MICROBIOLOGICAL ANALYSIS OF BACTERIAL PATHOGENS IN POULTRY FEEDS AND WATER RESOURCES IN BLOUBERG POULTRY VALUE CHAIN PROJECT, LIMPOPO PROVINCE, SOUTH AFRICA

LLOYD NGWENYA

MARCH, 2019

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BY

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[redacted]

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MARCH, 2019

DECLARATION

I declare that this mini-dissertation submitted to the University of Limpopo for the degree Master of Science in Agriculture (Animal Production) is my independent work and research, and that it has not previously been presented as a study for this university or elsewhere. I further declare that all sources have been duly acknowledged.

Name: Ngwenya Lloyd

Signature…… Date……

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ABSTRACT

Poultry is a good source of animal protein for many households due to its affordability. However, it is prone to bacterial infections which can be passed on to consumers, hence chickens that are reared without constant health checks present a potential health threat to humans. The objective of the study was to identify the zoonotic bacterial pathogens in poultry feeds and water resources in Blouberg poultry value chain project. A total of 88 samples comprising of 14 feed samples, 14 water samples, 60 mouth and rectal swab samples were collected from the farms. The samples were screened for the presence of *Escherichia coli, Salmonella* spp*.* and *Shigella* spp*.* through selective cultivation. Only coliforms and the dominant isolates were identified as *Escherichia coli, Klebsiella* spp., and *Enterobacter spp*., *Salmonella* and *Shigella* spp. were not detected in all the samples. *E. coli* strains that were isolated from the water sources and mouth and rectal swabs of the chickens showed a significant resistance to gentamycin, neomycin, penicillin, streptomycin, tetracycline, erythromycin, nalidixic acid, ciprofloxacin and ampicillin ($p<0.05$). *Klebsiella pneumoniae* showed resistance to neomycin; penicillin; erythromycin ($p<0.05$) while *K. oxytoca* and *E. absuriae* showed similar antibiotic resistance profile as penicillin, erythromycin, nalidixic acid and ampicillin. *E. coli* and *K. pneumonia* are mostly implicated in poultry disease outbreaks and they are enteric pathogens in humans as well. The presence of pathogens in poultry presents a great risk of secondary infection in humans and this will lead to socio-economic problems for the affected communities. The information generated in this study will guide the relevant stakeholders who handle poultry feeds and water resources in following good management practices.

Keywords: Food-borne disease; zoonotic pathogens; antibiotic resistance; bacterial isolates

TABLE OF CONTENTS

List of tables

List of figures

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Poultry is produced by large scale commercial farmers and small scale farmers for eggs and meat. It is regarded as one of the cheapest sources of meat (Mack *et al.,* 2005). The poultry industry prides itself on the fact that it feeds the nation, as more poultry products are consumed every year than all the other animal protein sources (SAPA, 2014). The South African poultry industry continues to dominate the agricultural sector, providing 63.1 % of animal protein down from 63.5 % in 2015. During 2016, consumption of poultry meat and meat products (including imports) amounted to 2.200 million tonnes and of eggs and egg products 0.465 million tonnes, giving a total of 2.665 million tonnes. Among poultry meat products, chicken carcasses, cuts, and processed products are the most consumed followed by turkey and to a lesser extent, duck (Nieminen, 2012). Vacuum packaging, chilling, or marinades are different practices for ensuring microbial quality during the storage of poultry cuts, and depend on consumer habits and countries (Rouger *et al.,* 2017). Smallholder farmers usually keep poultry under extensive low input farming systems characterised by poor housing, low quality scavenging feed sources and limited veterinary interventions (Gueye, 2005 & Mack *et al.,* 2005). The low input production system and limited biosecurity measures expose poultry to various contaminating pathogens (Mwale & Masika, 2011). Because of the relatively high frequency of contamination of poultry with pathogenic bacteria, raw poultry products are responsible for a significant number of cases of human foodborne diseases (Jorgensen *et al*., 2002; Teplitski *et al.,* 2009). Animal feed may serve as a carrier for a wide variety of microorganisms (Maciorowski *et al.,* 2006). In general, the amount of available water in the feed composition determines whether a microorganism grows or survives. Some microorganisms are adapted to the low amount of available moisture and grow actively within stored seeds and grains. Others enter survival state until the moisture is high enough for bacterial action. Finally, feed can act as a carrier for animal and human pathogens (Maciorowski *et al.,* 2007). The type of feed, processing treatments and storage conditions can all be factors that influence levels and types of microorganisms present. In South Africa, the most prevalent foodborne

pathogens in poultry carcasses and in poultry processing plants are *E. coli spp*., *Salmonella spp*., *Listeria monocytogenes and Campylobacter spp*. which all cause diseases in humans in the absence of proper hygienic conditions (Asiegbu *et al*., 2016; Christison *et al.,* 2008; Miettinen *et al*., 2002; Bartkowiak-Higgo *et al.,* 2006). A study by Voidarou et al. (2011) reviewed that food safety and shelf-life are both important microbial concerns in relation to broiler meat production. The study further reiterated that the focus should mainly be placed on the absence or control of potentially pathogenic microbes such as *Salmonella* spp. and *Campylobacter* spp. However, from the commercial point of view, other spoilage bacteria also play a role in posing potential threats (Ranjitkar *et al.,* 2016). Regarding food safety, the primary target should be the production of pathogen-free live animals, thus allowing slaughter plants to keep the processing line free of those microorganisms (Pacholewicz *et al.,* 2016). Consumers believe that quality of foods from organic production is superior to foods from conventional production. Several isolates of these contaminating pathogens from poultry carcasses and feeds have been reported to develop high resistance against the antimicrobials such as: amoxicillin, ceftriaxone, erythromycin, tetracyclines, ciprofloxacin, fluoroquinolones and sulphonamides (Picard, 2010; Henton *et al., 2011*).

1.2 Problem statement

Numerous articles have investigated the prevalence of various pathogens in poultry meat. Among the pathogens, Campylobacter, Escherichia coli and Salmonella make up the majority of human hospitalisations and poultry mortality reports. Other foodborne human pathogens present in various meat products have also been investigated, such as *Shigella* spp (Kirk *et al.,* 2017; Rossaint *et al.,* 2015; WHO, 2015). According to Grant *et al.* (2016), South Africa has been witnessing an increase in chicken meat consumption and a proportional increase in incidences of food-borne pathogens on chicken carcasses and chicken products. Shonhiwa *et al*. (2017) on a review of foodborne disease outbreaks reported to the outbreak response unit, National Institute for Communicable Diseases, South Africa, 2013 – 2017, has affirmed the detrimental effects of the latter pathogens outbreak. A high incidence of non-typhoidal Salmonella in poultry products was reported in the North West Province, South Africa (Roseline *et al.,* 2014) and post-evisceration

contaminations of broiler carcasses, ready-to-sell livers and intestines were reported for *Campylobacter jejuni* and *Campylobacter coli* in a high-throughput South African poultry abattoir (Bonsman *et al.,* 2014). Bacterial pathogens affect the quality of feeds, their organoleptic attributes, and nutritional quality (Stuper *et al*., 2013). Furthermore, microbiological metabolic activities may lead to spoilage and production of toxins and these pose a serious health risk to the chicken consumers (Bonsman *et al.,* 2014; Reischl *et al.,* 2002). On the other hand, unprotected drinking water has also been implicated in bacterial transmission and infections in poultry (Reitsma *et al.,* 2008). Water sources for poultry are generally open and there is no treatment at the point of consumption (Walters *et al.,* 2007). A serious challenge is the secondary infections and morbidity in humans which result from consumption of contaminated poultry products (Muvhali *et al.,* 2015; Nyamongo and Okioma, 2005). The challenge of contamination of feed commonly affects the small-holder farmers due to lack of resources and finances. Information on the microbiological quality of feeds and knowledge of common contaminants is important for improved monitoring and designing of quality control measures in the handling of feedstocks (Damerow, 2012).

1.3 Rationale of the study

Several studies reiterated the prevalence of foodborne diseases in South Africa, for example, Muvhali *et al.* (2015) reported that, *Salmonella Enteritidis* has become a significant foodborne pathogen in South Africa. The study further reported a high burden of morbidity and poultry mortalities of the pathogen outbreaks in different provinces of South Africa including Limpopo Province. Shonhiwa *et al.* (2017) reported the presence of foodborne pathogens such as *Salmonella* spp, *Clostridium perfringens, Bacillus cereus, Shigella* spp, *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli* O157:H7 and *Campylobacter* spp from farm products including poultry meat and poultry products. Nomsa *et al.* (2011) indicated the prevalence of *Escherichia coli* in poultry carcasses. Smith *et al.* (2007) reported that outbreaks of food-borne disease in humans are common in South Africa but rarely reported. Although food borne disease outbreaks are classified as a notifiable medical condition in South Africa, they are likely underreported (WHO, 2015). In some instances, community and household outbreaks occurred following

consumption of poultry meat from contaminated feeds and water resources (GERMS-SA, 2012). The study generated information on the potential zoonotic bacterial species in poultry feeds and water resources which may threaten the viability of the Blouberg poultry value chain project. This information will guide the relevant stakeholders who handle poultry feeds and water resources in following good management practices. This will subsequently lessen the common application of antibiotics which has become a worldwide problem that has led to high emergence of antibiotic resistant pathogens.

1.4 Aim and objectives

The aim of the study was to profile and characterise the zoonotic bacterial pathogens in poultry feeds and water resources in the Blouberg poultry value chain project, Limpopo Province, South Africa.

1.4.1 The objectives of the study were to:

i. Identify the potential zoonotic bacterial pathogens in poultry feeds and water sources, with specific interest to *E. coli, Salmonella* spp*.* and *Shigella* spp*.*
ii. Determine the type of bacteria present in the mouths and recta of chickens.
iii. Determine the antimicrobial resistance profiles of the isolated bacterial species.

1.5 Hypotheses

I. The water resources in Blouberg poultry value chain project are free from contamination by bacterial pathogens.
II. Mouth and rectal swab samples of chickens in Blouberg poultry value chain project are free from contamination by bacterial pathogens.
III. Bacterial pathogens isolated from the samples are resistant to the antimicrobials tested**.**

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Poultry play a vital and often under-valued role in rural development in many rural households and are a global asset for millions who live below the poverty line. They provide scarce animal protein in the form of meat and eggs (SAPA, 2015). South African poultry raw meat and meat products have previously been implicated as carriers of pathogenic bacteria such as *Staphylococcus aureus, Yersinia enterocolitica, Listeria monocytogenes, Campylobacter jejuni and Escherichia coli* (WHO, 2015). Rouger *et al.* (2017) affirmed that the constant increase in poultry meat consumption aggravates several foodborne disease outbreaks which include pathogenic species such as *E. coli*, *Salmonella* spp and *Campylobacter* spp. These are responsible for causing human gastroenteritis from consumption of contaminated poultry meat.

Feed and water of poor quality are excellent media for the growth of microorganisms and this is further influenced by storage conditions, deteriorating water content and alkaline pH (Damerow, 2012). Contamination of animal feed and water before arrival at the farm contributes to poultry infection and colonization of other food producing animals with bacterial pathogens (Asiegbu *et al*., 2016; Christison *et al.,* 2008). Pathogens are transmitted through the food chain to humans and cause human foodborne illnesses (Jorgensen *et al*., 2002; Teplitski *et al.,* 2009). *Campylobacter jejuni*, *Escherichia coli, Salmonella* spp*.* and *Shigella* spp. are the most commonly isolated pathogens in the poultry industry of South Africa (Jorgensen *et al.,* 2002; Bonsman *et al.,* 2014). These pathogens commonly result in high incidences of morbidity and mortality among chickens (Mwanza *et al*., 2011). Contamination of feeds and drinking water with pathogenic microorganisms may lead to low product yield (Nyamongo and Okioma, 2005). Bacterial pathogens are becoming drug resistant (Zhang and Sack, 2012). This results in antibiotic residues in animal derived products, and surging antibiotic resistance (Ayukekbong *et al.,* 2017 & Sahoo *et al., 2012*). Antibiotic resistance is of great public health concern because

the antibiotic-resistant bacteria associated with the animals may be pathogenic to humans, easily transmitted to humans via food chains, and widely disseminated in the environment via animal wastes. These may cause complicated, untreatable, and prolonged infections in humans, leading to higher healthcare cost and sometimes death (Ayukekbong *et al.,* 2017 & Williams-Nguyen *et al., 2016*). Therefore, in order to meet the increasing demand for safe, nutritious and high-quality animal derived foods at affordable prices, whilst maintaining the economic viability of the livestock industry, a number of contemporary challenges will have to be addressed. The challenges include biosecurity threats that leads to emergence of antimicrobial resistance.

2.2 Bacterial pathogens of importance to the South African poultry industry

2.2.1 *Escherichia coli*

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded animals. It has several pathotypes which are separated into different categories based on their associations with human disease. These include enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), enteroinvasive (EIEC), extraintestinal pathogenic (ExPEC), and diffuse adhering *E. coli* (DAEC) (Chandra *et al.,* 2013*)*. Amongst the latter pathotypes ETEC and EHEC remain the most common bacterial causes of shiga-toxin producing *E. coli* (STEC) strains which emerged as the vital zoonotic food-borne pathogens with the highest morbidity and mortality world-wide (Tam *et al.,* 2012). STEC strains are characterized by their ability to release different shiga-toxin subtypes that are identified as stx1, stx1c, stxfc, stx2, stx2e, stx2d and stx2g, which kills host cells in the intestine and enter the bloodstream to affect other organs, such as the kidneys and brain (Scallan *et al.,* 2011). One of the serogroups of STEC that has gained more attention and is considered to be a major public health concern is *E. coli* O157:H7. It has been implicated in outbreaks and cases of haemolytic uraemic syndrome (HUS) and haemorraghic colitis (HC) characterized by anaemia and kidney failure (Gould *et al.,* 2009).

Lupindu (2018) highlighted that Shiga toxin-producing *E. coli* (STEC) O157:H7 is responsible for intestinal and extra-intestinal disease syndromes in human. Isolation of the pathogen from animals, food, clinical samples and environment has been reported from all continents. Therefore, reports on isolation of pathogenic *E. coli* O157:H7 from all regions of the African continent (east, west, south, north and central) show that the pathogen is found throughout Africa. Shiga toxin-producing *Escherichia coli* O157:H7 isolation has been reported to be from humans, animals, food products and the environment. Abong'o and Momba (2008) reported 10.3 % prevalence of STEC O157:H7 from vegetable samples in Eastern Cape Province. Furthermore, poultry meat and meat products from the same location carried the pathogen at a proportion of 2.8 %. A total of 15 countries have reported recovery of pathogenic *E. coli* O157:H7 either from humans, animals, food products or the environment (Majowicz *et al.,* 2014). Furthermore, certain characteristics such as low infectious dose and ability to withstand extreme environments including low pH, as seen in certain foods and the gastrointestinal tract, contribute to its pathogenicity (Ferens and Hoyde, 2011). Carriers often experience symptoms associated with abdominal cramps, vomiting, and diarrhoea, which may progress to haemorrhagic colitis and about 30 % of confirmed cases require hospitalization (Byrne *et al.,* 2015a). The mode of transmission is via the fecal-oral route and is associated with the consumption of contaminated water or food (Hall *et al.,* 2008; Heiman *et al.,* 2015; Adams *et al.,* 2016).

2.2.2 *Salmonella* spp.

Salmonella infections are a worldwide major public health concern (Chen, 2013). The genus Salmonella is a Gram negative bacillus member of the Enterobacteriaceae family. Salmonella are essentially divided into two groups: those that cause typhoidal illness and the more common non-typhoidal (NTS) species. Salmonella are recognized as a major cause of foodborne disease in industrialized countries (Crump, 2015). In South Africa, the incidence of *S. enteritidis* escalated after the first poultry-associated outbreak reaching an incidence rate of 9.3 % (Picard *et al.,* 2010). Muvhali *et al.* (2015) reported Salmonella to be a major cause of morbidity and mortality in children under the age of five in most developing countries. South Africa has witnessed a tremendous increase in chicken meat consumption.

Concurrently, the Enteric Disease Reference Unit of the National Institute for Communicable Diseases noted an increasing number of NTS isolates despite the fact that human salmonellosis cases are rarely reported (NICD, 2010). Evidence of these occurrences are the outbreaks of food-borne illnesses in Mpumalanga Province of South Africa incriminating NTS serotypes. One of the outbreaks involved the consumption of meals prepared with poultry products. These outbreaks indicate the presence of NTS in South Africa, which may be an issue of public health concern (Smith *et al.,* 2007). A few investigations have been conducted in South Africa to ascertain the contamination of chicken carcasses and ready-to-eat foods from retail stores, with various pathogenic bacteria including Salmonella (Christison *et al.,* 2008). Threlfall (2002) reported that most waterborne diseases are propagated with *Shigella* spp. and *Salmonella* spp. The study further reported that, such waterborne from Salmonella outbreaks often implicate a considerable number of individuals being simultaneously affected, and in most cases the outbreak subsides when the water supply is adequately treated (Pillsbury, 2010). Facal shedding by food-producing animals is the leading source of contamination of water, and the environment, whereas intestinal carriage often leads to contamination of carcasses at slaughter (Abraham *et al.,* 2014). It is clear that Salmonella contamination in livestock and poultry has a direct effect on the global marketing of the respective food-producing animals and animal-derived food products (Abraham *et al.,* 2014; Magwedere *et al.,* 2015). The presence of these pathogenic bacteria poses severe threats to environmental and human health (Chapman, 2013). Niehaus *et al.* (2011), following an outbreak of food-borne salmonellosis after a school function in Durban, KwaZulu-Natal, reported that *Salmonella enteritidis* isolated from patients and food samples could not be distinguished phenotypically and genetipically. The authors suggested a point-source as the origin of the outbreak, with a possibility of continued transmission through the water supply.

Among non-typhoidal Salmonella infections, it is estimated that this pathogen causes about 93.8 million cases of gastroenteritis and 155,000 deaths annually worldwide (Majowicz *et al*., 2010). Salmonellosis is caused by non-typhoidal *Salmonella enterica* serotypes (*Salmonella typhimurium and Salmonella enteritidis*). *Salmonella enteritidis* is commonly associated with poultry and poultry products, whereas *Salmonella typhimurium* has a wider species range, including pigs and cattle as well

as poultry (Crump, 2015; Cosby *et al.,* 2015). Foods of animal origin, in particular contaminated poultry products (eggs and poultry meat) have been considered the main mode of Salmonella infection and symptoms are typically characterized by gastroenteritis syndrome manifesting as diarrhoea, fever and abdominal pain with an incubation period between 4 and 72 hours (Chen, 2013; Wasyl *et al.,* 2015). Salmonella species are able to adapt and survive in a wide range of stressful environments, such at pH between 3.9 and 9.5, media concentrations up to 4 % NaCl and temperatures as high as 54^0C or as low as 2^0C (Clemente *et al.,* 2015). Intervention through quality management practices is required to control the source of contamination and transmission of *Salmonella*. Keren *et al.* (2017) reported the detrimental effects of non-typhoidal Salmonella outbreaks amongst the nine provinces of South Africa to have increased from 45.80 million to 52.98 million over the period of 2003 to 2013. Olobatoke and Mulugeta (2015) on incidence of non-typhoidal Salmonella in poultry products in the North West Province, South Africa reported several outbreaks of non-typhoidal Salmonella diseases, which accounted for major mortalities amongst poultry farms. In South Africa, regulatory control measures of NTS in food-producing animals are targeted at *Salmonella enterica* subspecies *enterica* serotype *Enteritidis* in poultry under Section 31 of the Animal Diseases Act (Act 35 of 1984). These prescribed measures are supplemented by a movement control protocol that is triggered by an outbreak of *Salmonella enteritidis* infection in poultry or other birds (Magwedere *et al.,* 2015). In fact, Salmonella is now defined in the standard operating procedure for the microbiological monitoring of imported meat as a biological agent associated with serious illness or death, particularly those strains resistant to one or more critically important antimicrobials used in human medicine.

2.2.3 *Shigella* spp.

Shigella is a Gram-negative, facultative anaerobic bacterium (Scallen *et al.,* 2011). Multiple serotypes of *Shigella* have been reported. However, the most common pathogenic serotypes are *Shigella sonnei* which causes 77 % of shigellosis cases in developed countries and *Shigella flexneri,* which accounts for 60 % of shigellosis cases in African countries including South Africa (Woodward *et al.,* 2005). There is no animal reservoir for *Shigella*, and infection is transmitted person-to-person, via

fomites, and from ingestion of contaminated food or water. *Shigella* species outbreaks are associated with poor sanitation and hygiene and limited access to clean drinking water. Transmission control under these circumstances is made more difficult by the relatively low infectious dose of this pathogen. Humans are the main reservoir for shigellosis. The organism has been found in food products including beef, chicken, raw milk, and yoghurt (Ahmed & Shimamoto, 2014). Healthy individuals with mild infections usually recover without specific treatment. Antibiotic treatment is recommended for dysentery, severe shigellosis, and individuals with compromised immune systems (Mokhtari *et al.,* 2012). The emergence of multi-drug-resistant strains of *Shigella* further complicates antibiotic treatment, making prevention of infection critical. The global burden of shigellosis has been estimated to be 150 million cases, with one million deaths per year recorded in developing countries (Parsot, 2005; Schmid-Hempel and Frank, 2007). *Shigella* spp are normally found in water polluted with human excreta (Saha *et al.,* 2009). The presence of *Shigella* spp. in drinking water indicates human faecal contamination. This bacterium is of fundamental public health significance because of its great pathogenicity. Outbreaks of shigellosis have been associated with water treatment failures (at times inefficient treatment) in water supply systems (Karanis *et al.,* 2007).

2.3 Bacterial contamination of poultry feeds

Magwedere *et al.* (2015), indicated that foodborne and waterborne illnesses associated with *Salmonella* spp. were more commonly due to increased faecal pollution of feeds resources. The study also reiterated that the use of animal waste as fertilizer for crops or raw materials destined for producing animal feed is a common practice by some farmers. It is plausible that such raw materials may lead to subsequent contamination of the feed mill environment. Poultry feed is typically composed of maize and soybean meal mixtures, including several vitamins and minerals, and generally contains two or three medications; which comprises 68% of total production costs (Jones *et al.,* 2016). The microbial diversity found in different animal feeds is dependent on the water activity, oxygen concentration, pH and nutrient composition of the feed ration (Glen *et al.,* 2013; Kim *et al*., 2007). Feed materials are usually inoculated during growing, harvesting, processing, storage and dispersal of the feed (Jones *et al.,* 2016; Maciorowski *et al*., 2004). A soil mixed with

animal faeces can contaminate standing crops either by direct deposition or when used as fertilizer. Houseflies and cockroaches feeding on facal matter can act as both vectors and reservoirs for pathogens in the environment (Glen *et al.,* 2013; Kim *et al*., 2007; Maciorowskiet *et al.*, 2004). The majority of small animal feeds producing plants in South Africa have difficulty in providing adequate treatment and disinfection which result in animals and animal by-product consumers are at risk of foodborne diseases (Maciorowski *et al.,* 2007).

Sanderson *et al.* (2005) reported that coliform bacteria, including *Escherichia* spp., *Klebsiella* spp., and *Enterobacter* spp., are the most pathogens contaminating animal feeds. As such, they prompt zoonotic diseases outbreaks and secondary infections in humans resulting in major hospitalisation cases. Therefore, the ingredient quality control component of a poultry operation's feed mill is an important first step in preventing the contamination of birds on the farm. Many times the *Salmonella* serotypes found in feed ingredients are not the same as those commonly found in processed poultry (Jones, 2016). Heat treatment is the most effective control method used to inactivate feed pathogens. Reductions in bacterial contamination by heat depends on the temperature, treatment time and the moisture content of the feed (Maciorowski *et al.,* 2004). Chemical treatments such as organic acids alone or in combination with formaldehyde are sometimes used (Mekeer, 2015; Richardson, 2004). Pelleting is a process of pressing conditioned material with specific dimensions of openings and thickness. One of the supplementary benefits of pelleting is destruction of pathogenic and reduction of total microorganisms, due to the increased temperature during processing (Zimonja, 2009). Applications of steam and water in animal feed manufacturing have long been recognized as a good way to achieve production of high quality pellets (Sredanović *et al.,* 2005). Because feeds are a significant source of exposure for poultry to potential foodborne pathogens, therefore on-farm control efforts to decrease and to prevent feeds contamination need to be implemented (Maciorowski *et al*., 2004; Sanderson *et al.,* 2005). Improving biosecurity measures for feed storage at the feed mill or on the farm would likely be a more-cost effective risk management strategy to lower pathogens proliferation in animal feeds.

2.4 Bacterial contamination of poultry water resources

Water composition varies with geographical region and environmental conditions. Water contamination can occur if surface water drains into the well especially if the water source is exposed. Several researchers have demonstrated a positive association between drinking water contamination with *E. coli* O157:H7 and the presence of this organism in poultry faeces (Levantesi *et al.,* 2012; Saha *et al.,* 2009). Poor water quality may interfere with digestion and subsequent bird performance (Brain *et al*., 2014: Narita *et al*., 2014). The effectiveness of vaccines and medications administered through the water can be reduced when water quality is poor (Brain *et al*., 2014). Leaky water through nipples inside the poultry house will wet the litter and lead to increasing ammonia production and prompt micro-organism proliferation (Ashbolt, 2015). Therefore, control measures should be prioritized to prevent the occurrence of diseases that are spread through water, and would certainly result in great economical losses (Kostyla *et al*., 2015). In this regard, water is an excellent transmission route of agents responsible for human and animal diseases, mainly those in which faecal oral transmission occurs, since contamination of water supplies still gradually increases as a result of urban and rural activities (Brain *et al.,* 2013). Safe and sufficient water and sanitation would reduce animal mortalities and child deaths by 50 % and prevent 25 % of diarrhoea (Momba and Kaleni, 2003; Momba and Notshe, 2003).

In 2004, about four million people were still obtaining water from rivers, ponds and springs which were 13usually not treated and were faecally contaminated (Momba and Kaleni, 2003; Momba and Notshe, 2003). While the present South African Government has implemented many rural water supply schemes under the National Reconstruction and Development Programme, where rural water supplies do exist, drinking water is often of poor quality and considered unsafe (Momba *et al.,* 2003; 2004; 2006). Such waterborne outbreaks often lead to a considerable number of individuals being simultaneously affected, and in most cases the outbreak subsides when the water supply is adequately treated (Pillsbury, 2010). Momba *et al.* (2006) reported the abundance of pathogenic *Escherichia coli, Salmonella typhimurium* and *Vibrio cholerae* in both surface and groundwater sources in South Africa. The presence of these pathogenic bacteria in drinking water sources poses a serious health risk to consumers. Although eliminating *E. coli* O157:H7 from poultry drinking

water may be a meritorious goal, and an effective measure that would reduce poultry drinking water contamination with pathogenic bacteria (Bucher *et al.,* 2007).

2.5 Antimicrobial resistance

Antimicrobial resistance has drastically increased due to the global misuse and overuse of antibiotics (Fletcher, 2015). This is prompted by the high prevalence of foodborne zoonotic pathogens such as *Escherichia coli*, *Enterococcus spp*., *Staphylococcus aureus* as well as non-typhoidal Salmonella (NTS) and *Campylobacter* spp. (Landoni and Albarellos, 2015). The situation is aggravated in developing countries like South Africa, with an estimated 11 million bacterial infections per year (OECD, 2017). In severe cases, effective antimicrobial agents are essential but, the emergence of *Salmonella* strains that are resistant to ampicillin, chloramphenicol and trimethoprim sulfamethoxazole pose a challenge leading to morbidity and mortality (Crump, 2015; Chen, 2013; Wasyl *et al.,* 2014). An epidemiology of Global monitoring of Salmonella serovar reported the resistance of *E*. *coli* and Salmonella strains on ampicillin, chloramphenicol and trimethoprim sulfamethoxazole (Hendriksen *et al.* 2011; Eager *et al*., 2012) also confirmed that the application of antimicrobials in food animals, may lead to a development of resistant strains of bacteria, which propagates to infect both animals and man. Oguttu *et al*. (2008) on antimicrobial drug resistance of *Escherichia coli* isolated from poultry abattoir workers at risk and broilers on antimicrobials, has reported an antimicrobial usage in food animals to be increasing and resulting with development of antimicrobial drug resistant bacteria. It has been suggested that this resistant bacterial strains can be transferred to people working with such animals, e.g. abattoir workers. Antimicrobial drug resistance was investigated for *Escherichia coli* from broilers raised on feed supplemented with antimicrobials, and the people who carry out evisceration, washing and packing of intestines in a high-throughput poultry abattoir in Gauteng, South Africa. Khumalo *et al.* (2014) reported that *Salmonella enterica* is causing a public health concern because of its increasing prevalence and resistance to multiple antibiotics, with mostly animal-borne serovars being multidrug resistant. *Salmonella enteritidis* is one of the most prevalent serovars with an increased display of antimicrobial resistance globally (Hendriksen *et al.,* 2011; Okeke *et al.,* 2007; Parry 2013).

In addition to their specific effects, edible tissues of poultry might contain veterinary drug residues, which would cause hazardous health effects in humans, such as toxicological effects, hypersensitivity, allergic reactions, change of gut microflora, and increased bacterial resistance to antimicrobials (Bayene, 2016). Serious concerns are raised on the antimicrobial resistance in zoonotic enteropathogens (*Salmonella* spp., *Campylobacter* spp.), commensal bacteria (*Escherichia coli*, *Enterococci*), and bacterial pathogens of animals (*Pasteurella*, *Actinobacillus* spp.) (Stephano and Avalleno, 2014).

2.6 Conclusion

Microbial contamination of animal feed and water resources is a significant potential pathway for entry of pathogens into the human food supply chain. Ensuring that animal feeds and water resources are free from bacterial pathogens will help reduce human foodborne illness.

CHAPTER THREE

MATERIALS AND METHODS

All the procedures performed during the study which involved the handling of animals were approved by the University of Limpopo Animal Research and Ethics Committee.

3.1 Study site

The study was conducted at Blouberg poultry value chain project under the Department of Rural Development and Land Reform in Blouberg Municipality, Limpopo Province, South Africa (51.4818° N latitude, 7.2162° E longitude) co-ordinates 29°00'4.68". The project consists of 34 broiler farmers and 35 layer farmers. The Department of Rural Development and Land Reform supplied farmers with feeds and day-old chicks from one supplier. The beneficiaries of this project had different levels of training and expertise with regard to poultry management.

3.2 Acquisition of the study materials

All the materials and laboratory consumables used in this study were purchased from SIGMA-ALDRICH® Company.

3.3 Materials and sampling

A total of 88 samples comprising of 14 feed samples, 14 water samples, 60 mouth and rectal swab samples were collected from the randomly selected farms. Fifty millilitres of water samples were collected from the same bulk tank in duplicates and from the drinkers within each poultry house into 50 ml sterile bottles. Two hundred grams of feed samples were collected from the storage room and from the feeders in each house section in duplicates into 200 g sterile bottles. The samples were collected in one day. Mouth and rectal samples were collected from 10 % of the

randomly selected chickens. Collected samples were transported on ice to the University of Limpopo, Microbiology laboratory for analysis within three hours of collection.

3.4 Microbiological analyses

3.4.1 Preparation of samples for screening and enumeration of selected bacteria

3.4.1.1 Media preparation and characteristics of target bacteria.

Selective cultivation method was used for isolation and enumeration of zoonotic pathogens. MacConkey agar (SIGMA-ALDRICH ®), Xylose Lysine Deoxycholate (XLD) agar (SIGMA-ALDRICH ®), Rappaport Vassiliadis broth (SIGMA-ALDRICH ®).

MacConkey agar was used for the isolation of Gram-negative enteric bacteria. It was also used for the isolation of coliforms and intestinal pathogens in water, dairy products and biological specimens. Lactose fermenting strains grew as red or pink. Lactose non-fermenting strains, such as Shigella and Salmonella were colourless and transparent.

Xylose Lysine Deoxycholate (XLD) agar was used for isolation and differentiation of Salmonella and Shigella from both clinical and non-clinical specimens. Salmonella colonies form colonies with black centers and *Shigella* colonies formred colonies.

Rappaport Vassiliadis broth was a primary enrichment to isolate Salmonella species from food and environmental specimens. After incubation the broth was subcultured by streaking on to plates of X.L.D. Agar.

3.4.1.2 Processing of water samples

Water samples were mixed thoroughly. Serial dilutions were prepared with saline solution (0.9% NaCl) from 10^{-2} to 10^{-6} in a 2 ml Eppendorf tube. The mixture was mixed thoroughly by vortexing and 100 microliters of the mixture was plated on the agar plates of MacConkey, Rappaport Vassiliadis broth and XLD media. The plates were incubated according to the manufacturer's guidelines.

3.4.1.3 Processing of feed samples

A 100 mg of feed sample was weighed and suspended into a microcentrifuge tube containing 900 microliters of saline. The mixture was mixed by vortexing for about 30 seconds to one minute and left to settle. Hundred microliters of the mixture was serially diluted from 10^{-1} to 10^{-6} and inoculated onto the media plates (XLD media) and the broth (Rappaport Vassiliadis broth). The plates and the broth were incubated according to the manufacturer's instruction.

3.4.1.4 Processing of swab samples

Each swab was rolled onto the total agar surface of MacConkey and XLD media and incubated according to the manufacturer's instruction. The cotton bud of the swab was then cut and inoculated into the tubes containing the Rappaport Vassiliadis broth.

3.5 Colony morphology and Gram staining

The plates with colonies were observed, selected and counted. Enumeration was performed manually under white light on the media plates that contained total colony counts of 30-300. All the media cultures including the broth culture colonies were sub-cultured and Gram stained to check for purity and cellular characteristics of the isolates. The isolates were sub-cultured on MacConkey and XLD media for subsequent identification assay.

3.6 Identification of isolated organisms

Identification of the bacteria was done with a MALDI-TOF MS through a modified biotyping protocol that was provided by the manufacturer (Bruker).

A fresh bacterial colony was thoroughly suspended in 300 µl of deionized water in an Eppendorf microcentrifuge tube. Nine hundred microliters of absolute ethanol was added and mixed by vortexing. The Eppendorf tubes were centrifuged at a maximum speed of 13,000 rpm for 2 min. The supernatant was discarded, and all the residual of the absolute ethanol was carefully pippeted off without disturbing the pellets. The pellets were allowed to dry at room temperature for 2 to 3 minutes. After drying, 80 µl of 70 % formic acid was added and mixed thoroughly by vortexing. Eighty microliters of 70 % acetonitritate was then added, mixed by vortexing and centrifuged at a

maximum speed of 14 000 rpm for 2 minutes. One microliter of the supernatant was pippeted onto a MALDI target plate and allowed to dry at room temperature. Then 1 µl of matrix liquid was pippeted onto the dried spot of the supernatant. The MALDI target plate was loaded into the flex control MALDI-biotyper for identification.

NB: Sample identity refers to the identity codes assigned to the farmers. Culture source refers to where the samples were collected, poultry feeds, water resources and (mouth and rectal swabs). Colonial morphology is the morphology of the colonies identified as suggested by the media manufacture's guidelines. Cellular morphology by Gram staining refers to the morphology of the cells as determined through Gram staining. Identity, these are the results of the organisms obtained through MALDI-TOF MS identification and the score values.

3.7 Antibiotic sensitivity assay

Nine different antibiotics of varying strengths presented on discs (MASTIDISCS ^{TM}) were utilized to determine the sensitivity of the isolated bacteria. Antibiotic sensitivity test was performed following the Kirby Bauer disk diffusion method (Bauer *et al.,* 1966 and Jorgensen *et al.,* 2007). The antibiotic discs used were streptomycin (S_{10}), ampicillin (AP_{25}), penicillin (PG_{10}), tetracycline (T_{30}), nalidixic acid (NA_{30}), erythromycin (E_{15}), neomycin (NE_{30}), ciprofloxacin (CIP_5) and gentamycin (GM_{10}). The results were interpreted as susceptible, intermediate, or resistant according to the zone diameter interpretative standards suggested by the Clinical and Laboratory Standards Institute (CLSI, 2001). The multiple antibiotic resistance (MAR) index of each strain was also detected using the equation provided by Singh *et al.* (2010) as follows:

$$\text{MAR} = \frac{\text{number of antimicrobial drugs to which the bacterium is resistant}}{\text{total number of antimicrobial drugs}}$$

CHAPTER FOUR

RESULTS

4.1 Isolation of zoonotic bacterial pathogens in poultry swabs (mouth and rectal), feeds and water resources

The samples were analysed for their bacterial quality using the culture plate count method. Not all samples yielded an isolate and in such cases an attempt was made to re-culture them. Table 4.1 shows the identity of the bacterial isolates and their morphological characteristics.

Table 4.1 Culture characterisation and identification from poultry swabs (mouth and rectal) and water samples

Sample identity	**Culture: source**	**Colonial morphology**		**Cellular morphology by Gram staining**		**Identity**	
		XLD	**MacConkey**			**Organism (best match)**	**Score Value**
SEO	Rectal swab 1	Large, flat, yellow colonies	Pink coloured colonies	Rods	Negative Pink/red	Not reliable identification	1.637
	Rectal swab 2	Pink, flat, rough colonies	Pale transparent colonies	Rods	Negative Pink/red	***Escherichia coli***	1.995
	Mouth swab 1	Large, flat, yellow colonies	Pink coloured colonies	Rods	Negative Pink/red	***Escherichia coli***	1.848
	Mouth swab 2	Pink, flat, rough colonies	Pink coloured colonies	Rods	Negative Pink/red	***Escherichia coli***	1.893
MAT	Mouth swab1	Yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	***Escherichia coli***	2.071
	Mouth swab 2	Red colonies, black centers	Pale transparent colonies	Rods	Negative Pink/red	***Escherichia coli***	2.106
	Rectal swab 1	Large, flat, yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	No peaks found	

	Rectal swab 2	Large, flat, yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	No peaks found	
	Water sample 1	Large, flat, yellow colonies	Pink coloured colonies	Rods	Negative Pink/red	***Escherichia coli***	1.955
	Water sample 2	Large, flat, yellow colonies	Pink coloured colonies	Rods	Negative Pink/red	***Escherichia coli***	1.921
RAS	Rectal 1	Large, flat, yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	No peaks found	
	Rectal swab 2	Yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	***Escherichia coli***	1.858
	Mouth swab 1	Large, flat, yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	Not reliable identification	<1.590
	Mouth swab 2	Pink, flat, rough colonies.	Pale transparent colonies	Rods	Negative Pink/red	No peaks found	
RAM	Rectal swab 1	Large, flat, yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	No peaks found	
	Rectal swab 2	Mucoid, yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	No peaks found	
	Rectal swab 3	Large, flat, yellow colonies	Pink coloured colonies	Rods	Negative Pink/red	No peaks found	
	Mouth swab 1	Yellow colonies	Pink coloured colonies	Rods	Negative Pink/red	***Escherichia coli***	2.071
	Mouth swab 2	Yellow colonies	Pink coloured colonies	Rods	Negative Pink/red	***Escherichia coli***	1.885
	Mouth swab 3	Large, flat, yellow colonies	Pink coloured colonies	Rods	Negative Pink/red	No peaks found	
	Water sample 1	Red colonies	Pink coloured colonies	Rods	Negative Pink/red	***Klebsiella oxytoca***	1.864
	Water sample 1	Red colonies	Pink coloured colonies	Rods	Negative Pink/red	***Klebsiella pneumoniae***	1.933

MAU	Rectal swab 1	Mucoid, yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	***Klebsiella oxytoca***	1,984
	Rectal swab 2	Large, flat, yellow colonies	Pink coloured colonies	Rods	Negative Pink/red	***Klebsiella pneumoniae***	2.127
	Rectal swab 3	Yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	***Escherichia coli***	1.994
	Rectal swab 4	Pink, flat, rough colonies	Pink coloured colonies	Rods	Negative Pink/red	***Klebsiella oxytoca***	1.757
	Mouth swab 1	Large, flat, yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	No peaks found	
	Mouth swab 2	Large, flat, yellow colonies	Pink coloured colonies	Rods	Negative Pink/red	No peaks found	
	Mouth swab 3	Large, flat, yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	No peaks found	
	Mouth swab 4	Red colonies	Pink coloured colonies	Rods	Negative Pink/red	***Klebsiella oxytoca***	1.988
	Water sample 1	Large, flat, yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	***Enterobacter asburiae***	1.738
	Water sample 1	Large, flat, yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	***Enterobacter kobei***	1.728
	Water sample 2	No growth	Pale transparent colonies	Rods	Negative Pink/red	No peaks found	
	Water sample 2	No growth	Pale transparent colonies	Rods	Negative Pink/red	No peaks found	
SEL	Rectal swab 1	Large, flat, yellow colonies	Pink coloured colonies	Rods	Negative Pink/red	No peaks found	
	Rectal swab 2	Yellow colonies	Pink coloured colonies	Rods	Negative Pink/red	No peaks found	
	Mouth swab 1	Large, flat, yellow	Pink coloured colonies	Rods	Negative Pink/red	Not reliable identification	1.649

		colonies					
	Mouth swab 2	Large, flat, yellow colonies	Pale transparent colonies	Rods	Negative Pink/red	Not reliable identification	1.692
	Water sample 1	Red colonies	Pink coloured colonies	Rods	Negative Pink/red	***Klebsiella pneumoniae***	2.121
	Water sample 2	Red colonies	Pink colonies	Rods	Negative Pink/red	***Klebsiella pneumoniae***	2.098
MAD	Rectal swab 1		Red colonies	Rods	Negative Pink/red	No peaks found	
	Rectal swab 2	Large, flat, yellow colonies	Large, flat, yellow colonies	Rods	Negative Pink/red	***Escherichia coli***	1.941
	Mouth swab 1	Large, flat, yellow colonies	Large, flat, yellow colonies	Rods	Negative Pink/red	No peaks found	
	Mouth swab 2	Large, flat, yellow colonies	Large, flat, yellow colonies	Rods	Negative Pink/red	Not reliable identification	1.544

*No peaks found means no identity
*Values between 2.300 - 3.000 means highly probable species identification.
*Values between 2.000 – 2.299 means secure genus identification, probable species identification.
*Values between 1.700 – 1.999 means probable genus identification.
*Values between 0.000 – 1.699 means not reliable identification.

Most isolates from the poultry swabs scored values between 1.700 – 1.999, which means that the organism could be identified up to a genus level. Very few isolates from water samples could be identified up to genus identification mostly could not yield reliable identification. Similarly, most of the isolates that were identified in poultry swabs from different farms were also obtained in water resources. There was a trend between characterisation and identification which is the prevalence and confirmation of the target organisms.

4.2 Colony counts in feed samples

Bacterial culture in feed samples with both XLD and MacConkey media could not yield any colonies indicating that there was no bacterial growth.

4.3 Colony counts in water samples

Figure 4.1 indicates the average number of colonies counted from water samples in both XLD and MacConkey media. Different colonies were obtained and counted from different selective media. The average colonies counted from the MacConkey media ranged from 66 to 87 cfu/ml. and the average colonies counted from the XLD media ranged from 226 to 261 cfu/ml. similar isolates were observed from the samples which indicated the positive presence of the target microorganisms.

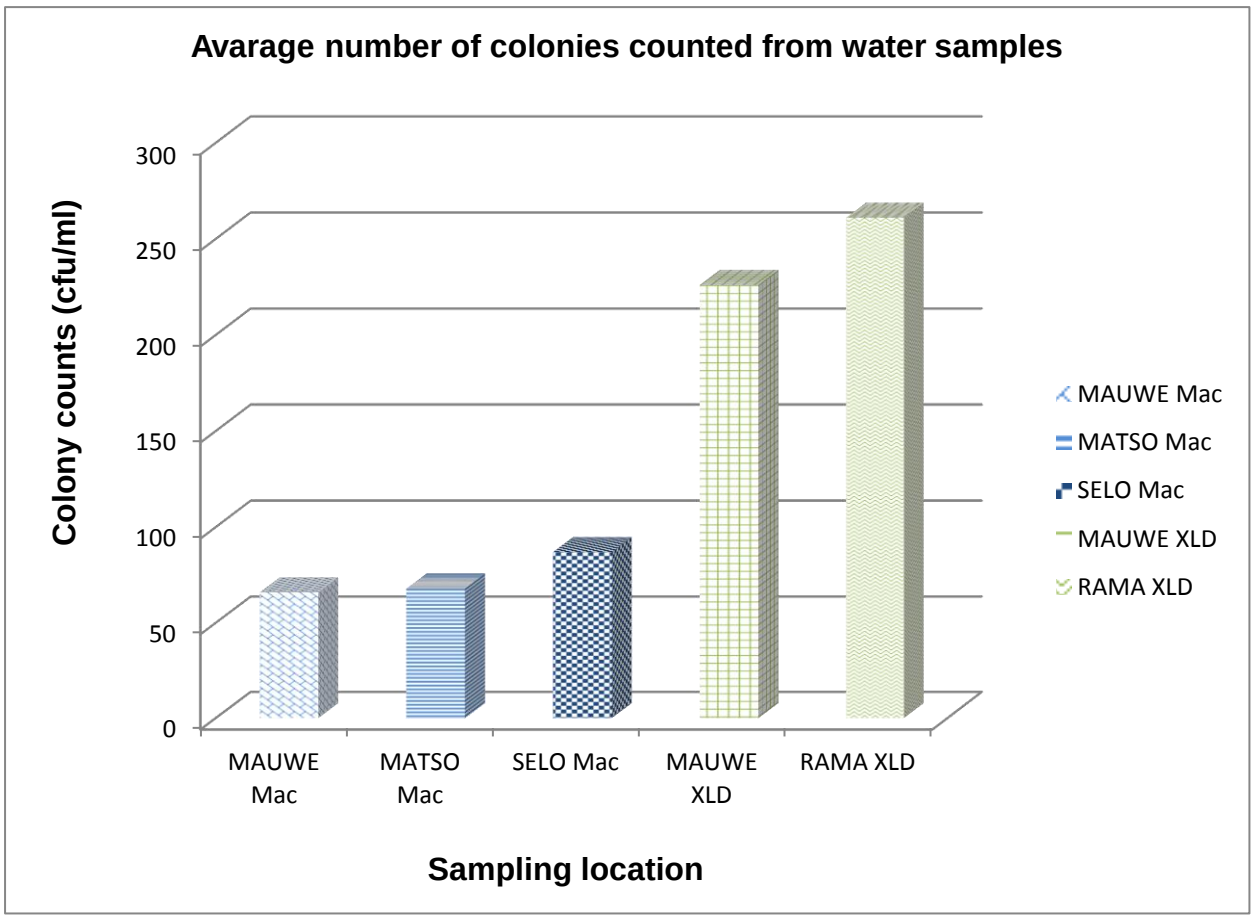

Figure 4.1 Average number of colonies counted from water samples

4.4 Antibiotic sensitivity test.

Table 4.2 and Table 4.3 show the results of the antibiotic sensitivity tests for the isolated organisms from each sample (mouth and recta) and water resources.

Great variation in the resistance to antibiotics was observed. A large number of isolates were resistant to most antibiotics. However, from the poultry mouth and rectal swabs, the highest resistance occurred in Farm 4 and Farm 5 with 89 % of

Escherichia coli resistant to gentamycin, neomycin, penicillin, streptomycin, tetracycline, erythromycin, nalidixic acid, ciprofloxacin and ampicillin. The lowest percentage of resistance was observed in Farm 1 with 33, 3 % of *Klebsiella pneumoniae* resistant to neomycin; penicillin; erythromycin and also in Farm 2 with 33, 3 % *Klebsiella pneumoniae* resistant to penicillin; erythromycin and ampicillin. Similarly, *Klebsiella pneumoniae* isolates in Farm 1 and Farm 2 were resistant to the same antibiotics. And the unreliable identified organisms in Farm 2 and Farm 3 were resistant with 67 % to the same antibiotics namely; penicillin, streptomycin, tetracycline, erythromycin, nalidixic acid and ampicillin. From the poultry swab samples in farm 2, three organisms were isolated namely: *Escherichia coli, Klebsiella oxytoca* and *Klebsiella pneumoniae* which showed almost similar antibiotic resistance phenotype. *Escherichia coli* was isolated from both swabs and water samples, which showed the highest antibiotic resistance phenotype with 89 % in farm 4 from the poultry swabs and 78 % of resistance in farm 3 from the water samples. *Klebsiella pneumoniae* showed similar antibiotic resistance phenotype of 67 % from swabs in farm 1 and farm 2 of the water samples. Similar resistance of 56 % was further observed in both swabs and water samples in farm 2 and 4.

Table 4.2 Antibiotic resistance phenotype percentage observed among the isolates from poultry swabs (mouth and recta) in the study sites

	SITE SAMPLE ID.		ORGANISM					NO OF ISOLATES	AB RESISTANT PERCENTAGE (%)	AB PHENOTYPE
			E. coli	*K.oxytoca*	*K. pneumonia*	No peaks found	Unreliable identity			
Farm 1	SEL	M1	-	-	+	-	-	1	33.3	NE-PG-E-
	SEL	R2	-	-	+	-	-	1	67	NE-PG-T-E-NA-AP
Farm 2	MAU	R1	-	+	-	-	-	1	56	NE-PG-T-E-AP
	MAU	R2	-	-	+	-	-	1	56	NE-PG-E-NA-AP
	MAU	M2	-	-	-	-	+	-	67	PG-S-T-E-NA-AP
	MAU	R3	+	-	-	-	-	1	33,3	PG-E-AP
	MAU	M4	-	+	-	-	-	1	67	NE-PG-T-E-NA-AP
Farm 3	MAT	M2	+	-	-	-	-	1	56	NE-PG-T-E-AP
	MAT	R2	-	-	-	+	-	1	67	PG-S-T-E-NA-AP
Farm 4	MAD	R2	+	-	-	-	-	1	89	GM-NE-PG-S-T-E-NA-AP
	MAD	M2	-	-	-	-	+	1	67	GM-PG-T-E-NA-AP
Farm 5	RAS	R2	+		-	-	-	1	89	NE-PG-S-T-E-NA-CIP-AP
	RAS	M2	-	-	-	+	-	-	56	NE-PG-T-E-AP
Farm 6	SEO	M1	+	-	-	-	-	1	56	PG-S-T-E-AP
	SEO	R2	+	-	-	-	-	1	56	PG-S-T-E-CIP
Farm 7	RAM	M1	+	-	-	-	-	1	56	GM-NE-PG-T-E-
	RAM	R3	-	-	-	+	-	-	56	PG-S-T-E-AP

Table 4.3 Antibiotic resistance phenotype percentage observed among the isolates from poultry water resources in the study site

	SITE SAMPLE ID.		ORGANISM					NO OF ISOLATES	AB RESISTANT PERCENTAGE (%)	AB PHENOTYPE
			E. coli	*K.oxytoca*	*K. pneumonia*	*E. kobei*	*E. absuriae*			
Farm 1	MAU	I1	-	-	-	-	+	1	44.4	PG-E-NA-AP
	MAU	I2	-	-	-	+	-	1	44.4	PG-T-E-AP
Farm 2	SEL	I1	-	-	+	-	-	1	56	NE-PG-T-E-AP
	SEL	I2	-	-	+	-	-	1	67	GM-NE-PG-E-NA-AP
Farm 3	MAT	I1	+	-	-	-	-	1	78	NE-PG-T-E-NA—CIP-AP
	MAT	I2	+	-	-	-	-	1	67	PG-S-T-E-NA-AP
Farm 4	RAM	I2	-	+	-	-	-	1	44.4	PG-E-NA-AP
	RAM	I2	-	-	+	-	-	1	56	NE-PG-E-NA-AP

* Streptomycin (S); Ampicillin (AP); Penicillin (PG); Tetracycline (T); Nalidixic acid (NA); Erythromycin (E); Neomycin (NE); Ciprofloxacin (CIP) and Gentamycin (GM) * *AB- Antibiotic;*
* (+) – means that the target organism was identified in that particular sample.
* (-) – means the sample was free from the target

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Most of the microorganisms isolated in this study are commonly associated with diseases of the poultry farm. Zoonotic pathogens such as *Salmonella* spp and *Shigella* spp were not detected except the prevalence of *Escherichia coli*. These results are similar to the findings of Voidarou *et al.* (2016) which indicated the presence of bacteria such as *Salmonella* spp., *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Campylobacter spp.,* and *Clostridia perfringens.* Agapi *et al.* (2012) on spoilage microbiota associated to the storage of raw meat in different conditions, also isolated *Salmonella* spp and *K. pneumoniae*, which contribute to poultry foodborne outbreaks and poultry carcasses spoilage. The contamination of poultry meat is mainly due to undesired microbial development during improper feed storage (Agapi *et al.*, 2012). The type of bacteria and their loads depend on the initial bacterial contamination, and proliferation of the bacterial pathogens might be aggravated by poor hygiene (Brightwell *et al.,* 2007). Ribot *et al.* (2006) reported that *E. coli* is distributed among poultry of all ages. The *Escherichia* bacterium is a natural inhabitant of the gut in poultry and in most other animals. Normally, it is kept in check by other bacteria in the gut, but if large colonies form or develop, then it can cause severe discomfort, illness, and mortality amongst the chickens (Chapman, 2013; Niehaus *et al.,* 2011). This explanation could be the reason why *Escherichia coli* identified in the mouth and rectal swabs could not be detected from the water samples.

Escherichia coli might have been introduced by air or faecal contamination or by any other contaminated substances to the water resources. On the other hand, the presence of *Klebsiella spp,* and *Enterobacter spp*. may be due to the exposure of the poultry drinkers and harvested rain to the unsanitary environment which allowed such organisms to proliferate and contaminate the water resources (Tzouvelekis *et al.,* 2012; Berendonk *et al.,* 2015). However, the results showed 2.3 % prevalence of the *Enterobacter spp*. As a result of their genetic and phenotypic relatedness, they are combined in the *Enterobacter* cloacae complex (Nyenje *et al.,* 2013). *Enterobacter cloacae* sub-species occur in the intestinal tracts of humans and

animals, seldom found in poultry, but in hospital environments, in water, sewage, soil and meat. It is reputed as a common cause of hospital-associated infection in humans, including urinary tract infection, bacteremia, pneumonia and surgical site infection (Manzur *et al.,* 2007). Bunkova *et al*. (2010) isolated *Escherichia coli*, *Proteus vulgaris*, *Klebsiella oxytoca* and *Klebsiella spp* from poultry carcass. These isolates are similar to the isolates obtained in the current study. The results of this study confirm the poor microbiological quality of the drinking water that is supplied to the farmers and chickens. The isolates from the mouth and rectal swabs are different to the isolates from the water samples. The epidemiology and ecology of *Klebsiella* spp. *Salmonella spp*. and *E. coli O157* suggest faecal contamination of feed or water may be a possible source of exposure of different microorganisms in poultry conventional houses and water resources (Bunkova *et al*., 2010). Poultry faeces promote a significant growth of foodborne pathogens. Dust has been associated with a long persistence of *E. coli* and *Salmonella spp.,* in the poultry houses. As the above pathogens have been found to be surviving in small pockets of fan dust, which had been left after cleaning and disinfection of the poultry house (Davis and Morishita, 2005). Dust could possibly act as a vector for pathogens spread from infected hens to healthy ones, through a potential airborne transmission (Sirsat *et al.,* 2009). Holt *et al.* (2007) and Umali *et al.* (2012) suggested that implementing strict biosecurity measures would limit the spread of airborne infection within flocks, and reduce the risk of potentially contamination.

The microbiological quality of drinking water supplied to the South African communities including Limpopo Province is poor (Momba *et al.* 2006). Obi *et al*. (2005) confirmed the presence of enteric pathogens such as *Salmonella enteritidis, Shigella dysenteriae,* and *verotoxigenic E. coli* in the water consumed by the communities in the Limpopo Province. The authors concluded that this prompt several foodborne diseases outbreak that result in devastating effects of mortalities in broilers and human hospitalisation. The poor microbiological quality of drinking water and especially the presence of pathogenic *E. coli* strains, and other pathogens in drinking water could explain how the variation amongst the prevalent organisms were attributed. The environment of the poultry house can act as reservoir for pathogens (Gast, 2007). Magwedere *et al.* (2015) isolated several bacterial spp., including *Escherichia* spp., *Klebsiella spp*., and *Enterobacter* spp., in poultry feeds

and water resources which are considered to be brought by faecal contamination in feeds and water. According to a study by Magwedere *et al.* (2015) these pathogens are also responsible for poultry mortalities and severe secondary infections in humans. This could explain why these similar isolates were obtained in our study from poultry water resources.

Hald *et al.* (2006) isolated an average of 45 % of *Salmonella, Candida albicans, Proteus* spp., *Escherichia coli* and *Pseudomonas* spp*.* from feeds. This is however not comparable with our study because of the differences in the microbial activities and environmental factors. In this study, feed samples did not yield any isolate even when re-culturing was attempted. This situation may be prompted by the selective media that were used or that the targeted organisms were very low to detect. Crumps *et al.* (2002) observed that Salmonella was the major contaminant of poultry feeds. Zimonja (2009) highlighted that pelleting feeds is associated with destruction of pathogenic and reduction of total microorganisms due to the increased temperature during pelleting. Applications of steam and water in animal feed manufacturing have long been recognized as a good way of reducing pathogens (Sredanović *et al.,* 2005*).* Conditioning in animal feed production is the process of converting mixed mash with the use of heat, water, pressure and time, to a physical state which is more suitable for compaction of feed mash*.* Properly conditioned feed mash give pellets good durability, hardness and hygienic quality, together with improved nutritional value of feed (Maciorowski *et al.,* 2007). The above reviews maybe some of the reasons on why the feed samples could not yield isolates.

High levels of bacteria in drinking water negatively impact productivity in poultry (Derouchey *et al.,* 2004). *Klebsiella pneumoniae* causes mortalities in broilers, indicating that transmission could be facilitated by close contact between broilers and humans (Eman *et al.,* 2016). Sadeyen *et al.* (2004) reported that persistent pathogenic strains in the digestive tract are caused by an immunodeficiency state of the animal, which results in diseases outbreak when the pathogens accumulate. *Klebsiella pneumoniae* has been proposed as a model organism for poultry diseases due to its presence in both the environment and in animal guts and in the development and spread of resistance (Tzouvelekis *et al.,* 2012; Berendonk *et al.,*

2015). The presence of this pathogen could be the cause of mortalities amongst the farms.

A study of antibiotic resistance of bacteria isolated in poultry environment by Amit *et al.* (2017) reported the highest resistance of more than 70 % of *E. coli* isolates to common antibiotics such as co-trimoxazole, doxycycline, amoxyclav, levofloxacin, ciprofloxacin, cefotaxime, penicillins, fluoroquinolones. The study also reported a low resistance percentage of less than 30 % to aminoglycosides such as ampicillin, amikacin and gentamicin. Berendonk *et al.* (2015) discovered the detrimental effects of *E. coli*, *Enterococcus*, *Salmonella*, and *Staphylococcus*, *Aeromonas*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* to both poultry and human population. According to Berendonk *et al.* (2015) this situation has been perpetuated by the development of antimicrobial resistance strains against common used antibiotics: amoxicillin, ampicillin, ciprofloxacin, erythromycin, tylosin, gentamycin, streptomycin, neomycin tetracycline, chortetracycline, oxytetracycline. This antimicrobial resistance results affirms similarity to the results obtained in our study. There is high prevalence of bacterial resistant strains in poultry environments (Furtula *et al.,* 2013; Laube *et al.,* 2014). Resistant bacteria proliferate and can also be transferred to humans through several routes such as direct contact of handlers, live animals and carcasses at poultry farms and slaughter houses. Besides resistant bacteria, antibiotic residues in environment and those entering into humans through consumption of food may also create selective pressure in bacteria.

From the poultry water resources the highest resistance was *Escherichia coli* with 78% resistance to neomycin, penicillin, tetracycline, nalidixic acid, ciprofloxacin and ampicillin. Similar findings were recorded in literature (Moon *et al.,* 2011; Soufi, 2009 and Makhol *et al.,* 2011). Oguttu *et al.* (2008) reported a high multidrug resistance of *E. coli* isolates from broilers to doxycycline, sulphamethoxazole, ampicillin, enrofloxacin fosfomycin and nalidixic acid which caused a public health concern. Téllez *et al.* (2015) also documented the multidrug resistance of Salmonella isolates recovered from chickens, eggs, and poultry derived products, as well as cross transmission of plasmids between animal and humans, which resulted in several mortalities in poultry. Tavakoli *et al.* (2015) recovered three tetracycline residues and 63 antibiotic-resistant Gram-negative bacteria that presented resistance percentage

of between 33.3 and 66.7 % to five well-known antibiotics used in livestock farming, tetracycline, chloramphenicol, nalidixic acid, sulphamethoxazole, and ampicillin.

Antibiotic resistance is of great public health concern, because the antibiotic resistant bacteria associated with the animals are mostly pathogenic to humans (Economou and Gousia, 2015; Friedman *et al.,* 2016). Friedman *et al.* (2016) and Moyane *et al.* (2013) showed that bacterial resistant strains are easily transmitted to humans via food chains, and widely disseminated in the environment via animal wastes. These may cause devastating, untreatable, and prolonged infections in humans, leading to higher healthcare cost and in severe cases death. However, the soil and water environment have been regarded as vital reservoirs and sources of antibiotic resistance (Uchil *et al.,* 2014). Founouet *et al*. (2015) affirmed that the public health consequences perpetrated by zoonotic pathogens are ever challenging to evaluate. The consequences involve complex production and distribution systems of food and animals, dissemination of resistance genes and bacterial clones, increased mortality and morbidity, resulting in higher costs of treating the disease. Antibiotic resistance limits the choice of antibiotics to be implemented in therapy and jeopardizes the chances of the effectiveness of the existing potent antibiotics in treatment regimens used for the eradication of serious common diseases (Da Costa *et al*. 2013). The rising level of antibiotic-resistant bacterial pathogens will eventually hamper future treatment and the prevention of infectious diseases in both animals and humans (Vincent *et al*., 2016). The incidence of antibiotic resistance is very critical to the immune-compromised population, since these individuals rely solely on the use of antimicrobials as a defense against pathogens (Jethwa, 2018).

5.2 Conclusions

The current study revealed the presence and prevalence of microbial pathogens in poultry and its environment. These zoonotic pathogens are important in the primary health care of humans due to potential secondary transmission. Of great concern is the level of antimicrobial resistance of these pathogens against the common antibiotics. This must prompt the poultry farmers and feed producers to implement good sanitary practices and biosecurity measures to prevent the proliferation of the pathogens from causing outbreaks of diseases. Good management strategies may

help to minimise economic losses and ensure the safety of broiler meat via the control and elimination of food-borne pathogens.

5.3 Recommendations

Genetic selection of resistant animals, hygienic practices, elimination of pathogens from feed and water, vaccinations and applications of suitable feed and water additives are recommended. Awareness of the implications of the development of resistance for humans and animals, as well as promoting elimination of antibiotics, is of utmost importance, especially in the animal feed industry. Globally, producers are moving towards implementing an antibiotic-free system.

CHAPTER SIX
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