigenous chickens after heavy rains of year 2018, in Nairobi.

One hundred and twenty (120) intestine samples of indigenous chickens from three slaughter houses in Nairobi, namely: Kariokor, Burma and Kangemi were used in this study. Bacterial quantification was done using the method given by Miles and Misra (1938) while bacterial isolation and identification were done using standard bacteriological methods and tests by Markey *et al.,* (2013). The three most isolated bacteria, *Escherichia coli*, *Staphylococcus* spp and *Streptococcus* spp, were further tested for antibiotic and disinfectant susceptibility using gel diffusion method. For antibiotic susceptibility, each of them was tested using 5 antibiotics as instructed by Clinical and Laboratory Standards Institute CLSI (2016); for disinfectant susceptibility, all the three were tested using the same 6 disinfectants, which are frequently used in poultry industry in Kenya.

Total bacterial counts from intestinal samples ranged between $1.92x10^4$ and $1.04x10^{12}$ colony-forming-units per milliliter (cfu/ml), with arithmetic means of $4.7x10^{11}$, $5.6x10^{11}$ and $1.3x10^{12}$ cfu/ml for Kariokor, Burma and Kangemi slaughterhouses, respectively.

Escherichia coli was isolated at 85.8%, *Staphylococcus* spp at 55% and *Streptococcus* spp at 43.3%. Other bacteria, which were isolated at lower rates, included: *Proteus* spp, *Listeria* spp, *Bacillus* spp, *Streptobacillus* spp, *Klebsiella* spp, *Campylobacter* spp and *Pseudomonas aeuroginosa*.

Escherichia coli isolates were resistant to the following antibiotics: Ampicillin at 100%, Sulphamethoxazole at 93.3%, Amoxicillin at 93.3%, Gentamycin at 13.3%; all were susceptible to Ciprofloxacin. *Staphylococcus* isolates were resistant to Clindamycin at 73.3%, Tetracycline at 46.7%, Chloramphenicol at 40% and were susceptible to Sulphamethoxazole and Erythromycin. *Streptococcus* isolates were resistant to Sulphamethoxazole, Clindamycin, Erythromycin, Tetracycline and Chloramphenicol at 93.3%, 86.7%, 60%, 60% and 53.3% respectively. The three tested isolates (*Escherichia coli*, *Staphylococcus* and *Streptococcus*) showed multidrug resistance at 100%; 46.7% and 93.3%, respectively.

At recommended user concentration, *E. coli* isolates were resistant to the following coded disinfectants with their active ingredients in bracket: F (3.85% m/v of Sodium Hypochlorite) at 100%, D (Chloroxylenol 4.8%) at 26.7%, A (Glutaraldehyde 15% v/v; Benzalkonium chloride 10% v/v) at 40% and C (Glutaraldehyde 15%w/v; Coco-benzyl-dimethyl-Ammonium Chloride 10%w/v) at 46.7%. *Staphylococcus* isolates were resistant to F and B (Didecyl dimethyl ammonium HCl 18.75 gram; Diotyl dimethyl ammonium HCl 18.75g; Octyl decyl dimethyl ammonium HCl 37.5 gram; Alkyl dimethyl ammonium HCl 50 gram and Glutaraldehyde 62.50 gram) at 93.3% and 6.7%, respectively; *Streptococcus* isolates were only resistant to F at 93.3%.

The isolates were susceptible to the remaining disinfectants. About seventy three percent (73.3%) of *E. coli* and 6.7 % of *Staphylococcus* isolates showed resistance to both antibiotic(s) and disinfectant(s).

This study has demonstrated high concentration of various bacterial types in the intestinal tract of the indigenous chickens sampled after the heavy rains of year 2018, and that the isolated bacteria were variously resistant to the tested antibiotics and disinfectants. This highlights the possibility of the chickens serving as sources of pathogenic bacteria that can be transmitted to other chickens and humans; if resistant to antimicrobials, it would be difficult treating the resultant diseases. It is, therefore, recommended that chicken meat should be cooked well and that care be taken, during slaughtering of chickens since cross-contamination can occur and lead to food contamination. It is also recommended that before dispensing an antibiotic or using a disinfectant, one ascertains its susceptibility by carrying out antibiotic/disinfectant susceptibility testing and, where possible, free-range indigenous chickens be confined during rainy seasons. This data will aid policy makers come up with guidelines on reduction of environmental contamination.

CHAPTER ONE: INTRODUCTION

1.1 General background

Poultry population in Kenya is currently at about 36 million; over 80% of them being of indigenous type (Justus *et al.*, 2013); kept under free-range system of management in villages (Kingori *et al.*, 2010). These chickens serve as a source of protein to humans in form of meat and eggs (Justus *et al.*, 2013; Njue *et al.*, 2002). Just like other animals and humans, chickens carry bacteria in their guts (Ngoc *et al.*, 2016; Devriese *et al.*, 1991), reproductive systems (Tino, 2017; Diarra *et al.*, 2007) and respiratory tracts (Mbuthia *et al.*, 2008; Ramasamy *et al.*, 2008), mostly as normal flora. These non-pathogenic bacteria help the birds in digestion and also in keeping pathogenic organisms under check (Rakoff-nahoum *et al.*, 2004). However, although non-pathogenic, when they are too many, as may occur at times of heavy rains, they may cause stress to the bird, leading to immune-suppression (Adesiji and Baba, 2013) which can give rise to some of the bacteria causing disease to the host and subsequent reduction of productivity (Elijah and Adedapo, 2006). Thus, during heavy rains, chickens may be exposed to excesses of bacteria, as well as others that they normally don't carry, some of which may be zoonotic (Karuppaiah and Sujayanad, 2012). During evisceration of such birds, at slaughter, the zoonotic bacteria may contaminate the meat and cause food poisoning to humans who consume the contaminated meat. If by any chance, the pathogens are resistant to antibiotics, it will be difficult to treat the resultant disease(s) (Talebiyan *et al.*, 2014; Nemati *et al*., 2008).

Free-range indigenous chickens are notorious as sources of infection for intensively-reared ones (which are mainly exotic) and for humans. Also, since the bird may be stressed as a result of extreme weather changes due to heavy rains (for example those that occurred between March and May 2018), the bird's bacterial load may increase; resulting in disease to the bird and/or increased

environmental contamination. Increase in the bird's bacterial load may also be as a result of environmental contamination as bacteria and antimicrobials are washed from other areas by the running water; and it may also be as a result of proliferation of various insects which may be carrying the bacteria and are eaten by the chickens. These bacteria may be carrying resistant genes to antibiotic(s) and/or disinfectant(s) which can be easily transferred to other bacteria (Talebiyan *et al.*, 2014).

This change/increase in bacterial load in chicken gut and respective antimicrobial profiles, as a result of heavy rains, has not been studied in Kenya before. This study was therefore conducted to address this; it was carried-out to determine the bacterial carriage (concentration and type) of birds from different slaughterhouses in the wake of the experienced climate change of year 2018. Antibiotic and disinfectant susceptibility testing was also carried out on selected bacterial isolates to establish their antibiotic and disinfectant susceptibility/resistance patterns. Disinfectant susceptibility testing is important because disinfectants are extensively used in poultry intensive farming and in health facilities; it is important to know the effective ones.

1.2 Objectives of the study

1.2.1 General objective

To determine bacterial carriage and respective antimicrobial susceptibility profiles, with respect to indigenous chickens marketed in Nairobi, Kenya; after heavy rains.

1.2.2 Specific objectives

1. To quantify bacterial carriage in intestines of slaughtered indigenous chickens from selected slaughterhouses, three months after heavy rains of 2018, followed by cold season; Nairobi, Kenya.
2. To identify bacteria isolated from the intestinal contents of the slaughtered indigenous chickens.

3. To determine antibiotic resistance patterns of the three most commonly isolated bacteria from the intestinal contents of the slaughtered indigenous chickens.

4. To determine disinfectant resistance patterns of the three most commonly isolated bacteria from the intestinal contents of the slaughtered indigenous chickens.

1.3 Hypotheses

1. Bacterial carriage (concentration and type) in Kenyan indigenous chickens; is not affected by heavy rains caused by change in weather.

2. Bacteria carried by Kenyan indigenous chickens after heavy rains are not resistant to various antibiotics and disinfectants.

1.4 Justification for the study

Since they roam about the village, defecating everywhere, free-range indigenous chickens are normally a source of contamination to the environment resulting in spread of bacteria, including disease-causing ones, and especially those that are zoonotic (Nyaga, 2007; Safalaoh, 1997). The situation is worsened if these bacteria are resistant to antibiotic(s) and/or disinfectant(s) as it will be difficult to treat the resultant disease(s). Heavy rains may contribute more towards environmental contamination and spread of disease-causing organisms, some of which may be antibiotic/disinfectant resistant. This study was carried out to establish if this is the case, and also to determine the antibiotic and disinfectant resistance patterns of selected isolates. Antibiotic resistance in bacteria has reached worrying proportions worldwide; to the extent that it is sometimes not possible to treat life-threatening diseases. Thus, knowing the antibiotics that are currently effective is important as it provides information in the community on the possible antibiotics to use in disease treatment in humans as well as in animals. Disinfectant susceptibility testing is important because disinfectants are extensively used in poultry intensive farming and in

humans; they are also used in food producing units, for example: dairies, slaughterhouses, and in food-selling units such as hotels and food kiosks which can easily be contaminated with bacteria from poultry. It therefore, serves as a guide towards selection of effective disinfectant(s) and concentration to be used in disinfecting utensils, and materials. Knowledge of the facts mentioned above helps in informing the relevant authorities for better planning of bacterial control systems which will benefit farmers and the community in general.

CHAPTER TWO: LITERATURE REVIEW

2.1 General overview of free-range indigenous chickens

Indigenous chickens are normally more resistant to local diseases than the exotic breeds and scavenge for their own feed with little or no supplementation (Bebora *et al.*, 2005). They, therefore, could appear normal while carrying bacteria that are pathogenic to exotic chickens and more seriously to humans. If these bacteria are resistant to antibiotics, it will be difficult to treat the resultant diseases.

The traditional free-range system is the least capital intensive system requiring minimal financial input, hence affordable to even the resource-poor persons (Kingori *et al.*, 2010); Free-range indigenous chickens serve an important multi-purpose function in the village economy. Chicken meat and eggs generate money through selling and can also be used for home-consumption; chicken manure is used in farms and gardens as fertilizer (Yongolo *et al.*, 2002; Anderson, 2003). In addition, indigenous chickens are also used for cultural functions and rituals. The sector provides employment to a number of groups such as farmers, traders, transporters, restaurants, hotels and eateries' workers in villages and towns (Kingori *et al.*, 2010; Yongolo *et al.*, 2002).

2.2 The heavy rains of year 2018 and their effects

Between March and May 2018, there were heavy rains in Kenya (countrywide) as well as in the neighboring countries (Uganda, Burundi, Rwanda, Ethiopia, Somalia); Records show that the Kenyan rains were significantly above (145%) the usual average (FEWS NET, 22 Mar 2018). The heavy rains, progressed up to June 2018; The Meteorological Department had given an alert of the same (UNICEF, 2018).

As reported by Meteorological Department (Kenya), the 2018 rains affected more than 29 countries, which made it to be described as an *El Nino* phenomenon because the rain fall recorded

from some local meteorological sectors surpassed the one recorded at the time of *El Nino* 1997-1998 (IFRC, 1 May 2018). *El nino* phenomenon is described as a weather associated event that happens due to disruption of the ocean atmospheric system in the tropical Pacific which affects the normal weather pattern and can lead to increase in rainfall or drought in different parts of the world (Soko Directory Team, 4 May, 2016). Examples of 2018 rainfall data include: (1) Makindu station recorded 596.8mm of rainfall against the 510.7mm recorded in 1968 which surpassed the one recoded in 1997; (2) From March to 10th May, Embu station recorded 971.5 mm of rainfall, which was 169% of the estimated long-term mean of 575.3 mm (Kwamboka, 12 May 2018). Figures 2.1 and 2.2; show differences in rainfall records of 2017 and 2018, between months March and May; the 2018 rainfall was much higher than that of 2017.

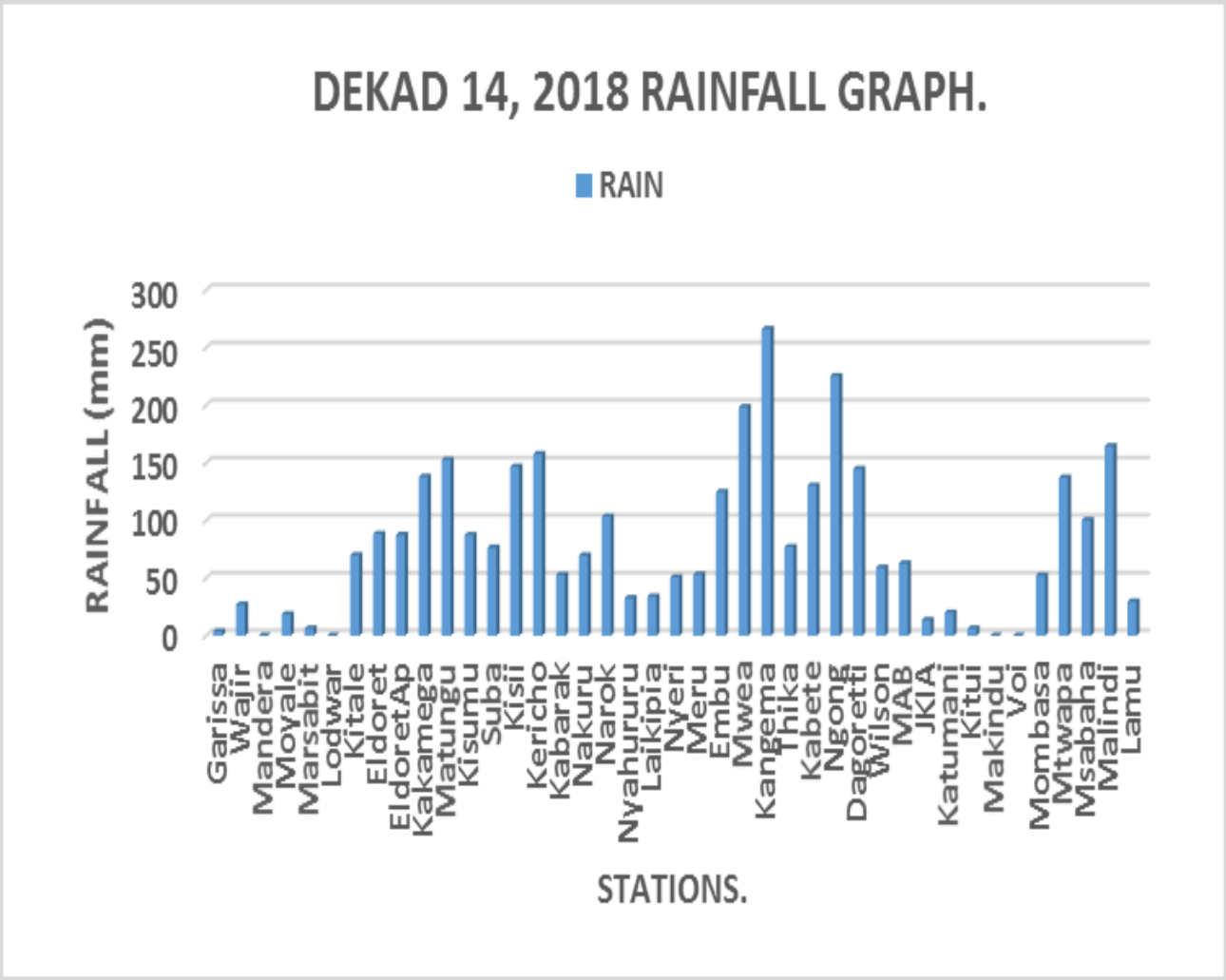

Figure 2.1: Rainfall records of different meteorological sectors of May 2018 in Kenya (http://www.meteo.go.ke/dekad/Dekad14-2018.pdf)

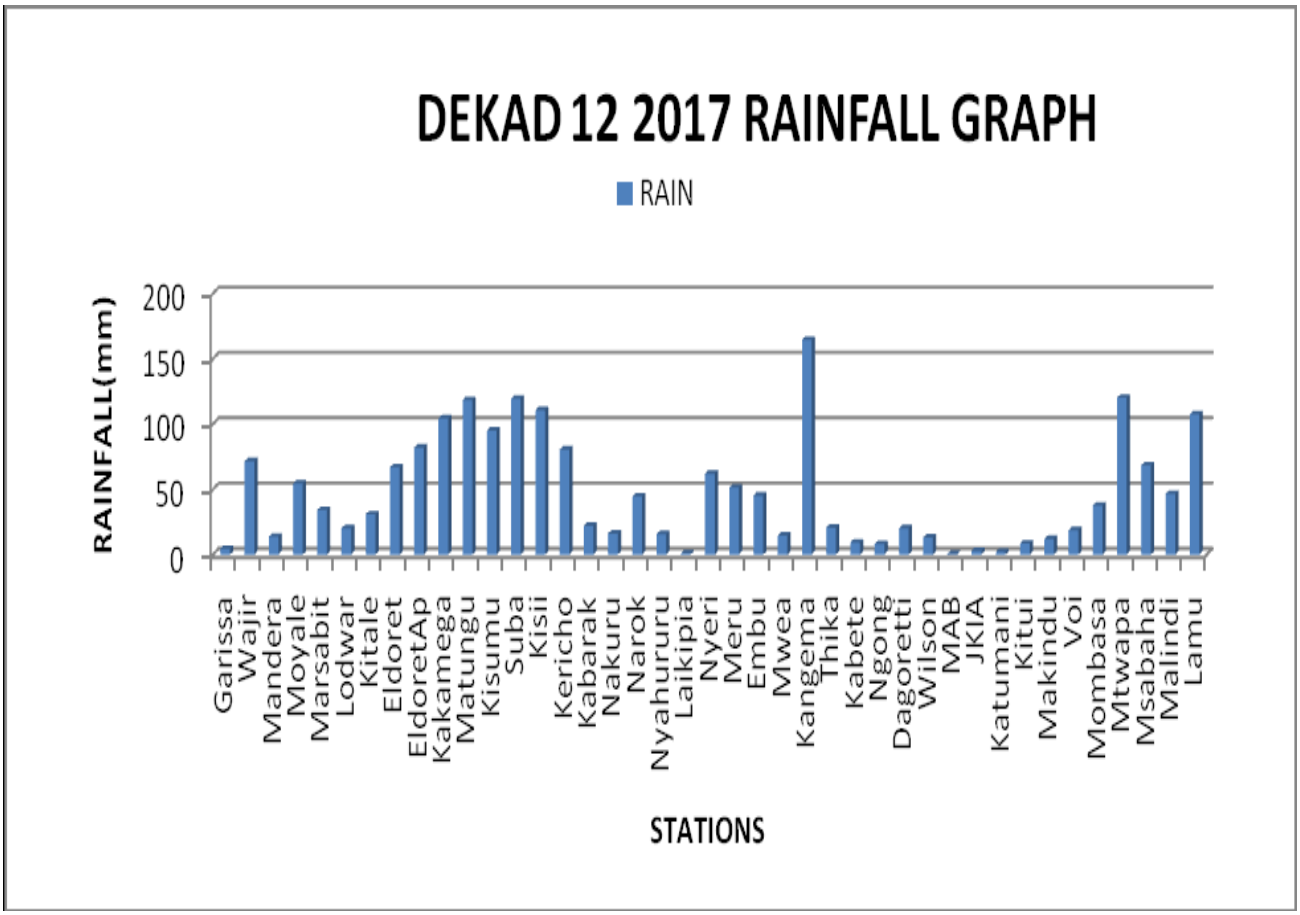

Figure 2.2: Rainfall records of different meteorological sectors of April 2017 in Kenya (http://www.meteo.go.ke/dekad/Dekad12-2017.pdf)

The heavy rains of March to May 2018, were supposed to come as a remedy for the inadequate water and food supply that was caused by the severe drought of year 2017 (Oxfam, 4 July 2017), but instead, they caused a lot of disaster and harm that affected lives of millions of people, livestock (including deaths), destruction of infrastructures, schools, health centers, roads and plants; due to massive flooding, landslides, overflow of several dams, that hindered transport of different things,/materials from various locations to others (OCHA, 25 April 2018).

The storm water caused by the rains was so strong that it overturned big cars (Figure 2.3) and destroyed well-constructed roads (Figure 2.4). Because of the intensity of flooded water, other

places which were receiving moderate rains suffered consequential damages. For example: the flooded Shebelle river which originates in Ethiopia caused a lot of damage through flooding in Somalia as well as in Kenya after bursting its banks (Patel, 2018).

In Kenya, it has been recorded that more than 186 people were killed by the floods; as a result of mudslides, houses collapsing and drowning; 800,000 people were affected; more than 300,000 people were displaced (Xinhua, 8 May 2018). Estimated cost of repair for roads that were destroyed by the heavy rains was $187 Million (The East African, 19 May 2018). Because of the severity of this disaster, different organizations such as UNICEF and Non-government organisations had to come-in to assist the vulnerable population.

Figure 2.3: Storm water overpowering vehicles in 2018, in Kenya. Source: The East African 19, May 2018

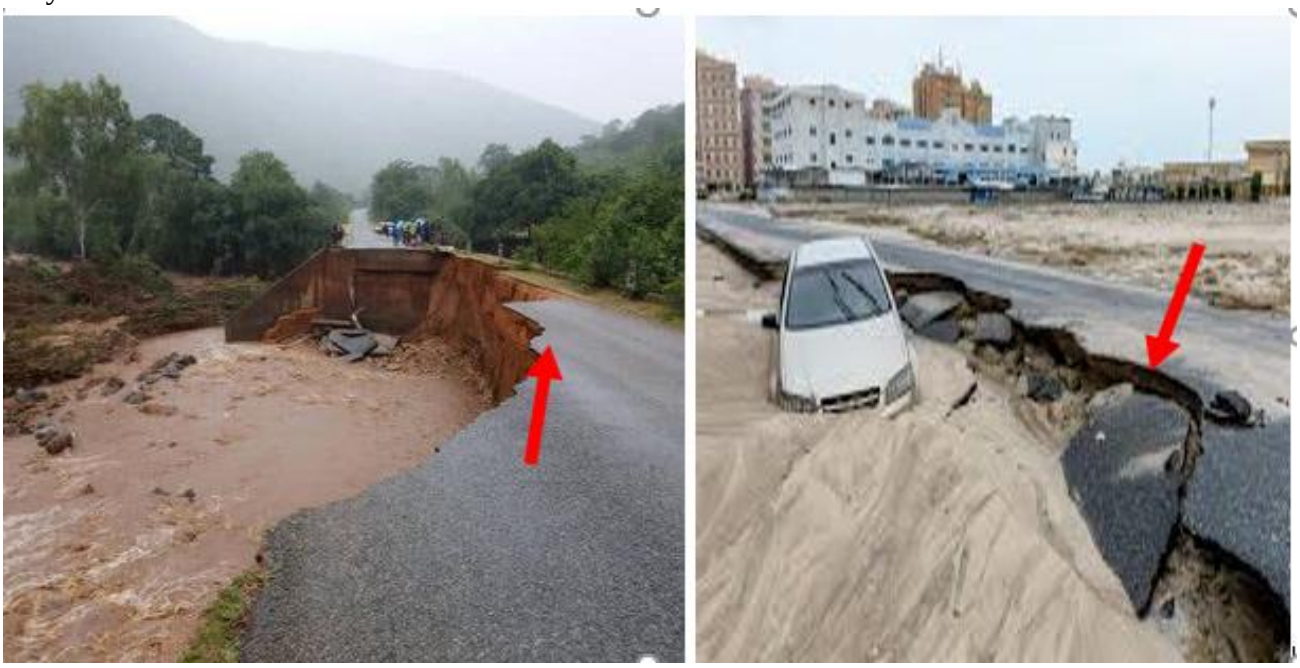

Figure 2.4: Destroyed roads (pointed by arrows) due to flooding caused by the heavy rain of 2018 in Kenya

Floods play a very big role in disease outbreaks which affect lives of animals and humans; they also cause environmental destruction. Flow of rain water from highlands to lowlands contributes to the spread of disease - carrying insects, a wide variety of herbs, among others (Opere, 2013); which can be harboring different types of organisms, including bacteria; the water can also pick and transport unattached bacteria, parasites, antibiotics, disinfectants, among other substances.

2.3 Heavy rains, antibiotics and antibiotic resistance

Antibiotic resistance is among the worldwide problems that need a lot of attention. Heavy rains contribute towards dispersing antibiotics in different locations and induce resistance in bacteria, which end up becoming a big problem; rains can also disperse antibiotic resistant bacteria. As it flows down from higher lands, some of the rain water ends-up in rivers, where it deposits some of the substances (antibiotics, disinfectants, bacteria) it has collected on its way. Presence of antibiotics in rivers has been documented severally; a few examples being: (1) A study done by researcher testing the presence of 144 antibiotics which are commonly used in 72 countries across continents, found 65% of the rivers to have antibiotic products exceeding the safe level, as defined by AMR (Antimicrobial resistance) Industry Alliance, by up to 300 times (Chiorando, 29 May 2019). Bangladesh, Kenya, Ghana, Pakistan, and Nigeria rivers were found to have the highest number and/or concentration of antibiotic(s) (Dazet, 29 May 2019). In China, Perl river was found to contain antibiotics (Yang *et al.*, 2010); There was presence of antibiotic sulfamethoxazole and trimethoprim at high concentration in the environment in Pakistan (Khan *et al.*, 2013), which can be washed off by water and end up in the river; A study done in Nairobi/Kenya, to test for presence of Sulfamethoxazole, Trimethoprim and Ciprofloxacin in Nairobi river basin, demonstrated presence of antibiotics at higher level than that present in waste water treatment plants (Ngumba *et al.*, 2016); (5) Within Lake Victoria Basin, Kenya, an investigation which was done to check

for presence of antibiotics in rivers, wastewater treatment plants (WWTPs) and hospital lagoons detected presence of antibiotics such as Ampicillin, Amoxicillin, Sulfamethoxazole, Chloramphenicol, and Ciprofloxacin, with Ampicillin being the highest in concentration (Kimosop *et al.*, 2016); (6) Investigation done from receiving rivers in the Nzoia Basin, Kenya, showed presence of Sulfamethoxazole which was detected at higher level compared to other antibiotics such as Metronidazole, Nalidixic acid, Sulfadocin (K'oreje *et al.*, 2018).

Environmental or river contaminations can occur through different means: it can be as a result of antibiotic disposal by pharmacies, hospitals or patients (K'oreje *et al.*, 2016); it can also be as a result of direct discharge of wastes from livestock, as manure or farm run-off sludges, containing antibiotics (Burkhardt *et al*., 2005; Managaki *et al.*, 2007). Antibiotic pollution in the environment and rivers is, therefore, a big problem which can contribute to increase in the level of antibiotic resistance especially in cases of heavy rains. The flooding rain water aids in washing the antibiotics and/or antibiotic-resistant bacteria into the rivers; and also, to other locations. As a long-term effect, the spread of antibiotics may aid in inducing respective antibiotic-resistances in exposed bacteria.

All these substances (bacteria, antibiotics, disinfectants and other substances), carried across different locations by rain water, can be picked by respective indigenous chickens as they feed; the chickens ending up being colonized by the various organisms. Also, due to cold weather (climate change), that normally stresses humans as well as animals, resulting in reduced immunity, bacteria get better chance to multiply and enhance their colonization of chickens.

It is therefore reasoned that, in this study, recording the rain situation in areas where the marketed birds came from will not help in explaining the situation in year 2018, since water flowed from highlands to lowlands (even from neighboring countries) and passed in areas where the rains were

not correspondingly high, for example, Lamu area at the Coast – it flooded so much that water got into houses, houses were damaged, toilets/septic tanks overflowed causing massive contamination and there were some deaths through drowning; also Nyando plains flood because of water coming from elsewhere. For both Lamu and Nyando plains, water came from highlands, passing through several areas before reaching them – carrying with it several substances it came across, including bacteria, antibiotics, disinfectants, insects (other types coming in; including increases in number of house flies), change of vegetation; providing plenty of food for various organisms, including bacteria and insects.

2.4 Weather change and chicken bacterial carriage

There are various aspects of weather change; this study was limited to wetness and temperature changes. While there are many studies that have been done on bacterial carriage in the intestine of chickens in Kenya, for example: *Escherichia coli* (Odwar *et al.*, 2014); *Campylobacter* spp (Ngoc *et al.*, 2016); *Listeria* spp (Njagi *et al.*, 2004)*; Pasteurella multocida* (Mbuthia *et al.*, 2008), none of them has addressed carriage after heavy rainy season.

Running (rainy) water tends to wash off bacteria, parasite eggs, any drugs that have been carelessly dispensed off to the environment, and these may reach where the birds are, leading to the birds picking them as they feed on the ground (Jones *et al.*, 2007). The possible occurrence of parasitism, both endo- and ecto-, can lead to discomfort and cause the birds not to eat well. This will worsen the immunity of already immune-suppressed birds (Haller and Jobin, 2004; Karuppaiah and Sujayanad, 2012; Sabuni, 2009; Kemboi, 2014).

The bacteria brought to the chickens by the storm water may be carrying resistance genes, which will end-up being transmitted to bacteria that the birds were carrying originally. As a long-term effect, the drugs that the birds may pick from the storm water (which will be at a lower dosage)

may end up giving rise to bacterial strains that are resistant to them. Even though initially the resistance may be in non-pathogenic organisms, it can easily spread to pathogenic ones (Kikuvi *et al.*, 2007), some of them causing life-threatening conditions. The same is possible for disinfectants (Spellberg *et al.*, 2008; Russell, 2002).

In year 2018, between March and May, there were excessive rains in Kenya which resulted in flooding and mud-slides; more than what was experienced in 1961, 1984, 2006 and 1984 *El Niño* (Amissah-arthur *et al.*, 2002). The current study aimed at establishing whether heavy rains affected the chickens' bacterial carriage, in terms of type and prevalence; that is: whether the extreme wetness and resultant coldness stressed the birds and reduced their immunity enough for bacteria to establish themselves (Adesiji and Baba, 2013; Elijah and Adedapo, 2006).

2.5 Effect of chicken bacterial carriage

Free - range indigenous chickens normally roam long distances, defaecating all over, thus disseminating bacteria further in the environment (Safalaoh, 1997; Nyaga, 2007; Kingori *et al.*, 2010). The excreted bacteria can end-up being picked by other chickens, which will either also become carriers and continue disseminating them further or, if susceptible, come down with disease. The excreted bacteria can also end-up contaminating vegetables which humans eat and could lead to infection or food poisoning; especially when undercooked (Bodhidatta *et al.*, 2013). Also, during slaughter of the birds, the intestinal contents could contaminate the meat, leading to food poisoning for the consumer, especially when the meat is undercooked. If the respective bacteria are resistant to antibiotic(s) and disinfectant(s), it will be difficult to treat the resultant disease(s) (Talebiyan *et al.*, 2014; Furtula *et al.*, 2010). Therefore, it is useful to determine the susceptibility of the isolated bacteria to antibiotics and disinfectants.

2.6 Microbial flora; their benefits and disadvantages to the host

Microflora coexist and play an important role to their hosts. They act as immune modulators; they have components which activate innate and adaptive immunity (Ivanov, 2012). Some play an important role in the maintenance of intestinal epithelial homeostasis through an interaction of Toll-like receptors (TLRs) with microflora ligands. Activation of TLRs is critical in protection against gut injury and associated mortality (Rakoff-nahoum *et al.*, 2004). Microflora also play an important role in suppressing pathogenic bacteria through competing for attachment site and nutrients (Bourlioux *et al.*, 2003; Levy, 2000).

It has been found that microflora have the ability to metabolize harmful chemical compounds and help in digestion (Haller and Jobin, 2004). However, under some conditions such as stress the flora balances may become upset and commensal bacteria may subsequently lead to disease/ inflammation or other negative impacts such as malaise that can reduce the feeding ability of the birds hence result in reduction of immunity for pathogenic bacteria to establish themselves (Haller and Jobin, 2004; Bourlioux *et al.*, 2003). This increased prevalence of pathogenic bacteria can cause disease to the birds and in case they are eaten undercooked they can lead to food poisoning (Bodhidatta *et al.*, 2013).

2.7 Bacteria that have been isolated from intestines of healthy appearing chickens

The gastrointestinal tract of chickens may contain several bacteria, both aerobic and anaerobic including: *Staphylococcus* spp (Nemati *et al.*, 2008), *Streptococcus* spp, *Campylobacter* spp, *Salmonella* serotypes, *Listeria* and coliforms (*E. coli, Klebsiella, Enterobacter*) (Zhao *et al.*, 2001). These bacteria tend to occur as commensals but some of them, for example: *Escherichia coli, Campylobacter* spp, *Listeria* spp and *Salmonella* serotypes, are of public health importance – they can cause disease in humans, depending on their pathogenicity and concentration/dose (Markey *et*

al., 2013). They are normally associated with gastro-intestinal upsets, causing diarrhea, but sometimes they can become septicemic (Shane, 2005).

Tino (2017) isolated bacteria from chickens that were brought to the poultry clinic, not necessarily suffering from bacterial disease; as well as those from a slaughterhouse and those from a farm: the most prevalent being organisms of the genus *Streptococcus* (40.7%), followed by *Escherichia* (31.4%), then *Staphylococcus* (26.2%), *Bacillus* (9.3) and *Proteus* (2.9%). Bebora (1979) working on indigenous chickens from farms and a slaughterhouse, isolated the following bacteria, respectively: from farms *Salmonella* serotypes (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 the slaughterhouse: *Salmonella* serotypes (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 farms: *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%). This shows that different bacterial types (pathogenic and non-pathogenic) can be isolated from healthy-appearing chickens as well as diseased ones.

2.8 Description and pathogenicity of some bacteria isolated from chickens' intestines

Chicken intestine harbors many bacterial types, some are pathogenic, others are not pathogenic (Bourlioux *et al*, 2003), while, some are opportunistic, meaning they can cause diseases under favorable condition(s). Chickens act as carriers of pathogenic bacteria which when they get access to humans, they can cause diseases (Odwar *et al.*, 2014). A few examples are given below:

2.8.1 *Staphylococcus aureus*

These organisms are Gram-positive cocci, normally arranged in clusters. They are aerobic facultatively anaerobic, catalase positive, urease negative, coagulase test variable (Markey *et al.*, 2013). They have been isolated from noses and cloacae of healthy-appearing chickens (Nemati *et al.*, 2008). *Staphylococcal* infections are widespread in poultry; infections being mainly caused by *Staphylococcus aureus* (Colombari *et al.*, 2007*;* Aarestrup *et al.*, 2000). Thus, *Staphylococcus aureus* can pollute food as a result of processed carcasses (Mead and Dodd, 1990). Commonly affected tissues in chickens are: bones, tendon sheaths, joints, articular coxo-femoral and tibio-tarsal joints (White *et al.*, 2003; Shane, 2005)**;** normally resulting in pyogenic conditions. *Staphylococcus aureus* can also cause serious infections such as septicaemia, pneumonia, or bone and joint infections in humans (Tong *et al.*, 2015). However, as the organisms can be passed to humans by chickens, it has been shown that *Staph. aureus* in animals, including chickens, can be from human activities such as domestication and industrialization (Fitzgerald, 2012).

2.8.2 *Streptococcus* spp

These organisms are Gram-positive cocci, normally arranged in chains. They are aerobic facultatively anaerobic, catalase negative, nitrate reduction negative, CAMP reaction variable (Markey *et al.*, 2013). *Streptococcus* occurs globally in chickens; it is associated with both chronic and acute (septicaemic) disease, causing mortality rates of between 0.5% and 50% in poultry (Dinev, 2007). In humans, it is known to cause respiratory tract infections such as acute sinusitis, acute otitis media, pharyngitis, community-acquired pneumonia, and acute bronchitis among others (Camara *et al.*, 2013). *Streptococcus agalactiae* is known to cause chronic mastitis in cattle, goats and sheep; in human it can cause neonatal septicaemia (Markey *et al*., 2013).

2.8.3 *Escherichia coli*

These organisms are Gram-negative short rods which are aerobic facultatively anaerobic, mostly motile. They are also oxidase negative, catalase positive, indole positive, methyl red positive. Voges Proskauer negative, citrate negative, urease negative, H_2S production negative (Sneath *et al.*, 1986; Cowan, 2003). They are always found in the intestinal tract hence 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 (Markey *et al.*, 2013; Zhao *et al.*, 2001). Although most of them are non-pathogenic, there are some that are pathogenic (example: serotype O157:H7) causing diarrhea and sometimes septicemia in both chickens and humans. Septicemia can result in various other manifestations depending on where the bacteria end-up being deposited.

2.8.4 *Salmonella* serotypes

These organisms are Gram-negative rods which are aerobic facultatively anaerobic; consisting motile and non-motile strains. They are also oxidase negative, catalase positive, indole negative methyl red positive, Voges Proskauer negative, citrate positive, urease negative, H_2S production positive (Markey *et al.*, 2013; Sneath *et al.*, 1986). Worldwide salmonellae are causative agents of most human food borne diseases; some serotypes are host-specific while some are not host-specific. The genus has only two species; the one which includes most of the pathogenic serotypes is *Salmonella enterica;* notorious at causing human gastroenteritis (Bodhidatta *et al.*, 2013). The other species is *Salmonella cholerae-suis.* In both chickens and humans, salmonellosis manifests itself as diarrhea or septicemia, which can result in various other manifestations depending on where the bacteria end-up being deposited (Sanchez *et al.*, 2002).

2.8.5 *Listeria* spp

Listeria are Gram positive short rods to coccobacilli, which are aerobic facultatively anaerobic, motile but only when incubated at room temperature (25 – 30^{o} C). They are also oxidase negative, catalase positive, CAMP positive (*L. monocytogenes*), urease negative, nitrate reduction negative (Markey *et al.*, 2013). They produce pin-point black colonies on Cystine tellurite blood agar. Holding the medium at 4° C (cold enrichment) increases the chances of isolating the organism since growth of most of the competing organisms is inhibited at this temperature while *Listeria* continues to multiply. Thus, the cold treatment is useful for culturing contaminated specimens (Schwaiger *et al.*, 2010).

Listeria organisms have been documented as one of the sources of human food poisoning in Kenya; the organism is normally found in soil, plants materials and silages - chickens getting infected through feeding on these. It was documented that healthy- appearing indigenous chickens can be the source of *Listeria monocytogenes, L. innocua, L. seligeri, L. grayi and L. murrayi* which can cause diseases in humans if consumed (Njagi *et al.*, 2004); the most pathogenic being *L. monocytogenes*, which mostly causes meningitis, but can also cause endocarditis (Srinivasan *et al.*, 2005).

2.8.6 *Campylobacter* spp

Campylobacter spp. are microaerophilic (require decreased oxygen), Gram-negative, curved rods with a single unsheathed polar flagellum; they are also highly motile, catalase positive, oxidase positive (Markey *et al.*, 2013).

Campylobacter jejuni and *Campylobacter coli* can colonise the intestinal tract of most mammals and birds and are the most frequently isolated *Campylobacter* species in humans suffering from gastroenteritis (Awad *et al.*, 2015; Ngoc *et al.*, 2016). Awad *et al*. (2015) have also found that 20%

of diarrheal cases in human patients are caused by *Campylobacter* spp. Most chickens are asymptomatic carriers of *Campylobacter* organisms; a carrier rate of up to 40% has been documented. Thus, they are a threat to human consumers (Engberg *et al.*, 2000). *Camp. jejuni* and *Camp. coli* can also cause Guillain-Barre´ syndrome and reactive arthritis (Zhao *et al.*, 2001).

2.9 Antimicrobials and their use in poultry

The term "antimicrobial" simply means "against microorganisms", not necessarily as a medicine/drug, used to treat microorganisms (which are of different types including: bacteria, viruses, parasites, fungi); This means that the term can also be used to describe disinfectants (www.reactgroup.org cited 2017 Jan 28; Siddiqui and Sarwar, 2013). Thus, in this study, substances used to treat diseases (consumed or topically applied), whether produced by microorganisms, their synthetic versions or chemo-therapeutic agents that inhibit or destroy bacteria (Siddiqui and Sarwar, 2013), will be referred-to as "antibiotics", in order to distinguish them from "disinfectants", which are mostly toxic to be used for treatment.

Antimicrobials are very important in clinical disease treatment against infections and in maintaining healthy and productive animals (Yang *et al.*, 2004). Cleaning and disinfecting are key components of biosecurity and biosafety in poultry farming (Segal, 2018), while antibiotics are being used in poultry to treat bacterial infections and as growth promotors (Diarra *et al.*, 2007; Kikuvi *et al.*, 2001). This study was limited to antibiotics and disinfectants among other antimicrobials.

2.9.1 Antibiotics

Effective antibiotics 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).

Different studies have demonstrated that use of antibiotics in poultry whether as health promoters (Fair and Tor, 2014) or in disease treatment and control increases the chance of creating resistant strains in the birds by creating selective pressure (Marshall and Levy, 2011); These strains may later on affect humans (Adelaide *et al.*, 2008). Use of the same antibiotics in humans and animals is the risk factor which increases the transfer of antibiotic resistance in humans and animals (Marshall and Levy, 2011).

Use of antibiotics in animals is inevitable, noting that food animals are important to human welfare; animal health being 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 (Diarra *et al.*, 2007; Furtula *et al.*, 2010) 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 antibiotic resistance, is based on the fact that same drugs/medicines are used in both humans and animals (Smith *et al.*, 2002; OIE, 2015) for treatment and prophylaxis, and a large percentage of bacteria are shared between the two groups.

Use of antibiotics in animal feeds alter the intestinal flora by favoring creation of resistant bacteria like resistant *E.coli* that can be transferred through the food chain and affect humans (Diarra *et al.*, 2007; Talebiyan *et al.*, 2014). Humans and chickens can also get antibiotic resistant strains from the environment, water as a result of poor sanitation or heavy rain (Furtula *et al.*, 2010).

Prudent use of antibiotics in animals is, therefore, important as it will control the transfer of antimicrobial resistance between bacteria in and across animals and humans (Kikuvi *et al.*, 2001; Helmuth and Hensel, 2004; Gelband *et al.*, 2015). 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 animal and back to human (Clifford *et al.*, 2018). In cases of antibiotic resistance, the resultant food-borne or animal-acquired illness in humans will be less responsive to treatment with respect to the particular antibiotic(s) (Fair and Tor, 2014).

Indiscriminate usage of antibiotics, for example, as growth promoters in veterinary medicine (Marshall and Levy, 2011; Hart *et al.*, 2004) should be discouraged. Antibiotics should not be used to offset the shortcomings of poor management or insufficient hygiene standards in farms (Marshall and Levy, 2011; Gelband *et al.*, 2015); this means that antibiotics 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 antibiotic cover (OIE, 2007; O'Neill *et al.*, 2016).

2.9.2 Disinfectants

Disinfectants are chemical agents which are used for decontamination of surfaces and other inanimate objects applied in different fields, including in poultry production (Siddiqui and Sarwar, 2013). They are used to kill pathogenic microorganisms or reduce them to acceptable levels. They are used for biosecurity and biosafety reasons; they help in controlling disease causing pathogens (Ayliffe, 1989). The European committee for the standardization of disinfectants has defined disinfection as "the selective elimination of certain undesirable organisms in order to prevent their transmission, achieved by action on their structure or metabolism, irrespective of their functional state" (Ayliffe, 1989; McDonnell and Russell, 1999). Disinfection does not necessarily kill all microorganisms but reduces them to a level acceptable for a defined purpose (Njagi *et al.*, 2005; Wirtanen and Salo, 2003).

Disinfectant susceptibility test is conducted to check for effective ones; even though the more active/effective disinfectants tend to be more toxic. Potentially toxic products can be applied to

inanimate objects or surfaces, whereas for disinfection of human tissues only the less toxic disinfectants (antiseptics) can be considered (Wirtanen and Salo, 2003). Disinfectants are used extensively in human activities for cleaning purposes and in intensive poultry farms as part of hygienic practices; for prevention of diseases (Payne *et al*., 2005).

Various techniques have been employed in the laboratory by microbiologists to gauge disinfectant efficacy since 19th century (Bergan and Lystad, 1971). For example, there is a dilution method, designed to determine the highest dilution of a disinfectant which kills the test organism within a series of time intervals under specified conditions (Bergan and Lystad, 1971; Spooner and Sykes, 1972). There is also an agar well method, where wells are cut into the agar and then filled with the respective disinfectant; allowing it to diffuse into the agar and produce a zone of growth inhibition (Njagi *et al*., 2005). Disinfectants range from phenolic compounds, alcohol, chlorhexidine, chlorine compounds, formaldehyde, glutaraldehyde, hydrogen peroxide, iodophore, peracetic acid, quaternary ammonium compounds (McDonnell and Russell, 1999). Table 2.1 gives the mode of action, advantages and disadvantages of some disinfectants used in poultry production.

Table 2.1: Mode of action, advantages and disadvantages of some disinfectants used in poultry production

Disinfectant category	Trade names	Mechanism of Action	Advantages	Disadvantages
Alcohols	Ethyl alcohol Isopropyl alcohol	-Precipitates proteins -Denature lipids	-Fast acting -leaves no residues	-Rapid evaporation -Flammable
Aldehydes	Formaldehyde Glutardehyde	-Denature proteins -Alkylates nucleic acids	Broad spectrum	-Carcinogenic -Mucous membrane and tissue irritation -Only used in well ventilated areas.
Biguanides	Chlorhexidine Nolvasan Virosan	-Alters membrane permeability	Broad spectrum	Only functional in limited PH range (5-7). -Toxic to fish
Halogens: Hypochlorites	Bleach	Denature proteins	-Broad spectrum -short contact time -inexpensive	-Inactivated by sunlight -requires frequent application -corrodes metals -mucous membrane and tissue irritation
Halogenes: Iodine compound	Betadyne Providone	Denature proteins	-Stable in storage -Relatively safe	Inactivated by Quaternary ammonium Compounds (QACs) -Requires frequent application
Oxidizing agents	Hydrogen peroxides Peracetic acis Virkon 5 Oxy-Sept 333	Denature proteins and lipids	Broad spectrum	Cause damage to some metals
Phenols	One-Stroke Environ Pheno-Tech II Tek-Trol	-Denatures proteins -Alters cell wall permeability	-Good efficacy with organic materials -non-corrosive -Stable in storage	Can cause skin and eye irritation
Quaternary Ammonium Compounds (QAC)	Roccal DiQuat D-256	-Denatures proteins -Binds phospholipids of cell membrane	-Stable in storage -non-irritating to skin -Effective at high temperatures and high pH (9-10)	Inactivated in low pH and by salts (Ca2+ and Mg2+), resistance development, ineffective against Gram-negative bacteria.

(Segal, 2018**;** Wirtanen and Salo, 2003)

2.10 Antimicrobial resistance

2.10.1 Antibiotic resistance

The term “antibiotic resistance” refers to the ability of microorganisms (bacteria) to grow in the presence of an antibiotic (drug) at a concentration that would normally kill them or inhibit their growth (www.reactgroup.org cited 2017 Jan 28). Antibiotic resistance can be genetically encoded or naturally obtained by the microorganism. Different genetic elements such as transposable elements, plasmids can be acquired by bacteria and render them resistant to antibiotic (Cavaleri *et al.*, 2005). Resistance can be to a single or several antibiotics. It can also be transferred by several means; for example by plasmid (resistance factor; R factor) since the plasmid can be easily transferred across bacteria (Cavaleri *et al.*, 2005).

Like other animals, chickens harbor different microflora in their guts which are not harmful. In cases of inappropriate use of antibiotics as growth promoters, there are high chances of bacteria developing resistance to the used-antibiotics; worst scenario is development of multi drug resistant bacterial strains which are excreted in faeces in high concentrations. They end up contaminating the environment and increasing their spread (Adelaide *et al.*, 2008; Marshall and Levy, 2011).

Antibiotic resistance acquisition can be by direct contact from animal to human especially to those people who work in slaughterhouses, veterinarians and farmers. There is also evidence that shows that the resistant bacteria and their genes can be transferred through consumption of contaminated feed materials (Marshall and Levy, 2011). In humans, it has been shown that the incidence of antibiotic resistance in zoonotic infections is directly connected to the presence of resistant bacteria and genes in animals used as food (Smith *et al.*, 2002).

Many studies have demonstrated transfer of antibiotic resistant bacteria from chickens to humans; one of them has shown transfer through use of chicken dung as fertilizer in the field (Marshall and Levy, 2011; State and The, 2015). Resistant bacteria have also been shown to spread among the chickens as a result of picking things from the ground which is contaminated with chicken faeces (Adelaide *et al.*, 2008). Plasmid transfer by conjugation has been demonstrated to be the commonest means of transfer of antimicrobial resistance among bacteria (Russell, 2002).

Another factor which can enhance the increase of resistant bacterial strains in indigenous chickens is their feeding habit. These chickens roam around large areas scavenging for food (picking from the ground) getting little or no supplementation (Kingori *et al.*, 2010; Safalaoh, 1997). They can pick cereals, herbs, insects, bacteria and so on. There has been a report on the presence of antibiotic resistance genes and resistant bacteria in aquatic environment as the result of hospital waste, food animal barn wastewater and manure; which are associated with insects and other animals that can act as reservoirs and thus infect other animals including chickens (Gebreyes *et al.*, 2017).

During the time of heavy rains, as the water flows from one location to another it tends to carry different substances including bacteria, plasmids and antibiotics which were carelessly disposed in the environments. Thus as they feed, there is high possibility of these birds picking the bacteria, some of which may be resistant to some antibiotics; plasmids, antibiotics, and other substances from the contaminated soil or from the contaminated water that they drink, which can increase the number of antibiotic resistant bacterial strains in their guts (Marshall and Levy, 2011). If the antibiotic resistant trait is transferred to pathogenic bacteria, it will be difficult to treat the disease caused by the particular bacterium (Aarestrup *et al.*, 2000).

It has been found that the increase in antibiotic resistance is one of the major reasons why bacterial infection threat has not been vanquished (Spellberg *et al.*, 2008); for example, multi-drug resistant

Escherichia coli were isolated from chicken meat sold in Nairobi/Kenya (Odwar *et al.*, 2014); methicillin-resistant *Staphylococcus aureus* have also been documented from different animals (Aires-de-Sousa, 2017). In such cases, the resultant food-borne or animal-acquired illness in humans will be less or not responsive to treatment with respect to the particular antibiotic(s) (Fair and Tor, 2014).

Determination of bacterial susceptibility/resistance to antibiotic (s) is essential for accurate management of bacterial infections and for comparative analysis of antibiotics. Antibiotic susceptibility test can be performed by using different methods, either phenotypic or genotypic (Cavaleri *et al.*, 2005*;* Jorgensen and Jane, 2009). Phenotypic methods are techniques used to demonstrate metabolic, physiological and biochemical characteristics of the respective microorganism (Weatherall and Hospital, 2001). They include: disk diffusion test; broth dilution test; E-test (AB Biodisk, Solna, Sweden) (commercially available test that utilizes a plastic test strip impregnated with a gradually decreasing concentration of a particular antibiotic); automated instrument systems, mechanism-specific tests. The choice of method to be used is determined by several factors including ease of use, flexibility, automation or semi automation for larger-scale operations, cost, reliability and accuracy (Markey *et al.*, 2013). However, the most used methods of antimicrobial susceptibility testing are disc diffusion, broth dilution and agar dilution.

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 also known as Kirby Bauer method (Bonev *et al.*, 2008). Disc diffusion is the simplest method to perform. It involves placing of discs impregnated with antimicrobial agents onto an agar plate seeded with the bacterium to be tested, producing confluent growth. The antimicrobial agent diffuses into the agar

creating a zone saturated with the agent, in which an organism susceptible to that agent will not grow (Markey *et al.*, 2013; Bonev *et al.*, 2008).

Minimum inhibitory concentration (MIC) is the lowest antibiotic concentration which inhibits the growth of bacteria. In broth dilution test, the MIC is determined by adding various dilutions of the test antibiotic into respective tubes containing broth culture of the same bacterial type and concentration after overnight incubation (Carson *et al.*, 2002; Andrews, 2001). Growth is indicated by turbidity of the suspension.

Genotypic methods are techniques used to identify the genetic make-up of resistant strains of microorganism (Weatherall and Hospital, 2001), for example Polymerase chain reaction (PCR), Pulsed-field gel electrophoresis of whole chromosomal DNA, Southern blotting and Restriction 3fragment length polymorphism (RFLP) and DNA sequencing (Olive and Bean, 1999; Tenover *et al.*, 1995).

2.10.2 Disinfectant resistance

Bacterial resistance to disinfectants can also occur and can also be towards a single disinfectant or to several ones as it happens for antibiotics. There is also a possibility of combined resistance to antibiotic and disinfectant, where both are carried on the same plasmid (Russell, 2002). Disinfectant susceptibility/resistance testing can be done using various methods as given for antibiotic susceptibility/resistance, for example agar well diffusion, the suspension test of the German Society for Hygiene and Microbiology (DGHM), the suspension test of the Committee on Phytopharmacy, the A.O.A.C. use-dilution method, the KELSEY SYKES test, the surface-disinfection test of the DGHM and a modified version of the latter (Wirtanen and Salo, 2003).

The one mostly used is diffusion technique, where wells are dug into the inoculated agar and are filled with the respective disinfectant (Gaudreau and Gilbert, 1997). Mueller Hinton Agar is used

and seeding is done the same way as for antibiotic susceptibility/resistance testing. Reading is also the same as that for antibiotic susceptibility/resistance testing, however, there are no established cut-off points; so the reading is done as follows: inhibition diameter less than 10 mm is taken as being resistant, while diameter more than 10 mm is taken as being susceptible (Njagi *et al.*, 2005).

2.11 Bacterial characterization

The word "characterization" with respect to bacteria, means describing the bacterium's character or traits (Winslow, 1914). It encampuses all aspects including: biochemical testing, serological testing, genotyping, phage typing, antibiotic susceptibility testing, disinfectant susceptibility testing. In order to carry out these tests one must have a pure culture; so, getting a pure culture through aseptic culturing is the most important part of bacteriology. It is also the only way to be sure that you are dealing with a live organism.

2.11.1 Bacterial culturing

Bacterial culturing is the golden method for bacterial isolation and characterization. It is used to obtain pure culture of microorganisms which are able to grow under laboratory conditions. In order to grow, bacteria require nutrition and optimal conditions such as oxygen, pH, temperature; requirements being different for different bacteria, hence different culture media and conditions for bacterial isolation (Markey *et al.*, 2013). Different methods can be used for bacterial culturing including streaking the agar plate, pour plating and inoculation into broth media (Markey *et al.*, 2013; Shane, 2005). After getting pure culture of individual bacterium, it can be further identified by other methods such as biochemical tests, staining and molecular techniques. This study was limited to biochemical tests and gram staining.

2.11.2 Biochemical tests

Biochemical tests are very important for further identification of isolated bacteria. Some of the biochemical tests used are: ability to ferment sugars; urease; citrate; indole; catalase; oxidase; Methyl red; Voges-Proskauer tests and motility (Cowan, 2003). Biochemical tests are sometimes used in combination to test multiple properties; for example: Triple sugar iron (TSI) agar and Sulfide indole motility (SIM) medium (Mahon *et al.*, 2014; Markey *et al.*, 2013).

Gram staining technique was done according to the guidance provided by Markey *et al.* (2013); The isolates were first confirmed to be pure culture, Gram positive or Gram negative before performing further biochemical tests.

CHAPTER THREE: QUANTIFICATION OF INTESTINAL BACTERIAL CARRIAGE OF SLAUGHTERED INDIGENOUS CHICKENS NAIROBI, KENYA AFTER HEAVY RAINS.

3.1 Introduction

Indigenous chickens may end up having increased bacterial load while feeding from the ground by picking bacteria directly, eating insects which may be carrying bacteria and/or drinking contaminated water (Jones *et al.*, 2007); as result of heavy rains, that brought flooding water from different places, carrying bacteria (Nyambura *et al*., 2014; Kim *et al*., 2001). Also, the resulting coldness and wetness affect chickens in a way that their immune system can become weaker when they are trying to adjust to the weather change. The resultant increased bacterial loads may affect immunity of the chickens and make them susceptible to disease (Adesiji and Baba, 2013; Haller and Jobin, 2004). The objective of this study was to establish the prevalence of bacteria (in quantity) in indigenous chickens after the heavy rains of the year 2018.

3.2 Materials and methods

3.2.1 Study area

The study was conducted in poultry slaughterhouses in Nairobi City and peri-urban areas of Kariokor, Burma and Kangemi. Figure 3.1 (A) shows location of Nairobi County and (B) shows the slaughterhouses from which the samples were collected. The slaughterhouses get chickens, indigenous ones, some spent layers and cockerels from various parts of Kenya. Kariokor slaughterhouse receives indigenous chickens mostly from Ukambani, Bomet, Kericho, Nyandarua and Kiambu. The birds are usually transported by buses or bicycles and are normally slaughtered the same day they are brought. There are no additional foods nor supplements given to the birds at the slaughter house. Burma slaughterhouse receives indigenous chickens from Kisii, Ukambani, Kitale, Kawanandi, Mbameti, Kericho, Nyahururu and Kiambu. Chickens normally stay at the

slaughterhouse for some days before they are bought. During this time, they are fed on grains only. Kangemi slaughterhouse receives indigenous chickens from Bomet and Narok. They are usually transported by buses or bicycles. Not all of them are sold the same day they are brought-in; sometimes they stay for few days or weeks at the market; being fed on cereals mainly maize.

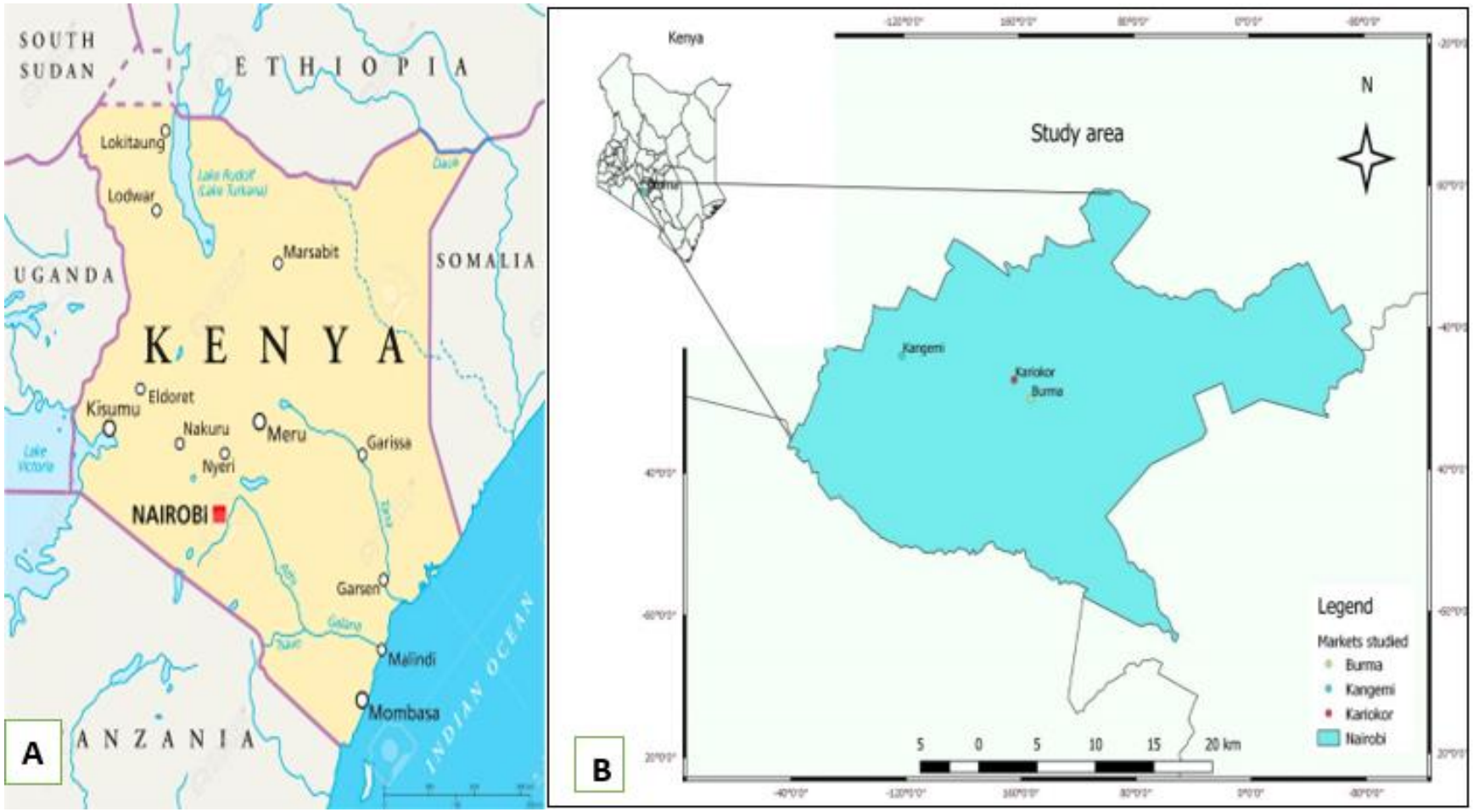

Figure 3.1: (A) Map of Kenya indicating position of Nairobi County and the respective slaughterhouses where samples were collected (B) https://www.google.com/imgres?imgurl

3.2.2 Study design

A cross sectional study was carried out, where intestines of slaughtered village chickens were bought from the respective slaughterhouses. The intestines were placed separately into sterile universal bottles, labeled and transported to Department of Veterinary Pathology, Microbiology and Parasitology in a cool box, where they were processed for bacterial counting. While buying and collecting the samples for testing, a questionnaire was administered to gather information on origins/ sources of the slaughtered birds (Appendix 1).

3.2.3 Sample size calculation

Sample size was calculated using the formula suggested by Martin *et al.* (1987), as given below:

$N = 4pq/L^2$

where N is the sample size; P is 50% i.e. 0.5 since the prevalence is not known; q= 1-p which is 1-0.5 =0.5; L is degree of accuracy/precision desired at 10%.

Hence the sample size was supposed to be $(4x\ 0.5^2)/\ 0.1^2$= 100 samples; as minimum number.

However, forty (40) samples were collected from each slaughter house, totaling to 40x3=120 samples.

3.2.4 Study population

The collected intestines were from indigenous village chickens, brought to the slaughterhouses from various parts of Kenya. The intestines were obtained from randomly selected indigenous chickens from the three slaughterhouses: Kariokor, Burma and Kangemi; 40 samples from each slaughterhouse as described in section 3.2.3.

3.2.5 Sample collection and handling

The intestines were obtained, placed separately into sterile universal bottles, labeled, sealed and transported in a cool box to the laboratory at the Department of Veterinary Pathology,

Microbiology and Parasitology, University of Nairobi, for direct isolation and then characterization of the isolates. For the total number of 40 per slaughterhouse, sampling was done in one or two visits, depending on availability of slaughtered indigenous chickens on the particular visit at the particular slaughterhouse; the intestines being collected directly from a slaughtered indigenous chicken. The intestinal contents collected from the three slaughterhouses were studied in turns (first batch from Kariokor then Burma, then Kangemi). Figure 3.3 (A) shows the intestines in the universal bottles ready for further processing and (B) shows the investigator collecting intestine from the slaughterhouse.

Figure 3.2. A: Samples (Intestines of indigenous chickens) collected in universal bottles from the slaughterhouse; B: Investigator collecting chicken intestines (as pointed by the yellow arrow) from a slaughtered indigenous chicken.

3.2.6 Determination of Bacterial counting

Total bacterial counting (cfu/ml) was done to enumerate the bacterial load using the method of Miles and Misra, (1938) as follows: one (1) gram of intestinal contents was placed in 9 ml of normal saline (0.85% sodium chloride); this was considered as 10^{-1} and mixed thoroughly to make smooth suspension by vortexing; then ten-fold serial dilutions were made (from 10^{-2} to 10^{-10}) in test tubes.

Then, using a dropper which drops 25 microlitres (equivalent to 40 drops to an ml; i.e. a drop represents $1/40^{th}$ of a ml), two drops from each dilution were dropped separately onto nutrient agar (in petri dish), which was divided into four quadrants, i.e: each plate accommodated four drops; two being duplicates of one dilution. The plates were then incubated at 37° C overnight prior to counting the resultant countable colonies; counts of the two drops from the same dilution were averaged. Quantity of bacteria for the original suspension was calculated using the formula:

$N \times 40 \times 10^{x}$

where "N" is the average number of counted colonies; "40" is the number of drops that make one ml, and "10^{x}" is the dilution factor (Miles and Misra, 1938). The resultant number was given as colony forming units (cfu) per ml/gm since each isolated colony was presumed to have originated from one bacterium and one ml was taken to be equivalent to one gram of intestinal content.

3.2.7 Data handling and analysis

Descriptive statistics was used to analyze obtained data (variables to be analyzed need to be included). Bacterial counts were analyzed by Analysis of Variance (NOVA) using Statistical Package for Social Sciences (SPSS) statistical program to compare the arithmetic means which were obtained by adding the sum of all individual counts from each slaughterhouse divide by the number of samples.

3.3 Results of total bacterial counting

Bacterial counting from the three slaughterhouses ranged from 10^4 to 10^{12} cfu/gm. Chickens from Kariokor slaughterhouse had lower bacterial carriage than those from other slaughterhouses. Detailed information can be found on Appendix 2. Countable colonies produced on Nutrient agar (NA) plates after overnight incubation, were as shown in Figure 3.4.

The birds had different intestinal bacterial concentrations. Some had low concentration, thus visible colonies could be counted at lower dilution, while others had higher bacterial concentrations so the colonies were countable only at higher dilution. Table 3.1 gives bacterial carriage distribution of the birds with respect to the initial dilutions giving countable colonies. Details of arithmetic means are given in Appendix 3.

Table 3.1: Bacterial carriage distribution of the birds with respect to the initial dilutions giving countable colonies

Slaughterhouse	Total number of samples used for counts	Number of birds that had countable colonies per dilution						
		10^4	**10^6**	**10^8**	**10^9**	**10^{10}**	**10^{11}**	**10^{12}**
Kariokor	38	4/38	4/38	1/38	1/38	3/38	22/38	3/38
Burma	36	-	-	3/36	1/36	19/36	11/36	2/36
Kangemi	40	-	-	1/40	12/40	15/40	8/40	4/40

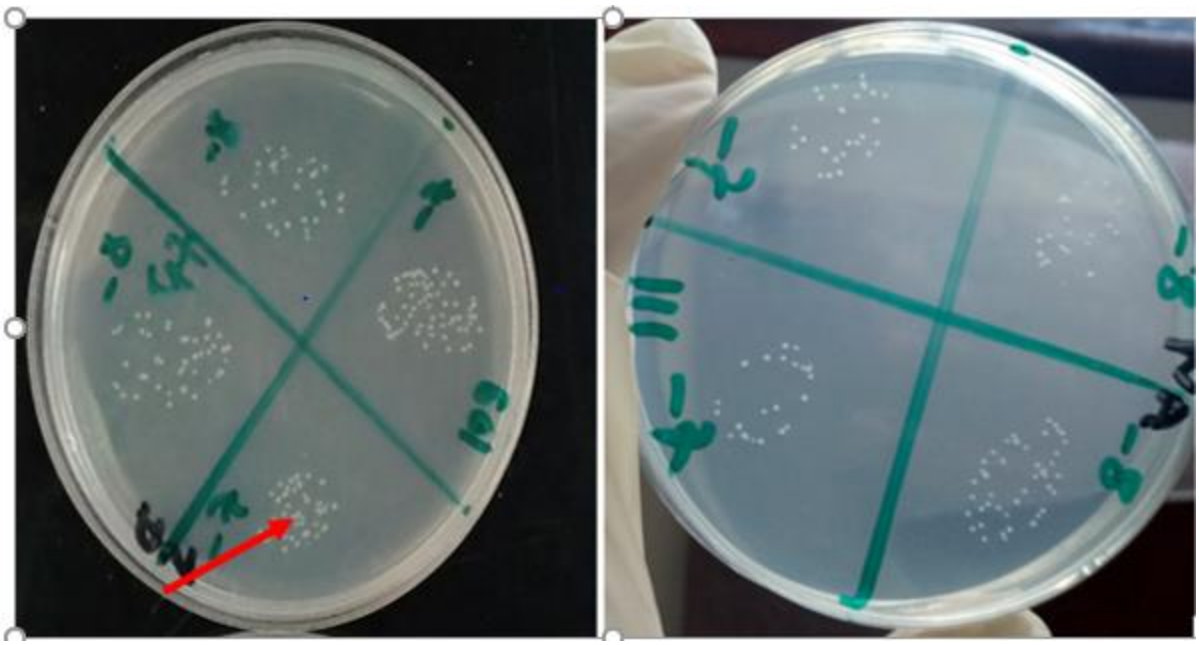

Figure 3.3: Countable colonies on NA agar; the one shown by the red arrow, drop from a 10^{-7} dilution.

The means results of total counting from the three slaughterhouses were significantly different (statistically) with p-value of 0.0266. Results of homogeneity test showed that the counts from Kariokor (4.7×10^{11}) and Burma (5.6×10^{11}) slaughterhouses were homogeneous but different from the counts from Kangemi slaughterhouse (1.3×10^{12}) as shown in Appendix 3, which shows the output of Analysis of Variance (ANOVA) after analyzing arithmetic means of the counts.

3.4 Discussion

Bacterial carriage of the test chicken intestines ranged between 10^4 and 10^{12} colony forming units per 1 gm of intestinal contents. The results have shown no difference in counts from Kariokor and Burma slaughter houses and the counts from Kangemi were higher compared to the other two slaughter houses as shown by ANOVA test. Mean counts from Kariokor and Kangemi were 4.7×10^{11} and 5.65×10^{11} cfu/gm, respectively, while the one from Kangemi was 1.32×10^{12} cfu/gm. This difference can be explained by a number of factors, for example: It may be that the indigenous

chickens from Kangemi were exposed to higher number of bacteria before being transported to the market (while feeding from contaminated environment) (Kim *et al*., 2001) or the birds may have acquired more bacteria at the slaughterhouse as a result of poor holding conditions, because the chickens normally stay there for long before being sold (though this could even happen in dry season); there could have been cross-infections among them.

A study done by Proietti *et al.*, (2006) has demonstrated bacterial counts from chicken intestines of $nx10^6$cfu/gm. That study showed a lower count compared to the one obtained in the current study which had means of $nx10^{11}$ and $nx10^{12}$. As mentioned in literature review, the heavy rains of 2018, that happened between March-May in Kenya, caused destruction of toilets and over flooding of rivers; this could have contributed to the spreading of different materials that were containing bacteria from one location to another (OCHA, 25 Apr 2018); hence increase in bacterial counts; suggesting that the indigenous/village chickens were feeding from highly contaminated environment. Also, there is high probability that the indigenous chickens were having reduced immunity as the result of climate change (due to wetness and cold) and became vulnerable to bacterial attack and multiplication (Elijah and Adedapo, 2006) hence increase in the bacterial carriage.

The findings from the current investigation were not different from the one got by Smith and Crabb (1961), even if it was not mentioned whether that study was conducted during rainy seasons or not; Their study had shown the total bacterial count in chicken feces to be between 10^3 to 10^{10} cfu/gm. However, there is minimal literature on intestinal bacterial counts and all of them are from other countries; this is the first study done on total intestinal bacterial counts from indigenous chicken in Kenya. Thus, where possible, free-range indigenous chickens should be confined during

rainy seasons to minimize the environmental exposure. It is also important that policy makers come up with guidelines on reduction of environmental contamination.

CHAPTER FOUR: IDENTIFICATION OF BACTERIA ISOLATED FROM THE INTESTINES.

4.1 Introduction

Food borne diseases are of public health concern due to their negative effect on the lives of humans (Zhao *et al.*, 2001); most of the food borne diseases are caused by bacteria. It is, however, interesting to note that, despite the fact that chicken meat is known to be a source of bacteria associated with food-borne diseases, there is an increase in consumption of poultry meats and their products worldwide (Tresse, 2017). Chickens have been shown to act as carriers of harmful bacteria such as *Salmonella*, *Listeria*, *Campylobacter*; causing diseases to respective consumers (Njagi *et al.*, 2004; Siringan *et al.*, 2014). As they roam over places, scavenging for food, indigenous chickens excrete bacteria through defecation; they also pick other bacteria from the soil/environment (Adelaide *et al.*, 2008; Kingori *et al.*, 2010).

Studies have shown that bacteria from chickens, such as *Salmonella* serotypes, *Campylobacter* spp; pathogenic *Escherichia coli* including *E. coli* O157:H7, *Listeria* spp, *Staphylococcus* spp*; Streptococcus* spp*; Pseudomonas* spp*; Aeromonas* spp, among others, cause diseases which can be mild or severe in humans; thus they can cause complications in patients (Zhao *et al.*, 2001; Tresse, 2017; Njagi *et al.*, 2004). During and after slaughtering, microbiota of the chickens can contaminate the slaughterhouse environment and utensils; resulting in their transfer among the carcasses and meat contamination (Tresse, 2017; Odwar *et al.*, 2014). This study has endeavored to characterize bacteria that were isolated from intestinal contents of indigenous chickens slaughtered at three slaughterhouses in Nairobi, Kenya.

4.2 Materials and methods

4.2.1 Study area

This was as given in Section 3.2.1

4.2.2 Study design

This was as given in Section 3.2.2, except for the bacterial counting. In this case, at the laboratory, intestinal contents which were brought-in in a cool box, were inoculated onto growth media, colonies observed and bacterial isolates characterized using standard tests (Markey *et al.*, 2013; Shane, 2005), as expounded below.

4.2.3 Study population

This was as given in Section 3.2.4

4.2.4 Sample size calculation

This was as given in 3.2.3

4.2.5 Sample collection and handling

This was as given in Section 3.2.5

4.2.6 Bacterial isolation and identification

4.2.6.1 Bacteria culturing

Bacterial culturing was done using different growth media: General medium used was blood agar, and selective and/or differential media were Mannitol salt agar for *Staphylococcus* spp, MacConkey agar for members of family Enterobacteriaceae, Salmonella-Shigella agar for *Salmonella* serotypes and *Shigella* spp isolation, Cystine tellurite blood agar for *Listeria* spp, Sodium azide crystal violet blood agar for *Streptococcus* spp, Thiosulphate Citrate Bile Salts Sucrose Agar (TCBS) for *Vibrio* spp, Camp Karmali for *Campylobacter* spp; To screen for *E. coli* O157:H7, MacConkey Sorbitol agar was used; the suspected colonies were typed using respective

antiserum Prolex ^{TM} *E. coli* 0157. Enrichment media were used: Selenite broth for *Salmonella* serotypes and Alkaline peptone water for *Vibrio* spp. To increase chances of isolating *Listeria* spp, samples were subjected to cold enrichment at 4°C overnight (Holt and Williams, 1989; Markey *et al.*, 2013). The media used were products of Oxoid Ltd (Wade Road, Basingstoke, Hants, United Kingdom).

4.2.6.2 Biochemical tests

Biochemical tests used to identify the isolated bacteria were as given by Bergey's manual for systemic bacteriology by Holt and Williams (1989) and Cowan (2003). Among others, the commonly used were: Oxidase, Catalase, Indole, Methyl red, Citrate, Urease, reaction on triple sugar iron agar, reaction on Sulphur indole motility medium. Other tests were also involved for further characterization; they included CAMP test for *Listeria monocytogenes* and *Streptococcus* spp and hanging drop motility test. Details of specific reactions are given in Appendix 4; some of the bacteria were identified to species level, for example: *Streptococcus agalactiae; Staphylococcus aureus; Listeria monocytogenes,* some were identified up to genus level, while others could not be identified with respect to the tests that were used. The processes of isolation and characterization for bacteria are demonstrated in Figure 4.1.

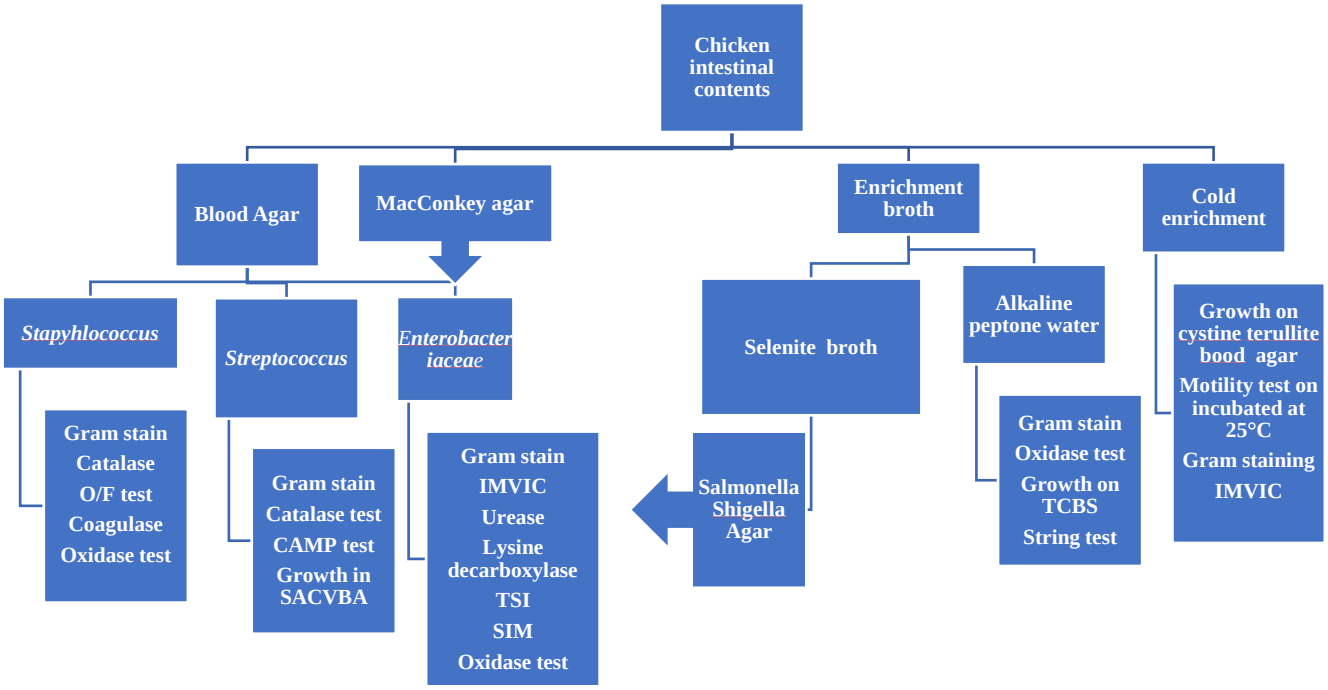

Figure 4.1: Diagrammatic representation of the isolation and identification pattern for bacteria
The diagram shows the connected chart flow in stepwise manner, from isolation to identification. The one with arrow shape, is pointing to the next further steps.

4.2.7 Data analysis

The results were analyzed by Chi square using SPSS statistical program, to check the association of the isolates from the three different slaughter houses.

4.3 Results of Bacterial isolation

From the 120 intestinal samples collected (40 per slaughterhouse), thirteen genera were identified among others.

Bacteria isolated from Kariokor slaughterhouse were as follows: the most prevalent was *Escherichia coli* (34/40; 85%), followed by *Staphylococcus* spp other than *Staph. aureus* at 55% (22/40); *Streptococcus* spp at 40% (16/40); *Staph. aureus* and *Bacillus* spp at 25% each (10/40); *Listeria* spp other than *Listeria monocytogenes* at 12.5% (5/40); *Listeria monocytogenes* at 7.5% (3/40); and lastly *Streptobacillus* spp at 2.5% (1/40).

The isolates from Burma slaughterhouse were as follows: the most prevalent was *E. coli* (34/40; 85%); followed by *Bacillus* spp at 65% (26/40); *Streptococcus* spp *at* 52.5% (21/40); *Proteus* spp at 50% (20/40); *Listeria* spp at 31.7% (12/40); *Staphylococcus* spp at 12.5% (5/40); *Staph. aureus* at 10% (4/40); *Klebsiella* spp at 5% (2/40); while the least isolated were *Campylobacter* spp*, Pseudomonas* spp*; Listeria monocytogenes* and *Streptococcus agalactiae* at 2.5% each (1/40).

Bacteria isolated from Kangemi slaughterhouse showed prevalence as follows: the most prevalent was *E. coli* (35/40; 87.5%); followed by *Listeria* spp at 52,5 % (21/40); *Staphylococcus* spp at 40% (16/40); *Streptococcus* spp at 37.5% (15/40); *Bacillus* spp at 35% (14/40); *Staph. aureus* and *Proteus* spp at 22.5% (9/40); *Klebsiella* spp at 17.5% (7/40); *Listeria monocytogenes* at 10% (4/40); *Campylobacter* spp at 5% (2/40); while the least isolated were *Streptococcus agalactiae* and *Pseudomonas aeruginosa* at 2.5% each (1/40). Details on bacteria isolation are given in Appendix 5.

Figures 4.2 and 4.3 give the prevalence rates of the isolates per slaughterhouse and overall picture of the bacteria that were isolated from the three slaughterhouses respectively; Figures 4.4 and 4.5 give colonial morphologies of some of the isolates; Figure 4.6 gives CAMP reactions for *Streptococcus* and *Listeria* isolates; Figure 4.7 gives some of the biochemical reactions demonstrated by some of the isolates; Figures 4.8 and 4.9 show the oxidase and catalase reactions

and the investigator doing microscopic observation respectively. Figures 4.10 to 4.12 give microscopic morphologies (Gram staining) of some of the isolates.

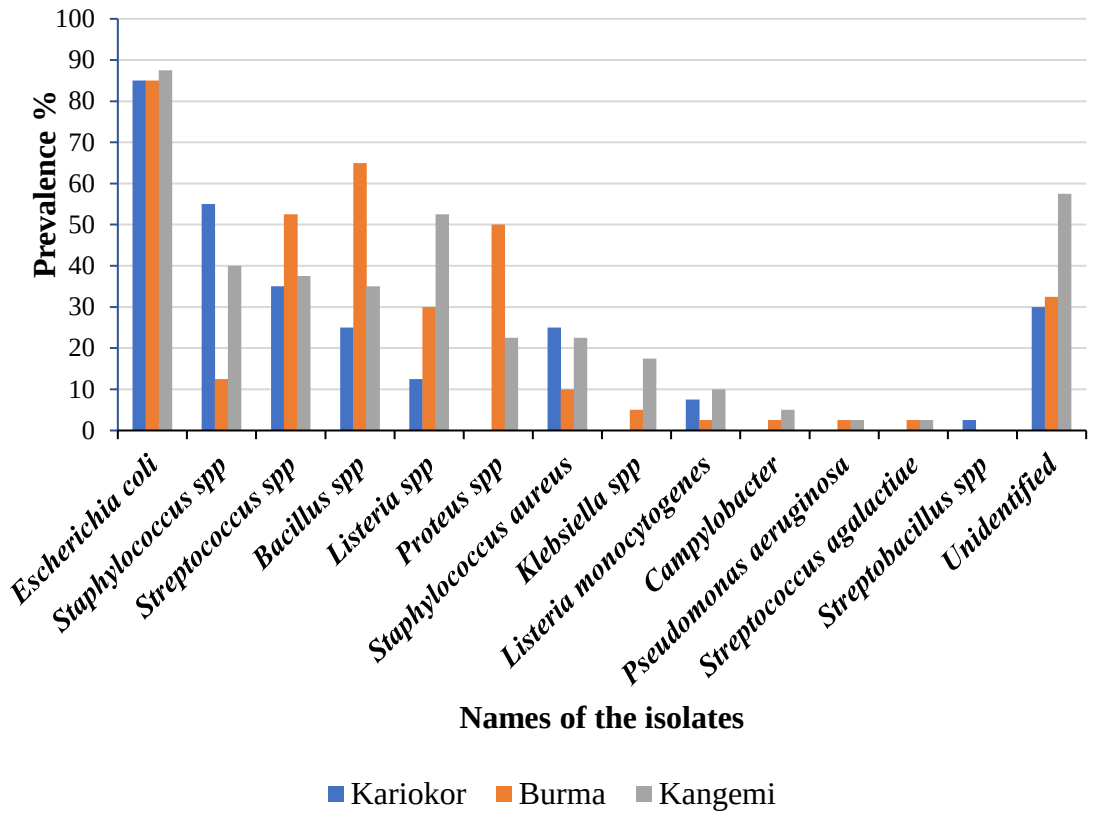

Figure 4.2: Prevalence rates of isolates per slaughterhouse

As mentioned in the Materials and Methods section, efforts were made to isolate *Vibrio* spp (TCBS media), *Salmonella* serotypes, *Shigella* spp (SSA media) but the bacteria were not isolated. *E. coli* isolates produced pale colonies on Sorbitol MacConkey but, on typing with respective antiserum, they were not serotype O157:H7. Also, there were isolates that could not be identified by the biochemical tests used.

Overall, *E. coli* was the highest isolated at 85.8%; followed by both *Bacillus* spp and *Streptococcus* spp other than *Strept. agalactiae* at 41.66% each; *Staphylococcus* spp other than *Staph. aureus* at

34.16%; *Proteus* spp at 24.16%; *Listeria* spp other than *L. monocytogenes* at 31.7 %; *Staph. aureus* at 17.5%; *Klebsiella* spp at 7.5%; *Listeria monocytogenes* at 6.66 %; *Campylobacter* spp at 2.5%; *Streptococcus agalactiae* at 1.66 %*; Pseudomonas aeruginosa* at 6 % and lastly *Streptobacillus* spp at 0.83%. These bacteria were isolated from healthy appearing indigenous chickens which were brought to the slaughter house for selling as meat prior to consumption.

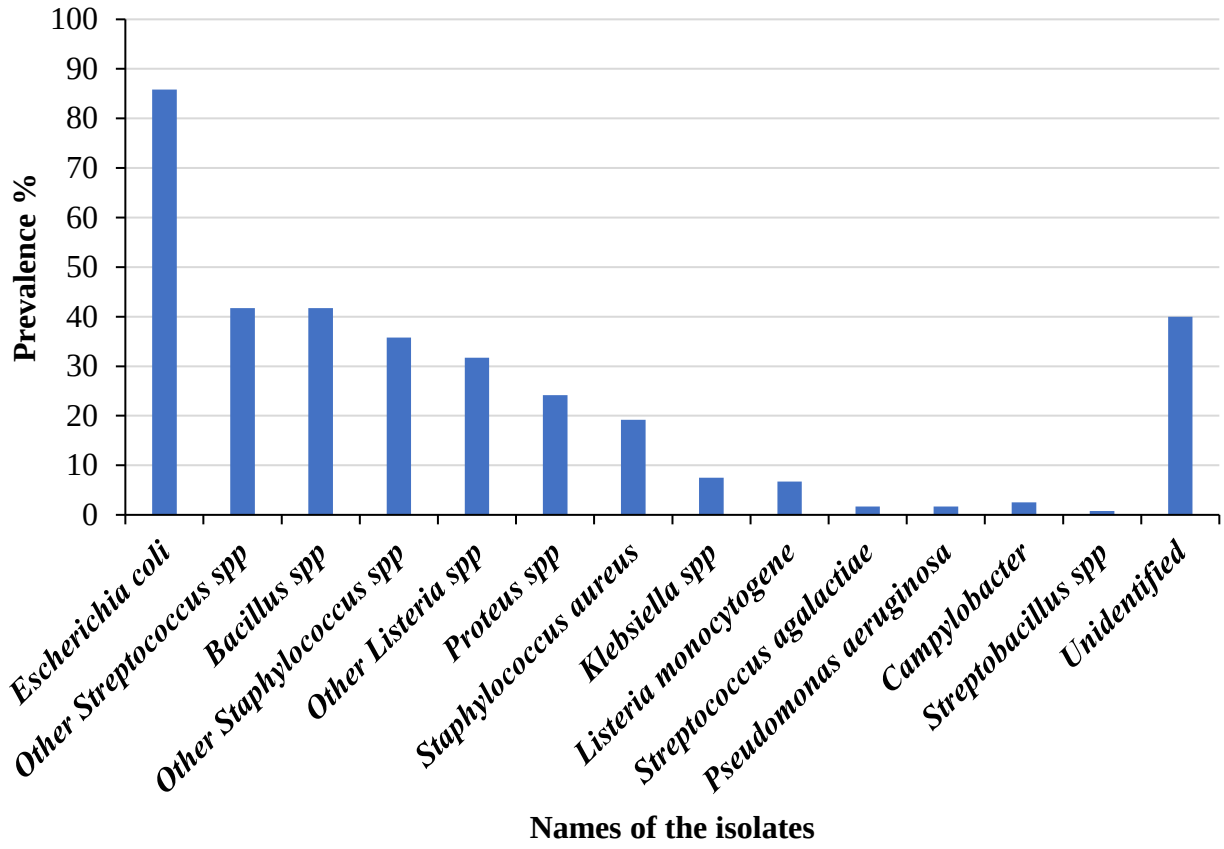

Figure 4.3: Overall prevalence of bacteria isolated from indigenous chickens intestines

Table 4.1 shows the prevalence of isolates per slaughterhouse and their Chi-square results. Detailed information on the statistical output can be found in Appendix 6.

Table 4.1: Prevalence of bacteria isolated from Kariokor, Burma and Kangemi slaughter houses and their Chi square analysis

Bacteria isolated	**Number of the isolates**	**Kariokor (%)**	**Burma (%)**	**Kangemi (%)**	**P-values**	χ^2
Escherichia coli	103 (85.8 %)	34 (85%)	34 (85%)	35 (87.5%)	0.8	0.46^{NS}
Proteus spp	29 (24.16 %)	0 (0%)	20 (50%)	9 (22.5%)	0.00	23.23^{***}
Staphylococcus aureus	23 (19.16 %)	10 (25%)	4 (10%)	9 (22.5 %)	0.19	3.34^{NS}
Other *Staphylococcus* spp	43 (35.83%)	22 (55%)	5(12.5%)	16 (40 %)	0.00	23.42^{***}
Streptococcus agalactiae	2 (1.66 %)	0 (0%)	1 (2.5%)	1 (2.5%)	0.60	1.03^{NS}
Other *Streptococcus* spp	50 (41.66)	14 (35%)	21 (52.5%)	15 (37.5%)	0.17	4.05^{NS}
Listeria monocytogenes	8 (6.66 %)	3 (7.5%)	1 (2.5%)	4 (10%)	0.40	1.88^{NS}
Other *Listeria* spp	38 (31.7 %)	5 (12.5%)	12 (30%)	21 (52.5%)	0.001	14.7^{***}
Pseudomonas aeruginosa	2 (1.66%)	0 (0%)	1 (2.5%)	1 (2.5%)	0.60	1.02^{NS}
Streptobacillus spp	1 (0.83 %)	1 (2.5%)	0 (0%)	0 (0%)	0.37	2^{NS}
Bacillus spp	50 (41.66%)	10 (25%)	26 (65%)	14 (35%)	0.00	19.38***
Klebsiella spp	9 (7.5%)	0 (0%)	2 (5%)	7 (17.5%)	0.009	9.37^{***}
Campylobacter	3 (2.5%)	0 (0%)	1 (2.5 %)	2 (5 %)	0.36	2.051^{NS}
Unidentified	48 (40%)	12(30%)	13 (32.5%)	23 (57.5%)		-

Legend: NS means no significant difference of the isolates in the markets, *******Means that there is significant difference, with respect to the isolates number among the markets at P-value of 0.05.

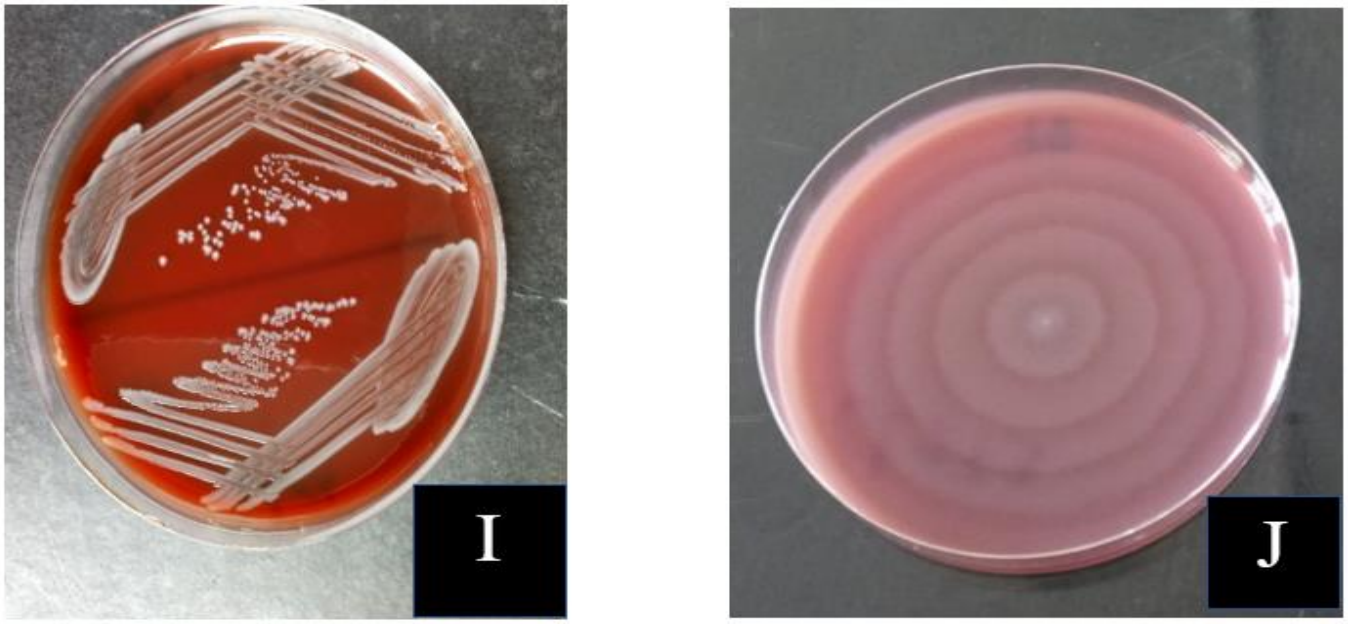

Figure 4.4: Photograph of *E. coli* colonies on Blood Agar media (I) and one showing the swarming motility of *Proteus spp* on Blood agar media(J)

Figure 4.5: CAMP test results of *Listeria monocytogenes* (G) and *Streptococcus agalactiae* (H) isolates against *Staphylococcus aureus*.

where: G shows the shovel shape of *Listeria monocytogenes* as pointed by the blue arrow (A) and H shows the arrow shape of *Strep. agalactiae* as pointed by the yellow arrow (B).

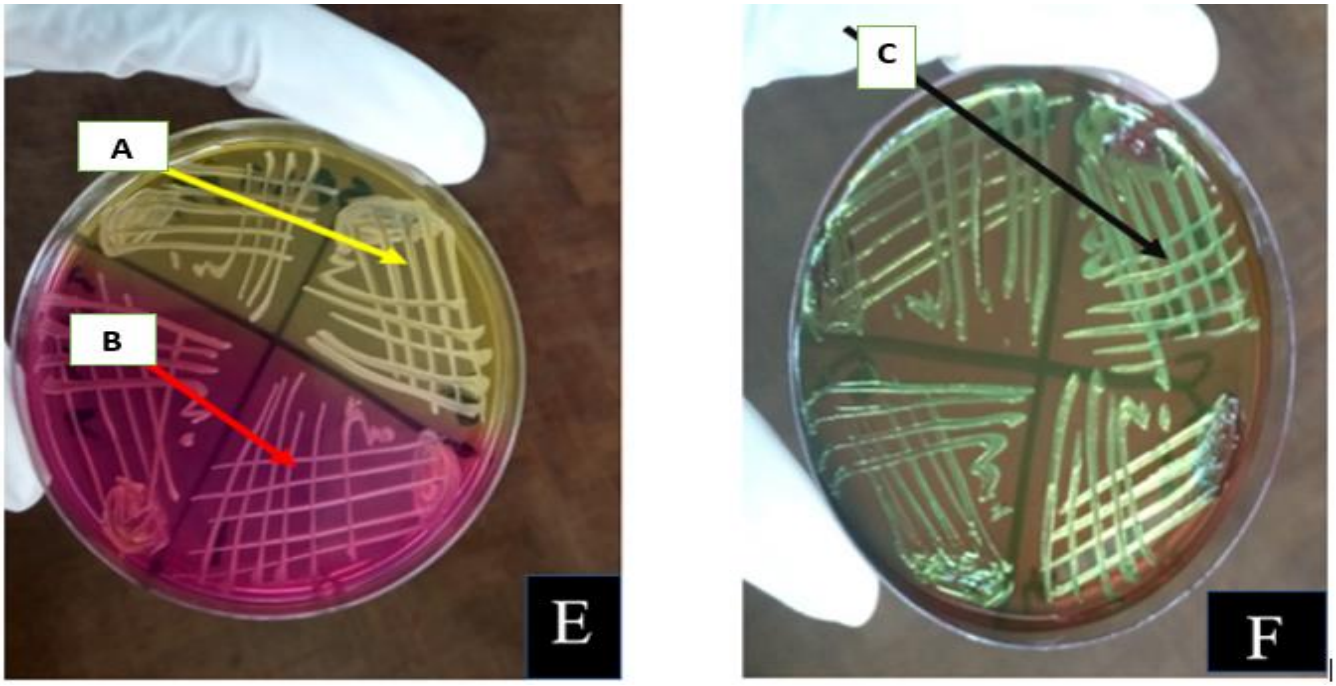

Figure 4.6: Photograph E showing the appearance of *Staphylococcus* spp on Mannitol Salt Agar (MSA) medium and; F is showing the metallic sheen of *E. coli* on Eosin Methylene Blue (EMB) medium.

The yellow arrow (A) points on the colonies of *Staph. aureus* (yellow in appearance) on MSA; pink represents the other *Staphylococcus* spp as pointed on by Red arrow (B) and the black arrow (C) point on the metallic sheen of *E. coli* on EMB.

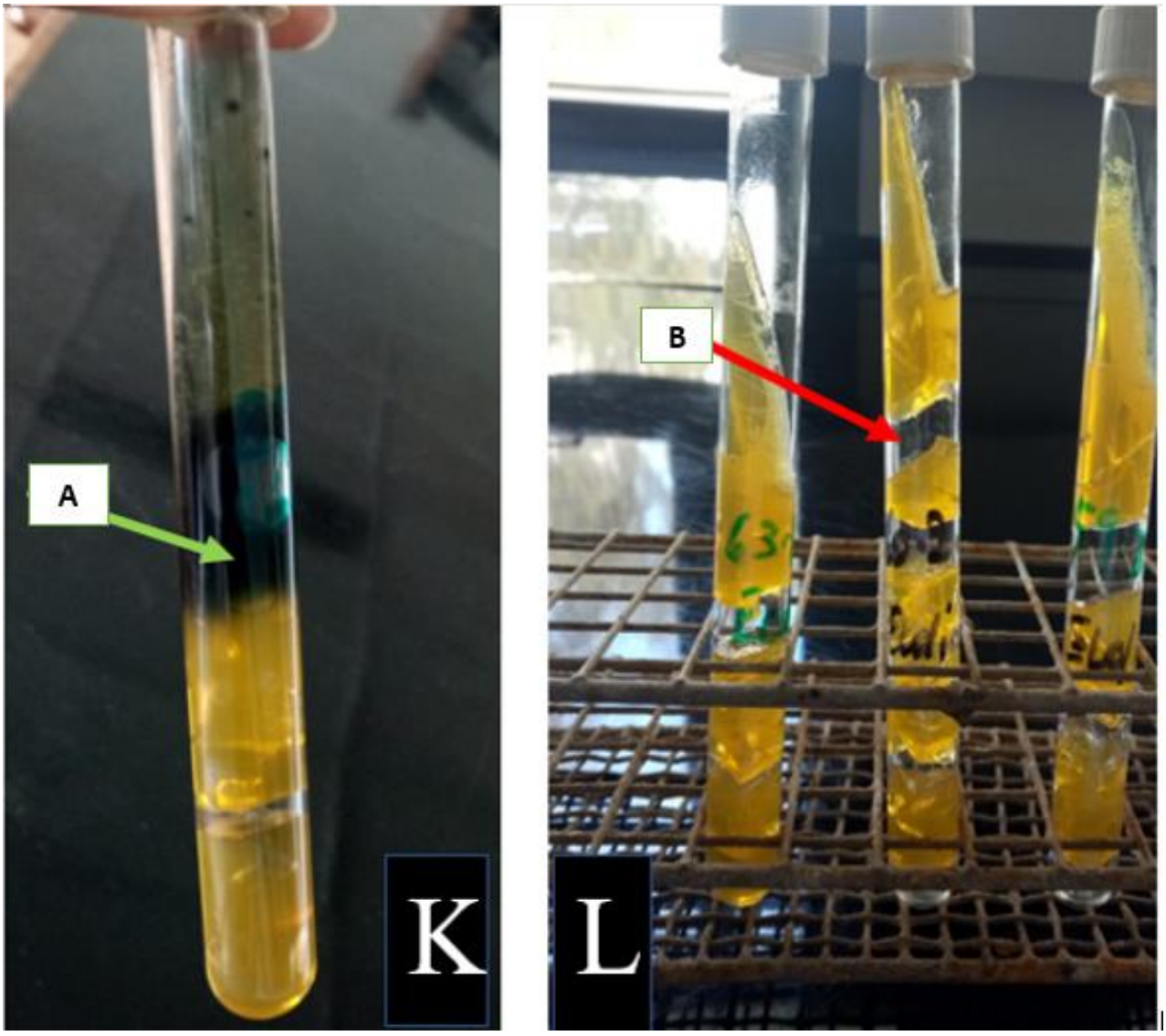

Figure 4.7: Reaction of *Proteus* spp on Triple Sugar Iron (TSI) agar (K)with the production of Hydrogen sulphide and L: represents the reaction of *Escherichia coli* on TSI with production of plenty of gas (cavitation)

The green arrow (A) and the Red arrow (B) shows the H_2S by *Proteus* spp and the production of gas by *E. coli* respectively.

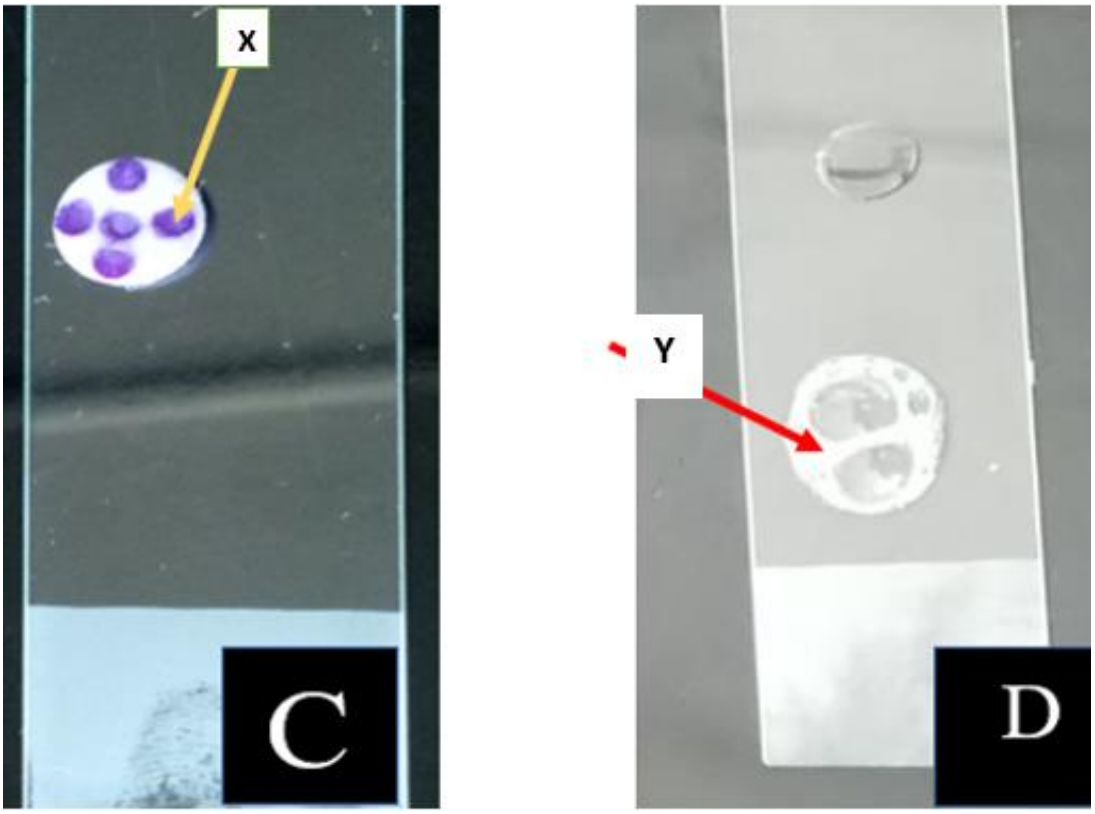

Figure 4.8: Oxidase positive reaction (C) and D is catalase positive
The yellow arrow (X) points on the oxidase positive reaction (Purple) and the red arrow (Y) point on the catalase positive reaction (effervescence).

Figure 4.9: The investigator doing microscopic observation

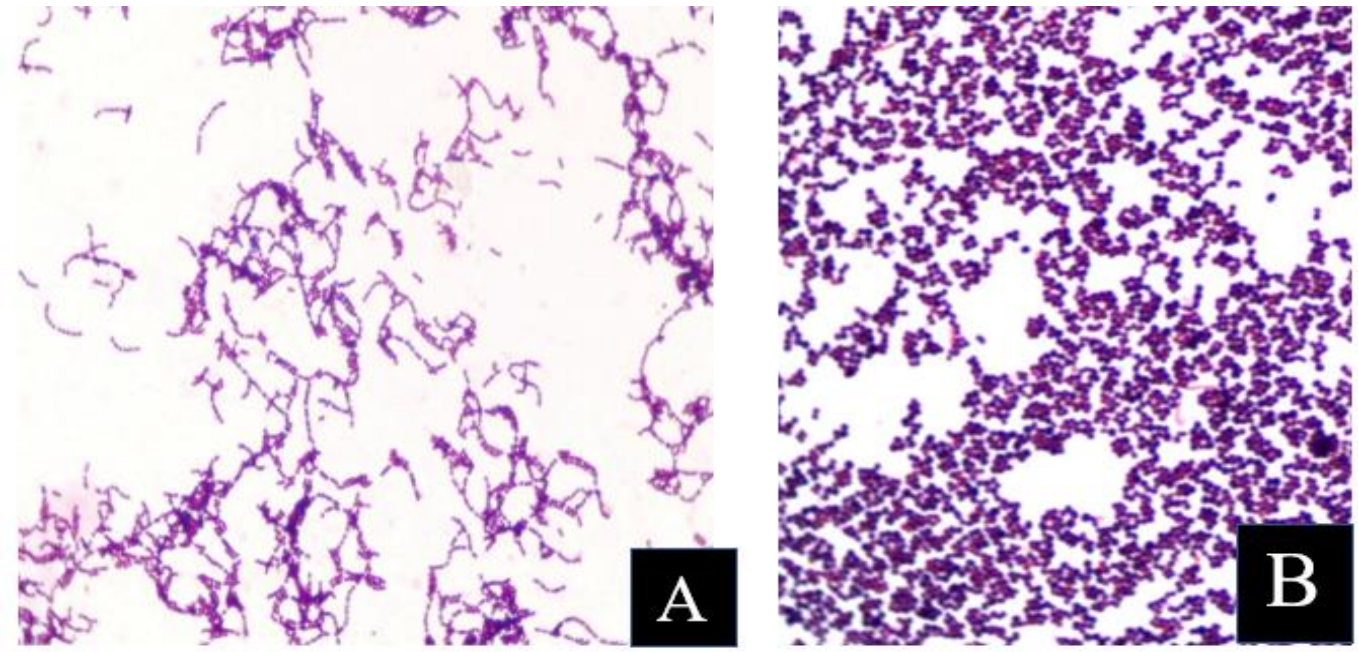

Figure 4.10: Gram positive cocci in chains (A) and Gram-positive cocci in clusters (B)

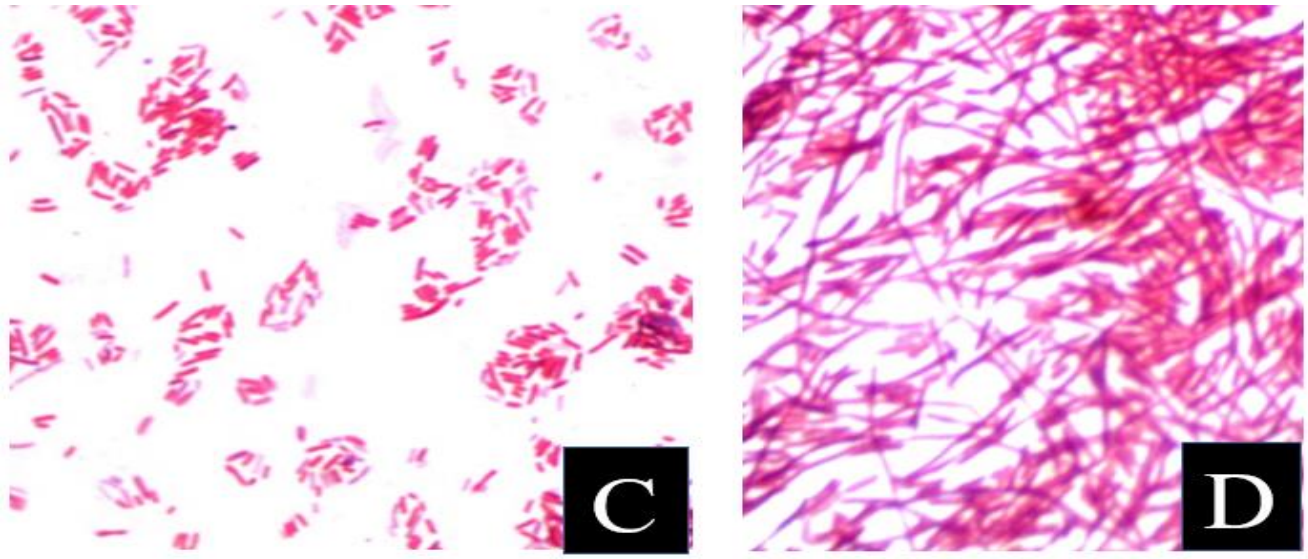

Figure 4.11: Photograph of Gram- negative staining where: C is showing Gram negative rods and D Gram negative filamentous bacteria

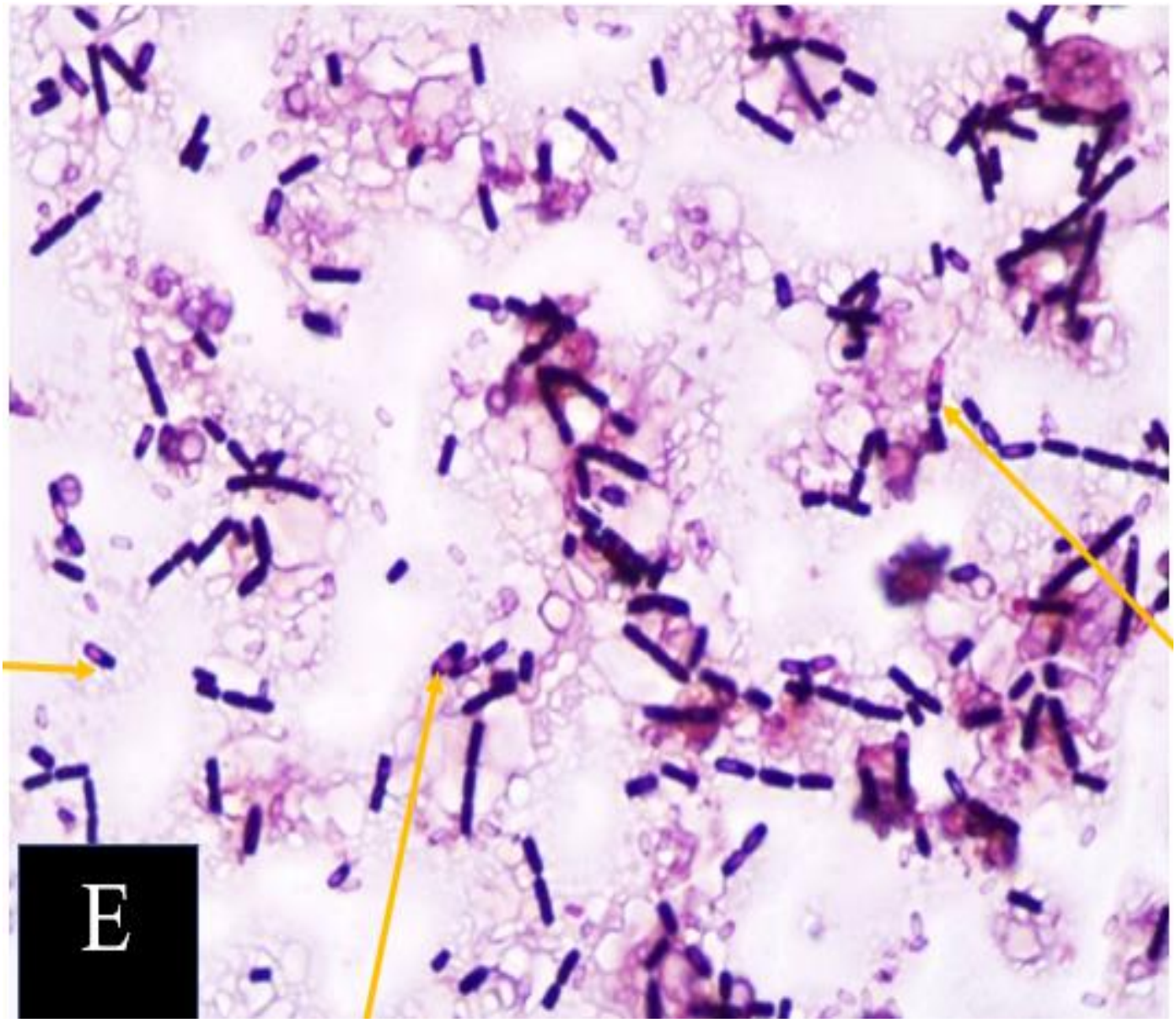

Figure 4.12: Gram positive Bacillus spp rods with spores (unstained). The yellow arrows are pointing to the spores produced by the bacteria.

4.4 Discussion

Among the identified bacterial isolates, *E. coli* was the most prevalent at 85.8 %; followed by *Staphylococcus* spp at 55%; *Streptococcus* spp at 43.3%; *Bacillus* spp at 41.66%; *Listeria* spp at 38.3%; *Proteus* spp at 24.16%; *Klebsiella* spp at 7.5%; *Campylobacter* spp at 2.5%; *Pseudomonas aeruginosa* at 1.66 %; and lastly *Streptobacillus* spp at 0.83%. There are other researchers who have isolated bacteria from market chickens (intestinal contents or cloacal swabs). Bebora (1979) recorded bacterial prevalence rates from intestines of chickens slaughtered at Kariokor slaughterhouse in Kenya as follows: *E. coli* (81.5%), *Proteus* spp (17.7%), *Streptococcus* spp (4.2%) and *Staphylococcus* spp (4.8%). While the current prevalence (85.8%) and the one of that study (81.5%) were almost equal, with respect to *E. coli* carriage, the present study has shown increased prevalence of *Staphylococcus spp* at 66% and *Streptococcus spp* at 43.3%.

Being a normal habitant of human and animal gastrointestinal tract (Markey *et al.*, 2013), having *E. coli* as the most isolated organism (at 85.5%) is not surprising because faecal material normally has high loads of *E. coli*. The study done by Furtula *et al.,* (2010) has demonstrated presence of high numbers of *E. coli* in chicken litter, this shows that isolation of high number of *E. coli* from Intestinal contents of chickens is normal. There was no difference in isolation rates of *E. coli* among the three slaughterhouses (p= 0.8).

Streptococcus spp was also isolated at a fairly high rate (41.66%), with *Streptococcus agalactiae* isolated at 1.66%. Isolation of *Streptococcus* spp in chicken intestines is normal as documented by Devriese *et al*., (1991) who showed presence of *Streptococcus* spp in intestines of healthy appearing chicks of ages 3 weeks (30%) and 12 weeks (27 %), respectively. In this study, there was no significant difference between isolation rates of *Streptococcus* organisms in general (p-

value of 0.2) and *Strep. agalactiae* in particular (p-value of 0.6) among the three study slaughterhouses.

Staphylococcus spp are normal flora of many animals including chickens; the organisms are however, known to be opportunistic and can cause serious disease under adverse circumstances. *Staphylococcus aureus* is known to cause intoxication in humans after consuming contaminated food (Aires-de-Sousa, 2017). They can also cause skin infections and life-threatening conditions like endocarditis, toxic shock syndrome and necrotising pneumonia (Fitzgerald, 2012). In this study, *Staphylococcus* spp were isolated at 55% (*Staph. aureus* at 19.16 % and other *Staphylococcus* spp at 35.83%). In previous studies *Staph. aureus* was isolated from chicken at 22 % (Sharma and Chattopadhyay, 2015), which is slightly different from this study's finding. The isolation rate of *Staphylococcus aureus* among the slaughterhouses was not significantly different (p- value= 0.2). *Staphylococcus* spp were lowest isolated at Burma slaughterhouse at 12.5%; meaning that the chickens sold there were minimally exposed to the organism. The organisms were mostly isolated from Kariokor slaughterhouse at 55%.

In this study, *Listeria monocytogenes* was isolated at 6.7%; isolation rates not being different among the three slaughterhouses (p- value 0.4). A study done by Njagi *et al.*, (2005) documented presence of *Listeria* spp in slaughtered indigenous chickens at 12.5%; which is different from what was found in this study though not significant. Furthermore, in the current study, other *Listeria* spp were also isolated at 31.7% with significant differences from the slaughterhouses (P-value = 0.001). Also, a study done in Germany has shown presence of *Listeria* spp in healthy chickens (Schwaiger *et al.*, 2010). *Listeria* spp's isolation rate at Kariokor slaughterhouse was lower than that of the other two markets; meaning that the chickens sold here were minimally exposed to the organism.

Being zoonotic, *Listeria monocytogenes* and *Campylobacter* spp, especially *Campylobacter jejuni* and *Campylobacter coli,* are of public health importance; they can cause food poisoning to the consumers. *Campylobacter jejuni* and *Camp. coli* are known to cause human gastroenteritis worldwide (Siringan *et al.*, 2014); while *Listeria monocytogenes* is known to cause meningitis and endocarditis among other symptoms (Srinivasan *et al.*, 2005). Chickens are known to be a major source of campylobacter infection with a carrier rate of 40% (Awad *et al.*, 2015); thus they can harbor the organisms without showing any clinical sign. A study done by Zhao *et al.*, (2001) has documented prevalence of *Campylobacter* spp of 70.7% in chickens from Greater Washington, D.C. In this study *Campylobacter* spp were isolated at low rate of 2.5% not because the chickens were free from the organism but may be because of the isolation conditions used which were not providing the favorable environment, for example not providing the required supplementation of CO_2 for the growth (Markey *et al.*, 2013).

Other bacteria isolated in this study were: *Pseudomonas aeuroginosa* (1.7%); *Klebsiella* spp (7.5%); *Streptobacillus* spp (0.83%). It has been recorded in literature that these bacteria can be isolated from chickens (Kilonzo-Nthenge *et al.*, 2008; Sharma and Chattopadhyay, 2015). They can even cause disease in the host if found in large amounts; *Pseudomonas aeuroginosa* can cause corneal ulcers if the eyes got infected by the organism (Karthikeyan *et al.*, 2013).

In this study, *Bacillus* spp and *Proteus* spp were isolated from chicken at 41.66% and 24.16 %, respectively. It is common to isolate these species from poultry; the study done by Kim and others has reported isolation of different *Bacillus* spp from chicken waste (Kim *et al.*, 2001); also Bebora (1979) has isolated *Bacillus* spp from market chickens. Most of *Bacillus* spp are harmless and /or opportunistic pathogens (*B. cereus, B. licheniformis*) which can cause food-borne diarrhea in humans with exception of some which are very harmful, for example *B. anthracis*. There is

evidence that some *Bacillus subtilis* strains are used for control against *Clostridium perfringens* infection in chickens (Teo and Tan, 2005) and *B. circulans* has inhibition activity to *Campylobacter jejuni* (Svetoch *et al.,* 2005).

Proteus mirabilis which has been isolated at 85% from chicken meat in Hong Kong (Wong *et al.,* 2013), can cause respiratory tract and wound infections as an opportunistic bacterium. From this study isolation rates of *Bacillus* spp and *Proteus* spp among the three slaughterhouses were significantly different (P= 0.000).

As shown in Table 4.1 and Figure 4.2, total bacterial prevalence was high in Burma and Kangemi slaughterhouses. The difference in prevalence can be pegged on the mode of selling/ keeping of chickens at the slaughterhouses or could be because chickens were brought from different places as mentioned before. As the questionnaire revealed, for the two markets, there are few customers; thus, the chickens are brought in and stay at the slaughterhouses for some time/days before getting a buyer – at Kangemi, birds can stay for more than a week without being sold. On the other hand, at Kariokor, slaughterhouse, there are many customers; the chickens are brought to the slaughter house and sold the same day. There is high probability that when the chickens stay for long in the market before being bought, contamination of the feed and water takes place, through defaecation or oro-pharyngeal excretions; leading to cross-infections among the birds.

There is also a high possibility that the birds came-in already carrying heavy loads of the respective bacterium/a; indigenous chickens scavenge for food; picking from the ground. So, if the environment is heavily contaminated; the bird's bacterial carriage can be high. As it happens in the times of heavy rains like what happened in year 2018 in Kenya, the flooding could have carried bacteria to different locations and got picked by indigenous chickens. Also, as mentioned earlier due to reduced immunity as the result of stress (from wetness and coldness), bacteria could have

established themselves in the birds easily. Due to change in climate, some insects which may harbor different bacteria migrate from one location to the other while looking for comfort or were washed there by the rain water and can end up being consumed by chickens; increasing their bacterial carriage. Though the bacterial types isolated and identified in this study were not different compared to what other investigators found, they were in large number in the chickens as detailed in the previous Chapter (Chapter 3). This shows that the rains could have contributed to increase in bacterial carriage of the indigenous chickens, hence it is recommended that the birds are monitored during the period of heavy rain; like keeping them indoors and feeding them inside rather than leaving them free the whole day in wet and cold environment.

CHAPTER FIVE: ANTIBIOTIC RESISTANCE PROFILES OF *STAPHYLOCOCCUS*, *ESCHERICHIA* AND *STREPTOCOCCUS* ISOLATED FROM THIS STUDY.

5.1 Introduction

Antibiotics are essential for human and animal health, but need to be used cautiously, noting that food animals are important to human welfare and animal health (ensure food safety) (OIE, 2015; Adelaide *et al.*, 2008).

Antibiotic resistance has been among the top global health challenges for the past years and is becoming worse as the years go by (Marshall and Levy, 2011; Fair and Tor, 2014). Resistance can be to a single antibiotic or to several antibiotics. It can also be transferred by several means; the worst being by a plasmid (resistance factor) since the plasmid can be easily transferred across bacteria (Cavaleri *et al.*, 2005). If the resistant trait(s) is/are transferred to pathogenic bacteria, it will be difficult to treat infection(s) caused by the particular bacteria. Prudent use of antibiotics in animals is, therefore, important as it will control the transfer of antibacterial resistance between animals and humans (Kikuvi *et al.*, 2007).

Antibiotic resistance in bacteria can be detected using either phenotypic or molecular methods (Jorgensen and Jane, 2009).This study has determined antibiotic resistance patterns/profiles of three bacterial types that were mostly isolated in Chapter 4, using disc diffusion technique.

5.2 Materials and methods

5.2.1 Test bacteria and reference strains

The antibiotic susceptibility testing was done on three bacterial types that were mostly isolated in Chapter 4: *Staphylococcus* spp, *Streptococcus* spp and *Escherichia coli*. Five isolates of each of the bacterial types were chosen randomly for each slaughterhouse; thus, the total number of isolates tested was 45. The reference strains used included: *Staphylococcus aureus* (ATCC 25923),

used for Gram positives and *Escherichia coli* (ATCC 25922) used for Gram negatives. They were obtained from Department of Public Health Pharmacology and Toxicology (PHPT), University of Nairobi; the original source being from University Boulevard; United States of America.

5.2.2 Medium and antibiotic discs used

Both the media and antibiotic discs used were manufactured by the Oxoid company (from United Kingdom). The medium used was Mueller Hinton agar and the antibiotics used were guided by recommendations of Clinical and Laboratory Standards Institute (CLSI, 2016) as follows: for Gram negative bacteria the drugs tested were Gentamycin (CN), Amoxycillin (AMC), Ciprofloxacin (CIP), Sulphamethoxazole (RL), and Ampicillin (AMP); for Gram positive bacteria the drugs tested were: Sulphamethoxazole (RL), Erythromycin (E), Clindamycin (DA), Chloramphenicol (C) and Tetracycline (TE).

5.2.3 Antibiotic resistance testing procedure

Antibiotic susceptibility testing was done using disc diffusion on Mueller-Hinton (MH) agar (Oxoid, Basingstoke, United Kingdom) according to the method given by the Clinical and Laboratory Standards Institute CLSI, (2016) and Balouiri *et al.*, (2016). Respective bacterial suspension was prepared by inoculating Nutrient broth and incubating overnight at 37° C; after which the suspension was adjusted to match the turbidity of 0.5 MacFarland nephelometer tube (equivalent to $1.5x10^8$ cfu/µl) using sterile normal saline. Sterile cotton swabs were then separately dipped into the suspensions, excess fluid squeezed out by pressing the dipped swab on the tube side and streaked on the surface of Mueller Hinton agar (Oxoid, Basingstoke, United Kingdom) three times while rotating the plate 60 degrees, to produce confluent growth (Hudzicki, 2016). Respective antibiotic discs were then placed on the inoculated surface and the plate incubated up-side-down at 37^o C overnight.

Susceptibility to the drug was indicated by the size of respective growth inhibition zone; measured in millimetre (mm) using a ruler; the size of the inhibition zone being directly proportional to the susceptibility of the organism to the particular antibiotic (Coyle, 2005). The interpretation of the test, based on the inhibition diameters, was done following the guidelines provided by CLSI (2016).

In this study, only two criteria were used: susceptible and resistant. Bacteria were concluded to be susceptible if the inhibition diameters were above or equal to the susceptible measurement given by CLSI (2016). If the measurement fell in the intermediate and resistant range, they were considered to be resistant; details of the interpretation for the three organisms that were tested are given in Table 5.1 which is extracted from CLSI (2016) guidelines; it gives interpretation of inhibition zones in terms of Susceptible, Intermediate and Resistant.

Table 5.1: Clinical and Laboratory Standards Institute (CLSI) interpretation of disc inhibition zones in terms of antibiotic susceptibility and resistance

Organisms	**Antibiotics**	S	I	R
E. coli	AMP (10µg)	≥17	14-16	≤13
	CN (10µg)	≥15	13-14	≤12
	CIP (5µg)	≥21	16-20	≤15
	AMC (30µg)	≥17	14-16	≤13
	RL (23.75µg)	≥16	11-15	≤10
Staphylococcus spp	E (5µg)	≥23	14-22	≤13
	TE (30µg)	≥19	15-18	≤14
	DA (2µg)	≥21	15-20	≤14
	C (30µg)	≥18	13-17	≤21
	RL (23.75µg)	≥16	11-15	≤10
Streptococcus spp	E (15µg)	≥21	16-20	≤15
	TE (30µg)	≥23	19-22	≤18
	DA (2µg)	≥19	16-18	≤15
	RL (23.75µg)	≥19	16-18	≤15
	C (30µg)	≥21	18-20	≤17

Legend: S means Susceptible; **I**: Intermediate and **R**: Resistant; **CN:** Gentamycin; **CIP:** Ciprofloxacin; **RL**: Sulphamethoxazole; **AMC**: Amoxycillin; **AMP:** Ampicillin; **E**: Erythromycin; **DA:** Clindamycin; **C:** Chloramphenicol; **TE:** Tetracycline (CLSI, 2016).

5.2.4 Data analysis

Data were analyzed using SPSS statistical software. Chi square was used to test the association of drug resistance strains with their respective slaughter houses.

5.3 Results

5.3.1 Overall antibiotic resistance patterns

Tested isolates showed different resistance patterns against the antibiotics; detailed results are given in Appendix 7. Figure 5.1 shows one of the Muller-Hinton plates used to test for antibiotic susceptibility of *Staphylococcus* isolate.

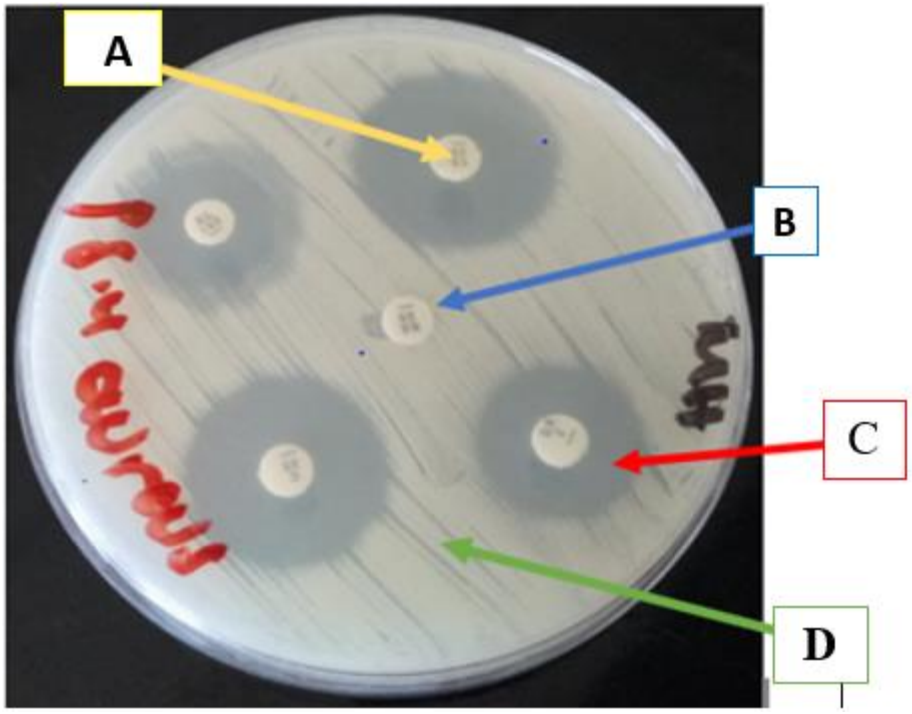

Figure 5.1: Photograph showing disc diffusion antibiotic susceptibility results: red arrow (C) is showing an inhibition zone caused by the antibiotic in the disc and the blue arrow (B) is showing a resistance case (no inhibition zone); green arrow (D) is showing confluent growth of the isolate and yellow arrow is showing antibiotic disc (A).

5.3.2 Antibiotic resistance profiles for *E. coli* isolates

Five randomly chosen *E. coli* isolates from each slaughterhouse, making a total of 16 including the reference strain, were tested for antibiotic resistance using the five antibiotics given in Section 5.2.2 and interpreted as given in Section 5.2.3. The tested isolates showed resistance to Ampicillin at 100%; Sulphamethoxazole at 93.3%; Amoxicillin at 93.3%; Gentamycin at 13.3% and were susceptible to Ciprofloxacin at 100%. All the tested isolates were multiply resistant (to 2 or more antibiotics). *E. coli* reference strain, ATCC 25922 was resistant to Amoxycillin, Ampicillin and Sulphamethoxazole and highly susceptible to Ciprofloxacin and Gentamycin. Figure 5.2 shows antibiotic resistance patterns of *E. coli* isolates per slaughterhouse. Differences between susceptibility/resistance of the tested *E. coli* isolates to the antibiotics, among the slaughterhouses,

were not statistically significant, with p- values of 0.6, 0.3 and 0.3 for Gentamycin, Sulphamethoxazole and Amoxicillin respectively.

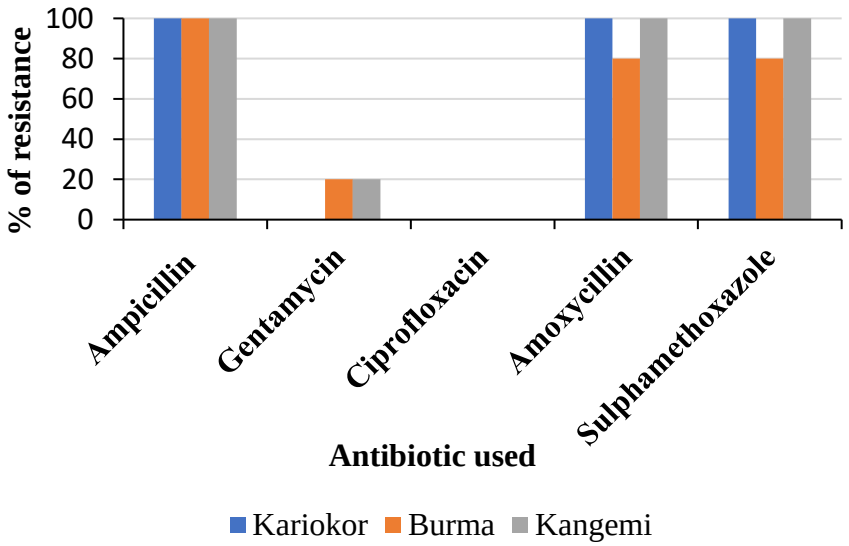

Figure 5.2: Antibiotic resistance patterns of *E. coli* isolated from the three slaughterhouses

5.3.3 Antibiotic resistance profiles for S*taphylococcus* isolates

Five randomly chosen *Staphylococcus* isolates from each slaughterhouse, making a total of 16, including the reference strain, were tested for antibiotic resistance using the five antibiotics given in Section 5.2.2 and interpreted as given in Section 5.2.3.

Resistance to Clindamycin was at 73.3%, Tetracycline at 46.7%, Chloramphenicol at 40% and all were susceptible to Sulphamethoxazole and Erythromycin. About 47% (46.7%) of them showed resistance to two or three antibiotics while 13.3% were susceptible to all the antibiotics used. Difference between resistance of the tested *Staphylococcus* isolates to the antibiotics, among the slaughterhouses, were not statistically significant; for both Chloramphenicol and Clindamycin p-value was 0.2; for Tetracycline p-value was 0.4. *Staphylococcus aureus* reference strain ATCC

25923, was susceptible to all antibiotics tested. Figure 5.3 shows antibiotic resistance patterns of *Staphylococcus* isolates from the three slaughterhouses.

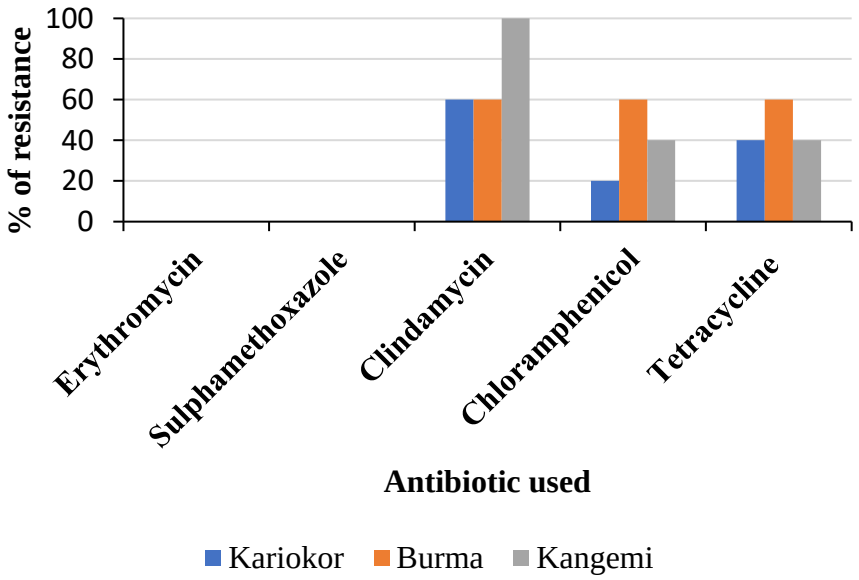

Figure 5.3: Antibiotic resistance patterns of *Staphylococcus* isolated from the three slaughterhouses

5.3.4 Antibiotic resistance profiles for *Streptococcus* isolates

Five randomly chosen *Streptococcus* isolates from each slaughter house; making a total of 16 including the reference strain, were tested for antibiotic susceptibility using the five antibiotics given in Section 5.2.2 and interpreted as given in Section 5.2.3. The tested isolates were resistant to the antibiotics as follows: Sulphamethoxazole at 93.3%; Clindamycin at 86.7%; Erythromycin at 60%; Tetracycline at 60%; Chloramphenicol at 53.3%. About 93% (93.3%) of the isolates showed resistance to more than two antibiotics, where 13.3% were resistant to all the antibiotics used.

Differences between resistance of the tested *Streptococcus* isolates to the antibiotics, among the slaughterhouses, were not statistically significant for Sulphamethoxazole, Erythromycin, Chloramphenicol, Tetracycline and Clindamycin p-values were 0.3, 0.4, 0.8, 0.4 and 0.1

respectively. *Staphylococcus aureus* ATCC 25923, was used as reference strain; it was susceptible to all the tested antibiotics. Figure 5.4 shows antibiotic resistance patterns of *Streptococcus* isolates from the three slaughterhouses. More details on statistical output of the isolates to the antibiotics used are shown in Appendix 8.

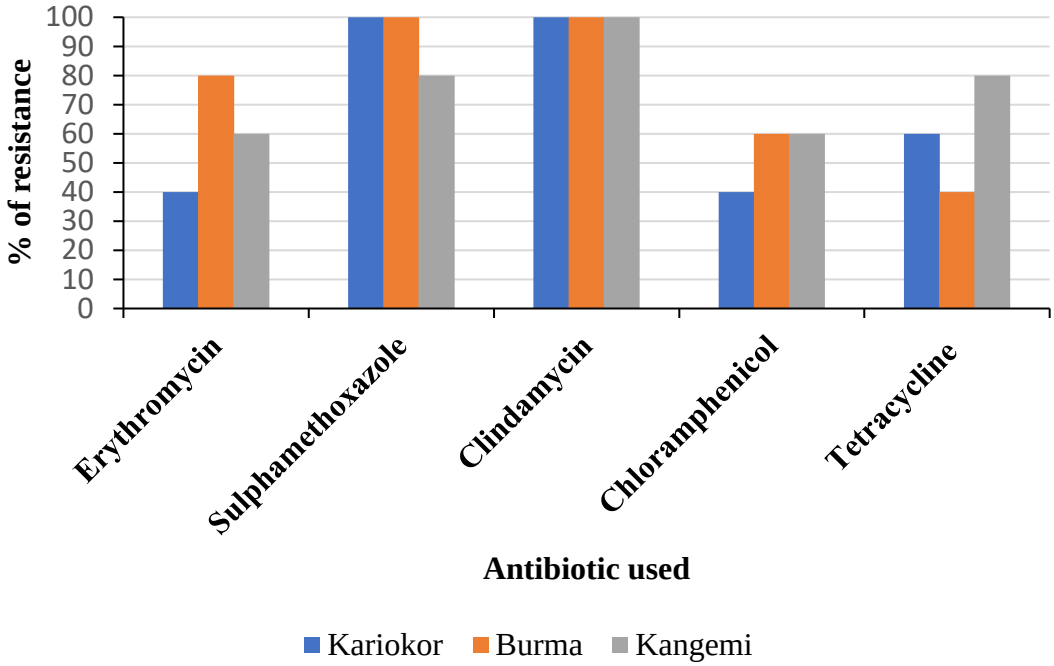

Figure 5.4: Antibiotic resistance patterns of *Streptococcus* isolated from the three slaughterhouses

5.3.5 Multi-drug resistance

Some of the isolates expressed multi-antibiotic resistance. Forty-six-point seven percent (46.7%) of *Streptococcus* isolates showed resistance to four antibiotics, while 6.7%, 26.7% and 13.3% showed resistance to two, three and five antibiotics, respectively. *E. coli* showed resistance to 2, 3 and 4 antibiotics at 13.3%, 73.3% and 13.3%, respectively. *Staphylococcus* isolates showed resistance to two and three antibiotics at 20% and 26.7% respectively. Figure 5.5 shows multidrug resistance patterns of isolates.

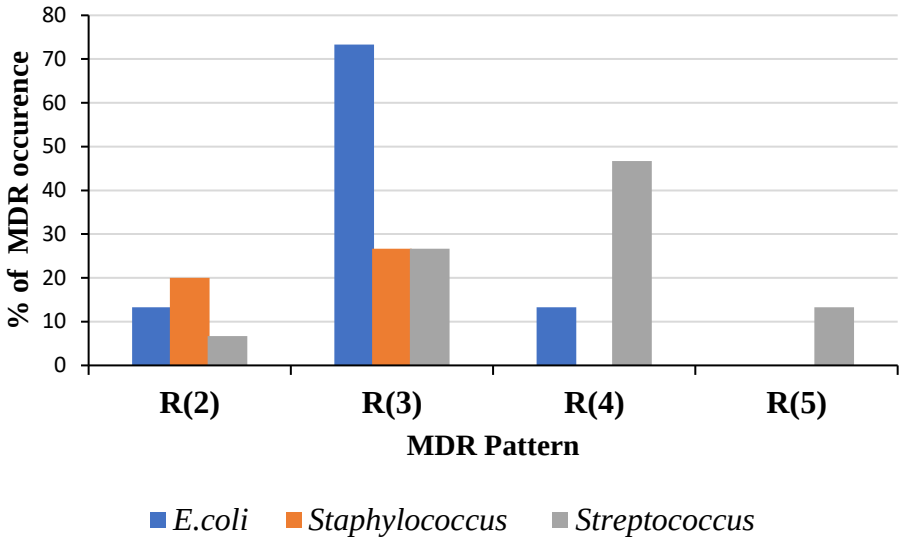

Figure 5.5: Multidrug resistance (MDR), with respect to number of antibiotics, for the three isolates. R(2), R(3), R(4) and R(5) refer to number of antibiotics the isolate proportion was resistant to

The combinations of antibiotics to which the isolates were multi-resistant were as follows: For *E. coli*: the combination AMC/AMP was at 6.7%; RL/AMP at 6.7%; AMC/RL/AMP at 73.3% and CN/AMC/RL/AMP at 13.3 %. *Staphylococcus* isolates showed MDR pattern of DA/C; DA/TE and DA/C/TE at 13.3 %, 6.7% and 26.7% respectively. *Streptococcus* isolates showed the following MDR patterns: RL/DA at 6.7%; RL/TE/E at 6.7%; RL/TE/DA at 13.3 %; RL/DA/ C at 6.7%; RL/DA/E/TE at 20%; RL/DA/E/C at 20%; R/DA/C/TE at 6.7% and RL/E/DA/TE/C at 13.3 %. Table 5.2 shows multi-antibiotic resistance patterns of the isolates and their frequencies.

Table 5.2: Multi-drug resistance patterns and respective frequencies

Bacteria	Antibiotic resistance pattern	Frequency of appearing
***E. coli* (n=15)**	AMC, AMP	1 (6.7%)
	RL, AMP	1 (6.7%)
	AMC, RL, AMP	11 (73.3%)
	CN, AMC, RL and AMP	2 (13.3 %)
***Staphylococcus* spp (n=15)**	TE	2 (13.3 %)
	DA	4 (26.7%)
	DA, C and TE	4 (26.7%)
	DA and TE	1 (6.7%)
	DA and C	2 (13.3 %)
***Streptococcus* spp (n=15)**	TE	1 (6.7%)
	RL and DA	1 (6.7%)
	RL, TE and E	1 (6.7%)
	RL, TE and DA	2 (13.3 %)
	RL, DA and C	1 (6.7%)
	RL, DA, E and TE	3 (20%)
	RL, DA, E and C	3 (20%)
	RL, DA, C and TE	1 (6.7%)
	RL, E, DA, TE and C.	2 (13.3 %)

Legend: **DA**: Clindamycin; **TE**: Tetracycline; **C:** Chloramphenicol; **E:** Erythromycin; **RL:** Sulphamethoxazole; **AMC:** Amoxicillin; **AMP:** Ampicillin; **CN:** Gentamycin

5.4 Discussion

There is increase in intensive use of antibiotics in poultry farming for treatment of bacterial infection and for maintaining healthy and productive birds (Yang *et al.*, 2004). In this study, *E. coli* showed high resistance to Ampicillin and Amoxicillin (100% and 93.3% respectively), yet it has been documented that these drugs are not used intensively in poultry production in Kenya (Adelaide *et al.*, 2008), however they are commonly used in humans. This means that there could be other factors that may have led to increase in resistance or the drugs are being used secretly, that is: they were not recorded (Managaki *et al*., 2007); which has resulted in bacteria developing

respective resistance. Other explanations to this could include: exposure to improperly disposed leftover drugs in the environment (Khan *et al*., 2013), or into the rivers (Ngumba *et al.*, 2016; Kimosop *et al*., 2016), which could have precipitated development of the resistance in in-contact bacteria, or the birds could have consumed (from the environment) already - resistant bacterial strains. There is a possibility that these bacteria or drug residues were washed to the area where the birds fed by rain water from even other countries.

Sulphonamides such as Sulphamethoxazole (to which *E. coli* and *Streptococcus* isolates showed resistance of 93.3%) are being used in prophylaxis of coccidiosis and different bacterial infections in chickens in Kenya (Adelaide *et al.*, 2008); this may have been the cause of increasing resistance to this antibiotic by the tested *E. coli* and *Streptococcus* isolates. It has also been documented that resistance to Sulfonamides can be as a result of ribosomal mutation in the chromosomal gene *rpsl* or by enzymatic modification of the drug (Guerra *et al*., 2003). However, *Staphylococcus* isolates from the study areas were 100% susceptible to this drug, which shows that there is another factor or other factors involved in the respective susceptibility/resistance, which could be intrinsic.

The presence of resistance to Chloramphenicol by *Staphylococcus* isolates (40%) and *Streptococcus* isolates (53.3%) is note-worthy because the use of this antibiotic in Kenya was outlawed since 2005 (Adelaide *et al.*, 2008). It is, however, documented that there may be an association between resistance to Chloramphenicol and resistance to other antibiotics such as Tetracycline and Clindamycin. It has been proven that resistance to aminoglycoside (Chloramphenicol in this case) can be due to enzymatic inactivation such as acetyltransferases, nucleotidyltransferases (adenylyltransferases) and phosphotransferases (Shaw *et al.*, 1993).

Erythromycin and Tetracycline, among other antimicrobials, are widely used in poultry production in treatment of staphylococcal and other infections (Nemati *et al.*, 2008, Aarestrup *et al.*, 2000;

White *et al.*, 2003); Tetracycline is also widely used as growth and production promoter in chickens. In this study, tested *Staphylococcus* isolates were resistant to Erythromycin and Tetracycline at 0% and 46.7% respectively; *Streptococcus* isolates were resistant to the drugs at 60% each. Since these drugs are widely used, there is possibility of resistance development towards them; however; the fact that *Staphylococcu*s isolates from the same study areas were 100 % susceptible to Erythromycin points to another possibility of it being a result of intrinsic factor(s). The study done by Aarestrup *et al.* (2000) in Denmark has, however, demonstrated resistance of *Staphylococcus* organisms to Erythromycin at 24%; it also demonstrated resistance of the organisms to Sulphamethoxazole at 19%.

Streptococcus isolates have shown higher resistance to antibiotics compared to *Staphylococcus* isolates; they showed high resistance to Sulphamethoxazole, Clindamycin and Tetracycline at 93.3%; 86.7% and 80% respectively. This resistance can be as a result of carrying R-plasmid by the tested *Streptococcus* isolates or due to genetic composition of *Streptococcus* organisms (Burdettt, 1980; Burdett *et al.*, 1982); these organisms also showed resistance to Erythromycin at 60%. It has been documented that resistance to Erythromycin by *Streptococcus* organisms is mainly mediated by two modes: target site modification and active efflux (Giovanetti *et al.*, 2002). In this investigation, there were cases of multidrug resistance (to more than one antibiotic). *E. coli* isolates showed multidrug resistance at 100%; *Staphylococcu*s isolates at 46.7% and *Streptococcus* isolates at 93.3%, with respect to the drugs used; there is a possibility that the resistances were combined; borne on a single plasmid.

The *E. coli* results are similar to those of Salehi and Bonab (2006) and Miles *et al.*, (2006) in Iran and Jamaica, respectively; they also found 100% multi-drug resistance in *E.coli* isolates.

White *et al*. (2003) reported multidrug resistance at 21% in *Staph. aureus* isolated from chickens in North-Eastern Georgia.

It has been shown that any bacterium, whether pathogenic or not, can be involved in transfer of antibiotic resistance genes; as long as they are carrying the respective resistance gene(s) (Abera and Kibret, 2011); These genes can be transferred easily among bacteria (Courvalin, 1994; Kikuvi *et al*., 2007). If the transfer is to a pathogenic bacterium, it will be difficult to treat the resultant infection(s).

This study has demonstrated the presence of antibiotic resistance in bacteria isolated from intestines of indigenous chickens. It is, therefore, recommended that, before dispensing an antibiotic, one ascertains its efficacy by carrying out antibiotic susceptibility testing and, where possible, free-range indigenous chickens be confined during rainy seasons, to protect them from ingesting potentially-harmful materials brought in from elsewhere by rain water. Measures towards prevention/reduction of development of antibiotic resistance need to be taken. One of these measures is creating awareness on the ills of antibiotic resistance and the factors that lead to its development, among people; this will contribute towards reduction of indiscriminate usage and disposal of antibiotics. The other one is formulation of effective policies which will safeguard proper usage of antibiotics; for example: discourage use of antimicrobials as food additives/growth promoters; prevent over-the-counter buying of drugs, and put in place effective ways of disposing remaining or expired drugs. It is also recommended that policy makers come up with guidelines on reduction of environmental contamination, especially during rainy season.

CHAPTER SIX: DISINFECTANT RESISTANCE PROFILES OF *STAPHYLOCOCCUS, ESCHERICHIA* AND *STREPTOCOCCUS* ISOLATED FROM THIS STUDY.

6.1 Introduction

The term "disinfectant" is generally used to describe products that are used on inanimate objects or surfaces to kill microorganisms; some can also destroy their spores (Siddiqui and Sarwar, 2013); those that are mild enough to be applied on the skin are referred-to as "antiseptics" (Siddiqui and Sarwar, 2013). There are different groups of disinfectants, based on their chemical constituents or structures: Alcohols, Glutaraldehyde; Peracetic acid; Hydrogen peroxide; Chlorine; Hypochlorite; Chlorine dioxide; Quaternary ammonium compounds; Iodophor; Ozone (Stringfellow *et al.*, 2005).

The use of disinfectants for cleaning in food industries including slaughterhouses is very important because it ensures that quantity of viable bacteria which can grow, multiply and contaminate food materials is minimized (McDonnell and Russell, 1999). However, this practice must be done prudently because the chemicals used as disinfectants can also cause harm. So, the selection of disinfectants to be used must depend on how powerful it works in killing or inhibiting the microorganisms, safety and rinsability. Use of disinfectants helps in reducing surface microorganisms hence reduce the chances of spreading foodborne illness (Wirtanen and Salo, 2003).

The common practice of using disinfectants in poultry production involves cleaning the surface first to remove the residues and physical matter then use the disinfectant. Disinfectants reduce microbial loads by working on different target sites resulting in membrane disruption, metabolic inhibition, and lysis of the particular cell (Payne *et al.*, 2005; Stringfellow *et al.*, 2005).

Disinfectant susceptibility test is conducted in order to select the effective ones; noting that, in most cases, the more active a disinfectant is, the more toxic it is (Wirtanen and Salo, 2003).

Resistance to disinfectants can also occur and can also be towards a single disinfectant or to several ones, as it happens for antibiotics. There is also a possibility of combined resistance to antibiotic and disinfectant, where both are carried on the same plasmid (Russell *et al.*, 1986; Russell *et al.*, 1999).

There are several methods used to test for disinfectant effectiveness, with respect to bacterial susceptibility, but the one mostly used is diffusion technique, where wells are dug into the inoculated agar and are filled with the respective disinfectant (Gaudreau and Gilbert, 1997). In this study, resistance patterns/profiles of three bacterial types that were mostly isolated in Chapter 4, to six commonly-used disinfectants, were determined using agar well diffusion method.

6.2 Materials and methods

6.2.1 Test bacteria and reference strains

Same bacterial isolates and respective reference strains as used in Chapter five, Section 5.2.1, were used for disinfectant testing.

6.2.2 Medium and disinfectants used

The medium used was Mueller Hinton agar manufactured by the Oxoid company while the disinfectants used were six, designated as A, B, C, D, E and F (real names withheld for ethical reasons); expansion of the respective active ingredients are given in Table 6.1. Disinfectant designates A; B and C are commonly used in poultry intensive production units/farms, and F; D; E are commonly used in hospitals, laboratories and for general hand washing. They were purchased from supermarkets and agrovets.

Table 6.1: Respective active ingredients of the tested disinfectants (in Coded names for Ethical purpose).

Disinfectants	Active ingredients of the disinfectant
A	Glutaraldehyde 15% v/v; Benzalkonium chloride 10% v/v
B	Didecyl dimethyl ammonium HCl 18.75 gram; Diotyl dimethyl ammonium HCl 18.75g; Octyl decyl dimethyl ammonium HCl 37.5 gram; Alkyl dimethyl ammonium HCl 50 gram and Glutaraldehyde 62.50 gram
C	Glutaraldehyde 15%w/v; Coco-benzyl-dimethyl-Ammonium Chloride (QAC) 10%w/v
D	Chloroxylenol 4.8%
E	Chlorhexidine gluconate 0.3 gram; Cetrimide 3.0 gram; N-propylalcohol 2.84% m/v
F	3.85% m/v of Sodium Hypochlorite

6.2.3 Dilution of the disinfectants for testing

Each disinfectant was diluted according to the manufacturer's recommended concentration (user dilution; given as concentration 3 in Table 6.2) and other concentrations above (x2 and x4 of the recommended user dilution; given as concentrations 4 and 5, respectively, in Table 6.2) and below (x ½ and x ¼ of the recommended user dilution; given as concentrations 2 and 1, respectively, in Table 6.2). Dilutions were made using sterile normal saline. Table 6.2 gives the respective concentrations used, pegged on the dilutions made.

Table 6.2: Concentrations of disinfectants used for susceptibility test

Disinfectant used	**Concentration of disinfectant in %**				
	1(x1/4)	2 (x1/2)	3 (3*)	4 (x2)	5(x4)
A	0.0625%	0.125%	0.25%	0.5%	1%
B	0.0625%	0.125%	0.25%	0.5%	1%
C	0.0625%	0.125%	0.25%	0.5%	1%
D	1.25%	2.5%	5%	10%	20%
E	1.5%	3%	6%	12%	24%
F	0.568%	1.135%	2.27%	4.54%	9.08%

Legend: **3*** is manufacturer's recommendation concentration; **1 (x1/4)** was the lowest concentration 3*x1/4; **2 (x1/2)** was the next lower concentration (3*x1/2); **4 (x2)** was twice the concentration of 3*; **5 (x4)** was the highest concentration (i.e fourth times the concentration of 3*). A, B, C D, E and F are designates given to disinfectants, active ingredients of which are given in Table 6.1

6.2.4 Preparation of bacterial suspension and seeding of plates for disinfectant testing

Same bacterial suspension made for antibiotic susceptibility testing (Section 5.2.3) was used to test for disinfectants and seeding of bacteria to produce confluent growth. However, more attention was placed on the depth of the agar medium, since well method was going to be used, rather than placing of antibiotic discs on the agar. In the well method, the agar depth had to be the same for correct comparison of the results (Lalitha, 2004). To standardize the agar depth, special/marked petri dishes that were made in China (brand name: Won) were used as shown on Figure 6.1; molten agar was poured up to the drawn line, which gave an agar depth of 6 millimeters.

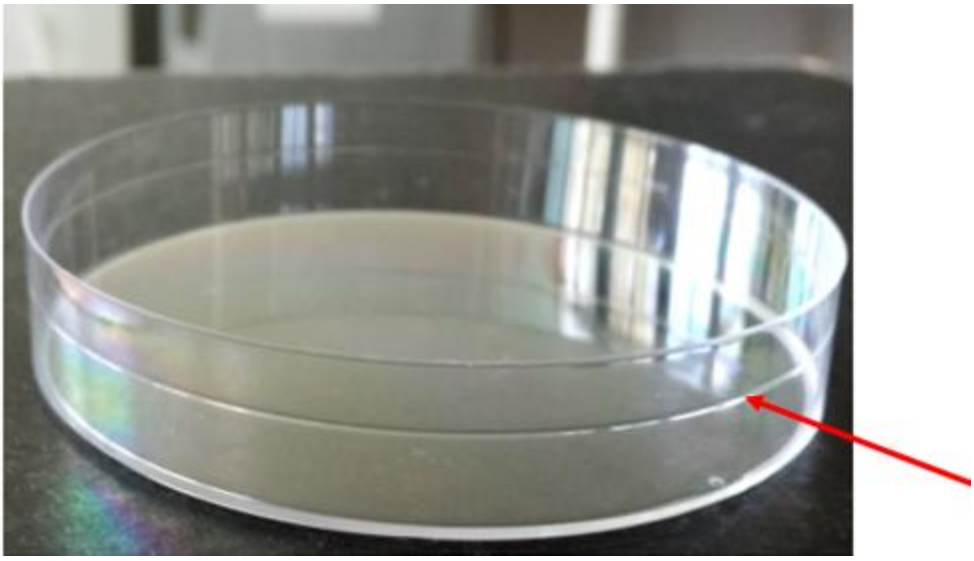

Figure 6.1: Photograph of the marked petri dish used for disinfectant susceptibility testing to ensure uniformity of depth of the medium used. The medium was filled to the line shown by the red arrow (the agar thickness/depth was confirmed to be 6 mm)

6.2.5 Disinfectant susceptibility testing procedure

Disinfectant susceptibility testing was done using the agar well method, as described by Spooner and Sykes (1972) and modified by Njagi (2003). After the molten Mueller Hinton agar was poured into the marked petri dishes, it was allowed to solidify, after which, seeding for confluent growth was done in the same way as given on Section 5.2.3. Wells were then dug using 4 mm diameter digger, plates were labelled according to the tested disinfectant, while each well was labelled according to the respective dilution. The wells were then filled with respective disinfectant/ concentration and incubated up-side-up overnight at 37 °C.

Susceptibility to the particular disinfectant was indicated by the size of respective growth inhibition zone measured in millimeters (mm). Reading of the inhibition zone sizes was done as for antibiotic susceptibility testing; the size of the inhibition zone being directly proportional to the susceptibility of the organism for the particular disinfectant (Lalitha, 2004). However, there

was a slight difference in interpretation of the readings; since there are no established cut-off points for the specific disinfectants. The inhibition diameters were interpreted according to Njagi (2003). Diameter measurements below or equal to 10 mm were considered as resistant (R); those beyond 10 mm were considered susceptible (S).

6.2.6 Data and statistical analysis

Data were analyzed using SPSS statistical software. Chi square was used to test the association of disinfectant resistant strains with their respective slaughter houses.

6.3 Results of disinfectant susceptibility test

Differences in resistance after doing susceptibility test of the isolates to the used disinfectants as shown by chi square analysis, were noted. More information on disinfectant resistance by the tested isolates can be found in Appendix 9. Statistical analysis showed that the activity of disinfectant designates F and E to the tested isolates (*E.coli*, *Staphylococcus* and *Streptococcus*) were not significantly different, with p-values of 0.7, 0,2 and 0.02 respectively; however there were differences in the effect of disinfectant designates D, A, B and C to the isolates as shown by the p-value of 0.03, 0.003, 0.008 and 0.02, respectively. Comparing the effectiveness of disinfectant per slaughterhouse, there was no statistical differences in the activity of disinfectant designates F, D, E, A and C to the isolates with p-values of 0.3, 0.2, 0.5, 0.4 and 0.4 respectively except for designate B with p-value of 0.02. Details of statistical analysis results can be found in Appendix 10.

6.3.1 Resistance of the test isolates to disinfectant designate A (expounded in Table 6.1)

Figure 6.2 shows resistance patterns of the test isolates with respect to various concentrations of disinfectant designate A, *Escherichia coli* isolates showed resistance rate of 60% at the lowest concentration used (1/4 of the manufacturer's recommended user concentration); 33.3% at 1/2 the recommended concentration; 13.3% at recommended concentration; 13.3% at twice the recommended concentration and 0% at 4 times the recommended concentration. *Staphylococcus* isolates were all susceptible at recommended concentration and at 1/2, twice and 4 times the concentration; they showed 6.7% resistance at 1/2 the recommended concentration. All the *Streptococcus* isolates were susceptible to disinfectant designate A at all test concentrations. Reference strain *E. coli* ATCC 259222 was resistant to 1/4 and 1/2 the recommended concentrations; however, it was susceptible to the recommended concentration and higher ones. Reference strain *Staphylococcus* ATCC 25922 was susceptible to disinfectant designate A at all test concentrations.

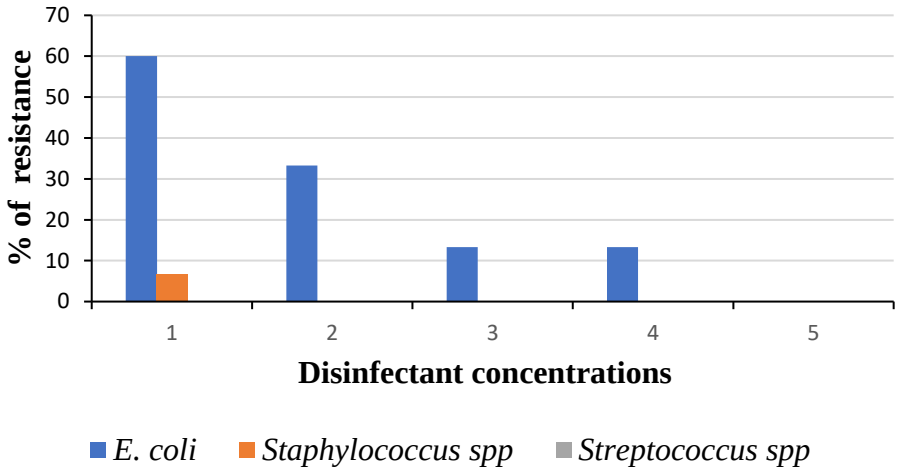

Figure 6.2: Resistance patterns of the test isolates with respect to various concentrations of disinfectant designate A [containing Glutaraldehyde and Benzalkonium chloride (Table 6.1 above)]
Legend: **3*** is manufacturer's recommended user concentration; **1 (x1/4)** was the lowest concentration (3*x1/4); **2 (x1/2)** was the next lower concentration (3* x 1/2); **4 (x2)** was twice the concentration of 3*; **5 (x4)** was the highest concentration (i.e four times the concentration of 3*).

6.3.2 Resistance of the test isolates to disinfectant designate B (expounded in Table 6.1)

Figure 6.3 shows resistance patterns of the test isolates with respect to various concentrations of disinfectant designate B. *Escherichia coli* isolates were resistant at 66.7% at 1/4 the manufacturer's recommended user concentration and 26.7% at 1/2 the recommended concentration; all were susceptible at recommended concentration and higher ones. All tested *Streptococcus* isolates were susceptible to all test concentrations. Fourteen (93.3%) of the tested *Staphylococcus* isolates were susceptible to all test concentrations; only one isolate (6.7%) was resistant to the recommended user concentration and at half and quarter of it. Reference strain *E. coli* ATCC 259222 was resistant to the recommended user concentration and half and quarter of it and susceptible to concentrations twice and 4 times the recommended concentration. *Staphylococcus* ATCC 25922 was susceptible to disinfectant designate B at all test concentrations.

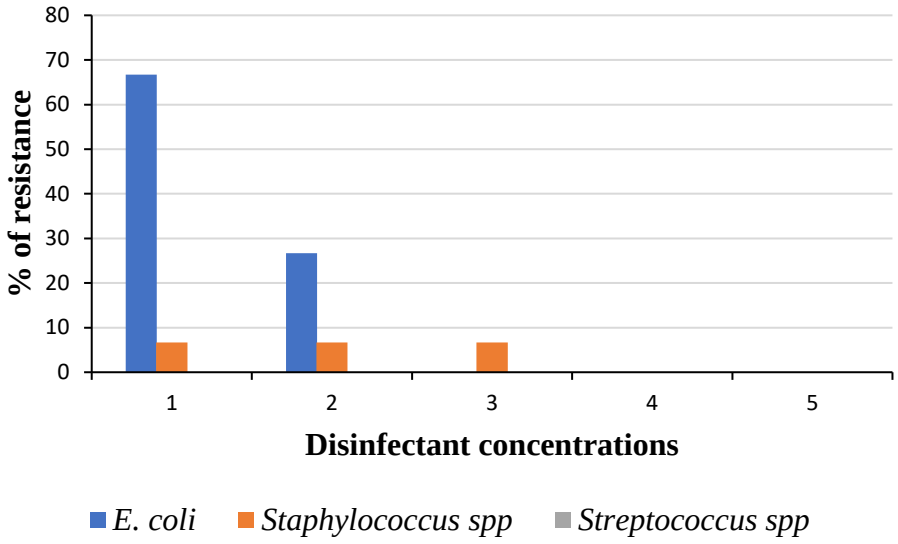

Figure 6.3: Resistance patterns of the test isolates with respect to various concentrations of disinfectant designate B [containing Didecyl dimethyl ammonium; Diotyl dimethyl ammonium, etc. (Table 6.1 above)]**.**
Legend: **3*** is manufacturer's recommended user concentration; **1 (x1/4)** was the lowest concentration (3*x1/4); **2 (x1/2)** was the next lower concentration (3* x 1/2); **4 (x2)** was twice the concentration of 3*; **5 (x4)** was the highest concentration (i.e four times the concentration of 3*).

6.3.3 Resistance of the test isolates to disinfectant designate C (expounded in Table 6.1)

Figure 6.4 shows resistance patterns of the test isolates with respect to various concentrations of disinfectant designate C. *Escherichia coli* isolates showed resistance of 100% to disinfectant C at 1/4 of the manufacturer's recommended user concentration; 60% at 1/2 the recommended user concentration; 46.7% at recommended user concentration; 20 % and 0% at concentration at twice and 4 times the recommended concentration, respectively. All tested *Staphylococcus* isolates were susceptible to disinfectant C at all concentrations. *Streptococcus* isolates were susceptible at all test concentrations except for 1/4 of the recommended concentration, where they showed resistance of 6.7%. Reference strains *E. coli* ATCC 259222 and *Staphylococcus* ATCC 25922 were susceptible to disinfectant C at all test concentrations.

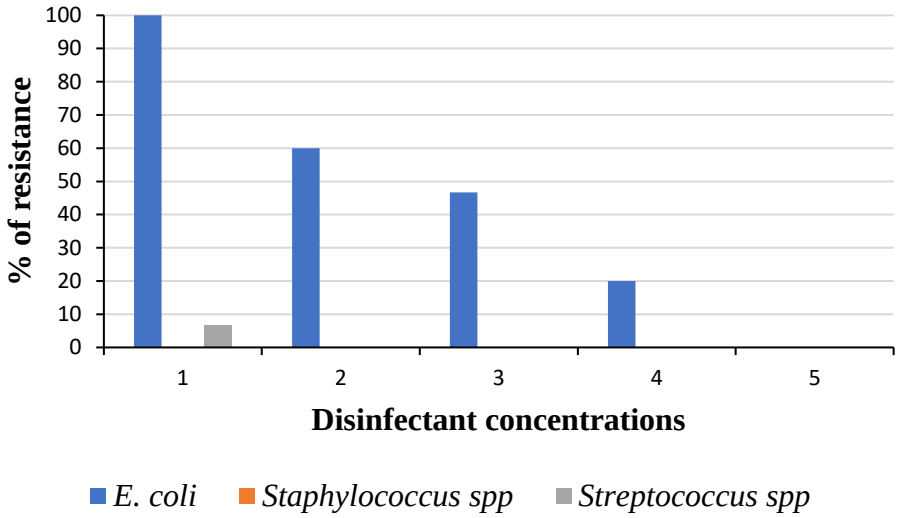

Figure 6.4: Resistance patterns of the test isolates with respect to various concentrations of disinfectant designate C [containing Glutaraldehyde and Coco-benzyl-dimethyl-Ammonium Chloride, (Table 6.1 above)].
Legend: 3* is manufacturer's recommended user concentration; **1 (x1/4)** was the lowest concentration (3*x1/4); **2 (x1/2)** was the next lower concentration (3* x 1/2); **4 (x2)** was twice the concentration of 3*; **5 (x4)** was the highest concentration (i.e four times the concentration of 3*).

6.3.4 Resistance of the test isolates to disinfectant designate D (expounded in Table 6.1)

At lowest concentration (1/4 of the manufacturer's recommended user concentration), *E. coli* isolates were resistant at 93.3%, *Staphylococcus* isolates were resistant at 20% and *Streptococcus* isolates were resistant at 60%; at 1/2 the recommended concentration, *E. coli* isolates were resistant at 66.7%; *Staphylococcus* isolates at 13.3% and *Streptococcus* isolates at 26.7 %. At the manufacturer's recommended user concentration, *E. coli* isolates were resistant at 26.7%; *Staphylococcus* and *Streptococcus* isolates were all susceptible. At higher concentrations (twice and four times the recommended concentration), all the isolates were susceptible. *Staphylococcus* reference strain ATCC 25922, was susceptible to disinfectant D at all test concentrations. *E. coli* reference strain, ATCC 259222, was resistant to disinfectant D at the lowest concentration (1/4 of the manufacturer's recommended user concentration) and susceptible to the other test

concentrations. Figure 6.5 shows resistance patterns of the isolates to disinfectant D at various concentrations.

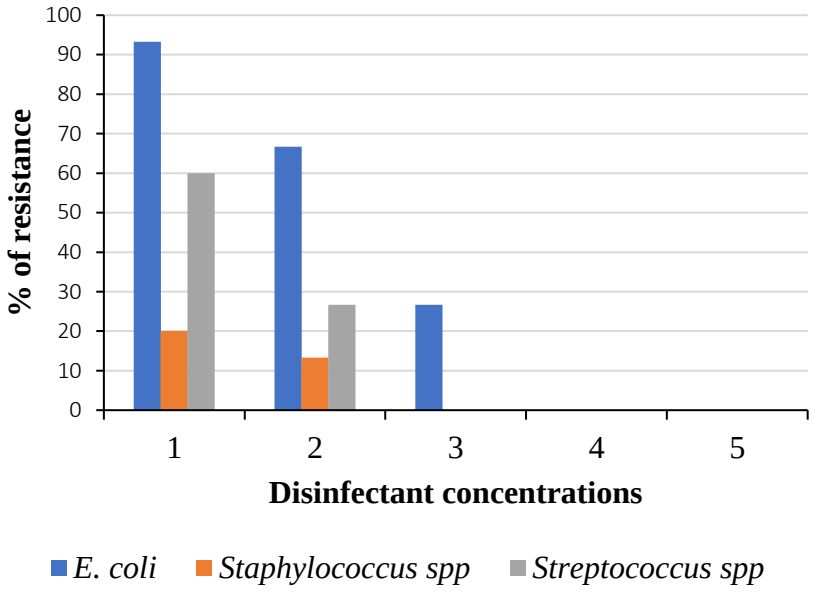

Figure 6.5: Resistance patterns of the test isolates with respect to various concentrations of disinfectant designate D [containing Chloroxylenol 4.8%, (Table 6.1 above)**].**
Legend: **3*** is manufacturer's recommended user concentration; **1 (x1/4)** was the lowest concentration (3*x1/4); **2 (x1/2)** was the next lower concentration (3*x 1/2); **4 (x2)** was twice the concentration of 3*; **5 (x4)** was the highest concentration (i.e four times the concentration of 3*).

6.3.5 Resistance of the test isolates to disinfectant designate E (expounded in table 6.1)

Figure 6.6 shows resistance patterns of the test isolates with respect to various concentrations of disinfectant designate E. All the tested *Staphylococcus* and *Streptococcus* isolates were susceptible to disinfectant E at all test concentrations; *E. coli*, showed resistance of 60% at lowest concentration (1/4 of the manufacturer's recommended user concentration) and 20% at 1/2 the recommended concentration. Since all other parameters were the same, zones of inhibition produced by disinfectant E were comparatively much larger than those produced by the other disinfectants (Figure 6.7); indicating that its activity on the respective bacteria was stronger than

that of the other disinfectants. Both reference strains (*E. coli* ATCC 259222 and *Staphylococcus* ATCC 25922) were susceptible to disinfectant E at all test concentrations.

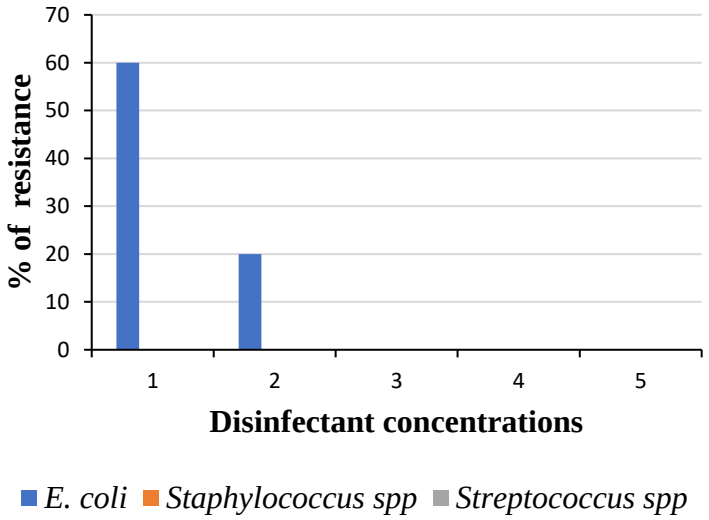

Figure 6.6: Resistance patterns of the test isolates with respect to various concentrations of disinfectant designate E [containing Chlorhexidine gluconate; Cetrimide and N-propylalcohol (Table 6.1 above)].
Legend: 3* is manufacturer's recommended user concentration; **1 (x1/4)** was the lowest concentration (3*x1/4); **2 (x1/2)** was the next lower concentration (3* x 1/2); **4 (x2)** was twice the concentration of 3*; **5 (x4)** was the highest concentration (i.e four times the concentration of 3*). Figure 6.7 shows disc diffusion susceptibility/resistance results of some disinfectants.

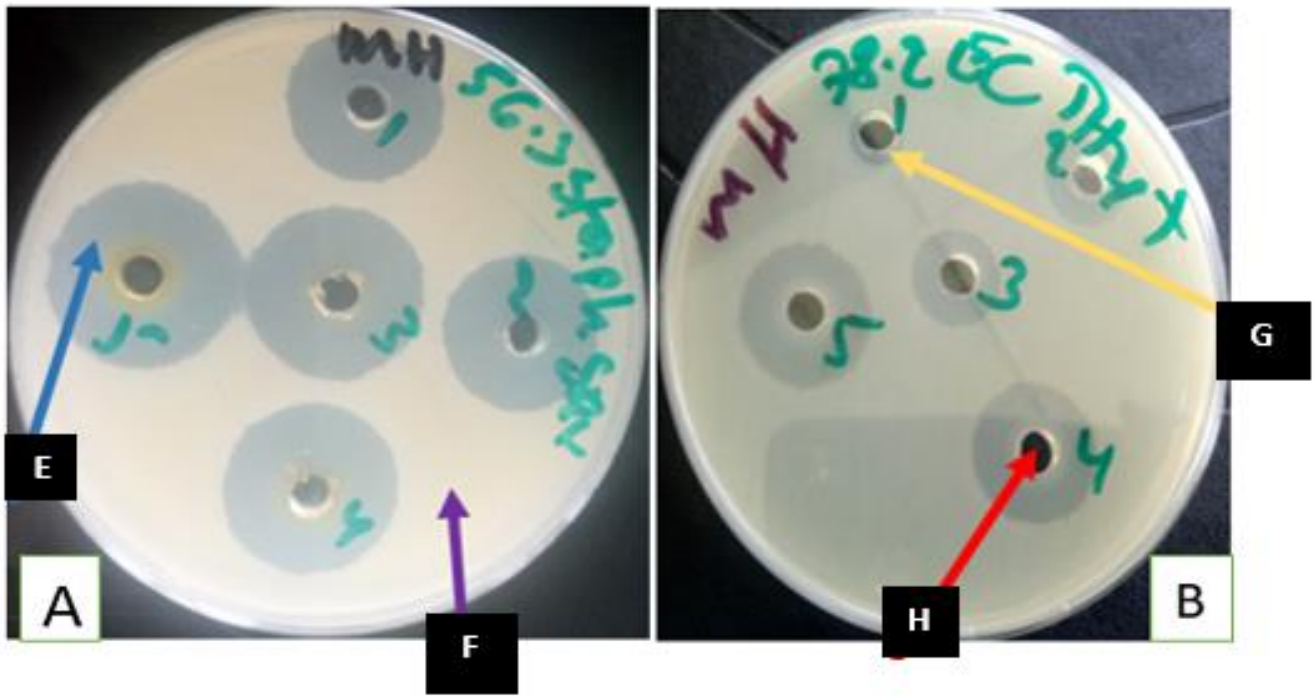

Figure 6.7: Photograph showing agar diffusion disinfectant susceptibility results. The disinfectant dilutions/concentrations were placed in the wells and allowed to diffuse through the agar, plated by the test organism. Inhibition zones (clear areas around the wells) produced by disinfectant as shown pointed by blue arrow (E) and Purple arrow (F) is showing confluent bacterial growth on a plate (shown by **fig 6.7.A**); and **(fig 6.7.B**) is showing the dug well as pointed by red arrow (H) and yellow arrow (G) is showing resistance (no inhibition zone; growth up to the well).

6.3.6 Resistance of the test isolates to disinfectant designate F (expounded in Table 6.1)

The isolates showed resistance to disinfectant designate F in the following pattern: at 1/4 and 1/2 of the manufacturer's recommended user concentration, all the three tested bacterial types (*E. coli*, *Staphylococcus* and *Streptococcus*) were resistant at 100%. At the manufacturer's recommended user concentration, all *E. coli* isolates were resistant (100%), while *Staphylococcus* and *Streptococcus* isolates were both resistant at 93.3%. At double the recommended concentration, *E. coli* isolates were resistant at 93.3%; *Staphylococcus* isolates at 86.7% and *Streptococcus* isolates at 93.3%; while at four times the recommended concentration, *E. coli* isolates were resistant at 46.7%; *Staphylococcus* isolates at 40% and *Streptococcus* isolates at 40 %. Figure 6.8 shows

resistance patterns of the test isolates with respect to various concentrations of disinfectant F. Reference strain *E. coli* ATCC 259222 was resistant to disinfectant F at manufacturer's recommended user concentration and 1/2 and 1/4 of it and susceptible at 2 times and 4 times the recommended concentration. Reference *Staphylococcus aureus* ATCC 25922 was resistant to F at recommended user concentration and 1/4, 1/2 and 2 times but susceptible to 4 times the recommended concentration.

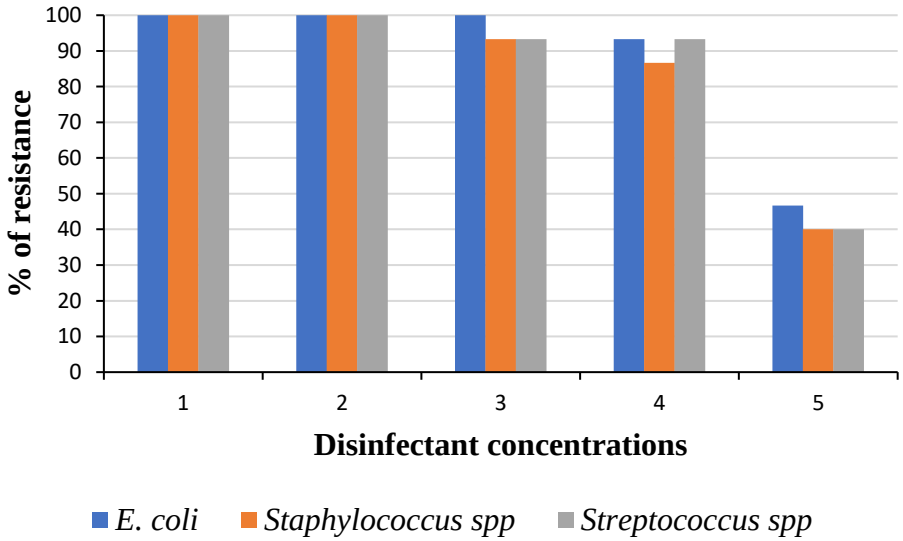

Figure 6.8: Resistance patterns of the test isolates with respect to various concentrations of disinfectant designate F [containing 3.85% m/v of Sodium Hypochlorite (Table 6.1 above)].
Legend: 3* is manufacturer's recommended user concentration; **1 (x1/4)** was the lowest concentration (3*x1/4); **2 (x1/2)** was the next lower concentration (3* x 1/2); **4 (x2)** was twice the concentration of 3*; **5 (x4)** was the highest concentration (i.e four times the concentration of 3*).

6.3.7 Summary of bacterial resistances to the test disinfectants at respective manufacturer's recommended user concentrations

At recommended user concentration, *E. coli* ATCC 259222 was resistant to disinfectants designated F and B; *Staphylococcus* ATCC 25922 was only resistant to disinfectant F. Among the tested disinfectants, disinfectant E showed the largest inhibition diameters against all the isolates, while F was the least effective. With respect to the manufacturer's user concentration, Figure 6.9

shows average of zone inhibition diameters for the six disinfectants per isolate; Table 6. 3 shows the percent resistance of the tested isolates to the test disinfectants per slaughterhouse; Figure 6.10 shows resistance patterns of the isolates to the test.

Isolates from Kariokor slaughterhouse showed resistance to disinfectants at recommended user concentration as follows: *E. coli* isolates were resistant to disinfectant F at 100%, to disinfectant D at 20%, to disinfectant A at 40%, and to disinfectant C at 100%; *Staphylococcus* isolates were resistant to disinfectant F at 100% and to disinfectant B at 20%, while *Streptococcus* isolates were resistant to only disinfectant F at 80%.

Isolates from Burma slaughter house showed resistance at recommended user concentration as follows: *E. coli* isolates were resistant to disinfectant F at 100%, to disinfectant D at 40%; to disinfectant A at 60% and to disinfectant C at 40%; *Staphylococcus* and *Streptococcus* isolates were 100% resistant to disinfectant F only.

The isolates from Kangemi slaughterhouse were resistant to disinfectant at recommended user concentration as follows: *E. coli* isolates were resistant to disinfectant F at 100%, to disinfectants D and A at 20%; *Staphylococcus* and *Streptococcus* isolates were resistant to only disinfectant F, at 80% and 100% respectively.

Detailed information of zones of inhibition induced by disinfectants are given in Appendix 9.

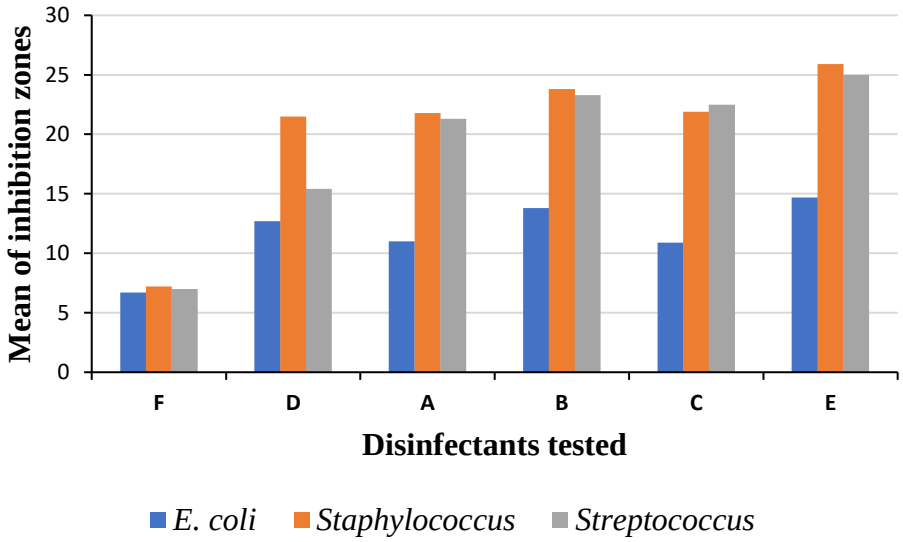

Figure 6.9: Mean zone inhibition diameters (in mm) for disinfectants per isolates at recommended user concentration

Legend: A, B, C, D, E and F are designates for disinfectants as expounded in Table 6.1

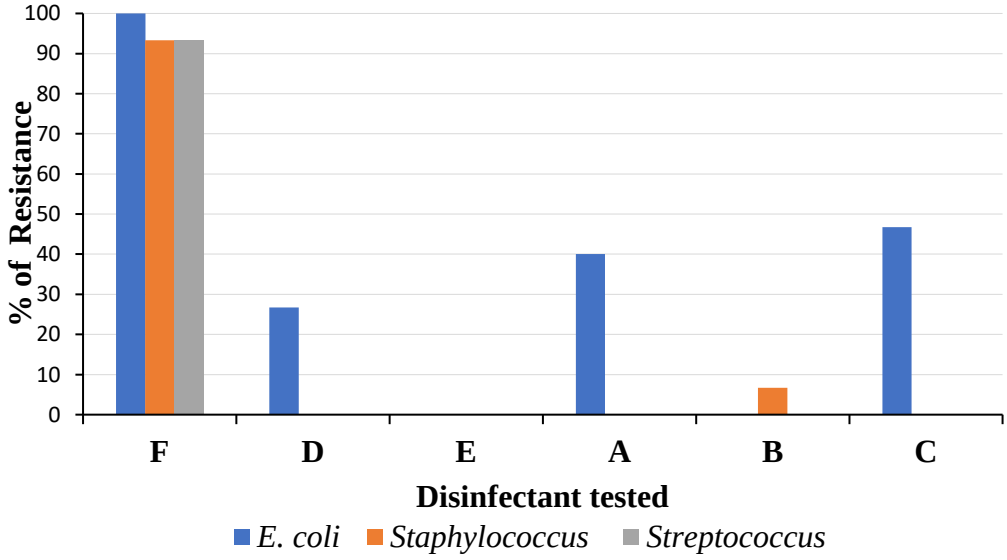

Figure 6.10: Resistance patterns of the isolates to disinfectants at recommended user concentration (3*)

Legend: A, B, C, D, E and F are designates for disinfectants as expounded in Table 6.1

Table 6.3: Percent resistance of the tested isolates to the test disinfectants, per slaughterhouse, at recommended user concentration; for n=5

		Disinfectants											
		F		**D**		**E**		**A**		**B**		**C**	
Isolates	**Slaughterhouses**	**n**	**R%**	**n**	**R%**	**n**	**R%**	**n**	**R%**	**N**	**R%**	**n**	**R%**
E. coli	Kariokor	5	100%	1	20%	0	0%	2	40 %	0	0%	5	100%
	Burma	5	100%	2	60%	0	0%	3	60%	0	0%	2	40%
	Kangemi	5	100%	1	20%	0	0%	1	20%	0	0%	0	0%
***Staphylococcus* isolates**	Kariokor	5	100%	0	0%	0	0%	0	0%	1	20%	0	0%
	Burma	5	100%	0	0%	0	0%	0	0%	0	0%	0	0%
	Kangemi	4	80 %	0	0%	0	0%	0	0%	0	0%	0	0%
***Streptococcus* Isolates**	Kariokor	4	80%	0	0%	0	0%	0	0%	0	0%	0	0%
	Burma	5	100%	0	0%	0	0%	0	0%	0	0%	0	0%
	Kangemi	5	100%	0	0%	0	0%	0	0%	0	0%	0	0%

Legend: **R%** (Resistance percentage); **n** in number of samples; A, B, C, D, E and F are designates for disinfectants as expounded in Table 6.1

6.3.8 Multiple resistance with respect to the disinfectants studied, as at manufacturer's recommended concentration

Some isolates expressed resistance to more than one disinfectant at recommended dilution. Eleven (11; 73.3%) of *Escherichia coli* isolates showed resistance to more than one disinfectant, 2 (13.3%) were resistant to 4 disinfectants. Only one (6.7%) *Staphylococcus* isolate showed resistance to more than one disinfectant. No *Streptococcus* isolate showed resistance to more than one disinfectant. Table 6.4 gives the respective multiple disinfectant combinations.

Table 6.4: Multiple-disinfectant resistance patterns/combinations and frequencies at recommended user concentration

Bacteria	Disinfectants at 3*	Frequency of appearing %
E. coli	F, A and C	2/15 (13.3%)
	F, D, A and C	2/15 (13.3%)
	F, C	3/15 (20%)
	F, A	2/15 (13.3%)
	F, D	2/15 (13.3%)
***Staphylococcus* isolates**	F, B	1/15 (6.7%)

Legend: A, B, C, D, E and F are designates for disinfectants as expounded in Table 6.1

6.4 Combined resistance to antibiotics and disinfectants

Some of the tested isolates showed resistance to both antibiotic(s) and disinfectant(s); *E. coli* isolates showed it at 73.3%; *Staphylococcus* isolates at 6.7%. Table 6.5 shows details of the isolates which expressed combined multi-resistance to antibiotic(s) and disinfectant(s) (at recommended user concentration) and their respective combinations.

Table 6. 5: Isolates which showed combined multi-resistance to antibiotics and disinfectants at recommended used concentration

Bacteria	Antibiotic resistance	Disinfectants resistance at recommended user concentration	Frequency of occurrence (%)
***E. coli* (n=15)**	CN, AMC, RL, AMP.	F, A and C	1(6.7%)
	AMC, RL, AMP	F, A and C	1 (6.7%)
	AMC, RL, AMP	F, D, A and C	1 (6.7%)
	AMC, AMP	F, D, A and C	1 (6.7%)
	AMC, RL, AMP	F and C	3 (20%)
	AMC, RL, AMP	F and A	2 (13.3%)
	RL, AMP	F and D	1 (6.7%)
	AMC, RL, AMP	F and D	1 (6.7%)
***Staphylococcus* isolates (n=15)**	DA, C, TE	F and B	1(6.7%)

Legend: CN: Gentamycin, **AMC:** Amoxycillin, **RL:** Sulphamethoxazole, **AMP:** Ampicillin, **DA**: Clindamycin, **C:** Chloramphenicol, **TE:** Tetracycline; A, B, C, D, E and F are designates for disinfectants as expounded in Table 6.1

6.5 Discussion on disinfectant testing (designations A, B, C, D, E and F, referred- to here, are expounded in Table 6.1)

In this study six different disinfectants that are being used in poultry production, other food-producing units, and humans were tested against three bacterial types that were most isolated: *E. coli*, *Staphylococcus* spp and *Streptococcus* spp. The isolates showed high resistance level/percentage to disinfectant designate F not only at and below the recommended user concentration but also at the two higher concentrations (twice and four times the manufacturer's user concentration). At 1/4 and 1/2 the manufacturer's user concentrations, all tested isolates (45) were resistant. At recommended concentration, *E. coli* isolates were resistant at 100%, *Staphylococcus* isolates at 93.3% and *Streptococcus* isolates at 93.3%. Even at the highest

concentration designated 5 (4 times the recommended concentration), *E. coli* isolates were resistant at 46.7%, while *Staphylococcus* and *Streptococcus* isolates were resistant at 40% each. This could have been precipitated by the fact that the disinfectant is frequently used or as the result of its chemical composition (3.85% m/v of Sodium Hypochlorite) which is less effective in killing the bacteria at low concentrations. This may not be surprising, since Njagi *et al.* (2005), did similar testing using a higher concentration of a similar disinfectant (10% Sodium hypochlorite) and showed that all (100%) the bacterial isolates they tested, which included *E. coli* were resistant to the disinfectant. It may, therefore, be advisable to use Sodium hypochlorite in combination with another disinfectant/other disinfectants or use it an even higher concentration so that it can give better results.

In the study done by Njagi *et al.* (2005), similar disinfectants to the ones used in current study were tested: they used Glutaraldehyde which is similar in composition to disinfectant designated C in this study and chloroxylenol which is similar to disinfectant D; however the results obtained back then are quite different from the current findings. In this study, *E. coli* was resistant at 46.5% to disinfectant C and at 26.7% to disinfectant D; *Staphylococcus* and *Streptococcus* isolates were 100% susceptible to disinfectants D and C at recommended user concentration. Njagi (2005)'s study showed the three isolates as being 100% resistant at recommended user concentration for both disinfectants. This can be explained in two ways: (1) that Njagi *et al*., (2005) could have used isolates that were already resistant to the disinfectants, or (2) that the particular disinfectants were being used more during Njagi *et al* (2005)'s time, which encouraged development of respective resistances in the bacteria. Concentrations higher than the recommended ones have shown high levels of effectiveness. In fact, in this study, the tested isolates were susceptible to higher concentrations (2 and 4 times the manufacturer's user concentration) to all disinfectants except

disinfectant F, with few exceptions of *E. coli* that was 20% resistant to disinfectant C and 13.3% to disinfectant A at concentration 4 that was two times the manufacturer's user concentration. Thus, in general, this finding shows that the higher the concentration of disinfectant, the more effective it is.

In this study, disinfectant designate E has shown high activity against the *Staphylococcus* and *Streptococcus* isolates which were susceptible at 100% for all the concentrations used (1/4, 1/2, 2 times and 4 times the recommended user dilution, including the recommended user concentration). *Escherichia coli* showed some resistance at concentrations lower than the recommended user one (1/4 and 1/2 the recommended concentration) at 60% and 20% respectively; while all the isolates were susceptible to the recommended user concentration and higher ones. This shows how powerful disinfectant E was against both Gram negative and Gram-positive bacteria. Its effectiveness can be due to its formulation which is a combination of Chlorhexidine gluconate 0.3 gram; Cetrimide 3.0 gram; N-propylalcohol 2.84% m/v.

Different factors that affect disinfectant action on bacteria have been documented, including: chemical composition of disinfectant and concentration, time of exposure, presence of interfering compounds i.e. of organic and inorganic matter, temperature, type of targeted microorganism (presence of biofilm or inoculum of the organism) and their concentration (inoculum), among others (Russell and Mcdonnell, 2000).

As found in this study, there is a difference in mode of reaction of Gram negative; *E. coli*, and Gram positive, S*taphylococcus* and *Streptococcus* isolates, to disinfectants; *E coli* were more resistant to the disinfectants compared to *Staphylococcus* and *Streptococcus* isolates. Investigations have shown that Gram negatives tend to be less responsive to disinfectant compared to Gram positive organisms, except for cases where the Gram positives form spores and where the

organism has complex cell-wall structure, like mycobacteria (Russell *et al.*, 1999). Explanation to this can be due to intrinsic factors, differences in cell wall structure between Gram negative and Gram-positive bacteria.

Different factors can cause development of disinfectant resistance in bacteria. Resistance to disinfectants can be genetically encoded to the organism (Leelaporn *et al.*, 1994), for example there is a finding of *E.coli* resistance to formaldehyde due to a formaldehyde resistance gene(s) that is located on a 4.6-kb BamHI fragment (Kümmerle *et al.*, 1996); there is another work that has proven that mercury resistance is inducible and can be transferred through conjugation because it is plasmid mediated (Shalita *et al.*, 1980). Another study has shown that there are resistant genes in *Staphylococcus aureus* that make them resistant to antimicrobials that have been found to be associated to multidrug resistance plasmid (Tennent *et al.*, 1989; Leelaporn *et al.*, 1994).

Another concern is that, the use of non-antibiotic antimicrobial agents has the ability to induce biocide resistant strains and selection of antibiotic resistant bacteria especially when used at lower concentration which is unable to kill them (Russell *et al.*, 1999). It is advisable to clean the surface where disinfectant is going to be used to remove debris or solid matter prior to application of disinfectant (Nyaga, 2007; Segal, 2018), as it was discovered that the presence of organic and inorganic matter can interfere with the effectiveness of disinfectant.

CHAPTER SEVEN: OVERALL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 Overall discussion

Heavy rains are among the factors that can increase bacterial prevalence and diversity in the indigenous chickens as a result of their feeding habit (picking from the environment) for example, they can pick up bacteria, insects, herbs that harbor bacteria or drink contaminated water carried by storm water; due to decrease in immunity of the chickens while trying to adjust to the climate change (wetness and coldness) bacteria can establish themselves and increase bacterial prevalence in the chickens. The worst case happens when they pick antibiotic/disinfectant resistant bacteria or genes, that can result in spreading of antibiotic/disinfectant resistance among the chickens, and worse case among the humans, leading to difficulty in controlling the agents.

From this study some isolates have shown resistance to disinfectants (at recommended user dilution) and antibiotics (Table 6.5); 73.3 % of *E. coli* and 6.1% of *Staphylococcus* isolates showed resistance to both antibiotic(s) and disinfectant(s). This is not surprising because the resistance can be due to same plasmid. Different findings have shown that isolates which are antibiotic resistant are frequently disinfectant resistant; therefore, there is some relationship between antibiotic and disinfectant resistance. There is possibility that resistance to antibiotic(s) can be carried by the same resistance gene as the one for disinfectant(s) (Townsend *et al.*, 1984).

During this investigation, there is a special case where a *Staphylococcus* isolate was multidrug resistant and showed resistance to a disinfectant that all other remaining *Staphylococcus* isolates and *Streptococcus* isolates were sensitive to. In this case there is high chance that this particular strain has a resistance gene to antibiotic(s) and disinfectant(s). Unfortunately, due to limited resources, this study didn't show the relationship. However, it is encouraging that, despite the fact

that all that 73.3% of *E. coli* which were resistant to more than one antibiotic and more than one disinfectant, were susceptible to Ciprofloxacin and disinfectant E. The same applies to the resistant *Staphylococcus* isolate; though it was multidrug resistant and resistant to more than one disinfectant, it was susceptible to Sulphamethoxazole, Erythromycin and disinfectant E, which can help in the control of *Staphylococcus* agents.

Similar to antibiotics, disinfectants also have target(s) on the microorganisms, some disrupt cell membrane integrity; others denature protein(s), interfering with enzyme functions, among others (Siddiqui and Sarwar, 2013). For example, autolysis brought about by phenolic compound is like the one induced by bacteria exposed to penicillin (Russell, 2002); uptake of Quaternary Ammonium Compounds into Gram-negative bacteria is similar to the one induced by streptomycin, gentamicin using a self-promoted entry system; inhibition of enoyl reductase in mycobacteria is affected by isoniazid (antibiotic) as well as triclosan (disinfectant); and filament induction in Gram-negative cells due to disinfectants (Acridines, Chloracetamidare) is similar to the one caused by antibiotic activity (Fluoroquilonones, β-Lactam) (Russell, 2002).

This study demonstrated higher concentration of various bacterial types in the intestinal tract of the market indigenous chickens sampled after the heavy rains of year 2018, and that the three mostly-isolated bacterial types were variously resistant to the tested antibiotics; some of them were multi-resistant. This highlights the possibility of the chickens serving as sources of pathogenic bacteria and/or resistance genes to other chickens and humans.

7.2 Conclusions

i. Indigenous chickens harbor different bacteria in terms of prevalence and types in number and diversity. Bacterial counts from intestinal contents of indigenous chickens were high and diverse in types where some were normal flora of chickens while others can be zoonotic to humans once consumed.

ii. Increased level of resistance of bacteria to the antibiotics was documented especially in *E. coli* followed by *Streptococcus* then *Staphylococcus*. Multi drug resistance was also observed in this study. If the resistance gene got transferred to humans, it will be difficult to control the agents.

iii. The test bacteria were shown to have developed resistance to disinfectants as they did to antibiotics. Some showed resistance to both disinfectants and antibiotics. This could pause problem towards control of the respective agents.

7.3 Recommendations

i. Precaution must be taken while slaughtering and preparing chickens for consumption; also, where possible, free-range indigenous chickens should be confined during rainy seasons to reduce their exposure to contaminated environment.

ii. Awareness on antibiotic resistance and/or disinfectant resistance can be made to people especially farmers so that they can use the antimicrobials accordingly, to reduce creation of resistant bacteria strains.

iii. When using disinfectants, the following should be put into consideration; the type of disinfectant, its formulation and concentration. The higher the concentration the better the results.

iv. As being done to antibiotics, other antimicrobials like disinfectants should be given a lot of attention, because the bacteria can develop resistance to them in the same way they do to antibiotics. Cut off points can be developed by research institution for disinfectants to help in comparing obtained results by different researchers.

v. It is also recommended that, as it is done for antibiotics, disinfectant susceptibility test be done first before use or recommending a particular concentration, so as to prevent unnecessary spread of pathogens or creating disinfectant resistant microorganisms, as shown that using inadequate concentration can induce resistance in bacteria.

vi. Precaution must be taken while disposing expired antimicrobial products, because they can be picked by the feeding chickens and induce resistance to the normal flora components of the chickens.

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APENDICES

Appendix 1: Questionnaire administered when collecting samples

- Date of collecting samples……
- Name of the slaughterhouse: ……
- Name of the person in charge: ……
- How was the rain fall pattern at the place where you got chickens for slaughter?.....
- How much time does it take to bring the chicken from the source to the slaughterhouse?.....
- Source of the chickens and relative number of birds taken from each source and mean of transport (by bus, motorbikes, in crates etc...)

Sources	**Number of birds/ week**	**Mean of transport**

- How long the birds have been in the market?.....
- Have the chickens been on treatment since reaching the market?
- If yes what kind of treatment?

Appendix 2:Results of bacteria counts and their logarithm 10 per slaughterhouse

Kariokor slaughterhouse			Burma slaughterhouse			Kangemi slaughterhouse		
Sample ID	**cfu/ml**	**Log10 of cfu/ml**	**Sample Id**	**cfu/ml**	**Log10 of cfu/ml**	**Sample Id**	**cfu/ml**	**Log10 of cfu/ml**
1	$2.8x10^{11}$	11.447	41	$2x10^{10}$	10.301	81	$4.8x10^{9}$	9.681
2	$8.4x10^{11}$	11.924	42	$8.8x10^{10}$	10.944	82	$4.48x10^{9}$	9.651
3	$5.6x10^{11}$	11.748	43	$4.8x10^{10}$	10.681	83	$1.2x10^{9}$	9.079
4	$6.8x10^{11}$	11.832	44	$1.2x10^{10}$	10.079	84	$3.6x10^{10}$	10.556
5	$4x10^{11}$	11.602	45	$5.6x10^{11}$	11.748	85	$6x10^{12}$	12.778
6	$3.4x10^{11}$	11.531	46	$3.6x10^{10}$	10.556	86	$2.4x10^{9}$	9.38
7	$2.56x10^{4}$	4.408	47	$6x10^{8}$	8.778	87	$9.2x10^{11}$	11.963
8	$6.4x10^{11}$	11.806	48	$2x10^{10}$	10.3	88	$2x10^{9}$	9.3
9	$6x10^{11}$	11.778	49	$5x10^{10}$	10.698	89	$5.04x10^{9}$	9.702
10	$1.66x10^{11}$	11.22	50	$3.6x10^{8}$	8.556	90	$3.4x10^{12}$	12.531
11	$8.6x10^{9}$	9.934	51	$2.8x10^{8}$	8.447	91	$2.52x10^{11}$	11.401
12	$4..8x10^{11}$	11.681	52	$7.2x10^{11}$	11.85	92	$2.4x10^{9}$	9.38
13	$6.8x10^{11}$	11.832	53	$7x10^{10}$	10.845	93	$8.8x10^{9}$	9.944
14	$4x10^{11}$	11.602	54	$5x10^{10}$	10.698	94	$2.4x10^{12}$	12.38
15	$1.92x10^{4}$	4.283	55	$4.8x10^{9}$	10.681	95	$2.8x10^{12}$	12.447
16	$7.4x10^{11}$	11.869	56	$6.8x10^{10}$	10.832	96	$1.84x10^{8}$	8.264
17	$2.92x10^{11}$	11.465	57	$4x10^{10}$	10.602	97	$2.2x10^{9}$	9.342
18	$7.6x10^{11}$	11.88	58	$1.48x10^{11}$	11.17	98	$4.8x10^{10}$	10.681
19	$1.24x10^{10}$	10.093	59	$2x10^{10}$	10.3	99	$2.8x10^{10}$	10.447
20	$6.4x10^{11}$	11.806	60	$8x10^{11}$	11.903	100	$5.28x10^{9}$	9.722
21	$3x10^{11}$	11.477	61	$2.2x10^{11}$	11.342	101	$8.6x10^{10}$	10.934
22	$2x10^{10}$	10.301	62	$2x10^{10}$	10.3	102	$6.8x10^{10}$	10.832
23	$1.2x10^{11}$	11.079	63	$3.6x10^{10}$	10.556	103	$1.3x10^{10}$	10.113
25	$8x10^{10}$	10.903	66	$4.2x10^{10}$	10.623	104	$8.2x10^{10}$	10.913
26	$1.04x10^{12}$	12.017	67	$1.4x10^{11}$	11.146	105	$1.14x10^{10}$	10.05
27	$1.4x10^{8}$	8.146	69	$2.08x10^{12}$	12.318	106	$8.2x10^{10}$	10.913
28	$5.8x10^{11}$	11.763	70	$9x10^{10}$	10.954	107	$5.44x10^{9}$	9.735
29	$2.4x10^{11}$	11.38	71	$9.6x10^{10}$	10.982	108	$1.86x10^{11}$	11.269
30	$1.92x10^{4}$	4.283	72	$4.8x10^{11}$	11.681	109	$1.68x10^{11}$	11.225
31	$1.56x10^{6}$	6.193	73	$9.8x10^{10}$	10.991	110	$2.2x10^{9}$	9.342
32	$7.6x10^{6}$	6.88	74	$3.2x10^{10}$	10.505	111	$9x10^{10}$	10.954
33	$6.2x10^{11}$	11.792	75	$3.28x10^{11}$	11.515	112	$1.2x10^{10}$	10.079
34	$8x10^{4}$	4.903	76	$2.46x10^{12}$	12.39	113	$4.88x10^{10}$	10.688
35	$2.24x10^{6}$	6.35	77	$8.8x10^{11}$	11.944	114	$1.4x10^{10}$	10.146
37	$2x10^{12}$	12.301	79	$1.88x10^{11}$	11.274	115	$1.92x10^{11}$	11.283
38	$7.2x10^{11}$	11.857	80	$1.8x10^{11}$	11.255	116	$1.54x10^{10}$	10.187
39	$2x10^{6}$	6.301				117	$1.02x10^{10}$	10.008
40	$1.8x10^{12}$	12.255				118	$2.38x10^{11}$	11.376
						119	$1.56x10^{11}$	11.193
						120	$3.94x10^{11}$	11.595

Appendix 3: Statistical analysis output for Bacterial counting.

1. Output of ANOVA at P- value of 0.05 showing homogeneity in means of the counts

SOURCE	DF	SS	MS	F	P
SOURCE	2	6.989E+24	3.495E+24	3.85	0.0266
ERROR	61	5.534E+25	9.071E+23		
TOTAL	63	6.232E+25			

Grand Mean 6.67E+11

2. Homogeneity of the means of the isolates

Source	**Means**	**Homogeneous Groups**
Kangemi	1.32E+12	A
Burma	5.65E+11	B
Kariokor	4.69E+11	B

Legend: DF: degree of freedom; SS: Sum of Square; MS: Mean of Square; F: and P: P-value, it shows that there is difference among the groups.

Appendix 4: Identification criteria of various bacteria isolated from slaughter houses

Name of the bacterium	Colony morphology On solid media	Gram staining	Catalase	Oxidase	Simmon Citrate	Urease	Indole	Methyl red	Motility	Other tests
Escherichia coli	Medium size colony, lactose fermenter on MacConkey agar	Gram -ve short rods	+ve	-ve	-ve	-ve	+ve	+ve	-	Metallic sheen on EMB media and produce acid butt and slant on TSI agar with gas
Staphylococcus aureus	Creamy white/ yellow and have β hemolysis on sheep BA; medium size	G +ve cocci in clusters	+ve	-ve	v	v	-ve	+ve	-	Ferment mannitol (yellow colonies on MSA media)
Other *Staphylococcus spp*	Creamy white/ yellow ; medium size	G +ve in clusters	+ve	-ve	V	v	-ve	+ve	-	Don't ferment mannitol (pink to white on MSA media)
Streptococcus agalactiae	Small pin point colonies; Hemolytic on sheep blood agar	G +ve cocci in chains	-ve	-ve	-ve	-ve	-	-	-	CAMP (+ve) with *Staph.aureus* arrow shape

Other *Streptococcus spp*	Small pin point colonies; some hemolytic others not on sheep blood agar	G +ve cocci in pair, triplets or chains	-ve	-ve	-ve	-ve	-	-	-	CAMP –(ve)
Bacillus spp	Large giant colonies rough with irregular shape.	G+ve long rods with spores	+ve	-ve	+ve	-ve	-ve	-ve	motile	Some are beta haemolytic others were not
Proteus spp	Swarming colonies on sheep blood agar; Non lactose fermenter on MacConkey, produce pale colonies on SSA with black center	G-ve rods	+ve	-ve	v	+ve	-ve	+ve	motile	Spread over the surface of BA media and produce H_2S on TSI
Pseudomonas aeruginosa	Medium sized colonies. hemolytic on sheep BA; greenish colonies on NA with sweet smell	G-ve rods	+ve	+ve	+ve	-ve	-ve	-ve	-	Doesn't ferment carbohydrates;
Listeria monocytogens	Small colonies, β hemolytic on sheep blood Agar. Pin point black on CTBA	G +ve short rods (cocobacilli)	+ve	-ve	-ve	-ve	-ve	+ve	tumbling motility'	CAMP +ve with *Staph. aureus* (Shovel shaped)
Other *listeria spp*	Small colonies on BA and NA; black pin point colonies on CTBA	G+ve short rods (cocobacili)	+ve	-ve	-ve	-ve	-ve	+ve	tumbling motility'	CAMP -ve
Streptobacillus spp	medium white non-hemolytic on sheep blood agar	G -ve filaments	-ve	-ve	Late +ve	-ve	-ve	+ve	-	Lactose fermenter on MacConkey agar
Klebsiella spp	Medium sized colonies on BA, Large LF, mucoid on MacConkey agar	Gram -ve rods	+ve	-ve	+ve	+ve	-ve	-ve	-	Acid slant and butt on TSI
Campylobacter spp	Tear drop like medium sized colonies (white on Camp Karmali media)	Gram -ve rods(polymorphic)	+ve	+ve	-	-	-	-	-	Produced H2S on TSI; grew after 48 hrs of incubation at 37°C

With BA: Blood agar; MSA: mannitol salt agar, EMB: Eosin Methylene blue; G +ve: Gram positive; G -ve: Gram negative; +ve: positive; -ve: negative; V: variable; TSI: Triple sugar iron.

Appendix 5:Bacteria isolated from the three different slaughterhouses

A) Isolates from Kariokor slaughterhouse

Sample Id	Bacterial isolates
1	*Staphylococcus spp; Staphylococcus aureus; Escherichia coli*
2	*Staph. aureus; E. coli* +unidentified
3	*E. coli; Staphylococcus spp; Streptococcus spp*
4	*E. coli; Staphylococcus spp*
5	*Staphylococcus spp; E. coli* +Unidentified
6	*E. coli; Staphylococcus spp; Listeria monocytogenes*
7	*Bacillus spp; Streptococcus spp*
8	*E. coli*
9	*Staphylococcus spp* +Unidentified
10	*E. coli*
11	*E. coli; Staphylococcus spp* +unidentified
12	*E. coli; Listeria spp; Staphylococcus spp*
13	*E. coli; Staphylococcus spp*+ unidentified
14	*E. coli; Streptococcus spp; Listeria spp; Staph. aureus*
15	*Bacillus spp; Staphylococcus spp*
16	*E. coli; Staph. aureus;* other *Staphylococcus spp*
17	*Staph. aureus; Listeria monocytogenes; Streptococcus spp; E. coli*
18	*E. coli; Staphylococcus spp*+ unidentified
19	*Listeria monocytogenes; Staphylococcus spp; E. coli* +unidentified
20	*E. coli; Streptococcus spp* +unidentified
21	*Staphylococcus spp; Streptococcus spp* + unidentified
22	*Streptococcus spp; Staphylococcus spp; E. coli*
23	*Staphylococcus spp*+ unidentified
24	*E. coli; Streptobacillus spp*
25	*E. coli; Streptococcus spp*
26	*Streptococcus spp; E. coli; Staph. aureus*
27	*Listeria spp; E. coli* +unidentified
28	*Staphylococcus spp; E. coli*
29	*E. coli; Staph. aureus; Staphylococcus spp; Bacillus spp*
30	*Staph. aureus, E. coli; Bacillus spp*
31	*Bacillus spp; E. coli*
32	*Bacillus spp; Staph. aureus; E. coli*
33	*Staphylococcus spp; E. coli; Streptococcus spp*
34	*Staph. aureus; E. coli*
35	*E. coli; Streptococcus spp* +unidentified
36	*E. coli; Listeria spp; Staphylococcus spp*
37	*E. coli; Streptococcus spp; Bacillus spp*
38	*Staphylococcus spp; E. coli*
39	*Staphylococcus spp; E. coli; Streptococcus spp.*
40	*Listeria spp; E. coli*

B) Isolated bacteria from Burma slaughterhouse

Sample Id	Isolates
41	*Bacillus spp; Staphylococcus aureus; Escherichia coli.*
42	*Bacillus spp E. coli; Listeria spp*
43	*Bacillus spp* +unidentified
44	*E. coli; Bacillus spp; Listeria monocytogenes; Staphylococcus spp* and *Streptococcus spp.*
45	*Bacillus spp; E. coli*+ Unidentified
46	*Bacillus spp; E. coli; Proteus spp*
47	*Bacillus spp; E. coli; Proteus spp*
48	*Bacillus spp; E. coli; Staphylococcus spp*
49	*Bacillus spp; Proteus spp; E. coli*
50	*Bacillus spp; E. coli*+ Unidentified
51	*E. coli; Streptococcus spp; Listeria spp; Staphylococcus spp*
52	*Staph. aureus; E. coli*
53	*Bacillus spp; Staphylococcus spp; E. coli*
54	*Bacillus spp; E. coli*+ Unidentified
55	*Bacillus spp; E. coli; Proteus spp; Streptococcus spp; Staphylococcus spp*
56	*Bacillus spp; Streptococcus agalactiae; Staph. aureus; E. coli; Proteus spp*
57	*E. coli; Listeria spp*
58	*E. coli; Klebsiella spp; Listeria spp* +Unidentified
59	*E. coli; Pseudomonas aeuroginosa; Streptococcus spp; Proteus spp; Listeria spp*
60	*E. coli; Streptococcus spp; Listeria spp*
61	*E. coli; Streptococcus spp; Proteus spp*
62	*E. coli; Bacillus spp; Streptococcus spp*
63	*E. coli; Streptococcus spp; Bacillus spp; Proteus spp.*
64	*E. coli; Streptococcus spp*+ Unidentified
65	*E. coli; Proteus spp, Streptococcus spp*
66	*E. coli; Streptococcus spp; Proteus spp*
67	*Bacillus spp*+ unidentified
68	*E. coli; Streptococcus spp; Listeria spp*
69	*Bacillus spp; E. coli; Streptococcus spp; Listeria spp; Proteus spp*
70	*Bacillus spp; Proteus spp; Streptococcus spp*+ unidentified
71	*E. coli; Streptococcus spp; Proteus spp; Klebsiella spp*
72	*E. coli; Bacillus spp;* + unidentified
73	*E. coli; Staph. aureus; Bacillus spp; Streptococcus spp; Proteus spp; Listeria spp*
74	*E. coli; Streptococcus spp; Listeria spp*
75	*Streptococcus spp; Bacillus spp; Proteus spp; E. coli* +unidentified
76	*E. coli; Streptococcus spp; Proteus spp; Listeria spp*
77	*Bacillus spp; E. coli; Proteus spp; Listeria spp*
78	*E. coli; Bacillus spp; Streptococcus spp; Proteus spp* +unidentified
79	*Bacillus spp; Streptococcus spp; Proteus spp* +unidentified
80	*Bacillus spp; E. coli; Proteus spp* +unidentified

C) Isolates from Kangemi slaughterhouse

Sample ID	Isolates
81	*Listeria monocytogenes; Staphylococcus spp; E. coli; Proteus spp; Bacillus spp* + unidentified
82	*Staph. aureus; E. coli; Streptococcus spp* +Unidentified
83	*Proteus spp; Listeria spp; Staphylococcus spp;*
84	*E. coli; Bacillus spp; Staph. aureus*+ Unidentified
85	*E. coli; Streptococcus spp*
86	*Streptococcus spp; Bacillus spp; E. coli*+ Unidentified
87	*E. coli; Bacillus spp; Listeria spp; Staph. aureus*+ Unidentified
88	*Streptococcus spp; Bacillus spp; Listeria spp; E. coli;* +Unidentified
89	*Staphylococcus spp; Listeria spp; Staph. aureus* +Unidentified
90	*E. coli* +Unidentified
91	*E. coli; Staphylococcus spp; Proteus spp; Streptococcus spp*
92	*E. coli* +unidentified
93	*Streptococcus spp; E. coli; Bacillus spp; Staphylococcus spp; Listeria spp*+ Unidentified
94	*E. coli; Bacillus spp; Staph. Aureus; Streptococcus spp* +unidentified
95	*E. coli; Streptococcus spp*
96	*Streptococcus agalactiae; Streptococcus spp; E. coli; Staphylococcus spp; Staph. aureus; Klebsiella spp* +Unidentified
97	*E. coli; Listeria monocytogenes; Staphylococcus spp; Streptococcus spp;*
98	*Bacillus spp; E. coli; Staph. aureus;* + unidentified
99	*E. coli* +unidentified
100	*Staphylococcus spp; E. coli; Proteus spp; Streptococcus spp*+ Unidentified
101	*E. coli; Listeria spp*
102	*Listeria monocytogenes; Bacillus spp; Staph. aureus; E. coli*+ Unidentified
103	*E. coli; Listeria spp* +Unidentified
104	*E. coli; Listeria monocytogenes; Staphylococcus spp; Klebsiella spp;* other *Listeria spp* +Unidentified
105	*E. coli; Staphylococcus spp*+ Unidentified
106	*E. coli; Streptococcus spp; Listeria spp*+ Unidentified
107	*E. coli; Staphylococcus spp; Streptococcus spp; Listeria spp*+ Unidentified
108	*Proteus pp; Klebsiella spp; E. coli; Listeria spp*
109	*Listeria spp; E. coli*
110	*E. coli; Klebsiella spp; Listeria spp*
111	*Bacillus spp; Streptococcus spp; Listeria spp*
112	*Bacillus spp; E. coli; Listeria spp*
113	*Bacillus spp; Klebsiella spp; Listeria spp; Staphylococcus spp; E. coli*+ Unidentified
114	*E. coli; Staphylococcus spp; Klebsiella spp; Proteus spp*
115	*E. coli; Listeria spp; Staphylococcus spp*
116	*Bacillus spp; Listeria spp; Staphylococcus spp; Proteus spp; Pseudomonas aeruginosa*
117	*Streptococcus spp; Listeria spp*
118	*Klebsiella spp; Bacillus spp; Listeria spp*
119	*Staph. aureus; E. coli; Streptococcus spp; Listeria spp; Proteus spp*
120	*E. coli; Proteus spp +Unidentified; Listeria spp*

Appendix 6: Statistical analysis of the isolates with respect to their slaughterhouses

***E. coli* Vs Slaughterhouses**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.457[a]	2	.796
Likelihood Ratio	.460	2	.795
Linear-by-Linear Association	.113	1	.736
N of Valid Cases	120		

***Staphylococcus* aureus Vs Slaughterhouses**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.335[a]	2	.189
Likelihood Ratio	3.625	2	.163
Linear-by-Linear Association	.080	1	.777
N of Valid Cases	120		

***Staphylococcus* spp other than *Staph.aureus* Vs Slaughterhouses**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	16.050[a]	2	.000
Likelihood Ratio	17.159	2	.000
Linear-by-Linear Association	3.258	1	.071
N of Valid Cases	119		

***Streptococcus agalactiae* Vs Slaughterhouses**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.030[a]	2	.597
Likelihood Ratio	1.656	2	.437
Linear-by-Linear Association	.785	1	.375
N of Valid Cases	119		

***Streptococcus* spp other than *Strep. agalactiae* Vs Slaughterhouses**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.587[a]	2	.166
Likelihood Ratio	3.576	2	.167
Linear-by-Linear Association	.205	1	.651
N of Valid Cases	120		

***Listeria monocytogenes* Vs Slaughterhouses**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.875[a]	2	.392
Likelihood Ratio	2.113	2	.348
Linear-by-Linear Association	.199	1	.655
N of Valid Cases	120		

***Listeria* spp other than *L. monocytogenes* Vs Slaughterhouses**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	14.700[a]	2	.001
Likelihood Ratio	15.026	2	.001
Linear-by-Linear Association	14.280	1	.000
N of Valid Cases	120		

***Klebsiella* spp Vs Slaughterhouses**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.369[a]	2	.009
Likelihood Ratio	10.953	2	.004
Linear-by-Linear Association	8.755	1	.003
N of Valid Cases	120		

***Proteus* spp Vs Slaughterhouses**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	23.226[a]	2	.000
Likelihood Ratio	30.255	2	.000
Linear-by-Linear Association	5.758	1	.016
N of Valid Cases	120		

***Bacillus* spp Vs Slaughterhouses**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	19.376[a]	2	.000
Likelihood Ratio	19.987	2	.000
Linear-by-Linear Association	2.549	1	.110
N of Valid Cases	120		

***Pseudomonas aeuroginosa* Vs Slaughterhouses**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.017[a]	2	.601
Likelihood Ratio	1.639	2	.441
Linear-by-Linear Association	.756	1	.384
N of Valid Cases	120		

***Campylobacter* spp Vs Slaughterhouses**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.051[a]	2	.359
Likelihood Ratio	2.824	2	.244
Linear-by-Linear Association	2.034	1	.154
N of Valid Cases	120		

***Streptobacillus* spp Vs Slaughterhouses**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.992[a]	2	.369
Likelihood Ratio	2.197	2	.333
Linear-by-Linear Association	1.481	1	.224
N of Valid Cases	119		

Appendix 7: Results of Antibiotic susceptibility test of the isolates

A) Antibiotic resistance/susceptibility profile of tested *E. coli* isolates

Sources	Sample ID	CN (S ≥15)	AMC (S ≥17)	CIP (S ≥21)	RL (S ≥16)	AMP (S ≥17)
	E. coli ATCC 25922	23 **(S)**	10 **(R)**	37 **(S)**	11 **(R)**	6 **(R)**
Burma	78 *E. coli*	20 **(S)**	15 **(R)**	40 **(S)**	6 **(R)**	9 **(R)**
	69 *E. coli*	19 **(S)**	15 **(R)**	40 **(S)**	6 **(R)**	10 **(R)**
	80 *E. coli*	20 **(S)**	18 **(S)**	38 **(S)**	6 **(R)**	10 **(R)**
	62 *E. coli*	19 **(S)**	16 **(R)**	36 **(S)**	20 **(S)**	8 **(R)**
	51 *E. coli*	20 **(S)**	6 **(R)**	35 **(S)**	6 **(R)**	6 **(R)**
Kariokor	6 *E. coli*	17 **(S)**	13 **(R)**	25 **(S)**	13 **(R)**	9 **(R)**
	20 *E. coli*	19 **(S)**	10 **(R)**	32 **(S)**	6 **(R)**	8 **(R)**
	25 *E. coli*	18 **(S)**	15 **(R)**	30 **(S)**	12 **(R)**	9 **(R)**
	26 *E. coli*	18 **(S)**	14 **(R)**	30 **(S)**	14 **(R)**	9 **(R)**
	4 *E. coli*	16 **(R)**	13 **(R)**	32 **(S)**	9 **(R)**	10 **(R)**
Kangemi	82 *E. coli*	16**(R)**	6 **(R)**	26 **(S)**	6 **(R)**	6 **(R)**
	97 *E. coli*	18 **(S)**	11 **(R)**	34 **(S)**	6 **(R)**	15 **(R)**
	107 *E. coli*	20 **(S)**	11 **(R)**	33 **(S)**	7 **(R)**	9 **(R)**
	114 *E. coli*	18 **(S)**	10 **(R)**	34 **(S)**	10 **(R)**	7 **(R)**
	101 *E. coli*	20 **(S)**	11 **(R)**	30 **(S)**	8 **(R)**	8 **(R)**

B) Antibiotic resistance/susceptibility profile of tested *Staphylococcus* isolates

Sources	Sample ID	RL (S ≥16)	E (S ≥23)	DA (S ≥21)	C (S ≥18)	TE (S ≥19)
	Staph. aureus ATCC 25923	26 **(S)**	28 **(S)**	30 **(S)**	20 **(S)**	28 **(S)**
Burma	51 *Staphylococcus spp*	6 **(S)**	12 **(S)**	6 **(R)**	15 **(R)**	11 **(R)**
	53 *Staphylococcus spp*	6 **(S)**	15 **(S)**	6 **(R)**	16 **(R)**	7 **(R)**
	56 *Staph. aureus*	21 **(S)**	23 **(S)**	17 **(R)**	16 **(R)**	21 **(S)**
	41 *Staph. aureus*	18 **(S)**	26 **(S)**	25 **(S)**	21 **(S)**	27 **(S)**
	73 *Staph. aureus*	12 **(S)**	10 **(S)**	26 **(S)**	21 **(S)**	8 **(R)**
Kariokor	26 *Staph. aureus*	14 **(S)**	17 **(S)**	18 **(R)**	25 **(S)**	30 **(S)**
	15 *Staphylococcus spp*	6 **(S)**	25 **(S)**	27 **(S)**	24 **(S)**	6 **(R)**
	1 *Staph. aureus*	13 **(S)**	24 **(S)**	20 **(R)**	24 **(S)**	25 **(S)**
	2 *Staph. aureus*	28 **(S)**	27 **(S)**	30 **(S)**	30 **(S)**	32 **(S)**
	34 *Staph. aureus*	16 **(S)**	25 **(S)**	16 **(R)**	15 **(R)**	18 **(R)**
Kangemi	119 *Staph. aureus*	16 **(S)**	21 **(S)**	18 **(R)**	23 **(S)**	28 **(S)**
	87 *Staph. aureus*	17 **(S)**	18 **(S)**	15 **(R)**	13 **(R)**	10 **(R)**
	102 *Staph. aureus*	6 **(S)**	12 **(S)**	18 **(R)**	15 **(R)**	21 **(S)**
	98 *Staph. aureus*	19 **(S)**	23 **(S)**	18 **(R)**	22 **(S)**	22 **(S)**
	114 *Staphylococcus spp*	12 **(S)**	17 **(S)**	18 **(R)**	20 **(S)**	7 **(R)**

C) Antibiotic resistance/ susceptibility profile of tested *Streptococcus* isolates

Sources	Sample ID	RL (S ≥19)	E (S ≥21)	DA (S ≥19)	C (S ≥19)	TE (S ≥23)
Burma	79 S*treptococcus spp*	6 **(R)**	18 **(R)**	6 **(R)**	18 **(R)**	27 **(S)**
	64 *Streptococcus spp*	6 **(R)**	17 **(R)**	6 **(R)**	19 **(R)**	23 **(S)**
	75 *Streptococcus spp*	6 **(R)**	26 **(S)**	8 **(R)**	21 **(S)**	24 **(S)**
	56 *S. agalactiae*	6 **(R)**	18 **(R)**	7 **(R)**	19 **(R)**	13 **(R)**
	74 *Streptococcus spp*	6 **(R)**	16 **(R)**	6 **(R)**	21 **(S)**	6 **(R)**
Kariokor	17 *Streptococcus spp*	6 **(R)**	25 **(S)**	6 **(R)**	26 **(S)**	8 **(R)**
	22 *Streptococcus spp*	6 **(R)**	20 **(R)**	10 **(R)**	20 **(R)**	27 **(S)**
	35 *Streptococcus spp*	6 **(R)**	9 **(R)**	10 **(R)**	22 **(S)**	6 **(R)**
	25 *Streptococcus spp*	6 **(R)**	29 **(S)**	6 **(R)**	25 **(S)**	6 **(R)**
	26 *Streptococcus spp*	6 **(R)**	24 **(S)**	8 **(R)**	20 **(R)**	24 **(S)**
Kangemi	107 *Streptococcus spp*	27 **(S)**	22 **(S)**	26 **(S)**	24 **(S)**	13 **(R)**
	106 *Streptococcus spp*	6 **(R)**	12 **(R)**	6 **(R)**	13 **(R)**	6 **(R)**
	95 *Streptococcus spp*	6 **(R)**	21 **(S)**	17 **(R)**	19 **(R)**	8 **(R)**
	94 *Streptococcus spp*	6 **(R)**	18 **(R)**	6 **(R)**	6 **(R)**	25 **(S)**
	96 *S. agalactiae*	6 **(R)**	20 **(R)**	21 **(S)**	25 **(S)**	8 **(R)**

D) Resistance pattern of the tested organisms on antibiotics used in %

Bacteria	Antibiotics	Sources		
		Kariokor	Burma	Kangemi
E. coli	AMP	100	100	100
	CN	0	0	0
	CIP	0	0	0
	AMC	100	80	100
	RL	100	80	100
Staphylococcus isolates	E	20%	60%	80%
	RL	60%	60%	40%
	DA	60%	60%	100%
	C	20%	60%	60%
	TE	40%	60%	40%
Streptococcus isolates	E	40%	80%	80%
	RL	100%	100%	80%
	DA	100%	100%	60%
	C	40%	60%	60%
	TE	60%	40%	80%

Appendix 8: Statistical chi-square output of antibiotic resistance against the isolates

E. coli vs Gentamycin

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.154[a]	2	.562
Likelihood Ratio	1.772	2	.412
Linear-by-Linear Association	.000	1	1.000
N of Valid Cases	15		

E. coli vs Amoxycillin

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.143[a]	2	.343
Likelihood Ratio	2.344	2	.310
Linear-by-Linear Association	.000	1	1.000
N of Valid Cases	15		

***E. coli* vs Sulphamethoxazole**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.143[a]	2	.343
Likelihood Ratio	2.344	2	.310
Linear-by-Linear Association	.000	1	1.000
N of Valid Cases	15		

No statistical analysis made for CIP and AMP since the values were constant

***Staphylococcus* isolates vs Clindamycin**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.494[a]	2	.174
Likelihood Ratio	4.581	2	.101
Linear-by-Linear Association	3.261	1	.071
N of Valid Cases	15		

***Staphylococcus* isolates vs Chloramphenicol**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.403[a]	2	.182
Likelihood Ratio	3.555	2	.169
Linear-by-Linear Association	.697	1	.404
N of Valid Cases	15		

***Staphylococcus* isolates vs Tetracycline**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.808[a]	2	.405
Likelihood Ratio	1.861	2	.394
Linear-by-Linear Association	.075	1	.785
N of Valid Cases	15		

***Streptococcus* isolates vs Tetracycline**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.667[a]	2	.435
Likelihood Ratio	1.726	2	.422
Linear-by-Linear Association	.389	1	.533
N of Valid Cases	15		

***Streptococcus* isolates vs Chloramphenicol**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.536[a]	2	.765
Likelihood Ratio	.537	2	.764
Linear-by-Linear Association	.375	1	.540
N of Valid Cases	15		

***Streptococcus* isolates vs Clindamycin**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.615[a]	2	.099
Likelihood Ratio	5.050	2	.080
Linear-by-Linear Association	3.231	1	.072
N of Valid Cases	15		

***Streptococcus* isolates vs Erythromycin**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.667[a]	2	.435
Likelihood Ratio	1.726	2	.422
Linear-by-Linear Association	.389	1	.533
N of Valid Cases	15		

***Streptococcus* isolates vs** Sulphamethoxazole

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.143[a]	2	.343
Likelihood Ratio	2.344	2	.310
Linear-by-Linear Association	1.500	1	.221
N of Valid Cases	15		

Appendix 9: Results of Disinfectant susceptibility test of the isolates

A) Resistant/ susceptibility of the tested reference strains to disinfectants at all dilutions.

Sample Id	concentration	F	D	E	A	B	C
E. coli ATCC 259222	1	6 **(R)**	6 **(R)**	16 **(S)**	6 **(R)**	6 **(R)**	12 **(S)**
	2	6 **(R)**	12 **(S)**	16 **(S)**	6 **(R)**	6 **(R)**	14 **(S)**
	3	6 **(R)**	20 **(S)**	20 **(S)**	12 **(S)**	6 **(R)**	16 **(S)**
	4	14 **(S)**	22 **(S)**	32 **(S)**	14 **(S)**	16 **(S)**	20 **(S)**
	5	16 **(S)**	24 **(S)**	30 **(S)**	16 **(S)**	18 **(S)**	22 **(S)**
Staphylococcus ATCC 25922	1	6 **(R)**	13 **(S)**	18 **(S)**	18 **(S)**	19 **(S)**	17 **(S)**
	2	6 **(R)**	18 **(S)**	20 **(S)**	21 **(S)**	21 **(S)**	21 **(S)**
	3	8 **(R)**	19 **(S)**	22 **(S)**	22 **(S)**	24 **(S)**	22 **(S)**
	4	8 **(R)**	20 **(S)**	23 **(S)**	24 **(S)**	25 **(S)**	25 **(S)**
	5	11 **(S)**	21 **(S)**	25 **(S)**	26 **(S)**	26 **(S)**	26 **(S)**

B) Number of resistant isolates to disinfectants at different concentrations (1,2,3*,4 and 5) per slaughterhouse.

Site	Bacteria	concentration	F		D		E		A		B		C	
			R	S	R	S	R	S	R	S	R	S	R	S
Kariokor	*E. coli*	1	5	0	4	1	5	0	5	0	4	1	5	0
		2	5	0	1	4	2	3	4	1	1	4	5	0
		3*	5	0	1	4	0	5	2	3	0	5	5	0
		4	5	0	0	5	0	5	2	3	5	5	3	2
		5	4	1	0	5	0	5	0	5	5	5	0	5
	Staphylococcus	1	5	0	0	5	0	5	0	5	1	4	0	5
		2	5	0	0	5	0	5	0	5	1	4	0	5
		3*	5	0	0	5	0	5	0	5	1	4	0	5
		4	5	0	0	5	0	5	0	5	0	5	0	5
		5	2	3	0	5	0	5	0	5	0	5	0	5
	Streptococcus	1	5	0	4	1	0	5	0	5	0	5	0	5
		2	5	0	2	3	0	5	0	5	0	5	0	5
		3*	4	1	0	5	0	5	0	5	0	5	0	5
		4	4	1	0	5	0	5	0	5	0	5	0	5
		5	3	2	0	5	0	5	0	5	0	5	0	5
Burma	*E. coli*	1	5	0	5	0	3	2	5	0	4	1	5	0
		2	5	0	5	0	1	4	4	1	1	4	4	1
		3*	5	0	3	2	0	5	3	2	0	5	2	3
		4	5	0	0	5	0	5	0	5	0	5	0	5
		5	1	4	0	5	0	5	0	5	0	5	0	5
	Staphylococcus	1	5	0	3	2	0	5	1	4	0	5	0	5
		2	5	0	2	3	0	5	0	5	0	5	0	5
		3*	5	0	0	5	0	5	0	5	0	5	0	5
		4	4	1	0	5	0	5	0	5	0	5	0	5
		5	2	3	0	5	0	5	0	5	0	5	0	5
	Streptococcus	1	5	0	3	2	0	5	0	5	0	5	1	4
		2	5	0	2	3	0	5	0	5	0	5	0	5
		3*	5	0	0	5	0	5	0	5	0	5	0	5
		4	5	0	0	5	0	5	0	5	0	5	0	5
		5	1	4	0	5	0	5	0	5	0	5	0	5
Kangemi	*E. coli*	1	5	0	5	0	1	4	4	1	2	3	5	0
		2	5	0	4	1	0	5	1	4	2	3	0	5
		3*	5	0	1	4	0	5	1	4	0	5	0	5
		4	4	1	0	5	0	5	0	5	0	5	0	5
		5	2	3	0	5	0	5	0	5	0	5	0	5
	Staphylococcus	1	5	0	0	5	0	5	0	5	0	5	0	5
		2	5	0	0	5	0	5	0	5	0	5	0	5
		3*	4	1	0	5	0	5	0	5	0	5	0	5
		4	4	1	0	5	0	5	0	5	0	5	0	5
		5	2	3	0	5	0	5	0	5	0	5	0	5
	Streptococcus	1	5	0	2	3	0	5	0	5	0	5	0	5
		2	5	0	0	5	0	5	0	5	0	5	0	5
		3*	5	0	0	5	0	5	0	5	0	5	0	5
		4	5	0	0	5	0	5	0	5	0	5	0	5
		5	2	3	0	5	0	5	0	5	0	5	0	5

With 3* as the recommended dilution, S means susceptible and R means resistant

Appendix 10: Statistical chi-square output of disinfectants resistance against the isolates

Chi-square output of the isolates vs disinfectant A

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	26.227[a]	10	.003
Likelihood Ratio	29.832	10	.001
Linear-by-Linear Association	15.842	1	.000
N of Valid Cases	45		

Chi-square output of the isolates vs disinfectant B

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	20.618[a]	8	.008
Likelihood Ratio	23.756	8	.003
Linear-by-Linear Association	12.684	1	.000
N of Valid Cases	45		

Chi-square output of the isolates vs disinfectant C

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	21.167[a]	10	.020
Likelihood Ratio	23.240	10	.010
Linear-by-Linear Association	12.500	1	.000
N of Valid Cases	45		

Chi-square output of the isolates vs disinfectant D

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	20.052[a]	10	.029
Likelihood Ratio	22.904	10	.011
Linear-by-Linear Association	3.131	1	.077
N of Valid Cases	45		

Chi-square output of the isolates vs disinfectant E.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11.250[a]	8	.188
Likelihood Ratio	12.299	8	.138
Linear-by-Linear Association	5.739	1	.017
N of Valid Cases	45		

Chi-square output of isolates vs disinfectant F

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.086[a]	8	.748
Likelihood Ratio	5.778	8	.672
Linear-by-Linear Association	.090	1	.764
N of Valid Cases	45		

Chi-square output of the slaughterhouses vs disinfectant A

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.500[a]	10	.398
Likelihood Ratio	12.504	10	.253
Linear-by-Linear Association	.557	1	.455
N of Valid Cases	45		

Chi-square output of the slaughterhouses vs disinfectant B

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	18.382[a]	8	.019
Likelihood Ratio	20.949	8	.007
Linear-by-Linear Association	5.445	1	.020
N of Valid Cases	45		

Chi-square output of the slaughterhouses vs disinfectant C

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.100[a]	10	.432
Likelihood Ratio	10.760	10	.377
Linear-by-Linear Association	1.940	1	.164
N of Valid Cases	45		

Chi-square output of the slaughterhouses vs disinfectant D

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	12.983[a]	10	.225
Likelihood Ratio	15.873	10	.103
Linear-by-Linear Association	.000	1	1.000
N of Valid Cases	45		

Chi-square output of the slaughterhouses vs disinfectant E

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.050[a]	8	.531
Likelihood Ratio	8.263	8	.408
Linear-by-Linear Association	1.435	1	.231
N of Valid Cases	45		

Chi-square output of the slaughterhouses vs disinfectant F

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8.943[a]	8	.347
Likelihood Ratio	9.597	8	.294
Linear-by-Linear Association	.360	1	.549
N of Valid Cases	45		