UNIVERSITY OF GHANA

COLLEGE OF BASIC AND APPLIED SCIENCES

MICROBIOLOGICAL QUALITY ASSESSMENT AND CHARACTERISATION OF SALMONELLA SPP. ASSOCIATED WITH CHICKEN FROM DIFFERENT LIVE BIRD PROCESSING OUTLETS IN ACCRA, GHANA.

RICHARD YAW OTWEY

(10386880)

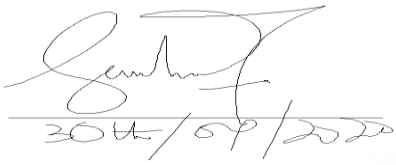
A THESIS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL FOOD SCIENCE

DEPARTMENT OF NUTRITION AND FOOD SCIENCE

JULY 2019

DECLARATION

This is to certify that this thesis presents the result of research undertaken by Richard Yaw Otwey, towards the award of Master of Philosophy in the Department of Nutrition and Food Science, University of Ghana under the supervision of Dr. Angela Parry-Hanson Kunadu and Dr. Lydia Mosi.



Date: ……30/04/2020…….

Richard Yaw Otwey

(Candidate)

Date: ……04/05/2020…….

Dr. Angela Parry-Hanson Kunadu

(Supervisor)

Date: ……03/05/2020…….

Dr. Lydia Mosi

(Co-Supervisor).

DEDICATION

I dedicate this work to the Almighty God, my parent and siblings.

ACKNOWLEDGEMENT

I wish to express my deepest gratitude to God for taking me through the program.

I would also like to thank the Department of Nutrition and Food Science the offering the opportunity to pursuit a Master's program in Food Science.

My appreciation again goes to IFS for funding my project, it was of great help.

To my wonderful supervisors Dr. Kunadu and Dr. Mosi God richly bless you and you families.

Dr. Emmanuel Kyere, Dr. Jocelyne Quansah and Ms. Eurydice Aboagye thank you very all for all you help and direction. Many thanks to Mrs. Mabel Preprah, Cecille Aboagye, Emmanuel Addo-Preko and Robert Ahenkorah for all your help in the lab.

Finally to Maame Kwansima Hayfron, God bless you richly.

ABSTRACT

Live bird markets (LBM) are generally known as a storefront or open market slaughter operations that slaughter, dress, and trade dressed or live poultry on demand. Poultry meat is a major vehicle for foodborne pathogens (*Salmonella, Campylobacter* and *E. coli O157:H7*) responsible for more than half of the global burden of foodborne illnesses. The activities of theses live bird operators are scarcely monitored hence many of them do not conform to regulations, increasing the risk of microbial contamination. The aim of this study was to use molecular and culture-based methods to identify and quantify significant microbiological hazards and characterize *Salmonella* spp. associated with broiler carcasses and processing environment at live bird markets in the Greater Accra Region of Ghana.

Samples (148) comprising of 60 broiler carcasses from LBM, supermarkets and cottage farms, and 33 bench-top swabs, 33 faecal samples and 22 rinse water samples from LBM were assessed for Aerobic Plate Count (APC), *Staphylococcus aureus*, *E. coli* and prevalence of *Salmonella*, *Campylobacter* and *E. coli* O157:H7 using standard culture-based methods. Presumptive *Salmonella, Campylobacter* and *E. coli* O157:H7 were confirmed with Oxoid Microbact. *Salmonella* isolates were further confirmed using *Salmonella ompC* gene amplification by conventional PCR. Traditional serological test was used in the determination of the serotypes of the confirmed *Salmonella* isolates. Biofilm formation ability of *Salmonella* isolates were determined using 96-well-plate-crystal violet assay. Lethal and sub-lethal concentrations of biocides were determined against *Salmonella* using the tube dilution method. Antimicrobial resistance (AMR) of *Salmonella* against 14 antibiotics was determined using disc diffusion assay and EUCAST breakpoints. Prevalence data for categorical variables were presented as frequencies and percentages of occurrences whereas One-way ANOVA was employed to test the significant difference between the means of the dependent (retail outlets,

serovars, environmental samples and biocide concentrations) and independent variables (log_{10}CFU, log_{10}CFU reduction, pH, and AMR breakpoints)

The mean microbial load (aerobic plate count, *S. aureus* and *Salmonellae*) on chicken carcasses from the live bird markets were significantly higher (P=0.0000) than those from the supermarkets and cottage farms except for *E. coli* counts, which was significant higher (P=0.0000) in the chicken samples from the cottage farms as compared to those from the supermarket and the live bird market. A total of 61% *Salmonella* prevalence was recorded for the chicken carcasses. The prevalence of *Salmonella* was also relatively higher in live bird market samples although none of the retail outlets was compliant with GSA standards. Nine non-typhoidal (*S.* Typhimurium, *S.* Enteritidis, *S.* Newport, *S.* Senftenberg, *S.* Agona. *S.* Infantis, *S.* Mississippi, *S.* Westhampton *and S.* Adelaide) and one typhoidal (*S.* Paratyphi B) *Salmonella* serotypes were identified from the carcass, faecal matter, bench surfaces and rinse water samples. The *Salmonella* serotypes isolated exhibited moderate to strong biofilm forming ability hence frequent and effective cleaning is required to prevent the formation of these biofilms. Biocide concentrations of 5% NaCl, 0.5% KOH, 0.5% Acetic acid, 3% Citric Acid and 1% H_2O_2 were able to cause an average of 4 log_{10}CFU/ml reduction. Most (93%) of the isolates were multidrug resistant; showing resistance against 3 to 9 antibiotics. Especially towards the antibiotics commonly administered to the birds for prophylactic purposes such as amoxicillin-clavulanic, oxacillin, erythromycin acid and tetracycline. Some of the *Salmonella* isolates recorded resistance against 3rd generation cephalosporins and fluoroquinolones prescribed for the treatment of invasive non-typhoidal *Salmonella* (iNTS) and typhoidal *Salmonella* (TS) infections in humans. There is therefore the need for regulators to ensure the implementation of good agricultural and hygienic practices coupled with food safety awareness training for both processors and consumers of the chicken.

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LIST OF ABBREVIATIONS

LBM – Live bird markets

APC – Aerobic plate count

PCR – Polymerase chain reaction

AMR – Antimicrobial resistance

EUCAST – European committee on antimicrobial susceptibility testing

HACCP – Hazard analysis and critical control point systems

ANOVA – Analysis of variance

GSA – Ghana standard authority

NTS – Non typhoidal *Salmonella*

iNTS – Invasive non typhoidal *Salmonella*

TS – Typhoidal *Salmonella*

MDR – Multidrug resistance

WHO – World health organisation

CDC – Centre for disease control and prevention

FAO – Food and agriculture organisation

VSD – Veterinary service directorate

USDA – United States department of agriculture

GAIN – Global agriculture information network

SRID-MoFA – Statistics research and information – Ministry of food and agriculture

PTFE – Polytetrafluoroethylene

MoH – Ministry of health

NAP – National action plan

MIC – Minimum inhibitory concentration

ISO – International organisation for standards

XLD – Xylose lysine deoxycholate

RV – Rappaport Vassiliadis

BHI – Brain heart infusion

CFU – Colony forming units

DNA – Deoxyribonucleic acid

CV – Crystal Violet

OD – Optical density

FSA – Food standards agency

FSAI – Food safety authority of Ireland

EFSA – European food safety authority

FSC – food safety criterion

GHP – Good hygienic practices

ICMSF – International commission on microbiological specifications for food

CHAPTER 1

1.0 INTRODUCTION

1.1 Background

Food safety awareness continues to be a major problem in informal food supply chains around the globe, especially in developing countries. In Ghana, as is typical of many developing countries, factors including lack of food safety awareness, education of processors and poor living standards translate into poor personal hygiene and poor food handling practices (Amanor-Boadu *et al.*, 2016; Odeyemi *et al*., 2019; WHO, 2015). Moreover, informal market structures make enforcement of food safety regulation difficult, which often results in the introduction of hazards into the food supply chain (Roesel & Grace, 2015).

Diarrhoea related diseases form more than half of the global burden of foodborne diseases resulting in over 500 million people reporting ill and an average of 230,000 people dying every year (WHO, 2015). WHO (2015) reported that norovirus, *Campylobacter* spp., non-typhoidal *Salmonella* spp. and pathogenic *E. coli* are the leading etiological agents of diarrhoea related diseases. These organisms are often associated with food products such as dairy, raw or undercooked meat and eggs.

Epidemiological studies also suggest that poultry products such as chicken and eggs are important vehicles for transmission of *Campylobacter* spp. and *Salmonella* spp., which are also the top two microbiological hazards associated with food (Gonçalves-Tenório *et al.*, 2018; Harb *et al*., 2018: WHO, 2015). Additionally, the European Food Safety Authority (EFSA) estimates that 20% to 30% and 50% to 80% of human cases of campylobacteriosis and salmonellosis respectively, occur in Europe as a result of handling or consumption of broiler meat (Gonçalves-Tenório *et al.*, 2018).

In Ghana, broilers are reared using intensive and semi-intensive systems on industrial, small and medium scale (Sonaiya & Swan, 2004). Some of these farmers also process and sell the dressed chicken at the farm gate or to supermarkets for retail as fresh cut chicken. However, the majority of the birds end up at live bird markets where they are either sold live or slaughtered and dressed on demand for clients (Amanor-Boadu, *et al.*, 2016; Ovai *et al.*, 2019). Many of these farms and live bird traders are small to medium scale and are unregistered, thus making it difficult for regulatory agencies to monitor their activities.

During primary production, unregulated doses of antibiotics are administered to the broilers to reduce infections and mortality, to prevent losses. Some studies suggest that the extensive use of antibiotics for therapeutic and prophylactic purposes in both humans and animals (in this case the birds) is a major contributor to the increased risk of the multidrug resistance of dangerous pathogens such as *Salmonella* spp. and *Campylobacter* spp. (Akbar & Anal, 2013; Tang *et al*., 2017).

Globally, *Salmonella* and *Campylobacter* are significant risk factors for chicken (WHO, 2007). Studies have shown that contamination of the broiler meat with *Salmonella* spp. can occur in day-old chicks, on the farm, during transportation, during dressing (defeathering, evisceration, washing, cutting), storage, meal preparation or could persist even to the point of consumption, depending on how the meal is prepared (Odeyemi & Bamidele, 2016; Stratev *et al.*, 2017).

In recent times, amoxicillin-clavulanic acid, ciprofloxacin, and extended-spectrum cephalosporins are the most commonly prescribed antibiotics for treating invasive *Salmonella* infections in humans due to the steady increase in antimicrobial resistance. The increasing rate of multidrug resistance (MDR) of *Salmonella* and *Campylobacter* is an emerging global public health problem (Akbar & Anal, 2013; Harb *et al*., 2018).

According to Chmielewski & Frank, (2003), given the right conditions such as the wet operations of poultry processing establishments, pathogens such as *Salmonella* spp. are able to survive, proliferate and even form biofilms which consequently lead to cross-contamination. Effective and regular cleaning in food processing facilities with the appropriate concentrations of sanitizers and disinfectants is required to reduce the risk of spreading MDR pathogens.

1.2 Rationale

Broiler meat in Ghana is traded predominantly in informal live bird markets by unlicensed traders and processors. The activities of these LBM operators are marginally regulated hence do not entirely conform to health and safety regulations (Ovai *et al*, 2019). This increases the risk of microbiological hazards associated with broiler meat. There is a lot of information on the microbiological quality of poultry and poultry product. However, there is limited data on the prevalence of microbiological hazards such as *Campylobacter*, *E.coli O157:H7* and *Salmonella* spp. at live bird markets in Ghana. *Salmonella* is said to be the highest contributor of poultry related foodborne illnesses (Zhu *et al*., 2017). Even so, studies that assessed prevalence of *Salmonella* rarely determine their concentrations which makes it difficult to quantify the risks and assess its impact on public health. This study, therefore, seeks to identify and quantify significant microbiological hazards associated with chicken processing at informal live bird markets in the Greater Accra region, Ghana. As well as evaluate and characterize *Salmonella* spp. based on their serotypes, biofilm formation ability, biocide tolerance, and antimicrobial resistance profile. The outcome of this research will provide data to help develop implementable interventions to make poultry meat safe for the final consumer.

1.3 Main Research Objective

To use molecular and culture-based methods to identify and quantify significant microbiological hazards and characterize *Salmonella* spp. associated with chicken processing at live bird markets in the Greater Accra region, Ghana.

1.3.1 Specific Objectives

1. To determine the prevalence of *Salmonella* spp., *E. coli* O157:H7 and *Campylobacter* spp. and the concentrations of microbiological hygiene indicators on informally processed fresh chicken carcasses and their processing environments from live bird processing operations (live bird markets, cottage farms and supermarkets) in Accra, Ghana.
2. To phenotypically and molecularly characterize *Salmonella* species using serological testing and *ompC* gene amplification respectively.
3. To characterize the antimicrobial susceptibility, biocide tolerance and biofilm formation ability of *Salmonella* species isolated from fresh chicken carcasses and their processing environments.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 The Global Burden of Food Safety.

Food safety is defined by Kiilholma (2007), as a system that safeguards food product from being hazardous to human health. Hazardous as used in this context refers to any physical, chemical or biological entity that possesses the potential to cause harm (FAO, 1998).

Food safety and quality continues to be a major problem across the globe in light of the constant discovery and reoccurrence of foodborne pathogens and other food-related hazards (WHO, 2015). According to the World Health Organisation (WHO, 2015), about 1 in 10 people fall victim to foodborne illnesses globally. Stratev *et al*. (2017), also reported that even in the face of numerous research and interventions towards food safety, an estimated 91 million people contract foodborne illnesses each year in underdeveloped and developing countries. Incidence of foodborne illnesses are more prevalent in such countries due to poor infrastructure, poor hygienic practices, lack of potable drinking water, contaminated and inappropriate food storage facilities and lack of food safety awareness (Odeyemi *et al.,* 2019). Other major contributors to the burden of foodborne illnesses include food handlers and consumers. Their food safety knowledge translates into how food is processed, handled after purchase, transported, prepared and even consumed (Sani & Siow, 2014).

Cases of foodborne illnesses are rarely reported formally to the hospital or public health facilities for treatment in developing countries. Accurate reports on the estimated burden and cost of foodborne illnesses are thus complicated and extremely difficult to obtain. To add to that, the WHO (2008) argues that not all cases of gastroenteritis are foodborne. Hence, the burden of foodborne diseases estimated by governments or research organisations with data available is most certainly underestimated.

2.1.1 Food Safety Risk Factors Associated with Poultry

There are three major food safety risk factors of concern to poultry production and consumption. The first includes microbiological hazards such as *Salmonella* spp., *E. coli* O157, and *Campylobacter* spp. The second set of food safety risks are chemical contaminants resulting from residues of natural toxins or environmental pollutants, pesticides, and veterinary medication excessively administered to the animals during production. Finally, physical hazards in poultry meat such as bones or foreign objects constitute the third set of food safety risk factors (FAO, 1998).

2.1.1.1 Microbiological Risk Factors

Broiler meat like all other forms of meat are extremely perishable and provide a conducive atmosphere for the growth of microorganisms (Dave & Ghaly, 2011; Jørgensen *et al*., 2002). Microbiological risk factors can be found in all poultry production systems and include pathogenic bacteria such as *Campylobacter* spp., *Listeria monocytogenes*, *Enterococci*, diarrhoeagenic *E. coli* and *Salmonella* spp. which are of public health interest. (Kiilholma, 2007).

There have been several studies that have focused on investigating the prevalence of pathogenic microorganisms in poultry meat; many of which have reported that *Salmonella* spp. and *Campylobacter* spp. were the most prevalent (Adzitey *et al*., 2015; Akbar & Anal, 2013; Carrique-Mas & Bryant, 2013; Henry, Reichardt *et al*., 2011; Jørgensen *et al*., 2002; Li *et al*., 2018; Rouger *et al*., 2017; Yao, 2015; Zwe *et al*., 2018). A weekly report on morbidity and mortality by the United States Centre for Disease Control and Prevention (CDC)'s Foodborne Diseases Active Surveillance Network (FoodNet), also cited Salmonellosis and Campylobacteriosis as the most frequently reported cases of foodborne illnesses in USA in

2016. Other reports included Shiga-toxin-producing *E.coli*, *Shigella*, *Cryptosporidium*, *Listeria*, *Vibrio*, *Yersinia and Cyclospora* mostly associated with the consumption of poultry meat and products (CDC, 2017).

2.1.1.1.1 *Salmonella spp.*

Salmonella spp. are Gram-negative, facultative anaerobic bacilli with flagella and mobility. It has about 2579 serotypes (Lamas *et al*., 2018). Serotypes are primarily specific variations within the species of the bacteria. In the case *Salmonella* spp. the determination of these serovars or serotypes is based on the differences in the flagella H antigen, the somatic O antigen and the phase-shift in the H antigen (Meneses-Gonzalez, 2010). This discovery was made by White and Kauffmann in the early 1900s. Serotyping makes it easy to understand the epidemiology of *Salmonella* infections hence makes it possible to track the source of contamination during an outbreak and the severity of the disease (Meneses-Gonzalez, 2010).

The most common and most studied *Salmonella* serotypes are those pathogenic to human beings. Serotypes such as Typhimurium, Enteritidis, Infantis, Typhi, Paratyphi A, B, and C are mostly studied because of their involvement in typhoidal and non-typhoidal outbreaks around the globe (Gal-Mor *et al.*, 2014). Immune response to *Salmonella* is dependent on the type of host and the serotype of *Salmonella* (Revolledo, 2012; van Immerseel *et al*., 2005). The route of *Salmonella* infection to poultry and other animals is oral. *Salmonella* spp. is not part of the original microbiota of poultry, however, they are ingested and readily colonize the gut. They tend to localize in the cecal tonsils and can also occur in the upper part of the small intestines and up to the gizzard and proventriculus (Revolledo, 2012).

Non-host specific *Salmonella* in poultry is mostly involved in food poisoning in humans. *Salmonella* spp. are known to be responsible for a range of clinical syndromes which include

typhoid fever, gastroenteritis, and bacteraemia. *Salmonella* infections may be severe especially amongst infants and immunocompromised persons (Kasturi & Drgon, 2017; Zhu *et al*., 2017; Zwe *et al*., 2018). According to the Centre for Disease Control and prevention (2015), *Salmonella* is responsible for over 1 million deaths in the United States. One of the most common foodborne illnesses is non-typhoidal salmonellosis caused by non-typhoidal *Salmonella* spp. (Kasturi & Drgon, 2017; Zhu *et al*., 2017). The most common vehicles for *Salmonella* infections are animal source products, such as poultry meat and eggs (Greig & Ravel, 2009). Concerns of the safety of poultry meat and product are constantly increasing owing to *Salmonella's* ability to reside in healthy chicken on the farm without any adverse repercussion on the health of the bird. *Salmonella* may, therefore, spread from the farm through to processing unnoticed thus increasing the risk of salmonellosis with the consumption of chicken and other poultry products (de Jong *et al*., 2014).

It is, therefore, an implausible to attempt eradicating *Salmonella* from poultry flock and their environment. However, good hygienic practices, on the farm and in the slaughterhouses are key to the reduction of cases of zoonosis (van Immerseel *et al*., 2005). Controlling *Salmonella* among broilers, however, depends on the knowledge of the source of infection. The feed, drinking water, littering material in coops, and the environment both in and out of the broiler house are all considered possible sources of *Salmonella* and much other bacterial contamination (FAO/WHO, 2009; Kiilholma, 2007).

Knowledge on the number and types of microorganisms found within the litter and feed are still not definitive, which shows that control strategies are poorly investigated. However, there have been a number of studies that have focused on the differences in the prevalence of *Salmonella* spp. at various stages of processing including, evisceration, scalding, chilling with or without additives (Dolberg, 2008; FAO/WHO, 2009; Mead, 2004; Ventura da Silva, 2013). In 2009, a review of scientific data on the occurrence and control of *Salmonella* and

Campylobacter found that, the apparent differences in reported prevalence levels was as a result of differences in the technique of sample collection as well as the methods of analysis (FAO/WHO, 2009).

2.1.1.1.2 *Campylobacter spp.*

Campylobacter is one of the most commonly diagnosed pathogens known to cause foodborne illness such as nausea, gastrointestinal pain, and diarrhoea (Kiilholma, 2007). In extreme and rare cases, *Campylobacter* can cause Guillain-Barre syndrome which is an immunological failure resulting in damage to part of the peripheral nervous system. The two most commonly diagnosed species of *Campylobacter* in human are *C. jejuni* and more rarely *C. coli*. The primary harbourage of *Campylobacter* is said to be in the alimentary canal of mammals and birds. The levels of *Campylobacter* spp. within the guts of these animals can be as high as 10^9 (Henry *et al.*, 2011; Ma *et al.*, 2014). According to Ma *et al.* (2014), the major route of Campylobacter infection is through the handling and consumption of *Campylobacter*-contaminated poultry meat, particularly chicken. Broiler chicken is considered one of the most susceptible animals to *Campylobacter* spp. and a common source for human *Campylobacter* spp. infections. Since studies have established that *Campylobacter* primarily resides in the gut of bird, the only means the carcass gets contaminated is if there is a leakage of gut/ faecal matter onto the meat. Literature has however reported differences in the levels of carcass contamination with regards to the source of purchase (Ma *et al.*, 2014; Reich *et al*., 2018; Vinueza-Burgos *et al*., 2018).

Epidemiological data and resources for the control of *Campylobacter* related zoonosis is lacking especially in developing countries. According to the WHO, *Campylobacter* is responsible for 37,600 deaths globally every year. Given the relatively low infective dose of *Campylobacter* spp. (≥500 cells) and the high consumption of poultry, the presence of these

organisms in poultry and poultry products raises very serious public health concerns (Vinueza-Burgos *et al*., 2018).

2.1.1.1.3 *Escherichia coli*

Escherichia coli is the most dynamic and one of the most studied bacteria in recent times. *E. coli* is a gram-negative bacterium and also a member of the family Enterobacteriaceae that is primarily known to be natural commensals in the digestive tracts of humans and animals, including birds (Abo-Amer *et al*., 2018). However, there are some strains of *E. coli* such as O157:H7, O103:H25, O111, and O26 that are of significant health concerns due to their intestinal and extra-intestinal pathogenicity. These pathogenic *E.coli* are often responsible for a wide range of infections spanning from self-limiting gastrointestinal infection, cramps, bloody diarrhoea to bacteraemia (Abo-Amer *et al*., 2018; Adu-Gyamfi *et al*., 2012; Hunt, 2010).

The avian gut is considered to be a reservoir of these organisms which can be transmitted when there is direct contact with their excrement or when the carcass is contaminated with faecal matter (Abo-Amer *et al*., 2018). The carcass of the bird is generally exposed to an increased potential of contamination at every step of processing, from within and outside of the animal. The presence of some organisms such as *E.coli*, therefore, reflects the hygienic condition under which the processing of the animal was carried out (Adu-Gyamfi *et al*., 2012).

2.1.1.1.4 *Staphylococcus aureus*

Staphylococcus aureus is described as an opportunistic pathogen that infects both humans and animals. *S. aureus* is primarily associated with food poisoning. It is also known as the third largest cause of foodborne illness following *Salmonella* spp. and *E. coli* (Akbar & Anal, 2013;

Ali *et al*., 2017). These organisms are broadly distributed in nature; they can be found in the air, water, feed, and even on the skin of humans. They are also known to be responsible for a wide variety of infectious diseases spanning from soft tissue infections, pneumonia, arthritis, mastitis, sepsis, and tissue and skin infections (Akbar & Anal, 2013; Li *et al*., 2018).

Generally, the presence of *S. aureus* on foods including retailed broiler chicken carcasses is as a result of poor handling practices. The prevalence levels of *S. aureus* in chicken meat ranges from 17.8% to 68% with a load of 10^2 to 10^4 CFU/g (Bortolaia *et al*, 2016).

2.2 The Ghanaian Poultry Industry

The term poultry generally comprises chicken, ducks, ostriches, quails, geese, turkeys, and guinea fowls. However, in Ghana poultry is synonymous with chicken because of chicken's dominance in the poultry sector (Amanor-Boadu *et al*., 2016). The poultry industry since the 1960s to date has gained a great deal of interest all around the world due to the increasing demand for poultry meat and products. This increase in demand of poultry products could be attributed to the rapid cultural integration, low prices and the nutritional characteristics of the product (Anang, Yeboah, & Agbolosu, 2013; Opoku-Mensah, Asare-Kyere, & Mensah, 2014).

The Ghanaian poultry industry also saw a massive boost in the 1960s when the government of Ghana gave incentives for the production of poultry which resulted in the crop up of poultry-related income generating ventures such as poultry slaughter, increased number of live-bird markets, and the market for eggs, within the country (Buamah, 1992). In recent years, the production of poultry within Ghana has realised a sharp decline from 80% of the market demand to 10%. Its growth has been fraught with several challenges which include the high cost of medication, expensive feed and competition with cheaper frozen chicken imported from other parts of the world (Anang *et al*., 2013; Schneider *et al*., 2010). There have been attempts

in the past to salvage the situation by imposing 20% taxes on all poultry imports to protect the local industry but compliance was very low. The local industry still continues to struggle with market share as the competition from imports remain a great threat to local poultry businesses (Banson *et al.*, 2015).

2.2.1 Primary Production and Commercialization of Poultry and Poultry Products

In an FAO review report, Ghanaian commercial poultry production has been categorised into large (> 10,000 birds) medium (5000 to 10,000 birds) and small scale , (<5000 birds) poultry businesses (Aning. 2006). The Ghanaian commercial poultry production has also been further categorized into formal and informal. However, poultry production is dominated by the informal sector making up about 80% of the total population (Kusi *et al*., 2015). Amanor-Boadu *et al.* (2016), also reported that the majority (58.1%) of commercial poultry farms are located in rural areas. Considering the resource investment (feed, medication, potable water, electricity, transportation, and market access) required in the production of poultry, production in the rural area would cost more.

Production of broiler chicken in Ghana has been reported to be expensive and not profitable. Close to 70% of the cost involved in a production is usually spent on expensive feed and medication (Aning, 2006; USDA-GAIN, 2017). This has led to farmers raising more layers compared to broilers as there is no competition with external market participants for chicken eggs as there is for chicken meat. The production of layers is not only profitable but also helps reduce dependency on imported eggs (USDA-GAIN, 2017).

Majority of broilers and layers traded on the Ghanaian live bird markets are sourced from both commercial and backyard/cottage system of raising poultry(Ovai *et al*., 2019).

2.2.2 Consumption of Poultry and Poultry Product

Poultry and poultry products are traded predominantly in informal markets where there is minimal enforcement of health and safety regulations. (I.e. markets where the majority of traders are not licensed). These include backyard poultry farms, live bird markets or wet market, and street food vendors.

Consumption of chicken continues to rise both within and outside Ghana. In 2017, poultry consumption was estimated to have gone up from 177,000 tonnes in 2016 to 193,000 tonnes in 2017 (USDA-GAIN, 2017). The consumption rates in urban areas within the country have doubled hence increasing the demand for less expensive imported frozen pre-cut chicken. It was also reported that there was a 10% increase in the importation of broiler meat to make up for the deficit created by the local industry's inability to meet with the high demand (USDA-GAIN, 2017). Chicken meat is generally accepted across religious groups and across cultures thus accounts for the rising consumption pattern worldwide because it is one of the cheapest sources of animal protein (Opoku-Mensah *et al*., 2014).

2.2.3 Poultry Processing Operations

The increasing demand for poultry meat has led to the introduction of automated processing lines which allows processors to process more birds up to about 13000 birds an hour. New and more sophisticated technologies are continually being introduced to simplify the processing of birds. However, informal processors in Ghana are not privileged with such infrastructure and the equipment to keep up with their competitors. (Fletcher, 2004; USDA-GAIN, 2017).

In recent times, plant designs are tailored towards the type of animal going to be processed. In a poultry (chicken) processing plant, the design is set up to include slaughtering, defeathering, evisceration, inspection, chilling and packaging. The structural design might differ slightly

from plant to plant with a few modifications in arrangement and add-ons with regard to unit operations (Barbut, 2002). Barbut (2015), further reported the existence of large variations with regards to the arrangement and techniques of processing which are a product of factors such as equipment, personnel, and target consumers (religious groups). Not only are processing techniques vital to the product quality but they are also critical to the microbial safety of the food product. The quality of processing techniques may increase or decrease the microbial load of the food product (Rouger *et al.*, 2017). Figure 1 is a depiction of the basic unit operations of a typical poultry processing system.

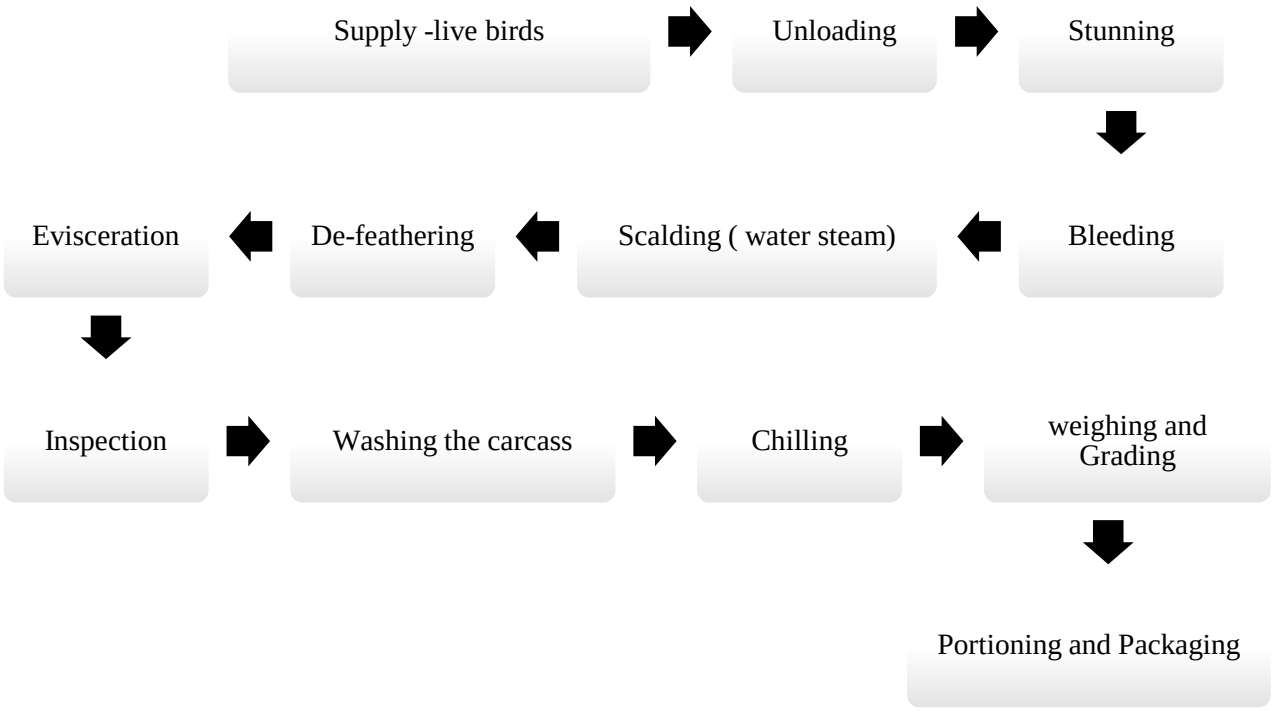

Figure 1. Standard Basic Unit Operation in Poultry Processing- *Adapted from* (Fletcher, 2004)

2.2.3.1 Unloading

In processing plants where the birds are not raised on site, the birds arrive in crates which are offloaded manually. In some sophisticated plants, automated unloading systems are used (Barbut, 2002).

2.2.3.2 Stunning

Stunning is a common practice employed to immobilize the birds prior to slaughter. However, in some religious groups, there are some considerations, examples being the Islamic and Jewish laws on animal slaughter known as Halal and Kosher respectively. There are a number of methods employed in stunning which include, electrocution or electrical stunning and controlled atmosphere stunning (requires the use of gases such as CO_2, argon or a mixture of gases to render the bird immobile). Stunning makes handling easier and further reduces bruising on the birds (Barbut, 2015).

2.2.3.3 Bleeding

In the bleeding process, a major blood vessel is opened to allow the bird bleed out. This usually takes about 2 to 5 minutes depending on the size and type of the bird (Barbut, 2015).

2.2.3.4 Scalding and Defeathering

Scalding is an important step employed to help loosen the feathers for defeathering. Traditionally, hot water scalding was used till recent times when the use of steam scalding has gained popularity. Three types of scalding commonly employed include:

a) Soft/ semi-scalding: often done for broilers and young turkeys for 1 to 3 min at 50℃–53℃
b) Sub/ medium scalding is done for matured birds at 54℃–58℃, also for 1 to 2 mins
c) Hard scalding is usually for hard skin birds and this is done at 59° C–61° C for up to 1.5minutes (Barbut, 2015).

The temperature-time heat treatment applied during scalding is dependent on the hardness of the skin and the need for efficient removal of the feathers. When the temperature is too low, the hair will not be able to loosen, conversely, when the water is too hot, the skin will cook and make it extra difficult to remove the feathers damaging to the muscle integrity (Irshad & Arun, 2013).

The temperature-time combination is also important in terms of food safety. How hot the scalding water is, reflects on the microbial load on the meat, and increases or decreases the tendencies for cross-contamination (Irshad & Arun, 2013). According to FSA (2016), the most critical steps where the meat is most vulnerable to contamination are scalding, defeathering, and evisceration. During traditional scalding and defeathering, the carcasses come in contact with other carcasses, processing equipments or bench surfaces, and processors where both pathogenic and non-pathogenic microorganisms get introduced to the meat (Fletcher, 2004; FSA, 2016; Irshad & Arun, 2013). A gram of dirt or faecal matter that may be attached to the feather of the bird is usually made of 10^8 to 10^9 CFU/g. It is thus important to minimise cross-contamination by keeping the scalding tank clean (Barbut, 2015).

2.2.3.5 Evisceration

This process is either done manually with a knife or scissors, semi-automatically or done using fully automated systems. Evisceration requires precision and careful removal of the viscera

from the body cavity. A piercing in the viscera can result in the spillage of the gut matter onto the meat, therefore exposing the meat to the high microbial content of the gut (Althaus, Zweifel, & Stephan, 2017; Barbut, 2015; Irshad & Arun, 2013). According to Barbut (2015), One gram of gut content contains about 10^9 bacteria. In some places, when there is a spillage, the contaminated part is rendered unfit and immediately discarded whereas, in some other places, the contaminated portions are trimmed off or washed.

2.2.3.6 Inspection

Inspection is often done after evisceration when all the parts are totally exposed. The exposed parts can give indications of sicknesses or any defect with the birds (Barbut, 2015). The inspections are usually carried out by an official from a government agency, preferably a veterinarian. This is to make sure only wholesome birds get to the markets for the purchase and subsequently consumption. Inspection requirements differ from country to country. It is, however, important to note that some countries do not have the requirement for such inspections (Barbut, 2015).

2.2.3.7 Chilling

Chilling is one of the most important unit operations in poultry processing. It is a requirement in most countries to chill and maintain a cold chain (4^{o}C) to reduce microbial proliferation. In some poultry processing plants, chilling is done prior to deboning whereas others do hot-deboning which is done before chilling. Chilling is done either by air chilling, cold water immersion (the carcass is immersed into cold water (<4^{o}C), spray chilling (sporadic spraying of water) or a combination of the mentioned techniques (Barbut, 2015).

2.3 Food Safety and Hygiene within the Value Chain

Activities within every stage of the poultry value chain have their ways of impacting the microflora of the broiler meat. Hence, there is a need to implement the principles of food safety at every stage. Some of the basic food safety principles include good hygienic practices such as cleaning and disinfection, monitoring, equipment design and conformance to food safety regulation. Good hygienic practices are key to the microbiological quality of poultry meat (CAC, 2005; Stratev *et al*., 2017).

The processing environment provides several harbourages for microorganisms and is a major source of contamination. The design and materials used in making working surfaces and processing equipment hinder the cleaning and disinfection thus, increasing the presence of spoilage microorganisms and pathogens. This increases the risk of foodborne illnesses associated with consumption of chicken (CAC, 2005). Food safety risk analysis often takes into consideration all the stages within the value chain i.e. from primary production through processing to consumption (Adonu *et al.*, 2017).

2.3.1 Efficacy of Biocides as Disinfectants

The ability of food-borne pathogens to persist in a food processing facility could result in the contamination of food products. Using cleaning agents or sanitizers that are efficient against microbes minimizes the contamination of food product (Cha *et al.*, 2016). Biocides are primarily chemical compounds that possess the ability to inactivate microorganisms. There are numerous commercial sanitizers and disinfectants on the market with varying active bactericidal agents and varying mechanisms of action. They are largely used in the cleaning of contact surfaces and also used to disinfect farm buildings to create barriers that ensure the restriction of infectious agents into the facility (Condell *et al*., 2012). The use of these biocides

however also comes with some risk. The overdependence and use of these chemical agents are gradually resulting in the emergence of tolerant phenotypes amongst major zoonotic pathogens (Condell *et al*., 2012). Antibiotic resistance as seen in bacterial cells are often as a result of target gene mutation whereas the tolerance of microbial cells occurs as a result of stress response mechanisms such as biocide efflux or changes in cell envelope permeability (Mavri & Moz, 2019).

The efficacy of all biocides is dependent on the concentration of the active ingredient, contact time and the target organism. All biocides have their minimum concentrations at which point they induce inhibition or reduce cell load. Some biocides tend to be more potent than others hence they are applied differently considering their minimum inhibitory concentration. It is also advised that cleaning is done prior to the use of disinfectant or sanitizers to get rid of biofilms formed in order to expose pathogens which allow for adequate disinfection (Jeffrey, 2007; Schmidt, 2018).

2.3.2 Biofilm Formation Ability

Microorganisms have always been described as planktons; free suspended cells, till the discovery by Antonie van Leeuwenhoek in the late 17th century where he first described the possibility of microorganisms to attach themselves and grow on an exposed surface. This discovery led to the study of biofilm formation abilities of various organisms which revealed that biofilms exhibited distinctive phenotypic characteristics (Merino *et al.*, 2018).

Biofilms are therefore defined as a community of surface-attached microbial cells surrounded by a self-produced extracellular matrix. This makes them extremely difficult to deal with as compared with their planktonic counterpart. As biofilms, they are able to easily exchange genetic information such as transfer of virulence genes and are able to resist biocides or

antimicrobial treatment more than they would have if they were pure free suspended cells (Iniguez-Moreno *et al.*, 2017; Merino *et al.* 2017; Paytubi *et al.*, 2017).

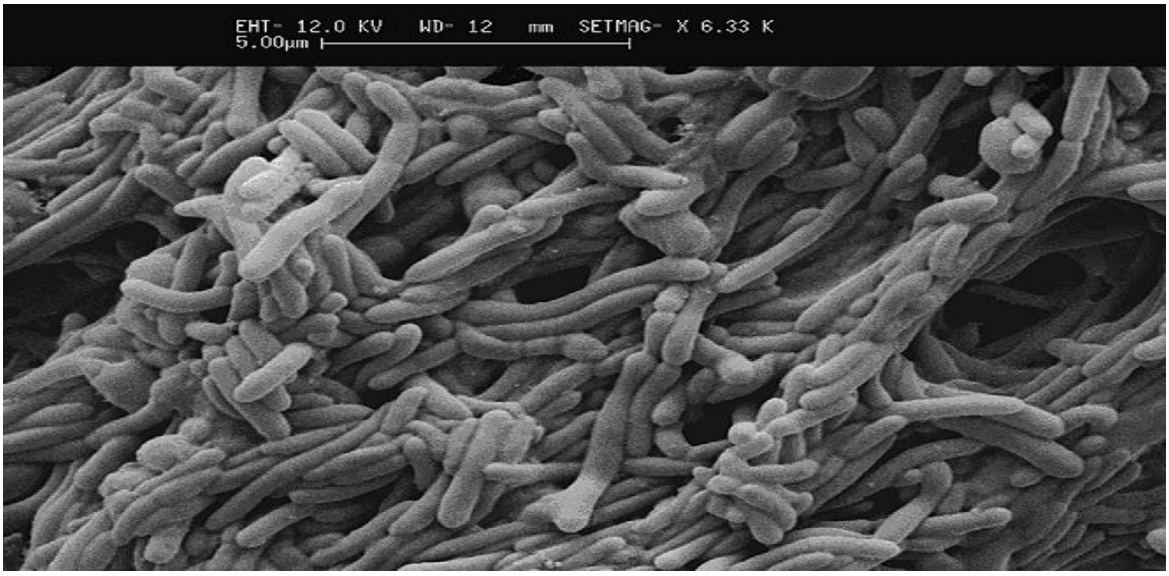

Figure 2. Scanning electron microscopy photomicrograph of a 6 day old *B. cereus* biofilm formed on a stainless steel surface (Simoes *et al.*, 2010).

2.3.2.1 Biofilm Formation Process

There are several proposed mechanisms for biofilm formation. One of the proposed processes governing biofilm formation comprises a series of steps which include (Bryers & Ratner, 2004):

1. Pre-conditioning: The process of microbial attachment is a complex process which begins either with the layering of a surface with macromolecules (such as dust, DNA, RNA) either present in the bulk liquid or already layered on the surface.
2. Transfer of planktonic or suspending cells from the bulk liquid to the surface;
3. Adsorption of cells at the surface;
4. The release of reversibly adsorbed cells;
5. Permanent adsorption of bacterial cells at a surface;
6. Production of a cell to cell signalling molecules;
7. Transport of substrates to and within the biofilm;
8. Substrate metabolism by the biofilm-bound cells and transport of products out of the biofilm. These processes are accompanied by cell growth, replication, and EPS production;

9. Detachment from Biofilm removal by detachment or sloughing

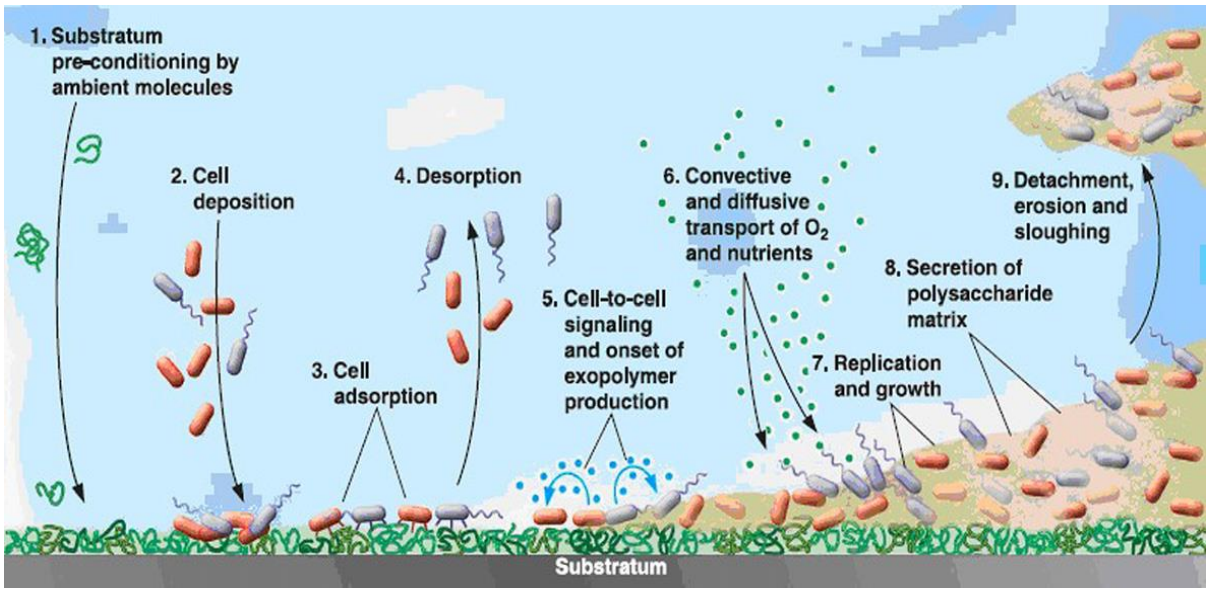

Figure 3. Processes governing biofilm formation (Bryers & Ratner, 2004)

2.3.2.2 Quorum Sensing

Biofilm cells generally have far more local cell densities than their planktonic counterparts. Due to this, there are elevated levels of metabolic by-product, secondary metabolic substances and diverse microbial secretions that biofilm cells are exposed to. Amongst these secretions are intercellular signalling or quorum sensing molecules (Parsek & Greenberg, 2005). Quorum sensing is, therefore, the use of chemical signals by bacteria to monitor the population density of their own species and also to regulate the expression of specific genes in response to population density. This phenomenon is most common amongst Gram negatives although some Gram positives express such characteristics (Hentzer *et al*., 2002).

2.3.2.3 Industrial Importance of Biofilms

Biofilm formation can have different consequences in different environments. Biofilms can practically form everywhere; on toilets seats, sinks, industrial water systems, and on medical devices. Biofilms continue to pose concerns to food manufacturers as they are the major reason

for limiting the run length of manufacturing plants and threatening the quality and safety of products. Biofilm can cause energy loss and blockade of pipes, water reservoirs and cooling towers (Chmielewski & Frank, 2003; Joseph *et al*., 2001; Møretrø *et al.*, 2012).

Contemporary food processing equipment are made with many different materials to which bacteria can attach such as glass, plastics. 316 and 304 stainless steel, rubber and even polytetrafluoroethylene (PTFE). Industry equipment may have large surface areas suitable for swift colonisation. The degree of hydrophobicity of the surface has often been cited as a major determining factor for attachment (Sinde & Carballo, 2000; Teixeire *et al.*, 2005) suggesting that, in aqueous solutions, adhesion is favoured by hydrophobic surfaces. PTFE is the most hydrophobic of the materials mentioned above and is often used as a 'non-stick' coating. Far from being 'non-stick' in relation to microbial adhesion, attachment of *Listeria* and *Salmonella* was found to be high (Sinde & Carballo, 2000).

Operations in a poultry processing establishment are mostly wet thus ideal for biofilm formation. *Salmonella* is isolated from poultry processing equipment and surfaces especially around the slaughter and evisceration points (Chmielewski & Frank, 2003). *Salmonella's* ability to adhere to food surfaces was the first to be reported on the foodborne bacterial biofilm phenomena and published by Duguid *et al.* (1966). The presence of components such as the flagella, fimbriae, and cellulose is necessary for making it possible for *Salmonella* to attach to surfaces easily (Ariafar *et al.*, 2016; Merino *et al.*, 2017). Many strains of *Salmonella* are able to form biofilms on all sorts of materials; plastic to metal sheets or surfaces and glass. *Salmonella* biofilms are able to survive harsh and unfavourable conditions such as the poultry dressing process which involves the use of high temperature (Iniguez-Moreno *et al.*, 2017).

In a food processing establishment, processing practices such as the quality and frequency of cleaning influences the microflora of processing surfaces hence the ability of biofilms to form.

Cleaning is the basic method of controlling biofilms. However, available commercial biocides were not entirely designed with biofilms in mind thus are not very effective and may require more efforts to control them (Joseph *et al.*, 2001; Paytubi *et al*., 2017).

2.4 Antimicrobial Resistance

Antimicrobial resistance of foodborne pathogens has become an important public health concern. Antimicrobial Resistance (AMR) is a situation where microorganisms such as viruses, bacteria, fungi, and some parasites are able to survive lethal doses of an antimicrobial agent, which consequentially results in a situation where standard treatments become ineffective; infections persist and may spread to others (MoH *et al*, 2017).

The upsurge of antimicrobial resistant strains has been associated with the constant use of antibiotics in both humans and animals for therapeutic and prophylactic purposes (Akbar & Anal, 2013; Kiilholma, 2007). In livestock especially, the use of antibiotics as growth promoters is a longstanding practice among agriculturalists and is unquestionably the primary cause of antimicrobial resistance in foodborne or livestock-related pathogens (Kiilholma, 2007; Zwe *et al*., 2018). These antimicrobial agents used in livestock production are administered at sub-lethal concentrations in livestock feed to enhance growth. The use of antibiotics or antimicrobials in general for nontherapeutic purposes has increased enormously. A report from the union of concerned scientists stated that 90% of antimicrobials used in the United States of America were used for nontherapeutic purposes (MoH *et al*, 2017).

In recent years, cases of antimicrobial resistance have been reported across the globe in both developed and developing countries. One of the most recent of such reports was in Singapore where there was an outbreak of salmonellosis from the consumption of meat contaminated with multidrug resistant *Salmonella* (Zwe *et al*., 2018). In Ghana, a study that was done in the

Ashanti region revealed that all sorts of antibiotics including 3rd and 4th generation cephalosporins were excessively used by farmers in the management of infections such as diarrhoea, coughs, and rashes. It was also revealed in the study that the storage and disposal practices for these medicines among the farms in Ghana were unsafe. Farmers misdiagnosed and overdosed in the administration of these antibiotics in the absence of a trained veterinarian. An Antimicrobial Resistance (AMR) National Action Plan (NAP) was therefore launched to help Ghana's desire to fight the emerging antimicrobial resistance in the country (MoH *et al*, 2017).

2.4.1 Multi-drug Resistance in Foodborne Pathogens

The increasingly emerging prevalence of multi-drug resistant *Salmonella*, particularly resistance to fluoroquinolones and third-generation cephalosporins is a major concern worldwide (Akbar & Anal, 2013). In the 1980s, conventional antimicrobial agents such as chloramphenicol, trimethoprim-sulphonamides, and ampicillin became inefficient against infections thus fluoroquinolones and extended-spectrum cephalosporins have been the antimicrobials of choice for severe infections (Akbar & Anal, 2013; de Jong *et al.*, 2014). Resistance to cephalosporin among Enterobacteriaceae is primarily as a result of the production of broad-spectrum b-lactams, such as extended-spectrum b-lactamases (ESBLs) and AmpC b-lactamases. Resistance to fluoroquinolones essentially results from point mutations in the quinolone resistance determining region of gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) genes (de Jong *et al.*, 2014). Efflux pumps are also known to be responsible for antimicrobial resistance in both gram-negative and gram-positive bacteria by way of ejecting the antimicrobial agent through the transmembrane proteins inserted in the cytoplasmic membrane (Ortez, 2005). Within the last decade, the expression of broad-spectrum

β-lactamases has often been confirmed in the microbiota of livestock reared for meat production (de Jong *et al.*, 2014).

Antimicrobial resistance or susceptibility can be measured either by disk diffusion where zones of inhibition determine the efficacy of the antimicrobial agent, or by the use of minimum inhibitory concentration (MIC) (Ortez, 2005). The use of PCR to target resistance-conferring genes are also being explored in recent research (Blaak *et al.*, 2014; de Jong *et al.*, 2014; González-lópez *et al.*, 2014; Phoba *et al.*, 2017).

CHAPTER 3

3.0 METHODOLOGY

3.1 Study Design and Sampling Plan

Information on the numbers and locations of live bird markets in Accra was obtained from the Veterinary Services Directorate (VSD) of the Ministry of Food and Agriculture and the Accra chapter of the National Poultry Association.

Out of 33 live bird markets in Accra, a cluster random sampling method was used to select the study sites. The known live bird markets in the Greater Accra region were divided into 4 clusters (North, South, East, and West). Twelve live bird markets were selected from those clusters for the microbiological survey. In addition to the 12 live bird markets, 4 supermarkets with abattoirs on site, 3 cottage farms and 1 research farm with poultry processing facilities were selected (Figure 4). The capacity and the operations of the research farm was similar to that of the cottage farms. All the live bird markets, supermarkets, and cottage farms were allocated numbers. “Random.org” (Cantu-Paz, 2002) was used to randomize and select 3 live bird markets, 1 supermarket and 1 cottage farm (1) for each of the four clusters.

Three freshly dressed broiler chicken carcasses were purchased from each of the 20 facilities on three different occasions. In all, 60 chicken carcasses were sampled for the microbiological analyses. Eleven out of the 20 live-bird facilities were conveniently selected based on those who allowed for the collection of environmental samples. Facilities that did not allow the collection of the environmental samples (rinse water, bench top swabs and faecal matter samples) were exempted in the environmental sample related analysis. At each live-bird market, environmental samples taken included 1 rinse water sample (≥200ml) after evisceration, 1 faecal matter sample (≥100g) and 3 bench top swabs (10 x 10cm). The 3 swab samples for each facility were however pooled for microbiological hazard assessment.

Replicates of each sample were collected. In all, 22 rinse water samples, 33 faecal matter samples and 33 bench top samples and 60 freshly dressed chicken carcasses were taken for microbiological analysis. This made a total of 148 samples analysed.

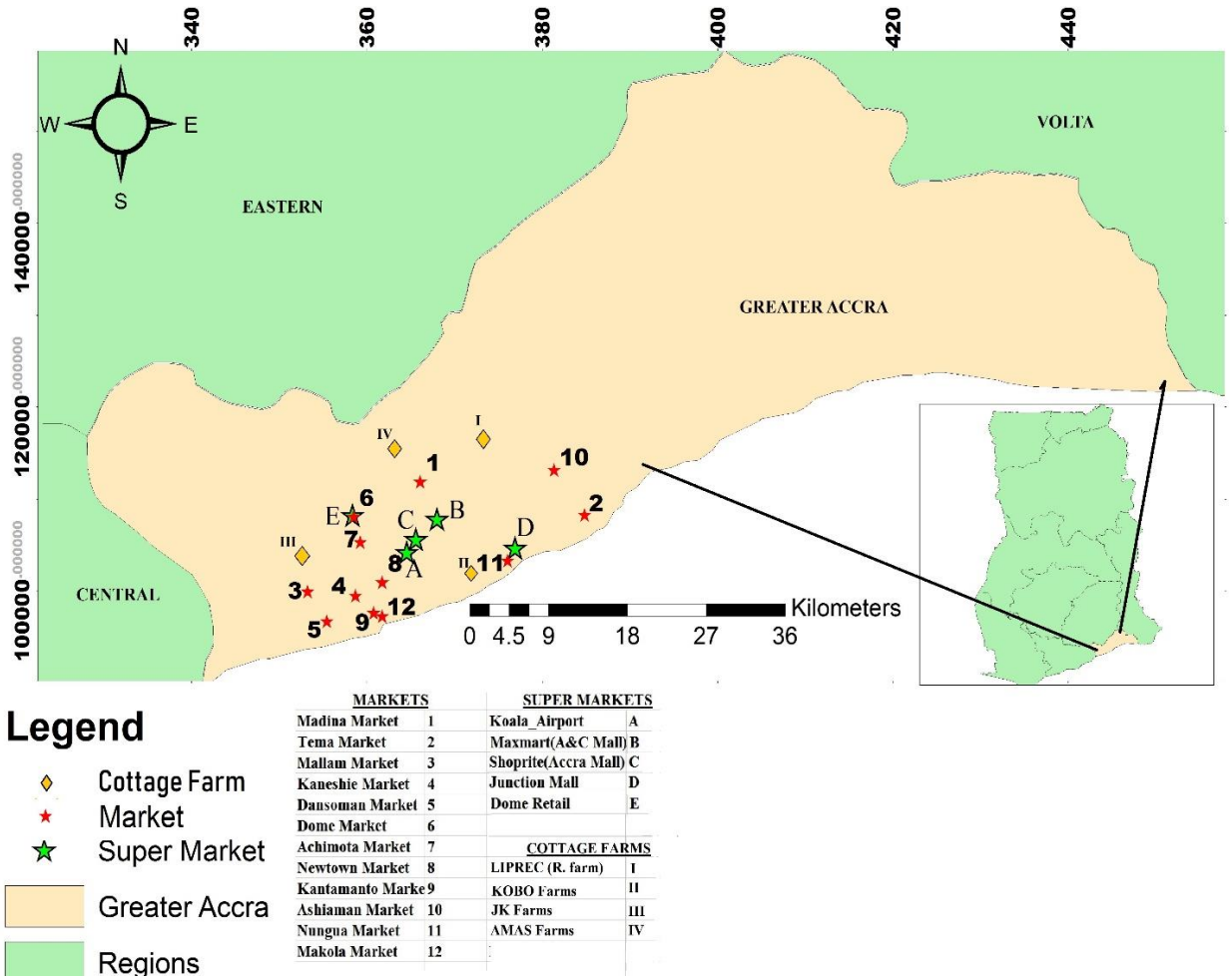

Figure 4. A diagrammatical representation of the study locations within the Greater Accra Region

3.2 Microbiological Analyses

At the live bird markets, live broilers were purchased and dressed at the market and was transported to the Microbiology Research Lab at the Department of Nutrition and Food Science, University of Ghana for microbiological analyses. All chicken samples were dressed individually and kept in separate sterile sample bags which were then placed in a thermal flask

with ice packs to ensure samples were transported chilled (4°C) within the two hrs of sampling. All samples were tested for concentrations of aerobic bacteria, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella*. Samples were also tested for the presence of *Salmonella* spp., *Campylobacter* spp. and *E. coli* O157:H7. All the microbiological protocols were the International Organisation for Standardization (ISO) protocols sourced from Hazeleger & Beumer, 2012.

3.2.1 Enumeration of Aerobic Plate Count

For aerobic plate count, 10g of the representative samples were transferred aseptically to stomacher bags and 90ml of sterile 0.1% peptone water was added. The mixtures were blended in a stomacher blender for 30 seconds. Subsequently, 0.1ml of appropriate serial decimal dilutions were plated on plate count agar (Oxoid, CM0325) using the pour plate technique. Plates were inverted and incubated under aerobic conditions at 37°C for 18 to 48 hrs. Colony counts were recorded as colony forming units per gram (CFU/g) (ISO 4833: 1991(E)) (Tsola *et al.*, 2008).

3.2.2 Enumeration of *E. coli*

To enumerate *E. coli*, 10g of the representative samples were weighed and transferred aseptically to stomacher bags and 90ml of sterile 0.1% peptone water was added. The mixtures were blended in a stomacher blender for 30 seconds. A hundred microliters (100 µl) of appropriate serial decimal dilutions were spread plated onto well-dried MacConkey Agar (Oxoid, CM0007) plates. Plates were incubated at 44°C for 24 to 48 hrs. Pink firm colonies on MacConkey were counted and recorded as presumptive *E. coli* counts. For confirmatory tests, presumptive colonies were sub-cultured by streaking on nutrient agar (Oxoid, CM0003) and

incubated at 37°C for 24 h. Isolated colonies were selected for the following biochemical tests: Triple Sugar Iron, Citrate Utilization, motility in SIM and Kovacs test. Isolates were further confirmed using Microbact 24E microbial identification system. Confirmed *E. coli* isolates were streaked on Sorbitol MacConkey agar (Oxoid) to determine the presence of sorbitol negative *E. coli* O157:H7 (ISO 4832) (Tsola *et al.,* 2008).

3.2.3 Enumeration of *S. aureus*

The concentration of *S. aureus* was determined by transferring 10g of the representative samples aseptically to stomacher bags and 90ml sterile 0.1% peptone water was added. The mixture were blended in a stomacher blender for 30 seconds. One hundred microliters of appropriate serial decimal dilutions were spread plated on well-dried plates of Baird-Parker agar (Oxoid, CM0275) supplemented with egg yolk tellurite (Oxoid, SR0054C) according to manufacturer's instructions. Plates were incubated at 37°C for 24 to 48 hrs. Typical colonies with black or grey, shining, convex morphologies were counted, selected and sub-cultured on nutrient agar (Oxoid, CM0003). Gram staining and catalase tests were performed along with coagulase test using plasma for the confirmation of the isolates (ISO 6888-1) (Kozačinski *et al.,* 2006)

3.2.4 Prevalence of *Campylobacter* spp.

To determine the prevalence of *Campylobacter* spp., 25 g of the representative chicken sample were transferred and homogenized in 225ml of Bolten Broth with Bolten broth supplement (Oxoid, code) and 5% lysed horse blood according to manufacturer's instructions. The pre-enriched cultures were incubated at 37°C for 4h and then at 41.5°C for 48 ± 4h under microaerophilic conditions. The inoculum was streaked on *Campylobacter* Agar base (Oxoid,

CM0689) supplemented with *Campylobacter* selective supplement (SR117) and 5% Laked Horse Blood (Thermo Scientific oxoid, SR0048C) and incubated for 24 to 48hrs at 42ºC under microaerophilic conditions. Grey-brown colonies were recorded as presumptive *Campylobacter* spp. Isolated colonies were streaked on Coloumbia Agar Base (Oxoid, CM0331) with 5% Laked Horse Blood (Thermo Scientific oxoid, SR0048C) and incubated for 24 to 48hrs at 42ºC under microaerophilic conditions. Presumptive cultures were stored in Brain Heart Infusion (BHI) with 20% glycerol for confirmation using Microbact 24E kit (ISO 10272) (Kozačinski *et al.*, 2006).

3.2.5 Prevalence, Identification and Enumeration of *Salmonella* spp.

3.2.5.1 Prevalence of *Salmonella*

Prevalence of *Salmonella* spp. was determined by homogenizing 25 g of chicken samples in 225ml of sterile buffered peptone water, and incubated for 18 to 24hr at 35˚C to recover injured cells. Subsequently, 1ml of the pre-enriched cultures were transferred into Rappaport Vassiliadis (RV) broth and incubated for 24 to 48 hr at 42ºC. A loop full of the enriched culture was streaked onto dried XLD (Oxoid, CM0469) agar plates and incubated for 18 to 24hr at 35ºC. Presumptive positive isolates were expected to be black with reddish surroundings. Isolates were confirmed using Oxoid Microbact 24E microbial identification system. Positive isolates were stored in BHI with 20% glycerol (ISO 6785) (Kozačinski *et al.*, 2006)

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3.2.5.2 Enumeration of *Salmonella* spp.

The concentration of *Salmonella* spp. was determined by transferring 25g of representative samples aseptically to stomacher bags and 225ml sterile buffered peptone water was added. The mixture was blended in a stomacher blender for 30 seconds. 1ml of appropriate serial

decimal dilutions were spread plated on well-dried plates of XLD (Oxoid, CM0469). Black colonies with red backgrounds were counted and recorded in CFU/g (ISO 4833: 1991) (Kozačinski *et al.*, 2006).

3.2.5.3. *Salmonella* Serotyping

Confirmed *Salmonellae* isolates were streaked on Nutrient Agar. Two to three matching isolated colonies were picked from each sample plate and suspended in 0.5ml of 0.85% saline. The suspension was mixed until homogeneous. A glass slide was divided into two with a "*Greek crayon"* to ensure there is no spillage. Two drops each of the homogeneous mixture was placed on both sides on the glass slide. A drop of the polyvalent O antiserum (Denka Seiken Co., LTD, Tokyo) was placed on the first sample whereas polyvalent O1 antiserum Denka Seiken Co., LTD, Tokyo) was dropped on the other. The reagent and the sample were mixed by tilting it back and forth for about 1 to 2 mins. Positive samples showed distinct agglutination whereas samples that did not show any form of agglutination within 3 mins were considered negative. Those that were positive to either of the polyvalent were further tested against their monovalent antisera in that group. The O-antigen formulae were then matched to the list on the Kauffman-White Scheme to determine the serotype (Grimont & Weill, 2007).

3.2.5.4 DNA Extraction

Salmonella spp. DNA extraction was carried out as described by Soumet *et al*. (1994) with modifications. Organisms were cultured in Muller Hinton Broth for 24hrs at 37ºC. Cultures were homogenized using a vortex. 1ml of each culture was pipetted into 1.5ml Eppendorf tubes and centrifuged at 13000rpm for 5mins. The resulting pellets were washed twice with 500µl 1x TAE buffer (pH: 8.0). The pellets were re-suspended in 200ml of sterile nuclease-free water.

The tubes were placed in a water bath to boil at 100^{o}C for 8mins to get the DNA. The DNA extracts were stored at -20^{o}C.

3.2.5.5. Amplification of the ompC gene

Amplifications were carried out in 25µl reaction volumes containing; 12.5µl of one Taq Quick-Load 2x Master Mix with standard buffer (New England Biolabs), 7.5µl of nuclease-free water, 1.25µl of each primer {Forward primer ompC-F (5′- ATC GCT GAC TTA TGC AAT CG -3′) and reverse primer; ompC-R (5′- CGG GTT GCG TTA TAG GTC TG -3′) – 204*bp*} and 2.5µl of the extracted bacterial DNA. The amplifications were done in 40 cycles with an initial denaturation step at 95 °C for 5 min, a denaturation step of 94 °C for 30s, primer annealing at 55°C for 1min and primer extension at 72°C for 3 minutes. Finally, an additional extension was done for 10 mins at 72°C. Five *µl* of the total reaction mixture was loaded on a 1.5% agarose gel stained with ethidium bromide and electrophoresed at 100 *V* at 70 *mA* for 60 *min*. The images for the amplified DNA bands were visualized by UV illumination after agarose gel electrophoresis (Jawad & Al-charrakh, 2016).

3.2.6 Statistical Analysis for Microbiological Data

Prevalence of *Salmonella* spp., *E. coli* O157:H7 and *Campylobacter* spp. were presented as frequencies of occurrences using Microsoft Office Excel 2013. A one-way Analysis of Variance (ANOVA) was employed to determine significant differences in the log CFU/g of hygiene indicators between the different groups (LBM, Cottage, and Supermarkets). This was done using STATGraphics centurion 16.1. Duncan's multiple range test was used to show the variation of the means from each other. Significance was set at $P<0.05$.

3.3 Biofilm Formation Assay

Biofilm formation assay was adapted from (Peeters *et al.*, 2008). The *Salmonella* cultures were revived in 5ml Tryptone Soya Broth (TSB) (Oxoid, CM129) for 24h. A 10-fold serial dilution was done using freshly prepared TSB to obtain a culture concentration of 10^8 CFU/ml. 100µl of diluted culture was pipetted into each well. The plates were then incubated for 48hrs at 37^{o}C. After the incubation period, the supernatant of the microtiter plate was removed and then the wells were rinsed with phosphate buffer saline. One hundred and twenty five millilitres of crystal violet solution was pipetted into each plate to stain the cell. The stained plate was incubated at room temperature for 15 mins. The plate was subsequently washed three to four times with sterile distilled water and inverted on a paper towel to dry for about 5 mins. Next, 125 microliters of 30% acetic acid were placed in each well to allow for the solubilization of the crystal violet (CV) retained by the cells. The solubilized CV stain was transferred a fresh flat bottom 96-well plate. The absorbance of dissolved stain was measured using Thermo-Scientific Varioskan™ LUX multimode microplate reader at 600nm.

3.3.1 Statistical Analysis of Biofilm Formation Data

Biofilm forming ability was categorized based on the ability of the organism to stain the walls of the test material as cited in (Díez-García *et al.*, 2012; Svabic-Vlahovic, 2000). The isolates were classified into weak, moderate and strong. Not biofilm formers (OD≤ ODc) where OD = optical density ODc = cut off OD, Weak biofilm formers (ODc < OD ≤ 2×ODc), moderate biofilm producers, when (2×ODc) < OD ≤ (4×ODc), or strong biofilm producers, when (4×ODc) <OD. Frequencies of each category were represented in a table. All qualitative data were represented as images and tables. One-way Analysis of Variance (ANOVA) was performed to establish significant differences in biofilms forming ability of the different serovars at a 95% confidence limit. Mean separations were obtained using Duncan's multiple

range test. Significance was determined at the $P < 0.05$ level using the STATGraphics software Centurion 16.11.1. (StatPoint Technology, Inc. Virginia). Standard curve trend illustrations were done using Microsoft Office Excel 2013.

3.4 Antimicrobial Resistance Testing

Isolated cultures were sub-cultured unto fresh plates XLD agar for 18 to 24hrs and further sub-cultured on Nutrient Agar, also for 18 to 24hrs to avoid testing mixed cultures. Two to three well-isolated colonies were picked using cotton swabs and suspended in Muller-Hinton Broth (Oxoid, CM0405). Suspensions were incubated to log phase which occurs between 2hrto 8hrs of incubation (Ortez, 2005). Following incubation, the turbidity was adjusted to match the 0.5 McFarland standard (corresponds to approximately 1.5×10^8 CFU/ml). The suspensions were vortexed to mix properly and streaked to cover the entire surface of a dry Muller-Hinton Broth (Oxoid, CM337) plates using sterile cotton swabs. Specific antimicrobial disks were applied, four disks to a plate and within 15mins of inoculation. Plates were inverted and incubated for 18 to 24hrs at 37℃. The diameters of the zones of inhibition were measured and compared to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables to confirm the susceptibility or resistance of the cultures tested (Ortez, 2005). Susceptibility, intermediate and resistance were presented as frequencies of occurrences between the antibiotics and the serovars recovered using Microsoft Office Excel 2013.

3.5 Determination of Lethal and Sub-lethal Doses of Biocides

Cultures of organisms were purified CM0129 Tryptic Soy Agar (TSA) for 18 to 24hrs to avoid testing mixed cultures. Two to three well-isolated colonies of the same morphology were picked and suspended in a 5ml CM0129 Tryptone Soy Broth (TSB) (Oxoid Ltd, United

Kingdom). Suspensions were incubated to Log Phase which occurs between 2hrs to 8hrs of incubation. Following incubation, the turbidity was adjusted to match that of a 0.5 McFarland standard (corresponds to approximately 1.5 X 10^6 CFU/ml). The appropriate amount of biocides were weighed and diluted to the required concentrations using TSB. An equal volume (1:1) of diluted culture added to biocide and incubated for 8hrs. After the incubation period, the optical densities of the samples inoculated with the different concentrations of biocides were measured (Ortez, 2005).

3.5.1 Construction of Standard Curve

A standard growth curve was prepared to help translate the optical densities into log CFU/ml. *Salmonella* cultures were prepared in Muller Hinton broth and incubated for 24 hours at 37˚C. Ten serial dilutions made in sterile Muller Hinton broth were incubated at 37˚C for 8 hours prior to enumeration by spread plating on pre-poured Muller Hinton agar plates. Cultures were plated in duplicates to obtain corresponding concentrations for each serial dilution. The optical densities of the dilutions were measured at 600nm with a JENWAY 6000 spectrophotometer. The standard curve was drawn using the log CFU/ml against the optical densities.

Table 1. **Biocide Concentration Used in the Determination of Lethal and Sub-lethal Doses**

Biocides	Concentrations Tested (%)	Incubation hours
Sodium Chloride	3, 5, 10, 15, 20	8
Citric Acid	0.1, 0.5, 1, 3, 5	8
Acetic Acid	0.1, 0.5, 1, 3, 5	8
Potassium hydroxide	0.5, 1, 2, 3	8
Hydrogen peroxide	1, 3, 5, 7, 10	8

Statistical Analysis for biocide tolerance data

A one-way Analysis of Variance (ANOVA) was employed to determine significant differences in the optical densities, pH measured and log reduction in relation to the different concentrations used. This was done using STATGraphics centurion 16.1. Duncan's multiple range test was used to show the variation of the means from each other. Significance was set at $P<0.05$. Standard curve trend illustrations were done using Microsoft Office Excel 2013.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Microbiological Quality of Broiler Carcass

Microbiological quality assessment of fresh raw chicken meat is considered important because chicken is susceptible to a variety of microbial contamination. This includes those capable of causing spoilage and pathogens which are capable of causing illnesses (Mead, 2004). Contamination of the chicken carcass may occur at various stages throughout the entire chain. Thus, the Codex Alimentarius Commission (CAC) has developed a code of hygienic practices, outlining the criteria for each step in the poultry value chain to ensure the safety of the chicken meat for consumers (ICMSF, 2011).

In this study, the general microbiological quality of raw chicken carcasses obtained from live-bird markets, super markets and cottage farms were compared. The aerobic plate counts (APC) for chicken carcasses from the live-bird market had the highest average count of 8.499 $\log_{10}$CFU/g, followed by the samples from the supermarkets with an average count of 6.394 $\log_{10}$CFU/g and the cottage farms with a mean count of 6.092 $\log_{10}$CFU/g (**Figure 5**). There was however no significant difference ($p = 0.0000$) between the counts for samples obtained from the supermarkets and that of the cottage farms. APC of the live-bird markets, on the other hand, was statistically higher ($p = 0.0000$) from that of the supermarket and cottage farms.

These results are consistent with Adu-Gyamfi *et al.*, (2012) who reported APC of 6 $\log_{10}$CFU/g on fresh chicken carcasses. The APC of chicken samples from the cottage farms and supermarkets were within the standard safe limit of <7 $\log_{10}$CFU/g (GS955: 2019) but those obtained from the live-bird markets exceeded this limit. On the other hand, these counts do not meet international standards such as the Food Standards Agency (UK) standard of <5$\log_{10}$CFU/g.

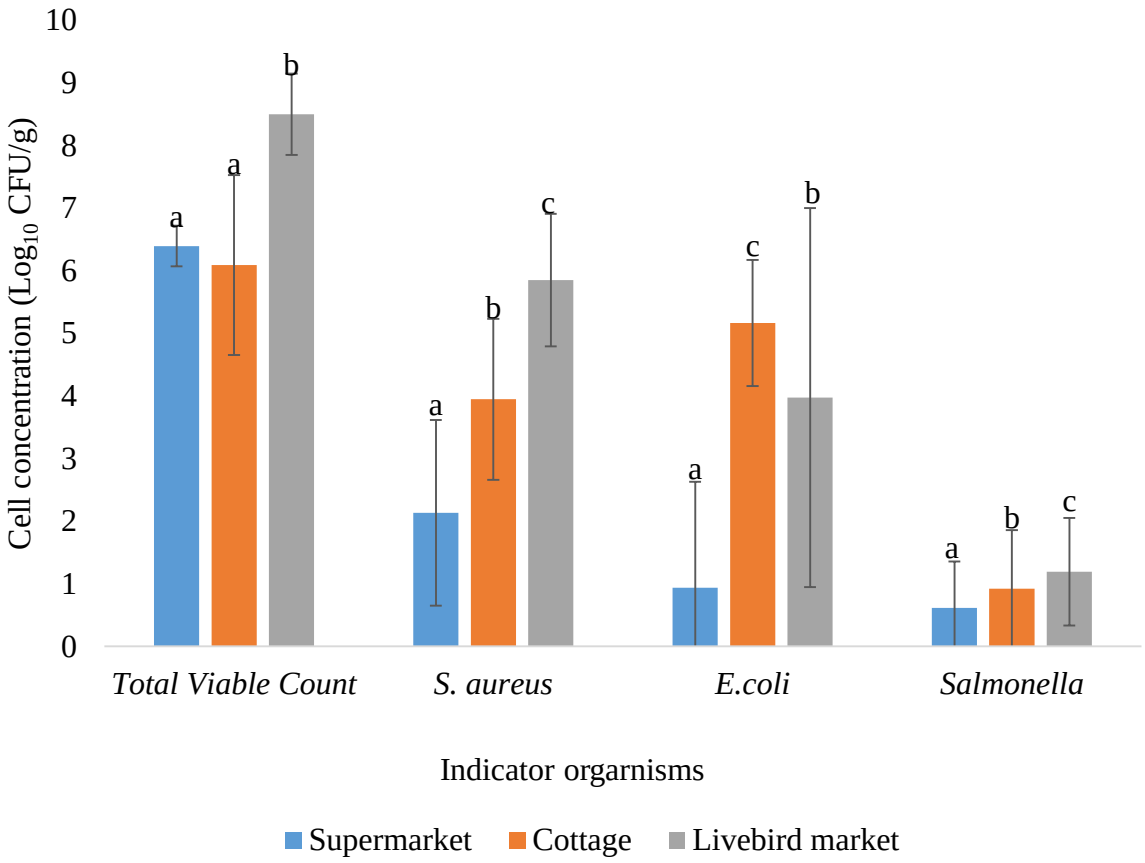

Figure 5. Microbiological quality of chicken obtained from retail outlets in Accra, Ghana.

Values with the different alphabets are statistically different from each other whereas those with the same alphabet are not statistically different.

The difference between the groups could be attributed to different conditions of processing. It is commonly known that most supermarkets have relatively better hygienic and processing conditions compared to the LBM. Most supermarkets have advanced chilling mechanisms and equipment that most of the LBM operators do not have. Chilling reduces the risk of proliferation of initial bacterial load (Nair & Kollanoor, 2019). This was confirmed with the quality of broiler carcass samples collected, which places emphasis on the lack of cold chain management at the live bird markets. The APC can only be minimized by observing good

hygienic and handling practices and strict adherence to cold chain integrity in order to be able to meet both local and international standards for raw poultry meat. Testing for psychrotrophic or mesophilic APC may also be useful for monitoring and the process control of key spoilage microorganisms (ICMSF, 2011).

There was a significant difference (p=0.0000) in *S. aureus* counts on chicken carcasses between the three freshly dressed chicken outlets. The counts ranged between 2.13 $\log_{10}$CFU/g to 5.85 $\log_{10}$CFU/g, with supermarkets having the least and the live-bird market having the highest *S. aureus* count. In relation to both the Ghana Standards Authority (GS955: 2019; Ghana) and the Food Standards Agency (FSA; United Kingdom) standards, none of the chicken carcasses from the poultry processing facilities met the local and international limit of <2 $\log_{10}$CFU/g. The supermarket processors came close to meeting the standards. However, this shows that generally, there is still some work to be done in order for fresh poultry meat vendors to meet the standards. Amongst the species of coagulase-positive staphylococci, *S. aureus* is known to be the most common foodborne pathogen. *S. aureus* produces enterotoxins in food, which causes food intoxications when the toxins are consumed. The production of the enterotoxin occurs when the number of *S. aureus* cells increases to about 4 $\log_{10}$ CFU/g or more (Mebkhout *et al.*, 2019). A break in cold chain temperature over a long period of time may encourage the growth of the organism, which will then produce the tasteless and scentless toxins (Medved'ová *et al.*, 2017). *S. aureus* can be found on the skins of many healthy people as well as the nose and throat (FSAI, 2016). This suggests that the presence of *S. aureus* is because of carcass handling. From observations made, most of the live bird processors did not wear gloves and hardly any other PPEs, which could have resulted in the high *S. aureus* contamination.

The presence of *E. coli* connotes faecal contamination as it is an indicator of poor hygienic processing. *E. coli* presence could also be attributed to evisceration contamination of the

chicken meat or poor hygiene of the live bird at the time of slaughter. The presence of *E. coli* O157:H7 raises concerns on the safety of the food as its presence can result in foodborne illness. According to GSA and the FSAI standards, *E. coli* limit on fresh chicken carcasses is <2 log_{10}CFU/g. The supermarkets were able to meet the limit with a mean count of 0.94 log_{10}CFU/g; the others, on the other hand, did not meet the standard. The prevalence of *E. coli* O157:H7 was 23%, 17%, and 8%, for live-bird markets, cottage farms (including the research farm) and supermarket respectively (**Figure *6***).

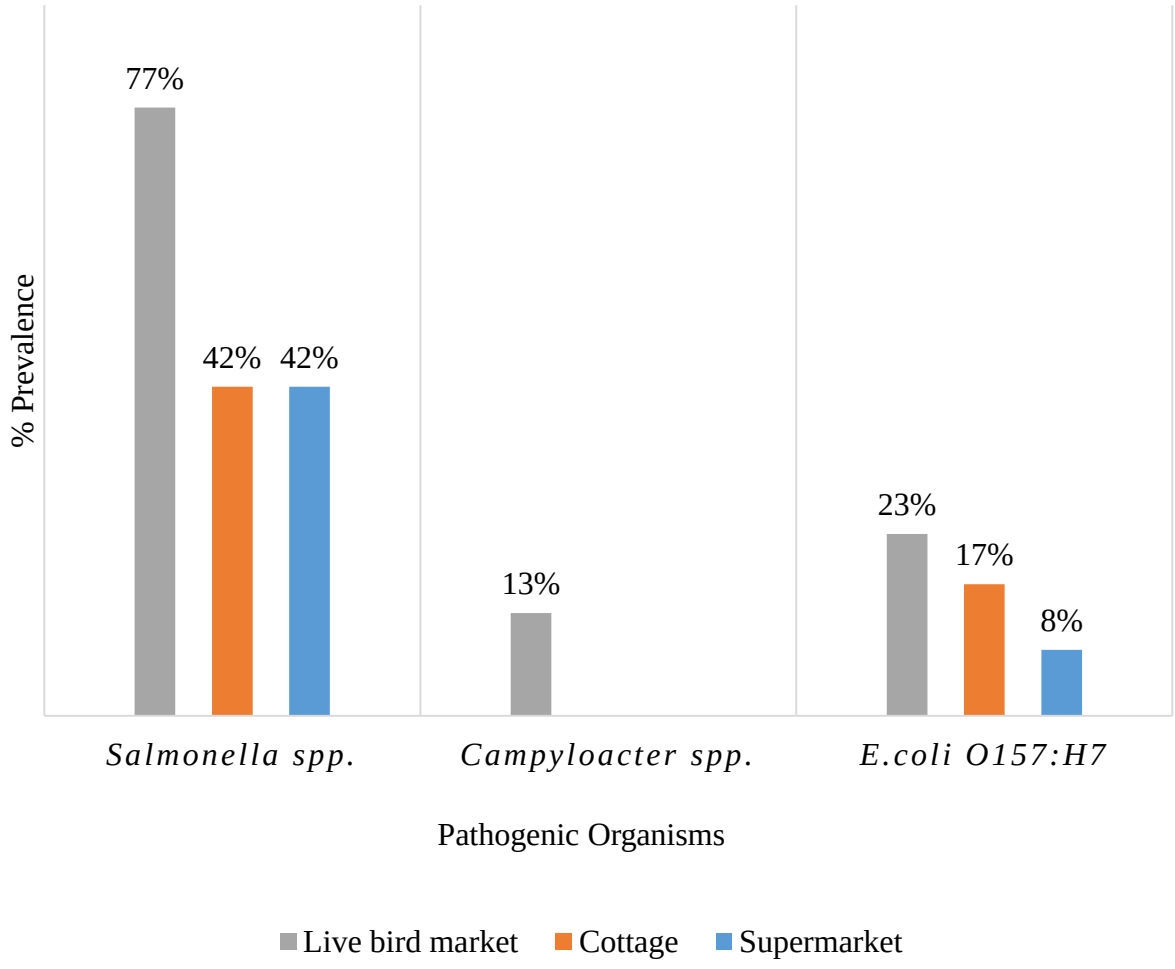

Figure 6. Prevalence of pathogenic microorganisms on chicken carcasses only

The presence of *Salmonella* spp. in raw chicken becomes a food safety issue when the chicken is undercooked or when cross-contamination occurs with other minimally processed or ready to eat foods. According to the European Food Safety Authority (EFSA), there is sufficient evidence that *Salmonella* is a foodborne hazard related to poultry meat. Studies show that cross-contamination from contaminated broiler meat to ready-to-eat foods often occur during grocery purchase or from using the same cutting surfaces for both minimally processed food and the chicken (Gonçalves-Tenório *et al.*, 2018; Luber, 2009). Prevalence of *Salmonella* spp. in all the chicken carcasses sampled was 61%. The prevalence of *Salmonella* on chicken carcasses from the live bird markets was 77% and 42% for both the supermarket and the cottage farms respectively. The high presence of *Salmonella* raises alarming food safety concerns of our meat production and processing techniques. According to the food safety criterion (FSC) for *Salmonella*, *Salmonella* should be absent in 25g of raw poultry meat intended to be eaten cooked (Manfreda & Cesare, 2012). This suggests that averagely none of the broiler outlets conformed to the standards. From Figure 5, all the sources recorded high count thus, none meets the recommended limits. The $\log_{10}$CFU counts for *Salmonella* spp. were 0.61, 0.92 and 1.19, for supermarkets, cottage, and the live-bird markets respectively. *Salmonella* was present in seemingly more hygienic processing facilities such as the supermarket who also refrigerate the meat.

The presence of *Salmonellae* may not necessarily reflect unhygienic handling of processors, although poor hygiene may contribute to its spread. This implies that the contamination is not restricted to unhygienic conditions only although unhygienic conditions help it spread but could also be contaminated from the animal itself (Cohen *et al.*, 2007). According to a study carried out by Corry *et al.* (2002), serotypes of *Salmonella* detected during primary production were usually also found in lesser concentrations on the birds on the day of slaughter and on the carcasses at various points during processing. From the *Salmonella* serovar data, serovars such

as *S.* Typhimurium, *S.* Agona, *S.* Infantis, *S.* Newport and *S.* Enteritidis that were recovered from the faecal matter samples were also present on the chicken samples (Figure 7). This supports the hypothesis that *Salmonella* contamination on chicken may have been as a result of faecal contamination. Cross-contamination could also have occurred from their working surfaces because most of the LBM processors did not have easy to clean and durable material which could serve as anchorage for these indicator organisms.

The *ompC* gene (204bp) was used as the target gene for the identification of the *Salmonella* genus in the sample. Almost all the isolates showed a strong amplification of the *ompC* gene except for sample 17, 22 and 55 which were faint but still positive (Figure 8). The *ompC* gene was however not serovar specific (Ahmed & Shimamoto, 2012; Alvarez *et al.*, 2004), thus the need for the serotyping to narrow it down to the actual serovars. This is a confirmation of the presumptive *Salmonella* isolated from the culture methods.

S. Typhimurium, *S.* Enteritidis, *S.* Agona, *S.* Infantis *S.* Paratyphi, *S.* Newport, *S.* Senftenberg, *S.* Westhampton, *S.* Mississippi and *S.* Adelaide were the *Salmonella* serovas identified in this study (Figure 7). Some of these serovars have been implicated in a number of Salmonellosis outbreaks. In some parts of Europe, serovar Infantis has been reported as the most prevalent in poultry and the third common cause of salmonellosis in humans; after Typhimurium and Enteritidis (Cetinkaya *et al*., 2008). A number of international outbreaks caused by different *Salmonella* serotypes have also been reported, most of which cited chicken as the major source of contamination (Edelstein *et al.*, 2004; FDA, 2018; Gymoese *et al.*, 2017). According to the United States Centre for Disease control and Prevention (CDC, 2019), *Salmonella* Infantis outbreak occurred in 32 states in the USA in 2018. 129 people were infected, 25 were hospitalized and 1 died. The outbreak was linked to raw chicken products including chicken carcasses, raw chicken pet food and live chicken. In 2019, *S.* Uganda, *S.* Concord, *S.* Newport have all been linked to outbreaks in the USA being associated various foods (CDC, 2019).

However, no major *Salmonella*e outbreak has been reported in Ghana featuring any of these serovars identified.

Some of these serovars like *S.* Typhimurium, *S.* Enteritidis, *S.* Newport, *and S.* Senftenberg are among the commonly isolated serotypes from poultry and farm environment (Andino & Hanning, 2015), hence not surprising to have recovered these serotypes in this study. Others such as *S.* Agona. *S.* Infantis, *S.* Paratyphi were commonly isolated from pigs and cows (Andino & Hanning, 2015), finding them in broilers suggests cross contamination either at the farm level or during processing, especially at facilities where other forms of meat are processed. *S.* Typhi and *S.* Paratyphi A and B are vastly host-adapted pathogens, with humans being the only natural host and reservoir of infection. (Levine *et al.*, 2011; Sanderson *et al.*, 2015). Hence, presence on chicken carcasses could be due to cross-contamination from processors who could be infected or asymptomatic.

Some serotypes isolated in this study, such as *S.* Typhimurium and *S.* Enteritidis have been implicated blood stream infections. According to (Albert *et al.*, 2019), non-typhoidal *Salmonella* (NTS) causes the majority of bloodstream infections. In Ghana *S.* Typhimurium was the most commonly isolated serotype, followed by *S.* Enteritidis and *S.* Dublin, in relation to blood stream infections (Dekker *et al.*, 2018).

Serovars such *S.* Westhampton and *S.* Senftenberg are novel serotypes whose host restriction status have not yet been determined. However, these have been cited to have been isolated from cockles (Doublet *et al.*, 2009) and from faecal matter from abattoirs (Ibrahim *et al.*, 2015; Motsoela *et al.*, 2002). The recovery of these serotypes from cockles suggests an association to water which could explain why they were seen in the rinse water. Serovar Adelaide is known to be common in fruits thus could possibly have been present in their feed and ended up in their faecal matter (CDC, 2018).

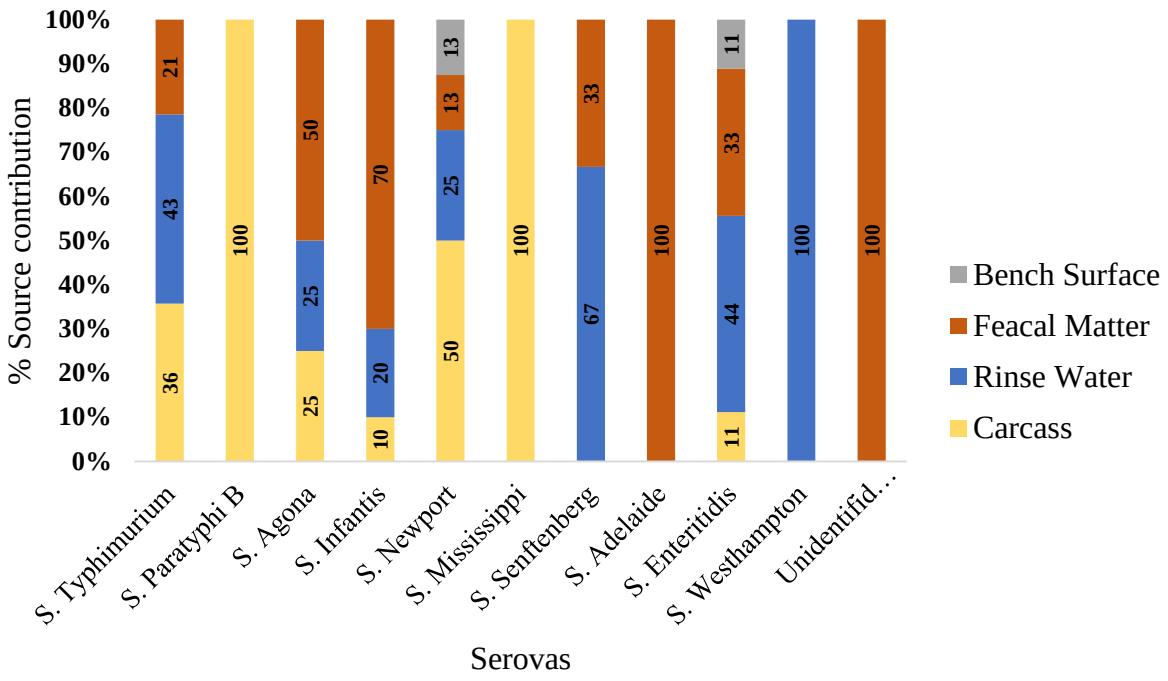

Figure 7. *Salmonella* serovars identified and the sources they were isolated from.

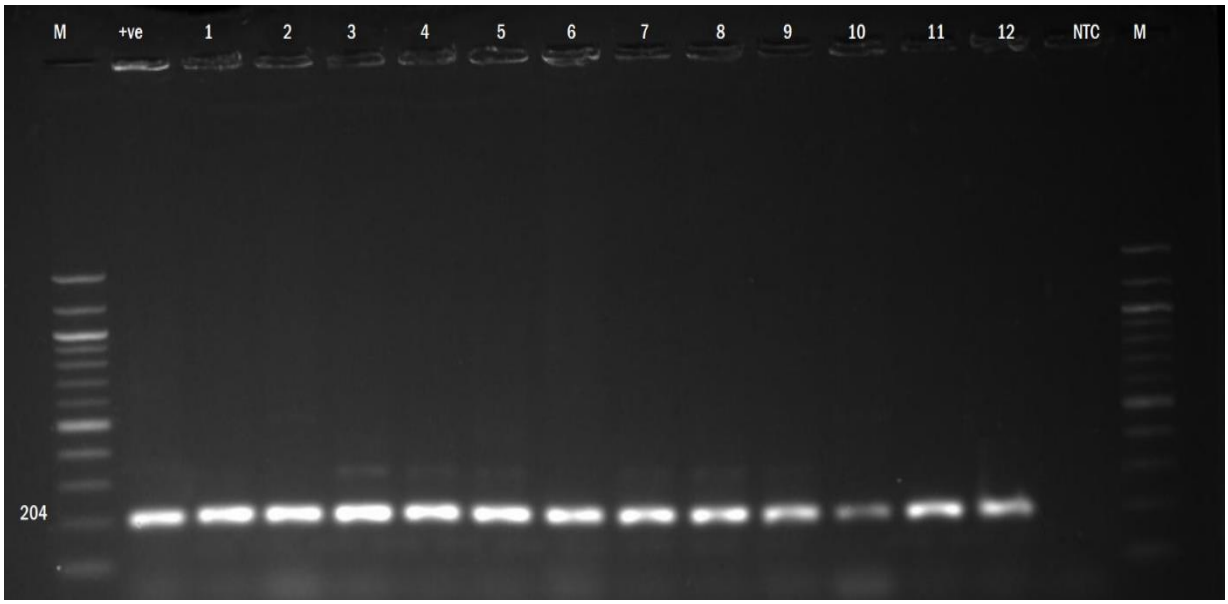

Figure 8. PCR amplification products of *Salmonella* spp. ompC gene using ompC-F and ompC-R primer pair.

Lane M=100 bp ladder; No template control, +ve = reference (204bp), lanes 1-12 =Sample lane

Campylobacter spp. had a prevalence rate of 13% on chicken carcasses from the live bird markets only. *Campylobacter* was not detected in samples from cottage and supermarkets. Comparing the prevalence of *Campylobacter* in this study to other literature, the prevalence in this study was very low. Usually, the prevalence rates recorded is >50% (FSA, 2002). According to the Food Safety Authority of Ireland (FSAI) (2016), *Campylobacter* is killed by normal cooking temperatures as well as freezing temperature. Considering that, scalding temperatures are not monitored in most of these establishments, the scalding water is hot, in some cases boiling hot or almost boiling. Hence, there is a possibility that most of the *Campylobacter* present were injured or killed which made them undetectable in the samples. This is evident in Figure 9, where the prevalence of *Campylobacter* was relatively higher in the faecal samples as compared to the carcass.

4.1.1 Possible Sources of Contamination or Cross-Contamination

To determine the contribution of faecal matter, rinse water, carcasses and bench surfaces to the contamination of chicken carcasses, the prevalence of the pathogens (*Salmonella*, *Campylobacter* and *E. coli* O157:H7) were categorized by these environmental sources (faecal matter, rinse water, carcasses and bench surfaces) (Figure *9*). These are the presumable sources of microbial contaminants of chicken meat during processing. The stages involved in poultry meat processing are good channels along which contamination and cross-contamination can occur. Since freshly dressed chicken processing does not include an intentional kill step, it is important to adhere to strict hygienic practices to prevent the introduction and proliferation of significant pathogens and spoilage organisms on raw chicken meat. The risk of foodborne illnesses as a result of consumption of *Campylobacter, Salmonella, Escherichia coli* O157:H7 in prepared or ready-to-eat foods is high when cross-contamination occurs during grocery shopping or food preparation in consumer kitchens.

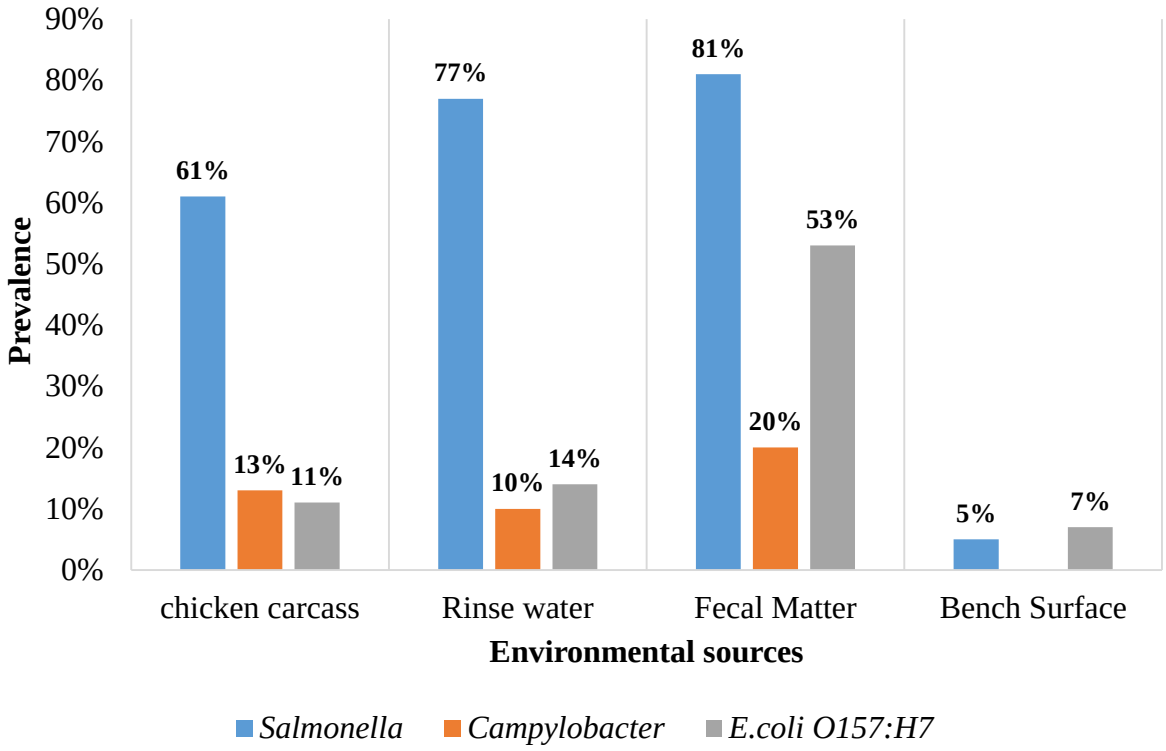

Figure 9. Prevalence of pathogenic microorganisms on chicken carcasses and environmental samples

Both *Salmonella* and *Campylobacter* colonize the gastrointestinal tract of poultry. Infected birds can shed large numbers of these organisms in their faeces without the birds being affected themselves. They are often referred to as having a commensal relationship with the birds (Meerburg & Kijlstra, 2007; Newell & Fearnley, 2003).

4.2 Biofilm Forming Ability of *Salmonella* spp.

Salmonella spp. have generally demonstrated their ability to form biofilms on several surfaces made of different materials including glass, polyethylene, polystyrene and even stainless steel. Biofilm formation favours the survival of the pathogen in a hostile environment such as processing plants and slaughterhouses given the right atmosphere and conditions to grow (Borges *et al*., 2018). The presence of a moist environment with nutrient to aid the growth of the organism are common in abattoirs and other food processing systems hence not surprising that contamination or cross-contamination keep recurring in the broiler meat.

All the *Salmonella* spp. tested exhibited biofilm forming ability but at a different level of intensities. Using the same principle of categorization based on the ability of the organism to stain the walls of the test material as cited in Díez-García *et al.*(2012) and Svabic-Vlahovic (2000), the isolates were classified into weak, moderate and strong biofilm formers (Table 2). Only 11% of the *Salmonella* isolates were weak biofilm formers with an average cell concentration of 2 $\log_{10}$CFU/ml (OD= 0.154). Seventy-three percent (73%) formed moderate biofilm whereas, 16% had strong biofilm forming abilities with an average cell concentration of 5.75 $\log_{10}$CFU/ml (OD of 0.467).

Table 2. *Salmonella* spp. categorized based on their biofilm forming ability

Biofilm forming ability	Prevalence	Average OD
Weak (<0.2)	11% (6)	0.154 ± 0.029^{a}
Moderate (0.2-0.4)	73% (23)	0.304 ± 0.021^{b}
High (>0.4)	16% (9)	0.467 ± 0.030^{c}

Not biofilm formers (OD≤ ODc), Weak biofilm formers (ODc < OD ≤ 2×ODc), moderate biofilm formers, when (2×ODc) < OD ≤ (4×ODc), or strong biofilm formers, when (4×ODc)<OD

OD = optical density

ODc = cut off OD

This suggests that if proper cleaning is not frequently done at the processing sites at the live bird markets and abattoirs, poultry meat could become contaminated with *Salmonella* spp. that can form biofilms on food contact surfaces.

Salmonella serotypes have peculiar inherent characteristics that influence its ability to form biofilms such as their ability to produce extracellular polymeric substances (EPS) which encapsulates the immobile cells to form a thicker biofilm (Banerjee *et al.*, 2015; Borges *et al.*, 2018). Hence, some serovars are able to form more biofilms than others. This is evident in the results obtained from this research as shown in Figure 10. There was a statistically significant difference ($p = 0.0003$) between the biofilm formed by the various serovars. This implies some although they can all form biofilms, some *Salmonella* species can form biofilms better than others.

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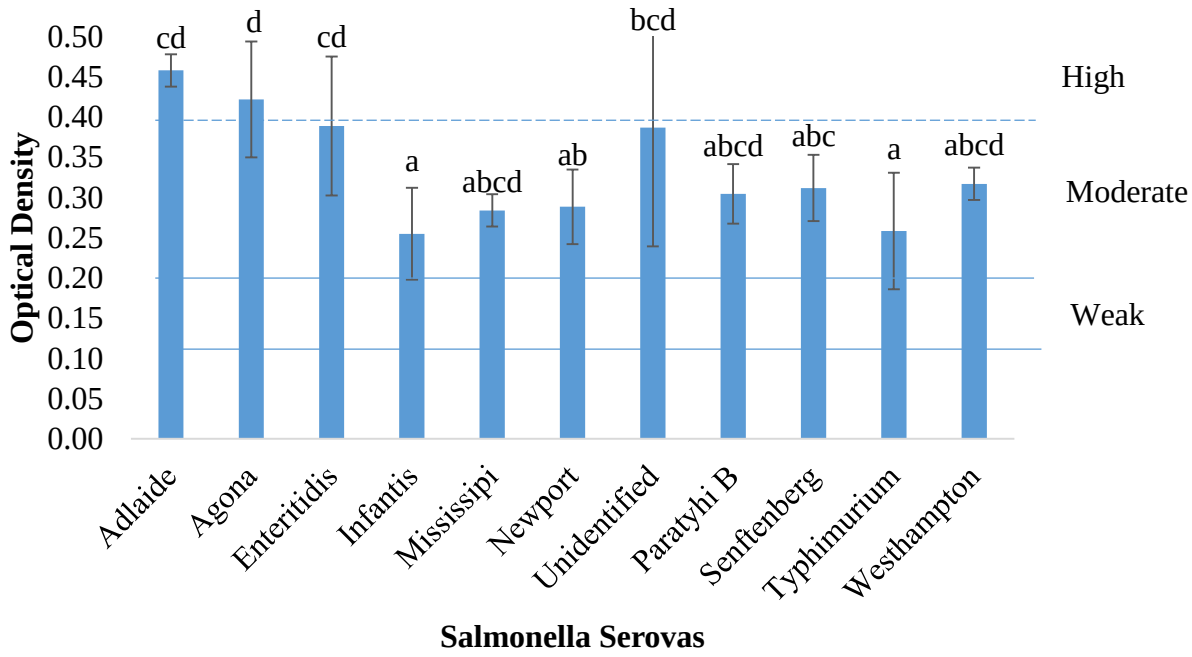

Figure 10. Biofilm forming ability of the different *Salmonella* serotypes isolated from broiler carcasses and their processing environment.

Categorizing them based on the serotypes, all the serovars tested appeared to be moderate to strong biofilm formers. Here, the weak biofilm formers are not seen because of the variations in within the individual serovars which is accounted for, by the standard deviations of the means. Serovars such as *S.* Adelaide and *S.* Agona led the chart with average ODs of 0.458 and 0.421 respectively which can be considered as strong biofilm formers. Whereas serovars such as *S.* Enteritidis, *S.* Senftenberg, *S.* Paratyphi *B*, *S.* Newport, *S* Mississippi, *S.* Typhimurium, and *S.* Infantis, fell in the moderate biofilm former category. There was no significant difference between the biofilm forming abilities of *S.* Typhimurium and *S.* Infantis. A similar trend was reported by Borges *et al.* (2018) where there was no significant difference between *S.* Typhimurium, *S.* Agona and *S.* Infantis. In their report, *S.* Enteritidis was also said to be a stronger biofilm former compared to *S.* Typhimurium. It is therefore not surprising that these serotypes are often implicated in foodborne diseases thus the need for effective and regular cleaning in food processing facilities.

4.3 Tolerance of *Salmonella* spp. to different concentrations of biocides

Effective implementation of GHP is principal in the determination of number and type of bacteria on raw poultry meat. The design of the equipment is particularly necessary to ease of maintenance and cleanability. Regular cleaning and disinfection of equipment and processing environment are required to maintain low levels of spoilage and pathogenic bacteria (ICMSF, 2011).

Biofilms are generally difficult to remove. Frequent and strict compliance to cleaning and sanitation regimen is required to prevent formation of biofilms on food contact surfaces (Schmidt, 2018). The lethal doses of biocides for various pathogens is dependent on the active ingredient in the biocide and the make-up of the target organism (Jeffrey, 2007). Biocides

tested included, sodium chloride (NaCl), potassium hydroxide (KOH), citric acid and hydrogen peroxide (H_2O_2) which are all active ingredients in commercial biocides used by local processors.

From Table *3*3, p = 0.0000 was observed for all the biocides. This suggests that there was a significant difference in the effect of the different concentrations of biocides on the *Salmonella* spp. For each biocide, there was an inverse relation where, as the concentrations increased the concentration of the organisms reduced. There was however no significant difference (p=0.2310) between the tolerance levels of the different serovars to the different biocide treatments.

Sodium Chloride (NaCl) is one of the most common traditional biocides. NaCl causes microbial inactivation by increasing solution salinity which then leads to water efflux from the organism. The efflux of water from the organism causes a counterbalance of a compatible solute such as glycine betaine, glutamate, proline, trehalose, and ectoine which increases the stress on cells hence damages the cell (Omotoyinbo & Omotoyinbo, 2016).

From the results, 3% and 5% NaCl showed a significant decrease in *Salmonella* spp. of 1.87 and 3.85 $\log_{10}$CFU/ml reduction respectively. At 10% and 15%, NaCl, the *Salmonella* cells were reduced to close to <1 $\log_{10}$CFU/ml which infers >8 $\log_{10}$CFU/ml reduction. Considering the concentrations of *Salmonella* (<2 $\log_{10}$CFU/g) determined from the enumeration data in Figure 5, 5% NaCl can be recommended as an effective dose for inhibiting or even disinfecting *Salmonella*e growth on bench surfaces. Common salt is readily accessible and cost effective thus a good option for the local poultry processors.

According to Raynor *et al.* (1996), KOH like NaOH are excellent saponifying and alkalizing agent. They result in the destruction of bacteria by saponifying their membrane lipids and also by solubilizing their cytoplasmic membrane proteins. Alkaline-based disinfectants such as

potassium hydroxide (KOH) and sodium hydroxide (NaOH) have previously been cited to be effective in reducing bacterial contamination in poultry meat and processing facilities. The report mentioned that 1% KOH was able to result in a microbial population reduction of about 2.5 log CFU/ml (Dickson *et al.*, 1994). However, there is a significant reduction of 4.43 log CFU/ml with 0.5% KOH (Table 3). This suggests that 0.5% KOH is a sub-lethal concentration against the *Salmonella* spp. whereas at ≥1% KOH can be said to be the lethal dose for these organisms.

Acetic acid and citric acid are both weak acid which inhibits growth through metal chelation and partial dissociation in the cytoplasm which acidifies the cell's interior hence inactivating the bacteria cell. These weak acids are generally regarded as safe (Mani-lópez, *et al.*, 2012). Concentrations of 0.1% to 5% were tested for both organic acids. A significant reduction of 2.3 $\log_{10}$CFU/ml was seen in acetic acid at 0.1% and further to about a 4 $\log_{10}$CFU/ml reduction at 0.5% acetic acid concentration. Citric acid comparatively had a weaker effect on the *Salmonella* spp., recording to 1.91 to 3.73 $\log_{10}$CFU/ml reductions at a higher concentration of 1% - 3%. Lio *et al.* (2003) and Mani-López *et al.* (2012), investigated the sub-lethal and lethal effects of acetic acid on 6 strains of *Salmonella* where cultures were exposed to different concentrations (0.06% to 3.0%). They also came to the conclusion that 0.1% of acetic acid was enough to inhibit the growth of *Salmonella* spp. and reported 2% acetic acid as a minimum bactericidal concentration. The sub-lethal dose for citric acid on the hand was pegged at 3% whereas the lethal concentration was said to be around 5%.

Hydrogen peroxide is one of the most oxidizing agents commonly used in industrial poultry operations for disinfecting surfaces because of their efficiency and ability to decompose into non-toxic by-products. They have low pH which makes it moderately corrosive. The mode of action of peroxide is to inhibit metabolism by acting on the enzymes and protein thiols groups

of the microorganism (Chima *et al*., 2012). Observations from the results suggest that 1% to 3% is capable of disinfecting bench tops and equipment.

Table 3. *Salmonella* spp. tolerance to different concentrations of biocides

Biocide	% Biocide used	Treatment time (hrs)	O.D. (600nm)	Log CFU/ml reduction	pH
NaCl	0%	8	1.248 ± 0.120^{e}	0.00^{e}	6.82 ± 0.01^{e}
	3%	8	0.994 ± 0.060^{d}	1.87^{d}	6.39 ± 0.01^{a}
	5%	8	0.725 ± 0.114^{c}	3.85^{c}	6.42 ± 0.00^{cd}
	10%	8	0.061 ± 0.012^{b}	8.74^{b}	6.43 ± 0.00^{d}
	15%	8	0.011 ± 0.018^{ab}	9.11^{ab}	6.40 ± 0.00^{ab}
	20%	8	0.000 ± 0.000^{a}	9.19^{a}	6.41 ± 0.00^{bc}
	P-value		**0.0000**	**0.0000**	**0.0001**
KOH	0%	8	1.248 ± 0.120^{e}	0.00^{e}	6.82 ± 0.01^{a}
	0.50%	8	0.647 ± 0.025^{d}	4.43^{d}	10.60 ± 0.15^{b}
	1%	8	0.202 ± 0.056^{c}	7.71^{c}	11.90 ± 0.00^{c}
	2%	8	0.085 ± 0.010^{b}	8.57^{b}	12.30 ± 0.06^{d}
	3%	8	0.005 ± 0.004^{a}	9.16^{a}	12.70 ± 0.06^{e}
	5%	8	0.000 ± 0.000^{a}	9.19^{a}	13.45 ± 0.15^{f}
	P-value		**0.0000**	**0.0000**	**0.0000**
Acetic Acid	0%	8	1.248 ± 0.120^{e}	0.00^{e}	6.82 ± 0.01^{f}
	0.10%	8	0.924 ± 0.056^{d}	2.39^{d}	3.65 ± 0.01^{e}
	0.50%	8	0.702 ± 0.094^{c}	4.02^{c}	2.86 ± 0.01^{d}
	1%	8	0.589 ± 0.011^{b}	4.85^{b}	2.52 ± 0.01^{c}
	3%	8	0.006 ± 0.004^{a}	9.15^{a}	2.03 ± 0.00^{b}
	5%	8	0.000 ± 0.000^{a}	9.19^{a}	2.00 ± 0.06^{a}
	P-value		**0.0000**	**0.0000**	**0.0000**
Citric	0%	8	1.248 ± 0.120^{e}	0.00^{e}	6.82 ± 0.01^{f}
	0.10%	8	1.174 ± 0.076^{d}	0.55^{d}	4.42 ± 0.01^{e}
	0.50%	8	1.002 ± 0.077^{d}	1.81^{d}	3.90 ± 0.01^{d}
	1%	8	0.989 ± 0.041^{c}	1.91^{c}	3.52 ± 0.01^{c}
	3%	8	0.741 ± 0.024^{b}	3.73^{b}	2.73 ± 0.00^{b}
	5%	8	0.030 ± 0.002^{a}	8.03^{a}	2.60 ± 0.06^{a}
	P-value		**0.0000**	**0.0000**	**0.0000**
H_2O_2	0%	8	1.248 ± 0.120^{c}	0.00^{c}	6.82 ± 0.01^{f}
	1%	8	0.761 ± 0.343^{b}	3.59^{b}	3.50 ± 0.06^{e}
	3%	8	0.131 ± 0.034^{a}	8.23^{a}	3.32 ± 0.01^{d}
	5%	8	0.011 ± 0.031^{a}	9.11^{a}	2.66 ± 0.00^{c}
	7%	8	0.001 ± 0.002^{a}	9.19^{a}	2.47 ± 0.00^{b}
	10%	8	0.000 ± 0.000^{a}	9.19^{a}	$2.1\ 0 \pm 0.00^{a}$
	P-value		**0.0000**	**0.0000**	**0.0000**

4.4 Antimicrobial Susceptibility of *Salmonella* Spp.

According to Popelka *et al.* (2007), the most commonly used antibiotics for both human and animal therapy are beta-lactam antibiotics. From observations made, antibiotics such as amoxicillin-clavulanic acid, penicillin, tylosin tartrate, oxytetracycline, and chlortetracycline were the most widely administered antibiotics to live birds at the live bird markets. In addition to the antibiotics, various supplements were given to the birds either through their feed or through their drinking water. These supplements contained active ingredients such as vitamins (A, D, E, K, B2, and B12), amino acids, nicotinic acid and calcium pantothenate alongside antibiotics like colistin, oxytetracycline HCl and neomycin sulphate.

The *Salmonella* spp. isolated in this study were observed to have developed various degrees of resistance to some of these antibiotics frequently administered to the broilers. Antibiotics such as amoxicillin-clavulanic acid and tetracycline which were regularly dosed in the drinking water, recoded high resistance as compared to the antibiotics only administered as a result for therapy.

Recent reports cited that in Ghana, third-generation cephalosporins and fluoroquinolones such as ciprofloxacin, cefuroxime, cefotaxime, and chloramphenicol are currently the most prescribed for the treatment of invasive non-typhoidal *Salmonella* (iNTS) and typhoidal *Salmonella* (TS) infections in humans (Aldrich *et al.*, 2019; Labi *et al.*, 2014).

The percentage prevalence of resistance and susceptibility of *Salmonella* spp. against 14 commonly used antibiotics was determined (Figure 11). Of the 14 antibiotics used in this susceptibility testing, 7 antibiotics recorded high levels of resistance. The *Salmonella* isolates exhibited complete resistance to oxacillin (OX), 95% resistance to erythromycin (E), 74% resistance to trimethoprim-sulfamethoxazole (SXT), 67% resistance to pefloxacin (PEF), 51% resistance to doripenem, 47% to amoxicillin-clavulanic acid, and 43% to tetracycline (TGC).

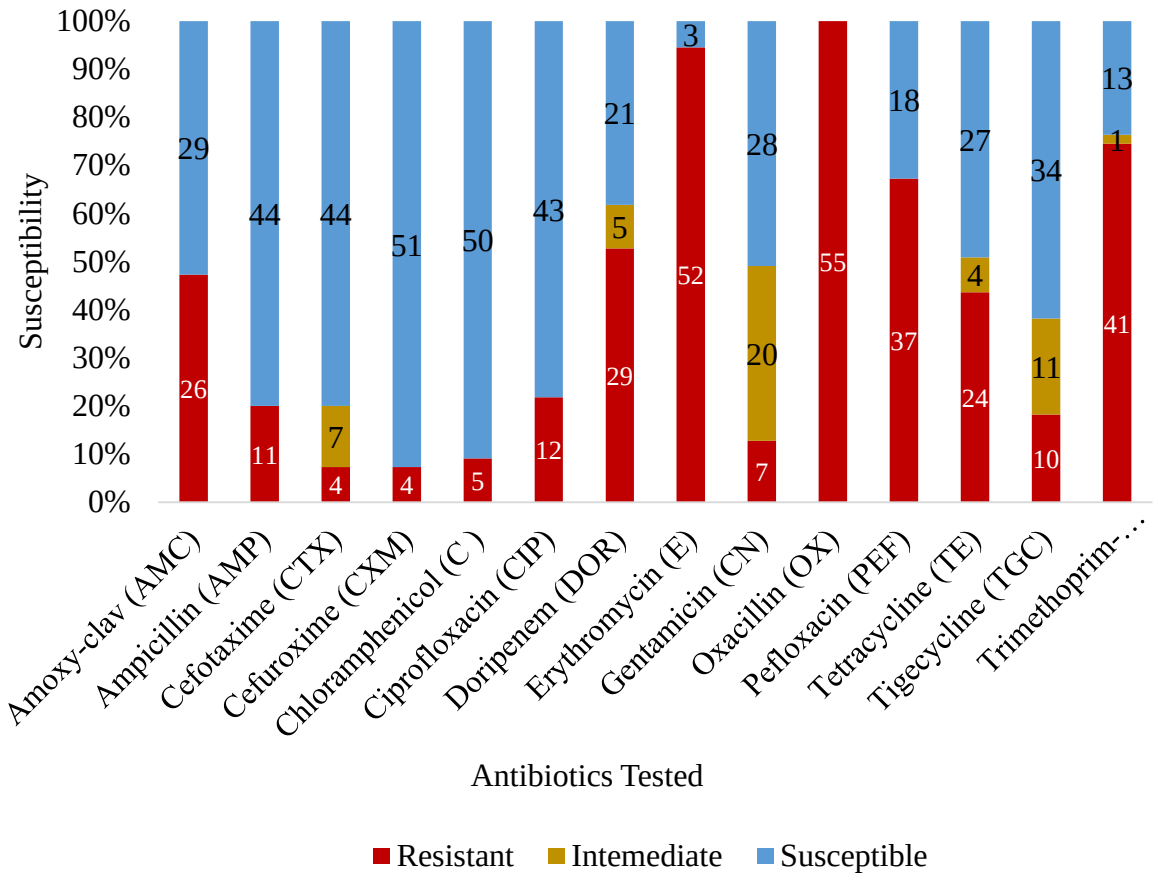

Figure 11. Antimicrobial susceptibility of *Salmonella* spp. isolated from poultry carcasses and environmental samples (N=55)

Antibiotics such as cefuroxime (CXM), cefotaxime (CXT), and chloramphenicol (C) recorded less than 10% resistance. Gentamicin, tigecycline, ampicillin, and ciprofloxacin also had 12%, 18%, 20%, and 21% resistance respectively. Susceptibility to these antibiotics was expected because they are broad spectrum antibiotics. Ciprofloxacin is a member of the fluoroquinolones whereas cefuroxime and cefotaxime form part of the third generation cephalosporins which are often referred to as last resort antibiotics. This is because they have the widest spectrum of activity. Hence, resistance to these antibiotics could suggest the production of broad-spectrum β-lactams, such as AmpC β-lactamases and extended-spectrum β-lactamases (ESBLs) (de Jong *et al.*, 2014). However, the multidrug resistance (MDR) could be attributed to a number of

reasons. Some of which are spontaneous mutation due to previous conditions (primary production and processing conditions), impermeability, overexpression of efflux pump genes and target gene modifications. These phenomena may occur singly or in varying combinations (Rawat & Nair, 2010; Webber & Piddock, 2003).

Intermediate resistance was also observed for some of the antibiotics as shown in Figure 11. The isolates in the intermediate category were not clearly resistant or susceptible and could have however been easily classified under resistance. This is because intermediate organisms may easily develop resistance with time if the appropriate doses are not administered. It is, therefore, advised that a patient who has an infection with a pathogen that shows intermediate resistance to broad spectrum antibiotics can be given high doses of the required antibiotic as though the result showed resistance (Adzitey *et al*., 2015).

The serovars identified in this study were compared to assess if there were significant differences in the levels of susceptibility to or resistance against the antibiotics used. Generally, there were no statistically significant differences between the different serovas.

Table 4. Antimicrobial resistance of *Salmonella* serotypes isolated from poultry carcasses and environmental samples (N=55)

Antibiotics	Adelaide	Agona	Enteritidis	Infantis	Mississipi	Newport	Paratyhi B	Senftenberg	Typhimurium	Westhampton	Unidentified *Salmonella* spp.
	n(N_o)	n(N_o)	n(N_o)	n(N_o)	n(N_o)	n(N_o)	n(N_o)	n(N_o)	n(N_o)	n(N_o)	n(N_o)
Amoxy-clav (AMC)	1(1)	1(4)	6(9)	1(10)	-	4(8)	1(2)	2(3)	6(14)	-	2(2)
Ampicillin (AMP)	1(1)	-	3(9)	1(10)	-	3(8)	1(2)	1(3)	2(14)	-	1(2)
Cefotaxime (CTX)	-	-	-	1(10)	-	-	-	-	1(14)	-	2(2)
Cefuroxime (CXM)	-	-	1(9)	-	-	-	-	-	2(14)	-	1(1)
Chloramphenicol (C)		2(4)	-	-	-	-	2(2)	-	-	-	1(2)
Ciprofloxacin (CIP)	-	2(4)	2(9)	-	-	5(8)	1(2)	-	2(14)	-	-
Doripenem (DOR)		3(4)	4(9)	6(10)		1(8)	1(2)	3(3)	6(14)	1(1)	2(2)
Erythromycin (E)	1(1)	4(4)	9(9)	10(10)	1(1)	8(8)	2(2)	3(3)	11(14)	1(1)	2(2)
Gentamicin (CN)	-	-	1(9)	-		4(8)	2(2)	-	-	-	-
Oxacillin (OX)	1(1)	4(4)	9(9)	10(10)	1(1)	8(8)	2(2)	3(3)	14(14)	1(1)	2(2)
Pefloxacin (PEF)	-	3(4)	6(9)	5(10)	-	6(8)	2(2)	2(3)	11(14)	1(1)	2(2)
Tetracycline (TE)	-	2(4)	5(9)	1(10)	1(1)	5(8)	1(2)	-	7(14)	-	-
Tigecycline (TGC)	-	1(4)	1(9)	3(10)		1(8)	-	-	1(14)	-	-
Trimethoprim-sulfamethoxazole (SXT)	1(1)	3(4)	9(9)	4(10)	1(1)	7(8)	1(2)	1(3)	12(14)	1(1)	-

n= the number of resistant isolates

N_o= the total number isolates within each serovar

N= total number of samples

The effectiveness of the antibiotics tested against each serotype of *Salmonella* is presented in Table 4. Cefotaxime, Cefuroxime, and chloramphenicol were the most effective antibiotics against *Salmonella* spp., recording less than 5 resistance each. Generally, some isolates showed more susceptibility than others, nevertheless 91% of the isolates tested were resistant to 3 to 9 antibiotics indicating evidence of multidrug resistance. Multidrug resistance is defined by the CDC as the situation where an organism is resistant to three or more classes of antibiotics. Categorizing the isolates under their serotypes; *S.* Paratyphi *B, S.* Enteritidis, *S*. Infantis and *S.* Typhimurium were relatively seen to show higher resistance to multiple antibiotics. Adzitey (2015), have also cited in their report the prevalence of multidrug-resistant (MDR) *Salmonella* spp. with resistance to up-to 6 antibiotics.

The categories of multi-drug resistance of *Salmonella* isolates are depicted in table 4. This helps in quantifying the extent of antibiotic resistance and associated health risk. Being infected by an organism that is resistant to multiple antibiotics primarily means the organism will be hard to treat, which in effect will result in increased mortality (Joseph *et al*., 2017). The increase in MDR has been attributed to the abuse of antibiotic usage (Davis & Brown, 2019). Especially with AMR isolates from livestock and poultry, a number of studies have suggested that the prophylactic and therapeutic use of these antibiotics is the main cause of AMR hence MDR (Akbar & Anal, 2013; Joseph *et al*., 2017; Kiilholma, 2007).

There is, therefore, the need to monitor and regulate the use of antibiotic from primary production to live bird market. Some countries, like Canada, Denmark, South Korea, Mexico and Australia have resolved this problem by banning the use of some antibiotics in animal husbandry. Some antibiotics can be used only when there is a veterinary prescription (Ballard *et al.*, 2015; Maron *et al.*, 2013). These strategies can be employed in Ghana in order to curb the indiscriminate use of antibiotics by some of these live bird farmers and traders.

Table 5 Multidrug resistance of *Salmonella* serotypes isolated from chicken carcasses and processing environment.

	Resistant isolates within a serovar			
Serovars (n)	(≤3) antibiotics	(3-4) antibiotics	(5-6) antibiotics	>6 antibiotics
Adelaide (1)	-	-	1	-
Agona (4)	-	-	3	1
Enteritidis (9)	-	1	4	4
Infantis (10)	2	1	7	-
Mississipi (1)	-	1	-	-
Newport (8)	1	1	1	5
Paratyhi B (2)	-	-	1	1
Senftenberg (3)	-	1	2	-
Typhimurium (14)	1	3	4	6
Westhampton (1)	-	-	1	-
Unidentified (2)	-	-	1	1
Salmonella spp				
Total	4	8	25	18

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The microbial load (aerobic plate count, *S. aureus* and *Salmonellae*) on chicken carcasses from the live bird markets were significantly higher (P=0.0000) than those from the supermarkets and cottage farms except for *E. coli* counts, which was significant higher (P=0.0000) in the chicken samples from the cottage farms as compared to those from the supermarket and the live bird market. A total of 61% *Salmonella* prevalence was recorded for the broiler carcasses. The prevalence of *Salmonella* was also relatively higher in the live bird market samples although none of the retail outlet was compliant with GSA standards. Zero *Salmonella* in 25g of raw poultry as recommended by regulatory authorities is not practical since there are no control strategies in place at the farm level.

Nine non-typhoidal (*S.* Typhimurium, *S.* Enteritidis, *S.* Newport, *S.* Senftenberg, *S.* Agona. *S.* Infantis, *S.* Mississippi, *S.* Westhampton *and S.* Adelaide) and one typhoidal (*S.* Paratyphi B) *Salmonella* serotypes were identified from the carcass, faecal matter, bench surfaces and rinse water samples. The overlap of some serotypes across the samples collected confirmed the hypothesis that cross contamination is a major contributor to the prevalence of pathogenic organisms on chicken meat.

The *Salmonella* serotypes isolated exhibited moderate to strong biofilm forming ability hence frequent and effective cleaning is required to prevent the formation of these biofilms. Biocide concentrations of 5% NaCl, 0.5% KOH, 0.5% Acetic acid, 3% Citric Acid and 1% H_2O_2 were able cause an average of 4 $\log_{10}$CFU/ml reduction therefore live bird processors can employ these biocides in sanitizing their bench surfaces and equipment to reduce the probability of cross-contamination.

Most (93%) of the isolates were multidrug resistant; showing resistance against 3 to 9 antibiotics. Especially towards the antibiotics commonly administered to the birds for prophylactic purposes such as amoxicillin-clavulanic, oxacillin, erythromycin acid and tetracycline. Some of the *Salmonella* isolates recorded resistance against 3rd generation cephalosporins and fluoroquinolones prescribed for the treatment of invasive non-typhoidal *Salmonella* (iNTS) and typhoidal *Salmonella* (TS) infections in humans. The high prevalence of multi-drug resistant *Salmonella* spp. is a concern for public health. This suggests an increased public health risk of invasive*Salmonella* infections through poultry carcasses and processing environments, which may be difficult to treat due to resistance to 3rd generation cephalosporins and fluoroquinolones used as therapeutic drugs for bacterial infections in Ghana..

5.2 Recommendation

There is therefore the need for regulators to enforce the implementation of good hygienic practices coupled with HACCP through frequent training programs for processors. Food safety awareness on handling and preparation for consumers could also help reduce the risk of these microbiological hazards.

To significantly reduce the high prevalence of AMR in *Salmonella*, there is the need to implement control strategies such as imposing bans on the use of antibiotics as growth promoters or its use without a prescription or veterinarian on site.

Live bird markets and other processing operations could consider saline wash of the carcass and other applications as sanitizers for food contact surfaces since these biocides are effective.

A full risk assessment can be carried out on the microbiological hazards identified in this study to characterize the risks they pose to public health.

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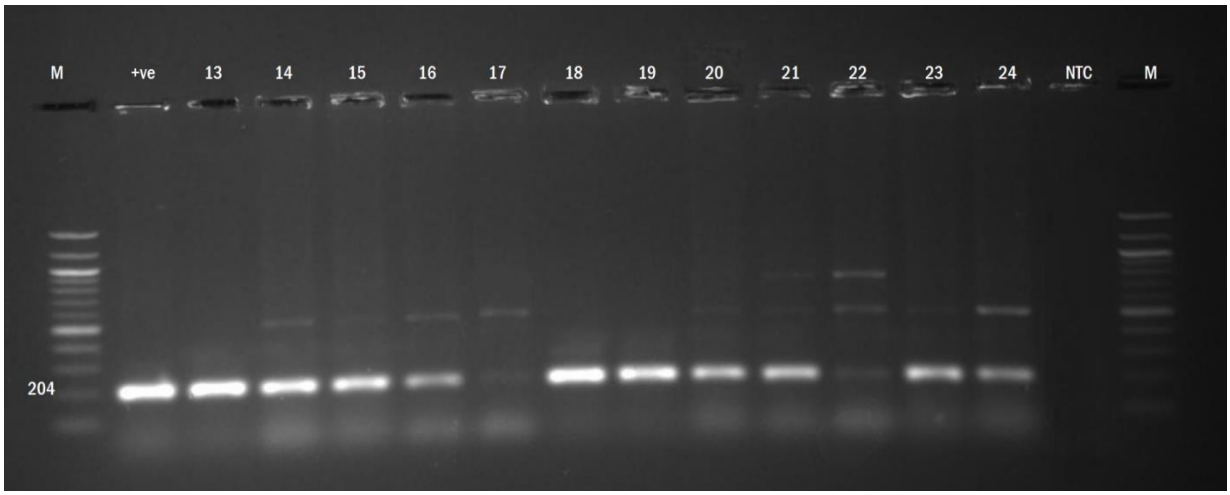
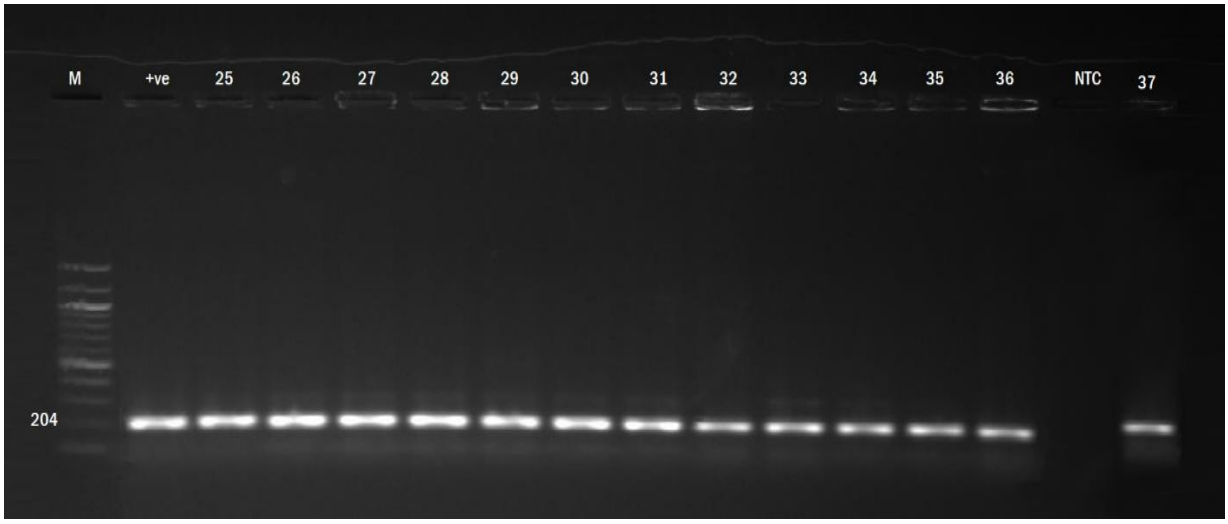
APPENDICES

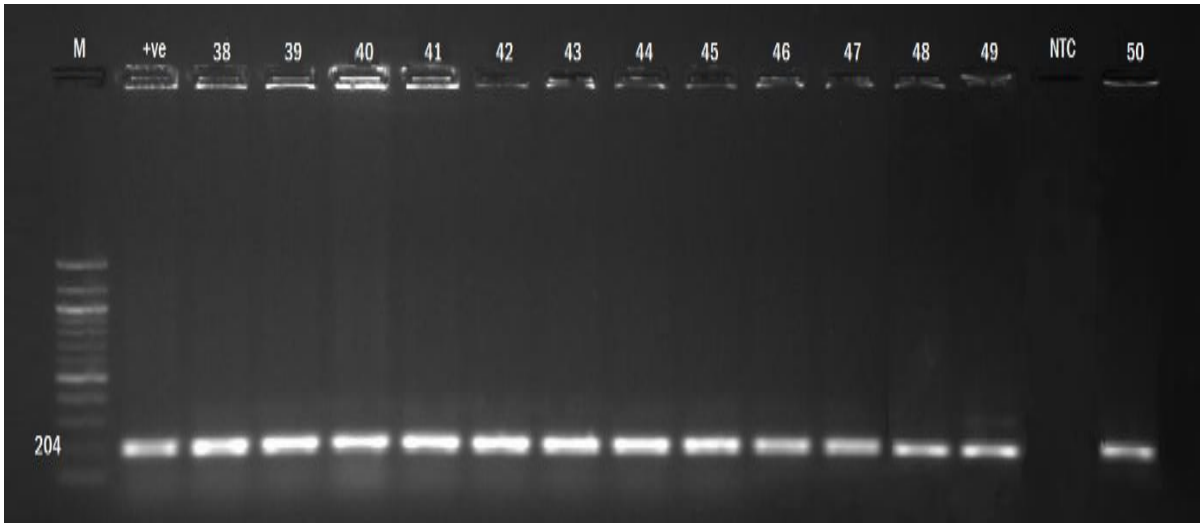
APPENDIX 1 (Figures)



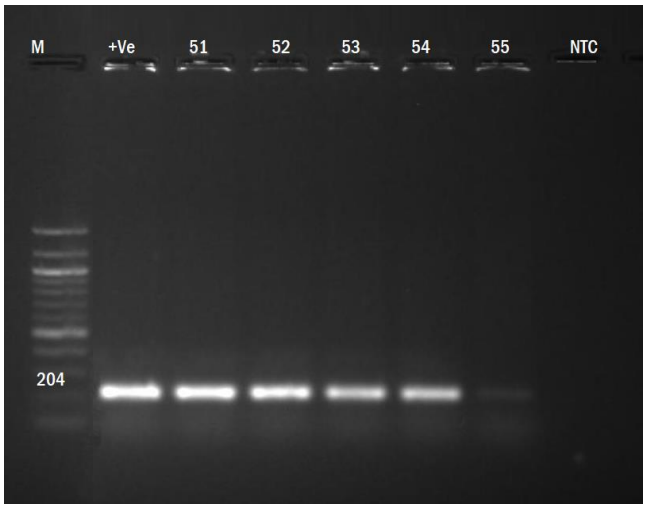
Appendix Figure **8** (B). *Lane M=100 bp ladder; NTC=No template control, +ve = reference (204bp), lanes 13-24 =Sample lanes*



Appendix Figure 8 (C) *Lane M=100 bp ladder; NTC=No template control, +ve = reference (204bp), lanes 25-37 =Sample lanes*



Appendix Figure 8 (D) *Lane M=100 bp ladder; NTC=No template control, +ve = reference (204bp), lanes 38-50 =Sample lanes*



Appendix Figure 8***12*** (E) *Lane M=100 bp ladder; NTC=No template control, +ve = reference (204bp), lanes 51-55 =Sample lanes*

APPENDIX 2 (Tables)

Appendix Table 1: ANOVA Table for TAC by category

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	104.364	2	52.1821	79.84	0.0000
Within groups	55.5557	85	0.653597		
Total (Corr.)	159.92	87			

Appendix Table 2: Multiple Range Tests for TAC by category

Method: 95.0 percent LSD

category	*Count*	*Mean*	*Homogeneous Groups*
cottage	16	6.09245	X
supermarket	16	6.39415	X
live bird market	56	8.49924	X

Appendix Table 3: ANOVA Table for S.aureus by category

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	186.912	2	93.456	66.62	0.0000
Within groups	119.234	85	1.40276		
Total (Corr.)	306.147	87			

Appendix Table 4: Multiple Range Tests for S.aureus by category

Method: 95.0 percent LSD

Category	*Count*	*Mean*	*Homogeneous Groups*
Supermarket	16	2.1349	X
Cottage	16	3.94671	X
live bird market	56	5.84956	X

Appendix Table 56: ANOVA Table for E.coli by category

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	155.882	2	77.9412	11.72	0.0000
Within groups	565.205	85	6.64948		
Total (Corr.)	721.088	87			

Appendix Table 67: Multiple Range Tests for E.coli by category

Method: 95.0 percent LSD

Category	*Count*	*Mean*	*Homogeneous Groups*
Supermarket	16	2.1349	X
live bird market	56	3.94671	X
Cottage	16	5.84956	X

Appendix Table 78: ANOVA Table for *Salmonella* by category

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	155.882	2	56.3652	12.09	0.0000
Within groups	565.205	85	4.6584		
Total (Corr.)	721.088	87			

Appendix Table 8: Multiple Range Tests for *Salmonella* by category

Method: 95.0 percent LSD

Category	*Count*	*Mean*	*Homogeneous Groups*
Supermarket	16	0.61462	X
Cottage	56	0.9203	X
live bird market	56	1.19	X

Appendix Table 9: *Salmonella* serovars identified and the sources they were isolated from.

	Carcass	Rinse Water	Feacal Matter	Bench Surface	Total
S. *Typhimurium*	5	6	3	-	14
S. Paratyphi B	2	-	-	-	2
S. Agona	1	1	2	-	4
S. Infantis	1	2	7	-	10
S. Newport	4	2	1	1	8
S. Mississippi	1	-	-	-	1
S. Seftenberg	-	2	1	-	3
S. Adelaide	-	-	1	-	1
S. Enteritidis	1	4	3	1	9
S. Westhampton	-	1	0	-	1
Unidentifid *Salmonella.* spp	-	-	2	-	2

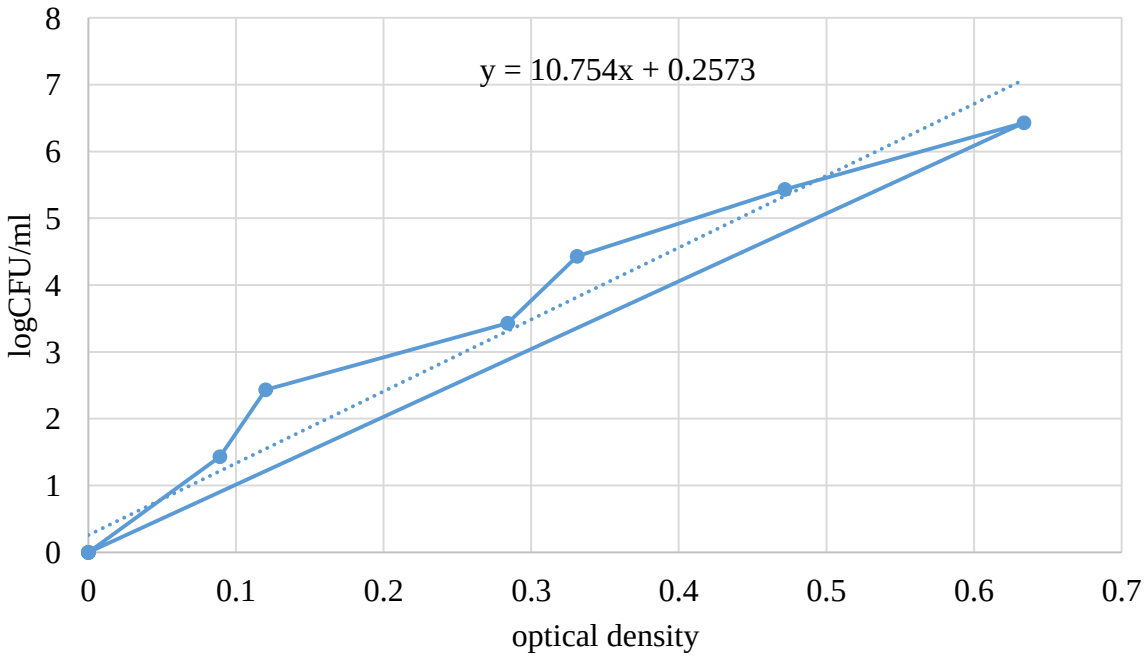


Figure 13. Growth curve for Salmonella spp.

Appendix Table 10: ANOVA Table for OD by serovars

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.21194	10	0.021194	4.35	0.0003
Within groups	0.214164	44	0.00486737		
Total (Corr.)	0.426104	54			

Appendix Table 11. Multiple Range Tests for OD by serovars

Method: 95.0 percent LSD

serovars	*Count*	*Mean*	*Homogeneous Groups*
Infantis	10	0.25498	X
Typhimurium	14	0.258357	X
Mississipi	1	0.284	XXXX
Newport	8	0.288562	XX
Paratyhi B	2	0.304617	XXXX
Senftenberg	3	0.311833	XXX
Westhampton	1	0.317	XXXX
O1	2	0.387	XXX
Enteritidis	9	0.388907	XX
Agona	4	0.421875	X
Adlaide	1	0.458	XX

Appendix Table 12: ANOVA Table for NaCl OD by NaCL Conc

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	8.7645	4	2.19112	645.04	0.0000
Within groups	0.15286	45	0.00339688		
Total (Corr.)	8.91736	49			

Appendix Table 139: ANOVA Table for KOH OD by KOH Conc

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2.90232	4	0.72558	951.34	0.0000
Within groups	0.034321	45	0.000762689		
Total (Corr.)	2.93664	49			

Appendix Table 14: ANOVA Table for Acetic OD by Acetic Acid Conc

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	6.9359	4	1.73398	214.68	0.0000
Within groups	0.363465	45	0.00807701		
Total (Corr.)	7.29937	49			

Appendix Table 1510: ANOVA Table for H2O2 OD by H2O2 Conc

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4.33067	4	1.08267	45.17	0.0000
Within groups	1.07849	45	0.0239664		
Total (Corr.)	5.40916	49			

Appendix Table 16*11*: ANOVA Table for NaCl Conc by pH

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.00246667	4	0.000616667	18.50	0.0001
Within groups	0.000333333	10	0.0000333333		
Total (Corr.)	0.0028	14			

Appendix Table 17: ANOVA Table for KOH CONC by pH

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	13.5373	4	3.38433	317.28	0.0000
Within groups	0.106667	10	0.0106667		
Total (Corr.)	13.644	14			

Appendix Table 18: ANOVA Table for Acetic CONC by pH

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	5.70764	4	1.42691	1659.20	0.0000
Within groups	0.0086	10	0.00086		
Total (Corr.)	5.71624	14			

Appendix Table 19: ANOVA Table for Citric Acid Conc by pH

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	8.7645	4	2.19112	645.04	0.0000
Within groups	0.15286	10	0.00339688		
Total (Corr.)	8.91736	14			

Appendix Table 20: ANOVA Table for Peroxide by CONC

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4.01609	4	1.00402	1394.48	0.0000
Within groups	0.0072	10	0.00072		
Total (Corr.)	4.02329	14			

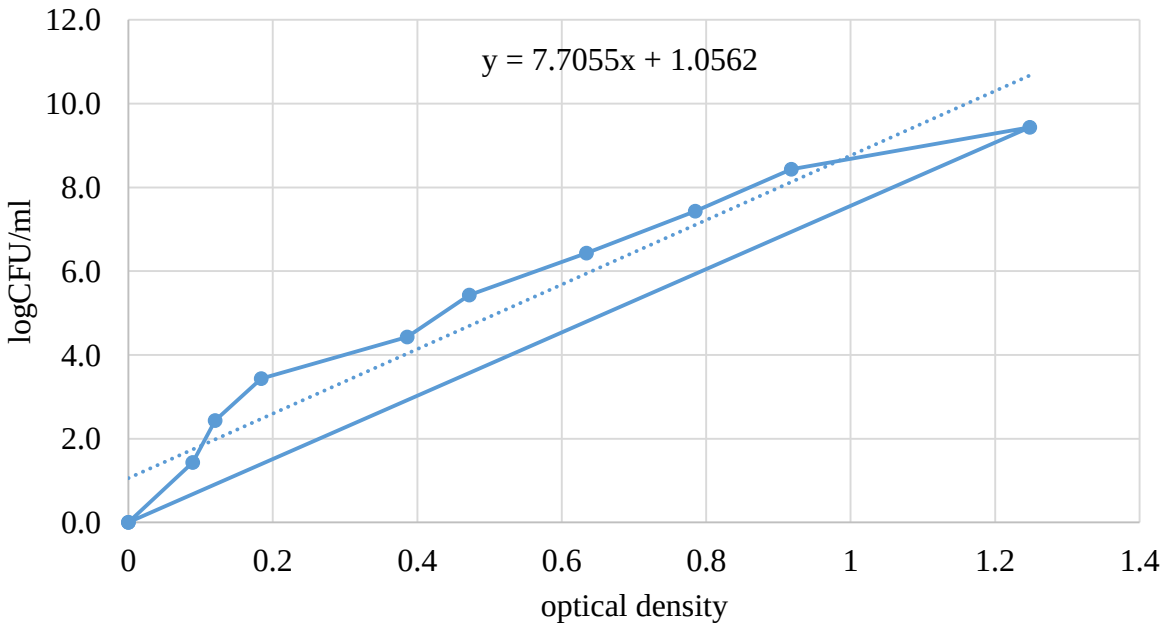


Figure 14. Growth for *Salmonella* spp. used for biocide tolerance determination

Appendix Table 21. Antimicrobial susceptibility of *Salmonella* spp. isolated from poultry carcasses and environmental samples (N=55)

Antibiotics	Resistant	Intemediate	Susceptible
Amoxy-clav (AMC)	26	0	29
Ampicillin (AMP)	11	0	44
Cefotaxime (CTX)	4	7	44
Cefuroxime (CXM)	4	0	51
Chloramphenicol (C)	5	0	50
Ciprofloxacin (CIP)	12	0	43
Doripenem (DOR)	29	5	21
Erythromycin (E)	52	0	3
Gentamicin (CN)	7	20	28
Oxacillin (OX)	55	0	0
Pefloxacin (PEF)	37	0	18
Tetracycline (TE)	24	4	27
Tigecycline (TGC)	10	11	34
Trimethoprim-sulfamethoxazole(SXT)	41	1	13

Appendix Table 22: ANOVA Table for Erythromycin by Serovar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	147.277	10	14.7277	1.13	0.3629
Within groups	573.704	44	13.0387		
Total (Corr.)	720.982	54			

Appendix Table 23: ANOVA Table for Chloramphenicol by Serovar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	179.547	10	17.9547	2.13	0.0421
Within groups	370.999	44	8.43179		
Total (Corr.)	550.545	54			

Appendix Table 24: ANOVA Table for Cefotaxime by Serovar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	74.0674	10	7.40674	0.65	0.7637
Within groups	502.042	44	11.41		
Total (Corr.)	576.109	54			

Appendix Table 25: ANOVA Table for Tigecycline by Serovar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	24.4447	10	2.44447	0.84	0.5956
Within groups	128.392	44	2.91799		
Total (Corr.)	152.836	54			

Appendix Table 26*12*: ANOVA Table for Doripenem by Serovar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	138.263	10	13.8263	1.50	0.1721
Within groups	405.846	44	9.22377		
Total (Corr.)	544.109	54			

Appendix Table 27*13*: ANOVA Table for Ampicillin by Serovar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	656.975	10	65.6975	1.33	0.2470
Within groups	2180.01	44	49.5456		
Total (Corr.)	2836.98	54			

Appendix Table 28*14*: ANOVA Table for Tetracyclin by Serovar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1184.92	10	118.492	1.46	0.1860
Within groups	3564.83	44	81.0188		
Total (Corr.)	4749.75	54			

Appendix Table 29: ANOVA Table for Gentamicin by Serovar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	51.187	10	5.1187	1.30	0.2625
Within groups	173.795	44	3.94988		
Total (Corr.)	224.982	54			

Appendix Table 30: ANOVA Table for Pefloxacin by Serovar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1052.92	10	105.292	1.32	0.2494
Within groups	3506.43	44	79.6916		
Total (Corr.)	4559.35	54			

Appendix Table *15*: ANOVA Table for Amoxy-Clav by Serovar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	521.449	10	52.1449	2.26	0.0310
Within groups	1014.48	44	23.0563		
Total (Corr.)	1535.93	54			

Appendix Table *16*: ANOVA Table for Cefuroxime by Serovar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	183.12	10	18.312	2.04	0.0519
Within groups	395.426	44	8.98695		
Total (Corr.)	578.545	54			

Appendix Table *17*: ANOVA Table for Ciprofloxacin by Serovar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	685.974	10	68.5974	1.76	0.0969
Within groups	1713.66	44	38.9469		
Total (Corr.)	2399.64	54			

Appendix Table *18*: ANOVA Table for Trimethoprim-Sulfamethoxazole by Serovar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1842.54	10	184.254	1.93	0.0663
Within groups	4200.3	44	95.4613		
Total (Corr.)	6042.84	54			