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MICROBIOLOGICAL ACCEPTABILITY OF SKINLESS CHICKEN BREASTS AND ANALYSIS OF ANTIMICROBIAL RESISTANCE IN *ESCHERICHIA COLI* ASSOCIATED WITH LEBANESE POULTRY

by
MARYA ELIE HARB

A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Science
to the Department of Nutrition and Food Sciences
of the Faculty of Agriculture and Food Sciences
at the American University of Beirut

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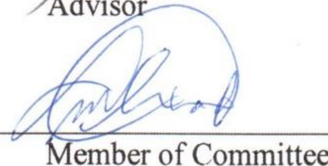

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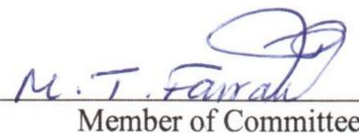

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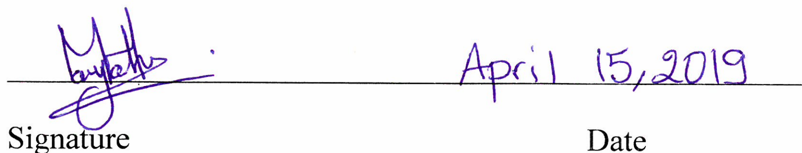

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AN ABSTRACT OF THE THESIS OF

Marya Elie Harb for Master of Science
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Title: Microbiological Acceptability of Skinless Chicken Breasts and Analysis of Antimicrobial Resistance in *Escherichia coli* Associated with Lebanese Poultry

The consumption of poultry meat has increasingly become popular worldwide. The poultry industry in Lebanon is considered more developed than other animal farming practices and a source that generates a vital economic profit for the country. Despite its importance, the safety of poultry meat is a major issue in Lebanon, because poultry is associated with a plethora of foodborne bacterial pathogens. Therefore, there is a need to assess the microbiological quality of poultry meat by testing for bacterial indicators such as total coliforms and *Escherichia coli*, because Lebanese poultry meat is hardly characterized and monitored. In addition, antimicrobial agents are used in poultry farming to control bacterial infections and increase productivity; however, the emergence of antimicrobial-resistance is a major challenge facing public health. Data is lacking regarding antimicrobial resistance in bacteria associated with Lebanese poultry meat. Lebanon will benefit greatly from this study that identifies, quantifies and characterizes bacterial contamination and antimicrobial resistance associated with poultry meat.

The overall objective of this national study in Lebanon was the microbiological analysis of post-harvest and pre-harvest poultry. The first objective was to analyze the prevalence and loads of fecal coliform and *Escherichia coli*, and antimicrobial resistance of *Escherichia coli* in Lebanese skinless chicken breast samples. The second objective was to analyze the resistance profiles of *Escherichia coli* isolated from poultry fecal matter. The third objective was to compare the resistance profiles of *E. coli* in pre- and post-harvest samples,

This study was conducted between February 2017 and July 2018. Post-harvest samples (n=151) and pre-harvest samples (n=183) were collected from major cities in Lebanon. The samples were transported to the laboratory within 24 hours, where analysis was performed on each sample. For the microbiological analysis, 25 grams of each sample were aseptically suspended in 225 ml of buffered peptone water (BPW) and homogenized for 90 seconds in a stomacher. The homogenate was serially diluted (10-folds) in BPW and 100 µL of each dilution were spread on RAPID'E. coli 2 agar plates for the selective isolation and enumeration of fecal coliforms and *Escherichia coli*. Antimicrobial susceptibilities testing was performed based on testing conditions stated in the Clinical and Laboratory Standards institute.

The prevalence and loads of fecal coliforms and *Escherichia coli* in skinless chicken breast samples was 100% and 80.79%, respectively. According to LIBNOR Standards, 129 (85.43%) out of 151 samples were labeled unacceptable. *Escherichia coli* isolates from fecal samples were resistant to ampicillin (95.44%), tetracycline (89.07%), ciprofloxacin (71.40%), gentamicin (56.65%), kanamycin (55.56%), chloramphenicol (83.42%), trimethoprim-sulfamethoxazole (75.59%), cefepime (21.49%), cefexime (33.33%), cefotaxime (37.70%), cephalexin (58.83%), and amoxicillin-clavulanate (47.54%). *Escherichia coli* isolated from skinless chicken breast sample were resistant to ampicillin (69.17%), tetracycline (67.50%), ciprofloxacin (59.17%), gentamicin (34.17%), kanamycin (38.33%), chloramphenicol (51.67%), trimethoprim-sulfamethoxazole (45%), cefepime (15.83%), cefexime (22.50%), cefotaxime (19.17%), cephalexin (25.83%), and amoxicillin-clavulanate (23.33%). Isolates (98.36%) from fecal samples were MDR (multi-drug resistant), while 70% of isolates from the skinless chicken breast samples were MDR.

The results show high prevalence and loads of bacterial indicators of fecal pollution. High prevalence of antimicrobial resistant *Escherichia coli* in Lebanese poultry meat was also documented. Therefore, efforts should be made to monitor the safety and microbiological quality of poultry meat in Lebanon, because it is important for the public health and country's economy.

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CHAPTER 1

INTRODUCTION

The increase in the world's population as well as the high demands on affordable, nutritious and safe foods have been exerting a pressure on the food production industry (Kassem et al., 2016; UN, 2017). This also applies to different agricultural practices, including the poultry production sector. The poultry industry is continuously growing worldwide, and it is estimated to dominate over half the growth of all meat products by 2025 (Ritchie and Roser, 2017; Kassem et al., 2016). Furthermore, the consumption of poultry meat has been increasing every year globally, because it is relatively an affordable source of nutrition (Mead, 2004; Farrell, 2013; Sofos et al., 2013). In Lebanon, it is estimated that around 30 kg of poultry are consumed per capita (Daou and Mikhael, 2016). For a relatively small country like Lebanon, poultry meat is appearing to be consumed in high quantities and is present in almost all food markets, households, and restaurants (Daou and Mikhael, 2016).

Lebanon harbors around 2000 poultry farms, eggs and broiler meat, and mainly has a significant contribution to the country's economy (Daou and Mikhael, 2016). However, it should be noted that poultry meat can be contaminated with a plethora of food-borne pathogens, including those that are considered major causes of gastroenteritis worldwide (Rivas et al., 2015; Zhao et al., 2001). Therefore, to maintain the safety and quality of poultry in Lebanon, there is a need to continuously assess and

monitor potential microbiological contaminants on poultry meat. The latter can be achieved by testing for bacterial fecal indicator microorganisms such as total coliforms and *Escherichia coli* (Rivas et al., 2015; Zhao et al., 2001).

Escherichia coli, a species of coliform bacteria, is a gram-negative bacterium found in the intestines of warm-blooded animals (Bélanger et al., 2011). *Escherichia coli* can contaminate poultry meat along several steps in the food chain such as during processing via fecal contact and contamination with polluted water or during handling, storing, and transporting the meat (CSIRO, 2002; Zhao et al. 2001). Monitoring of all potential fecal pathogens in fecally-contaminated poultry meat products is time-consuming, difficult and expensive. Therefore, testing for *Escherichia coli* serves as an indirect indication of possible presence of other fecal pathogens. Thus, *E. coli* is an indicator of fecal pollution and also can serve to reflect antimicrobial resistance in Gram-negative bacteria (CSIRO, 2002).

The poultry production environment is challenged by potentially several pathogens that pose a risk to flock and humans (Kassem et al., 2016). This affects the production, safety and quality of the meat and exerts a financial burden on producers. To control infections and improve productivity, the use of antimicrobial agents is crucial in food animals (Kassem et al., 2016). Antimicrobial drugs are used in animal farming to treat, prevent and control infections and to enhance growth performance (Page and Gautier, 2012). However, the misuse and/or overuse of antimicrobial drugs in humans and animals led to the emergence of antimicrobial-resistant bacteria (Marshall and Levy, 2011).

Antimicrobial resistance associated with food production has been recognized as a major challenge facing public health (Marshall and Levy, 2011). *Escherichia coli* can acquire antimicrobial resistance and transfer the resistance genes to other bacteria of the same or different species found in animals or nearby environments (Lindsey et al., 2011). Those antimicrobial-resistant bacteria can spread to humans via consumption of poultry meat or through contact with animals and environmental pathways, which leads to compromising the treatment of severe bacterial infections in humans (Lindsey et al., 2011). A more serious concern is the emergence of multiple drug resistant (MDR) human and animal pathogens (Hawkey and Jones, 2009).

In Lebanon, several food poisoning outbreaks have been reported and pathogenic microorganisms have been detected in products including poultry meat (El Jardali et al., 2014). Food safety is of great importance because it affects a country's economy and population's health (El Jardali et al., 2014). However, practices in the Lebanese food industry do not necessarily ensure the safety and might not comply with international standards (El Jardali et al., 2014).

To our knowledge and despite national awareness to food safety, research on bacterial indicators and foodborne pathogens has been rarely conducted in Lebanon. National efforts and data are lacking to monitor and quantify indicator bacteria such as *Escherichia coli*. Therefore, the purpose of this study is to conduct a nation-wide analysis of pre-harvest and post-harvest poultry in Lebanon. The main objectives are to 1) analyze the prevalence and loads of fecal coliforms and *Escherichia* in skinless chicken breast samples and to 2) analyze antimicrobial resistant phenotypes of *Escherichia coli* in Lebanese skinless chicken breast samples as well as fecal samples

from farms. The data collected in this study are essential and show the need to assess and enhance the safety of Lebanese poultry meat. This in turn will increase the competitiveness of the Lebanese poultry market via increased consumer confidence.

CHAPTER 2

LITERATURE REVIEW

2.1 Poultry Industry

2.1.1 The Poultry Industry: Growth in Brief

Since the 1800s, the world's population has increased by 7-folds, reaching ~ 7.6 billion people in 2017 (UN, 2017). It is predicted to reach 9.8 billion people in 2050 and to surpass 10 billion in 2100 (UN, 2017). As the population increases, so does the demands on affordable, wholesome, nutritious and safe foods, which place pressure on food industries to optimize their production practices (Kassem et al., 2016). This is exemplified by different modern food production systems, including poultry farming. There is a need to optimize the agricultural practices of poultry farming to accomplish continuous production, food safety and food security (Kassem et al., 2016). Poultry meat such as broiler chicken meat is popular worldwide, which led to a large and well-distributed poultry industry globally (Ritchie and Roser, 2017). Over the past 50 years, poultry production has increased rapidly, growing more than 12-folds since 1961 and is expected by 2025 to dominate over half the growth of all other meat products (Ritchie and Roser, 2018). This is due to several reasons including, being economically viable because of shorter growth cycles of poultry birds, having a high conversion rate of feed to meat and being relatively cheaper in comparison to other types of meat (Wahyono and Utami, 2018).

2.1.2 Poultry Production and Consumption

The continuous production of poultry meat has led to the industry to become the main driver of growth in total meat production globally (OECD and FAO, 2016). In 2018, the United States of America (USA) was the world's leading country in broiler meat production; with an amount of ~ 19 million metric tons (Statista, 2018). A recent census of Agriculture in the USA reported that $48.3 billion is generated yearly and 8.54 billion broilers are produced via 233,770 poultry farms (USDA, 2015). China produced 11.7 million metric tons, while countries in the European Union generated 12 million metric tons (Statista, 2018). Countries such as Turkey and Malaysia show a lower production with 2.3 million and 1.7 million metric tons, respectively (Statista, 2018).

The consumption of poultry meat worldwide has been also increasing throughout the years; from an estimated 9.7 kg/capita in 2000 to 13.8 kg/capita in 2018 (OECD, 2018). The USA and Saudi Arabia show the largest poultry meat consumption with 47.8 and 44.7 kg/capita, respectively (OECD, 2018). Countries in the European union show a consumption of 24.5 kg/capita (OECD, 2018). The lowest poultry meat consumption was reported in developing countries such as Nigeria with 0.9 kg/capita and Ethiopia with 0.5 kg/capita (OECD, 2018). In Lebanon, poultry is consumed at approximately 30 kg/capita (Daou and Mikhael, 2016). The difference in the amount of poultry meat consumption around the world is based on several factors which include the income, population size, price of poultry meat, and dietary preference (Wahyono and Utami, 2018).

2.1.3 Benefits of Poultry Meat in Human Nutrition

Chicken meat is characterized by having a white color in comparison to other types of meat such as beef, since its iron content (0.7mg/100g) is lower than that of beef (2mg/100g) (Farrell, 2013). Chicken meat is rich in vitamins, minerals and amino acids such as lysine, threonine, and tryptophan (Farrell, 2013). In comparison to other types of meat, chicken meat is considered healthier due to its low fat content (3g/100g) (Farrell, 2013). About half of the fat is monounsaturated fatty acids and poultry meat comparatively contains less trans fats that promote coronary heart disease (Farrell, 2013). In addition, chicken meat is a source of omega (n)-3 fatty acids, which is an essential polyunsaturated fatty acid (Farrell, 2013). Studies have shown that chicken meat can be enriched with important nutrients such as selenium that acts as an antioxidant and prevents some types of cancer (Yu et al., 2008).

2.1.4 The Poultry Industry in Lebanon

Poultry products, especially broiler chicken meat are very popular in Lebanon (Daou and Mikhael, 2016). In Lebanon, there are 10 large poultry producers and around 2000 poultry farms that work with the large poultry producers or independently (Daou and Mikhael, 2016). The poultry producers who have the largest shares in Lebanon are Hawa Chicken, Wilco, Tanmia and Shuman (Daou and Mikhael, 2016). They are fully integrated and cover all the steps of processing since they have their own farms, mills, slaughterhouses, and distribution channels (Daou and Mikhael, 2016). They produce 40 to 45 million birds out of the 150 million birds that Lebanon produces yearly (Daou and Mikhael, 2016).

Market studies show that the chicken breast is the most popular part for consumers, because it is affordable and nutritious (Daou and Mikhael, 2016). It is also vitally important to producers, because it generates more gains than other cuts (Daou and Mikhael, 2016). In 2016, broiler meat production had a significant effect on the economy of Lebanon; generating $350 million in revenue (Daou and Mikhael, 2016). Imports of poultry to Lebanon are estimated to cost around $17 million per year and consist of chilled and frozen products, processed chicken and live poultry (Daou and Mikhael, 2016). France is the main source of import of live poultry and, in 2015, these imports amounted to 79 tons and $4.98 million in cost (Daou and Mikhael, 2016). This shows that the poultry industry in Lebanon generates a vital source of nutrition as well as a considerable economic profit (Daou and Mikhael, 2016). In order to preserve competitiveness, raising and maintaining healthy flocks and generating a safe product according to food safety standards are necessary (Kassem et al., 2016). Nowadays, Lebanese consumers are more aware of their health and the need for safe products

(Daou and Mikhael, 2016), which will be main themes in shaping the future of the industry.

2.2 Food Safety and Poultry Meat

Food safety is a global issue that engages specific practices to ensure the production of safe food products and prevent foodborne illnesses in consumers (Wahyono and Utami, 2018). Food safety practices engage and monitor the entire food chain in order to eliminate the chance of contamination (biological/ chemical/ physical) and/or reduce the contaminants to a level that is safe for consumption (Wahyono and Utami, 2018). These practices apply also to poultry meat production from farm to fork (Wahyono and Utami, 2018).

Poultry meat can serve as a reservoir for a variety of food-borne pathogens (Rouger et al., 2017). The safety of poultry meat is a major issue for all stakeholders (Mead, 2004), because the consumption of contaminated food can cause severe and life-threatening illnesses, increasing the rates of morbidity and mortality, especially in developing countries where the public's health status is already compromised. (Mead, 2004). Foodborne diseases also result in an increase in healthcare and food production costs, which severely affect economies (Mead, 2004). Microbiological contamination of poultry meat can occur in several ways (CSIRO, 2002; Zhao et al. 2001) and can include important and well-established human pathogens such as Salmonella, *Campylobacter*, *Listeria monocytogenes*, *Clostridium perfringens* and *Escherichia coli.* These species are among the most recognized bacterial species that can contaminate poultry meat (Mead, 2004).

2.2.1 Sources of Contamination

Sources of contamination to poultry meat carcasses and cuts during slaughtering can occur from the animal itself or from the environment such as in slaughterhouses where bacteria can be found on surfaces and in air and in liquids (Rouger et al., 2017). Therefore, care should be taken during farming and processing to limit the contamination of the carcasses. Steps in poultry slaughtering differ from country to country and between large-scale and small-scale commercial slaughterhouses (Rouger et al., 2017). However, the main steps of poultry slaughtering and possible bacterial contamination routes are the same and are summarized in Figure 1 (Rouger et al., 2017).

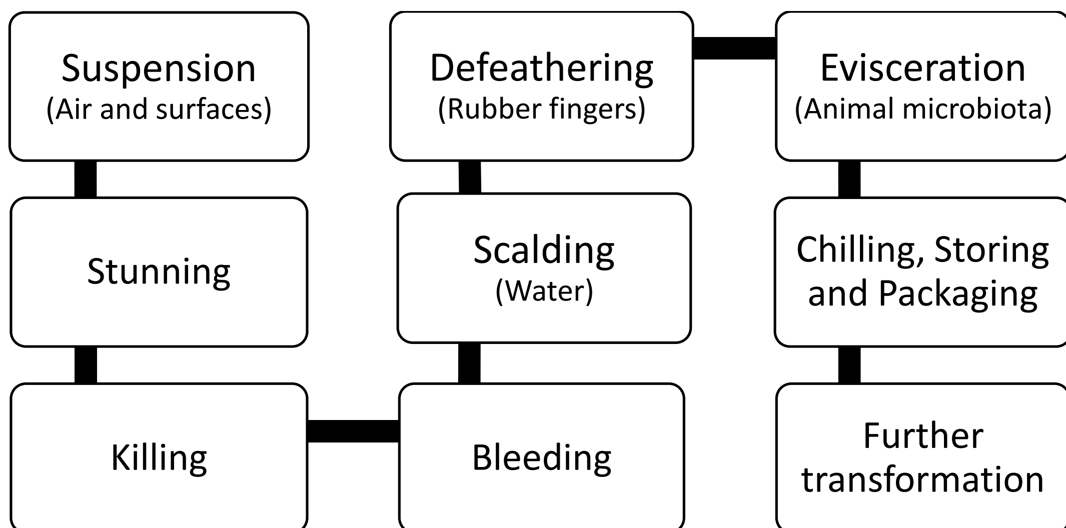

Fig. 1. Steps of poultry slaughtering and possible contamination sources (Rouger et al., 2017)

Poor slaughtering can result in the cross-contamination of those bacteria found in the intestines to surfaces of the carcasses (Rouger et al., 2017). Furthermore, bacteria from the equipment surfaces and those in air and water that are used during processing

can easily cross-contaminate poultry meat since they are in direct contact with the meat (Rouger et al., 2017). As a result, bacteria are mostly present on the surface of fresh meat (unprocessed) rather than on the inside (Rouger et al., 2017). In processed products, the bacteria can become mixed inside the meat (e.g. during mincing) (Rouger et al., 2017). Bacterial contamination can occur during the early phases in processing. Equipment surfaces such as conveyor belts and rubber fingers, that are used to remove the feathers, are sources of bacterial contamination to poultry carcasses and cross-contamination between carcasses (Rouger et al., 2017). Subsequent steps in the processing such as deboning, cutting, mixing, manipulators, air and water baths can also serve as sources of contamination (Rouger et al., 2017).

The levels of bacterial contamination throughout the process are dynamic (Rouger et al., 2017). The loads of bacteria decrease after evisceration when carcasses are immersed in cold water and chilled (Rouger et al., 2017). However, studies show that bacteria originating from poultry can persist even after washing and after 10 days of storing the carcass at refrigerated temperatures (Rouger et al., 2017). Bacterial counts might increase again along the end of the food chain if transportation, storage, handling and packaging are done under wrong conditions and practices (Rouger et al., 2017).

2.2.2 Bacterial Contaminants of Poultry Meat

Bacterial contaminants may be present on the product and can be characterized as either food-borne pathogens or commensal bacteria (Mead, 2004). The most important species of pathogenic bacterial contaminants in poultry meat are *Campylobacter*, *Salmonella*, *Clostridium perfringens*, *Listeria monocytogenes* and *Escherichia coli* (Mead, 2004).

2.2.2.1 *Salmonella* and *Campylobacter*

Salmonella and *Campylobacter* are the leading causes of gastroenteritis globally (Mead, 2004). Studies worldwide have associated both of these bacteria with contaminated poultry meat (Mead, 2004). It was reported that even at very low levels of *Salmonella*, contamination of food products could cause large outbreaks (Mead, 2004). *Salmonella* is also known for its ability to survive in the poultry environment and manure (Mead, 2004).

Campylobacter survive under conditions where there is high moisture and low oxygen (Mead, 2004). They can persist on poultry products such as chicken breasts through the entire supply chain and processing stages including scalding, washing, and water chilling, due to their strong attachment to poultry tissues (Mead, 2004). Infections caused by *Campylobacter* are known as campylobacteriosis and can occur at an infective dose of few hundred viable cells (Mead, 2004).

2.2.2.2 *Clostridium perfringens*

Clostridium perfringens is a spore-forming, obligate anaerobe that is capable of surviving in the environment and is found in low loads in the gastro-intestinal tracts of poultry (Mead, 2004). *C. perfringens* can survive germinate and grow to reach hazardous levels (Mead, 2004). Spores found in poultry product are heat resistant and cannot be easily destroyed. Therefore, most of the food poisoning outbreaks caused by *C. perfringens* involve heat-resistant spores (Mead, 2004).

2.2.2.3 *Listeria monocytogenes*

Listeria monocytogenes has been associated with poultry meat and affects mostly vulnerable groups of individuals with symptoms that can vary widely (Mead, 2004). Infections by *L. monocytogenes* are rare but have been increasing steadily in recent years (Mead, 2004). *L. monocytogenes* has the ability to persist under chilled conditions and cross-contaminate raw, cooked and/or ready to eat poultry products (Mead, 2004).

2.2.2.4 Indicator Bacteria

Total coliforms are a group of bacteria found in water and waste of humans and animals, and *Escherichia coli* is a species of coliform bacteria (CSIRO, 2002). *Escherichia coli* (also known as *E. coli*) is Gram-negative bacterium that can be found in the intestines of warm-blooded animals, including humans (Bélanger et al., 2011). Strains of *E. coli* can be either commensal or pathogenic (Bélanger et al., 2011). Poultry meat can be contaminated with *E. coli* and total coliforms, which can serve as bacterial indicator organisms (CSIRO, 2002). These indicators can be used to assess the quality and safety of the meat, because their presence is associated with likely contamination with enteric pathogens. Many food products, including poultry meat, have pre-established standards that rely on the quantity of bacterial indicators. Food products are either accepted or rejected based on the levels of indicator bacteria in the sample. (CSIRO, 2002).

2.3 *Escherichia coli*

2.3.1 History of Escherichia coli

In 1885, a German pediatrician and microbiologist, Theodor Escherich, was studying the role of disease and digestion in infants. He discovered a fast growing bacterium (Shulman et al., 2007), which he referred to as *Bacterium coli commune*. However, the bacterium was later renamed *Escherichia coli* after its original discoverer (Blount, 2015). By the 1940s, *Escherichia coli* have been used in many fundamental microbiological studies, which made it the bacterial model organism in biology (Blount, 2015). The latter was also due to *E. coli*'s several coveted characteristics such as the ability to grow and survive relatively easily under aerobic or anaerobic conditions, its comparatively unfastidious properties, and the early availability of information on its genomic sequences among others (Allocati et al., 2013; Yoon et al., 2009).

2.3.2 Characteristics of Escherichia coli in Brief

Escherichia coli, a non-spore forming, rod-shaped, Gram-negative bacterium, is normally found in the intestines of endotherms (Kaper et al., 2004; Tenaillon et al., 2010). *E. coli* typically has a length of 1 µm and a width of 0.35 µm; although dimensions can vary depending on the strain and growth conditions (Blount, 2015). *E. coli* has an optimal growth temperature of 37°C and can divide every 20 minutes under favorable conditions (Fotadar et al., 2005). *E. coli* can grow with or without oxygen; therefore, it is physiologically classified as a facultative anaerobe (Blount, 2015). If oxygen is available, *E. coli* will produce ATP aerobically, while when oxygen is absent, the bacterium will use fermentation pathways (Blount, 2015). Some strains of *E. coli* may have peritrichous flagella; whip like structures used for motility and/or pili; hair-

like structures used for surface attachments as shown in Figure 2 (Blount, 2015). *E. coli* belongs to the group of bacteria known as “coliforms” which are members of the Enterobacteriacae family and are closely related to pathogenic bacteria such as *Salmonella*, *Klebsiella*, *Serratia* and *Yersinia pestis* (Blount, 2015). Three major groups of *E. coli* exist based on their ability to be harmless or pathogenic.

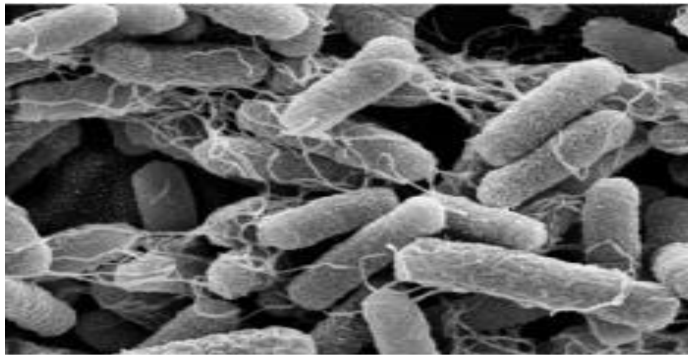

Fig. 2. Scanning electron micrographs of enteropathogenic strains of *Escherichia coli* that have hair-like pili (Nascimento et al., 2014)

2.3.3 Major Groups of Escherichia coli

E. coli can be classified as a commensal, an intestinal pathogenic, or an extraintestinal pathogen (Blount, 2015; Lukjancenko et al., 2010).

2.3.3.1 Commensal *Escherichia coli*

Commensal *E. coli* lack virulence factors and live in a peaceful coexistence in the intestinal tract of its hosts (Allocati et al., 2013). Many *E. coli* are commensal inhabitants of the mammalian gut microbiota, and they also found in the gut microbiota of birds, reptiles, and fish (Hartl and Dykhuizen, 1984; Leimback et al., 2013). *E. coli* is also found in food and on environmental surfaces and matrices, including soil and water

(Hartl and Dykhuizen, 1984; Leimback et al., 2013). The gut of mammals houses a very wide and diverse microbial community; mostly dominated by obligate anaerobes (Backhed et al., 2005). Notably, *E. coli* is the most common anaerobe (0.1-5%) that resides in the lower intestines of mammals; specifically, in the thin layer of mucus that lines the gut (Blount, 2015; Beloin et al., 2008). *E. coli* has a high population density in fecal matter; around $10^6 – 10^9$ cells/gram (Savageau, 1983; Chang et al., 2004). In the human gut, some strains of *Escherichia coli* reside for a long-term period, while other strains reside for a short while depending on the hosts' health, diet, exposure to antibiotics and on the bacterium's interactions with other gut microbes (Sears et al., 1950; Savageau, 1983).

E. coli can also have mutualistic interactions with its host (Allocati et al. 2013). Immunoglobulin A is normally secreted by the human gut since it plays an important role in the immunity of mucous membranes (Randal Bollinger et al., 2003) However, it has been found that Immunoglobulin A facilitates *E. coli*'s biofilm formation in the intestinal mucosa (Bollinger et al., 2003). Also, the human gut provides the nutrients and energy needed for *E. coli* to survive and grow (Blount, 2015). In return, *E. coli* can provide several benefits to its host such as the stimulating the production of vitamins that are need by mammals, including vitamin K and vitamin B12 (Bentley and Meganathan, 1982; Lawrence and Roth, 1996). *E. coli* can also consume oxygen that is entering the gut, which ensures the survival of important anaerobic bacteria in the gut (Blount, 2015). As importantly, *E. coli* in the gut can help to exclude pathogens from residing in this niche (Blount, 2015; Chang et al., 2004). For example, a study showed that a decrease in the levels of *E.coli* in the gut of newborns lead to the colonization

with *Staphylococcus aureus*, increasing the risk to several disorders (Lindberg et al., 2000; Neu and Rushing, 2011).

2.3.3.2 Intestinal Pathogenic *Escherichia coli*

Pathogenic *E.coli* are of great concern, because they can cause life-threatening diseases in humans (Allocati et al., 2013). The major route of transmission to humans is through the fecal-oral route by the ingestion of contaminated water or food (Allocati et al., 2013). Before virulence factors were identified, pathogenic *E. coli* strains were classified based on the antigens found on their membranes (Kaper et al., 2004). Those antigens included, O antigens which are found on the outer cell membrane that contains millions of lipopolysaccharide (LPS) molecules, K antigens which are found on the capsule, and H antigens which are found on the flagella (Kaper et al., 2004).

After virulence factors were identified, strains of *Escherichia coli* became classified into six pathotype groups according to their serological characteristics, types of virulence factors, and clinical symptoms in the host (Kaper et al., 2004). Table 1 shows the general description of the six pathotypes, which include, enterotoxigenic *Escherichia coli* (ETEC), enterohemorrhagic *Escherichia coli* (EHEC), enteroinvasive *Escherichia coli* (EIEC), enteropathogenic *Escherichia coli* (EPEC), enteroaggregative *Escherichia coli* (EAEC), and adherent invasive *Escherichia coli* (AIEC) (Kaper et al., 2004).

E. coli O157:H7 is the most well-known enterohemorrhagic strain worldwide. It can contaminate food products, because it can reside in certain food animals asymptomatically (Ferens and Hovde, 2011). Furthermore, this strain can be transferred via processing to consumed meat products (Ferens and Hovde, 2011). *E. coli* O157:H7

has caused several severe outbreaks that resulted in morbidity and mortality in humans (Scallan et al., 2011). It produces a shiga-like toxins that kill intestinal cells, attack blood vessels and cause hemolytic uremic syndrome and bloody diarrhea (Griffin et al., 1988).

Table 1. The general description of the six pathotypes of intestinal pathogenic *Escherichia coli* (Allocati et al., 2013)

Pathotype	Symptoms	Description
Enterotoxigenic *Escherichia coli* (ETEC)	Traveler's Diarrhea (Watery diarrhea and vomiting)	-ETEC adheres to intestinal mucosa using fimbriae adhesions - ETEC produces heat-labile and heat-stable enterotoxins
Enterohemorrhagic *Escherichia coli* (EHEC)	Hemorrhagic colitis and Hemolytic Uremic Syndrome	- EHEC uses bacterial fimbriae for attachment - EHEC has a phage-encoded Shiga toxin
Enteroinvasive *Escherichia coli* (EIEC)	Watery diarrhea and dysentery	- EIEC produces an infection that is identical to shigellosis
Enteropathogenic *Escherichia coli* (EPEC)	Diarrhea in children	- EPEC adhere to intestinal cells using intimin - EPEC has virulence factors similar to *Shigella*
Enteroaggregative *Escherichia coli* (EAEC)	Diarrhea with mucus and vomiting	- EAEC have fimbriae that aggregate tissue culture cells - EAEC produce hemolysin and heat-stable enterotoxin
Adherent Invasive *Escherichia coli* (AIEC)	Associated with Crohn disease	-AIEC invade intestinal epithelial cells - AIEC replicate intracellularly

2.3.3.3 Extraintestinal Pathogenic *Escherichia coli*

Extraintestinal pathogenic *Escherichia coli* (ExPEC) are capable of causing diseases outside the gastrointestinal tract, including peritonitis, colitis, meningitis, hemolytic uremic syndrome, bacteremia and urinary tract infections (Kaper et al., 2004). These diseases are estimated to kill around 2 million individuals every year and cost billions of dollars in prolonged therapy (Russo and Johnson, 2003). Some strains of ExPEC produce virulence factors and are classified into 3 pathotypes: uropathogenic *Escherichia coli* (UPEC), neonatal meningitis *Escherichia coli* (NMEC), and avian pathogenic *Escherichia coli* (APEC) (Allocati et al., 2013). UPEC is responsible for 80% of urinary tract and systemic infections, while NMEC is a major cause of neonatal bacterial meningitis (Johnson and Stell, 2000; Gaschignard et al., 2011). APEC might arguably constitute a source of human foodborne illnesses, because they are responsible for causing infections in a variety of avian species (Johnson et al., 2007; Allocati et al., 2013).

2.3.4 Escherichia coli in the Environment and Poultry Meat

E. coli has adapted very well to the conditions inside a host; however, it is regularly excreted in fecal matter into the surrounding environment (Blount, 2015). In the environment, despite challenging and fluctuating factors such as pH, nutrition, temperature, moisture, oxygen and resident microbes, *E. coli* can also survive and adapt considerably well (Savageau, 1983; Van Elsas et al., 2011). The metabolic flexibility *E. coli* permits the bacterium to survive for relatively long time in the environment; allowing the bacterium to persist as a member of the microbial community of soils, waters, and plants (Van Elsas, 2011; Blount, 2015).

Escherichia coli can be prevalent in food products such as poultry meat due to several steps along the food chain that could lead to contamination of the product (Rouger et al., 2017). Pathogenic serotypes of *Escherichia coli* have caused major food product recalls and foodborne intoxications (Vogt and Dippold, 2004). Taken together, these properties of *E.coli* facilitate its use as an indicator organism of fecal contamination in environmental samples and food products (Blount, 2015).

2.4 Antimicrobial Resistance

2.4.1 Antimicrobials and Antimicrobial Resistance

The discovery of antimicrobials was a revolutionary accomplishment in human and animal medicine and animal farming (Byarugaba, 2010). The term antimicrobial and antibiotic are used interchangeably in many occasions (Giguère, 2013). Antimicrobials are classified as natural or synthetic chemicals that kill or stop the growth of microorganisms (Giguère, 2013). Antibiotics are substances that are produced by microorganisms to specifically inhibit the growth or kill against other bacteria (Giguère, 2013).

In 1928, Alexander Fleming discovered the first antimicrobial agent, known as penicillin. Since then, antimicrobial agents were used extensively in treating bacterial infections and in other medical purposes in humans (Adenipekun et al. 2015). The popularity of antimicrobials was partly the result of a better knowledge and understanding of the pathophysiology of diseases and mostly, because of the rapid and simple manufacturing processes of new formulations of effective antimicrobials (Alanis, 2005). Antimicrobial agents are also used in animal farming to prevent and control infections and to enhance growth performance (Page and Gautier, 2012). Since the

introduction of antimicrobial agents, their misuse and overuse in humans and animals led to the rapid emergence and increase in antimicrobial-resistant bacteria (Marshall and Levy, 2011).

Antimicrobial resistant bacteria have the ability to resist the effect of these drugs. Hence, the drugs become ineffective in treating infections caused by those bacteria (Alanis, 2005). This is known to result in a longer duration of illness, a rise in rates of mortality and morbidity, and an increase in medical and treatment costs (Laxminarayan et al., 2014). A study combining results from animal farming in seven European countries showed that the emergence of antimicrobial resistant bacteria from animal sources was due to antimicrobial use (Chantziaras et al., 2014). Notably, the increase in antimicrobial resistance associated with food production has been recognized by the World Health Organization as a major threat facing public health in the 21st century (WHO, 2017; Marshall and Levy, 2011).

2.4.2 Antimicrobial Use in Poultry Farming

In food animal production, the global antimicrobial consumption was around 63151 (± 1560) tons in 2010 and is expected to rise by 67% in 2030 to reach 105596 (± 3605) tons (Van Boeckel et al. 2014). In 2010, the largest antimicrobial consumption for animal production was in China with 23%, followed by United States and Brazil with 13% and 9%, respectively (Kassem et al., 2016). The use of antimicrobials was crucial in food animals, because it limits infectious diseases in food animals as well as increases the efficiency of animal production (Kassem et al., 2016). In countries such as Brazil, Russia and India, the antimicrobial consumption for animals is estimated to increase by 99 % in 2030, while the human population is estimated to increase by only

13% (World Bank, 2016). Antimicrobial agents are important and highly used in poultry farming to treat infections, prevent and control animal diseases and for growth promotion purposes (Kassem et al., 2016). Therefore, the popularity and increase in poultry production worldwide have driven the use of antimicrobials in this sector, which, in turn, has contributed to the increase of antimicrobial resistant pathogens.

2.4.2.1 Antimicrobial Agents Used for Therapy

Therapeutic uses of antimicrobial agents in poultry farming are performed to treat infections (Kassem et al., 2016). The aim of giving antimicrobial agents for therapeutic purposes is to reduce the suffering of the affected poultry, kill the pathogenic agent responsible for causing the infection and prevent the transmission of disease to other healthy poultry in the flock (Kassem et al., 2016). Therapeutic uses of antimicrobial agents in poultry faming is also an important factor contributing to the increase in poultry economic profit and production, because infections in poultry can cause mortality and decrease the animals' performance (Kassem et al., 2016).

Antimicrobial agents given for therapeutic uses should be prescribed and overseen by a veterinarian (Kassem et al., 2016). In addition, only the drugs that are approved in a specific country should be prescribed when needed (Kassem et al., 2016). Some countries, such as the USA monitor the use and accumulation of antimicrobials in poultry and its products (Kassem et al., 2016).

2.4.2.2 Antimicrobial Agents Used for Prevention and Control

Animals at high risk of developing a disease can be given preventive therapeutic doses of antimicrobials as recommended by a veterinarian for a short period of time (McEwen and Fedorka-Cray, 2002). Prophylaxis is an example of prevention, where a single animal receives antimicrobials agents after a surgery or a trauma as a precaution against developing a disease (Kassem et al., 2016). Metaphylaxis involves administering antimicrobials to a whole flock or group of animals that were under unfavorable conditions or are at risk of exposure to an infection (Nickell and White, 2010).

2.4.2.3 Antimicrobial Agents Used for Growth Promotion

Antimicrobials are often used as feed additives in order to enhance the animals' physiological performance (Kassem et al., 2016). The mechanisms of facilitating growth performance in poultry are not well understood, but results show an increase in weight gain and an improvement in feed efficiency (Butaye et al., 2003; Graham et al., 2007; Dhama et al., 2014). The benefits of using antimicrobials in poultry production are to meet the consumers increasing demands for poultry meat protein and to increase economic gain in shorter growth cycles (Graham et al., 2007; Kassem et al., 2016).

Studies suggest that growth performance in poultry is enhanced by changes in the natural microflora in the intestines, which result in a better digestion of feed, an increase in metabolic uptake of nutrients, and in suppression of some pathogenic bacteria in the intestines (Izat et al., 1990; Dhama et al., 2014). After the introduction of antimicrobials, there was a 50% increase in broiler's weight and a 35% decrease in feed intake and growth period (Boyd, 2001). This results in an increase in the efficiency of

production, rendering the chicken product an affordable source of nutrition (Smith, 2002). Unlike other applications in poultry farming, antimicrobials given for growth promotion are not tightly regulated and sub-therapeutic doses are given for a relatively prolonged period of time (Graham et al., 2007; Kassem et al., 2016). In addition, most of the antimicrobial agents are used mainly for growth promotion and not for humane farming purposes (Graham et al., 2007). This is thought to exert a pressure on the natural microflora in poultry and their environment, which led to the emergence of resistance to multiple antimicrobial agents that are of great importance in treating human infections (Graham et al., 2007).

2.4.3 Antimicrobial Classes Used in Poultry Farming and their Mode of Action

Antimicrobial agents most commonly used in the poultry industry are important agriculturally and clinically (Levy, 1997). Classes of antimicrobial agents mostly given to poultry are aminoglycosides, polypeptides, cephalosporins, macrolides, quinolones, tetracyclines, streptogramins and lincosamides (Landoni and Albarellos, 2015). For example, chronic respiratory disease and salmonellosis in birds are treated using tetracycline and fluoroquinolones, while penicillin is used to treat necrotic enteritis (Agunos et al., 2012, Gouvêa et al., 2015). Classes of antimicrobials used for growth promotion include macrolides, streptogramins and polypeptides (Butaye et al., 2003). Resistance to classes of antimicrobials such as cephalosporins, aminoglycosides, and fluoroquinolones are of concern, because they are critically important in treating serious human infections (WHO, 2017). To better understand the mechanisms of antimicrobial resistance, it is necessary to analyze the chemical nature and mode of action of antimicrobial agents. Five different modes of action are available and include

inhibition of cell wall synthesis, inhibition of protein synthesis, inhibition of nucleic acid synthesis, inhibition of folate synthesis and inhibition of cell membrane functions (McDermott et al., 2003).

2.4.3.1 Inhibition of Cell Wall Synthesis

Certain antimicrobials such as β-lactam drugs and glycopeptides inhibit cell wall synthesis in bacteria (McDermott et al., 2003). β-lactam drugs are a class of broad-spectrum antibiotics that have a β-lactam ring. This molecular structure is the functional unit of this group, which includes antimicrobial agents in the classes of penicillin and cephalosporin (McDermott et al., 2003). β-lactams bind to penicillin-binding proteins (PBPs) and prevent the final step in the synthesis of the peptidoglycan layer (McDermott et al., 2003). They prevent cross-linking of the peptidoglycan, thus inhibiting cell wall synthesis (McDermott et al., 2003). The peptidoglycan precursors will accumulate in the bacterial cell and trigger autolytic hydrolases that will digest the existing peptidoglycan or lead to cell wall deficiency, which will eventually cause cell lysis due to osmotic pressure (McDermott et al., 2003).

2.4.3.2 Inhibition of Protein Synthesis

The ribosome is an organelle that contributes to protein synthesis in bacteria (McDermott et al., 2003). Antimicrobials such as macrolides, phenicols, tetracyclines and aminoglycosides inhibit protein synthesis in bacterial cells (McDermott et al., 2003). This is achieved by binding to structural subunits of the ribosome and interfering with its function (McDermott et al., 2003). Macrolides bind to the 50S subunit of the ribosome and cause the dissociation of tRNA molecules (McDermott et

al., 2003). Chloramphenicol inhibits protein synthesis by binding to the ribosome (McDermott et al., 2003). Chloramphenicol inhibits the reaction of peptidyl transferase, which allows the addition of amino acids to the growing peptide (McDermott et al., 2003). Tetracyclines and aminoglycosides bind to the 30S subunit of the ribosome (McDermott et al., 2003). Tetracyclines block the attachment site of tRNA, while aminoglycosides cause inaccurate mRNA translation by misreading or prematurely terminating it (McDermott et al., 2003).

2.4.3.3 Inhibition of Nucleic Acid Synthesis

Antimicrobials such as quinolones, fluoroquinolones and rifampicin act on bacterial cells by inhibiting their nucleic acid synthesis (McDermott et al., 2003). Quinolones and fluoroquinolones prevent the bacterial DNA from unwinding and duplicating by binding to the enzymes DNA gyrase and DNA topoisomerase IV (McDermott et al., 2003). The relaxation and supercoiling of DNA within bacterial cells is mediated by these two enzymes (McDermott et al., 2003). As a result, the binding of the antimicrobial agents to the enzymes inhibits DNA replication and transcription (McDermott et al., 2003). Rifampicin interferes with nucleic acid synthesis, especially the inhibition of RNA synthesis since it binds to DNA dependent RNA polymerase (McDermott et al., 2003).

2.4.3.4 Inhibition of Folate synthesis

Folate synthesis is inhibited by antimicrobials such as sulfonamides and trimethoprim (McDermott et al., 2003). Trimethoprim inhibits the enzyme dihydrofolate reductase in one of the steps in the biosynthesis of folate, while

sulfonamides inhibit the incorporation of para-aminobenzoic acid into the folic acid molecule by competing for the dihydropteroate synthetase enzyme (Mcdermott et al., 2003; Džidić et al., 2008). Most of the times a combination of these two antimicrobials are given to treat infections due to their synergistic effects (Smith and Powell, 2000).

2.4.3.5 Inhibition of Cell Membrane Function

The mechanisms of how antimicrobial agents such as polymyxin and daptomycin inhibit cell membrane function are not well defined (Džidić et al., 2008; Tenover, 2006). However, some studies show that polymyxins cause bacterial death due to the increase in membrane permeability that leads to leakage of bacterial contents (Džidić et al., 2008; Tenover, 2006). Daptomycin causes death of bacterial cells, because it binds to the cell membrane and triggers the efflux of potassium (Džidić et al., 2008; Tenover, 2006).

2.4.4 Mechanism and Modes of Resistance

Antimicrobial resistance in bacteria can be intrinsic or acquired (Blair et al., 2015). Bacterial species whose resistance to an antimicrobial agent does not involve the acquisition of genetic material from other species are labeled intrinsically resistant (Blair et al., 2015). This resistance is defined by the fact that the bacterial species lack the inherent functional or structural properties that are needed for the antimicrobial agent to act upon (Blair et al., 2015). For example, lipopeptide daptomycin is only effective against Gram-positive bacteria and not against Gram-negative bacteria due to their cell membrane structural differences (Randall et al., 2013). Most Gram-negative

bacteria are intrinsically resistant to many antimicrobial agents, because the agents are unable to cross the outer membrane (Blair et al., 2015).

Acquisition of resistant genes can also happen through horizontal gene transfer (HGT) (Huddleston, 2014). HGT can occur between bacteria of the same or different species (Huddleston, 2014). It is one of the most important processes of dissemination of antimicrobial resistant genes between bacteria (Huddleston, 2014). Genetic transfer between bacteria can occur in three major mechanisms and mobile genetic elements such as plasmids, integrons and transposons play a role in the transfer (Huddleston, 2014).

The first and most studied mechanisms known as conjugation involves the transfer of mobile genetic elements, that can carry resistant genes, from one bacterium to the other by direct contact regardless of their phylogenetic relation (Zechner et al., 2000; Huddleston, 2014). During the process of conjugation, a donor and recipient bacterial cells come in contact with each other (Huddleston, 2014). The donor bacterial cell will transfer its genetic material to the recipient via the formation of a channel by the pilus (Huddleston, 2014).

The second mechanism of gene transfer is known as bacterial transformation (Huddleston, 2014). Some bacteria have the ability to collect free linear DNA fragments that have been already dispersed from other bacterial cells into the environment (Huddleston, 2014). The DNA is then incorporated using homologous or illegitimate recombination to the recipient's own genome (Lorenz and Wackernagel, 1994). The recipient bacterial cells will then express the DNA that was incorporated (Lorenz and Wackernagel, 1994; Huddleston, 2014). The process of transformation is not fully understood; however, several reasons were shown as to why bacteria take up

dispersed DNA from the environment. Those reasons include using the DNA as a nutrient source or as a template for DNA repair or to increase genetic diversity and/ or survive under stressful conditions (Michod et al., 1988; Finkel and Kolter, 2001; Johnsen et al., 2009; Vos M, 2009; Bakkali, 2013; Huddleston, 2014). Transformation is common among several microorganisms and so is the third mechanism which is known as transduction (Huddleston, 2014).

Bacterial transduction involves bacteriophages that transfer DNA between bacterial cells using the lytic and lysogenic cycles (Balcazar, 2014). During the lytic cycle, bacteriophages attach to the surface of the bacteria and insert their genetic material into the cell, leading to the replication of the viral genome and the production of other materials needed for the development of new bacteriophages (Balcazar, 2014). During the process and in some cases, genetic material from the bacteria may be inadvertently encapsulated in the new phage (Huddleston, 2014). Lytic phages will burst the bacteria and spread to the environment where they can infect other bacteria, hence transferring the genetic material between different bacterial hosts (Schmeiger and Shicklmaier, 1999; Huddleston, 2014). The second process known as lysogenic cycle involves integrating the phages genetic material into the DNA of the bacteria, which can carry resistant genes (Balcazar, 2014; Huddleston, 2014). As the host multiplies, so does the phage's DNA (Balcazar, 2014; Huddleston, 2014).

Figure 3 shows the mode of action of and resistance to antimicrobial agents. Antimicrobial resistance occurs in three major processes which include, inactivation of the antimicrobial agent, altering or modification of the antimicrobial target and/or lowering the antimicrobial's intracellular concentration (Blair et al., 2015).

2.4.4.1 Inactivation of the Antimicrobial Agent

Inactivation is achieved by hydrolysis or modification of the antimicrobial agents through the use of bacterial enzymes (Blair et al., 2015). Thousands of hydrolytic enzymes were identified that are capable of inactivating antimicrobial classes such as macrolides, aminoglycosides and β-lactams (Livermore, 2008; Blair et al., 2015). For example, many Gram-negative bacteria produce enzymes known as β-lactamases that hydrolyze the β-lactam ring, resulting in the inactivation of the antimicrobial agent (Blair et al., 2015). Antimicrobial agents can also be inactivated by bacterial enzymes that transfer a chemical group to the binding site which modifies the site and prevents it from binding to its target (Blair et al., 2015). Several enzymes such as transferases transfer chemical groups such as phosphoryl, acetyl, ribitoyl and nucleotidyl groups to the antimicrobial agent's site (Wright, 2005). Antimicrobial agents such as tetracycline, chloramphenicol, macrolides, rifampicin and aminoglycoside can be inactivated by modification from these chemical groups (Džidić et al., 2008).

2.4.4.2 Modification of the Antimicrobial Agent's Target

While maintaining the normal cellular function of the target, mutational changes can alter or modify the target and prevent the binding of the antimicrobial agent (Blair et al., 2015). Several antimicrobial classes can be inhibited due to target alteration and those include aminoglycoside, fluoroquinolones, macrolides, tetracycline and β-lactams (Blair et al., 2015; Džidić et al., 2008; Mcdermott et al., 2003). For example, β-lactams normally target PBPs; however, mutational changes in the bacterial PBPs can lower their affinity of β-lactams and lead to resistance (Džidić et al., 2008).

2.4.4.3 Lowering the Antimicrobial's Intracellular Concentration

Minimizing the intracellular concentration of an antimicrobial agent can be achieved through efflux pumps or changes in the permeability of the outer membrane (Blair et al., 2015). Resistance to antimicrobial agents such as fluoroquinolones, phenicols, macrolides, tetracyclines and aminoglycosides can work in this way (Dever and Dermody, 1991; Mcdermott et al., 2003; Wright, 2011). For example, bacterial cells that have genes such as *tetA, tetB and tetC* minimize the concentration of tetracycline inside the bacteria via efflux pumps (Blair et al., 2015). Efflux pumps found on the bacterial membranes actively transport the antimicrobial agents out of the bacterial cells (Blair et al., 2015).

Changes in the permeability of the cell membrane is due to down-regulation or changes in porin channel expression, which affects the entry ability of antimicrobials to bacterial cells (Džidić et al., 2008). Antimicrobial agents such as chloramphenicol, fluoroquinolones and β-lactams need porin channels to enter Gram-negative bacteria. However, changes in the expression of these porin channels result in resistance, because the changes lead to lowering the intracellular concentration of the antimicrobial agents (Džidić et al., 2008).

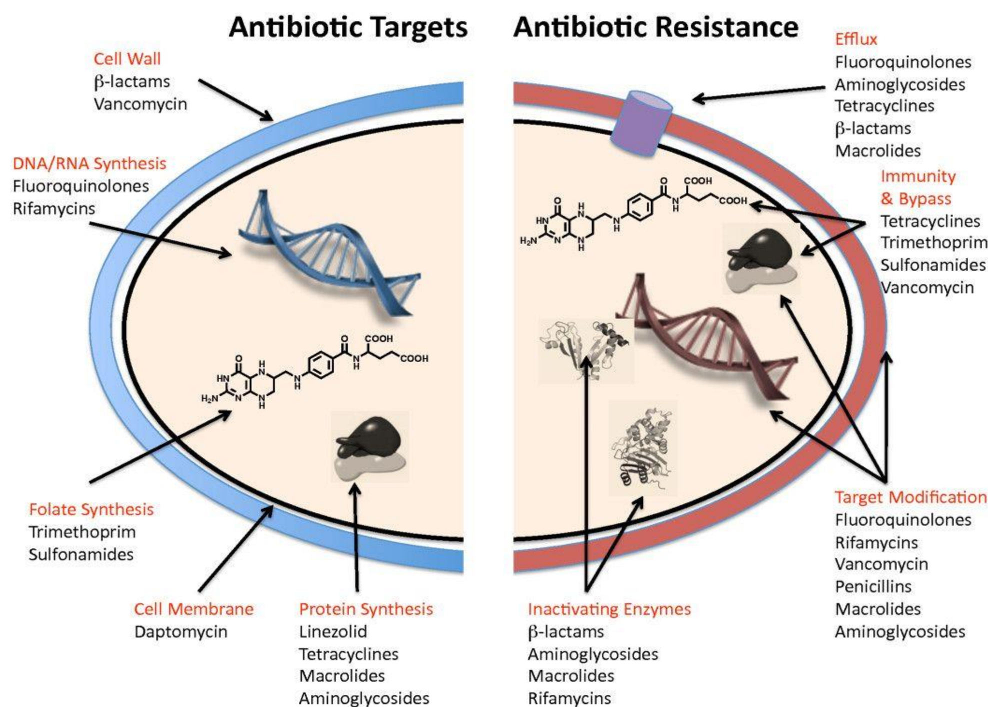

Fig. 3. The mode of action and resistance of antimicrobial agents (Wright, 2010)

2.4.5 Transfer of Antimicrobial Resistant Bacteria between Poultry and Humans

As mentioned previously, poultry are exposed to several types of antimicrobial classes, which results in the rise of antimicrobial resistant bacteria that can be transmitted to humans (Silbergeld et al. 2008). Antimicrobial-resistant bacteria and genes can spread to humans via direct or indirect pathways. Direct pathways involve direct contact between humans and animals, while indirect pathways include consumption of food or through environmental pathways (Marshall and Levy, 2011).

2.4.5.1 Direct Pathways

Transmission of bacteria between animals and human through direct pathways is often observed in farmers, veterinarians and individuals who live or work in close

contact with animals (Marshall and Levy, 2011). This was observed when comparing bacterial strains colonizing humans who handle animals in comparison to the general population (Marshall and Levy, 2011). Furthermore, this can result in the transfer of bacteria and resistant genes colonizing the animal into the community via the human workers (Marshall and Levy, 2011).

2.4.5.2 Indirect Pathways

2.4.5.2.1 Foodborne Transmission

Antimicrobial resistant bacterial strains can be transmitted indirectly via food (foodborne transmission) (Van den Bogaard et el. 2001). Research conducted in the USA shows the occurrence of antimicrobial resistant *E. coli* strains with different resistant patterns in retail meat (Zhao et al., 2012). Risk assessment analysis was conducted in Belgium to estimate the transfer of cephalosporin-resistant *Escherichia coli* to individuals consuming a meal containing chicken (Depoorter et al., 2012). The study revealed that 60% of the individuals were carrying the strain (Depoorter et al., 2012). Therefore, there is a risk of transmission that depends on the source of meat and the amount of contamination; however, the density needed for the transfer of antimicrobial resistant *Escherichia coli* from poultry to humans is still hard to estimate (Depoorter et al., 2012). However, it was proven that *Escherichia coli* from food animals could transfer their resistant genes to other bacterial species in the human's gastrointestinal tract when there is a significant amount of colony-forming unit upon ingestion (Smith, 1969).

2.4.5.2.2 Environmental Transmission

Transmission of antibiotic resistant bacteria and/or genes between the environment and humans is possible (Marshall and Levy, 2011). Several studies confirm the possibility of this transmission. For example, environmental transmission can occur from spreading manure on agricultural lands, where the survival of certain bacteria and the dissemination of their resistant genes to other soil bacteria are possible (Chee et al., 2009). Composting of the manure for a period of 5 weeks can decrease the colony-forming units of bacteria, however it was shown that resistant genes such as tetracycline or erythromycin will still be present and can be further transferred indirectly to humans when the manure is used as a fertilizer (Sharma et al., 2009). The use of manure and the leakage of storage pits for wastes also contaminate the groundwater as well as surface waters (Sapkota et al., 2007). A study done on water samples showed the prevalence of *Escherichia coli* and total coliforms near the surroundings of animal feeding operations (Sapkota et al., 2007). Those bacteria unlike isolates taken from far away were also resistant to certain antimicrobial agents such as erythromycin, clindamycin and tetracycline (Sapkota et al., 2007). Other studies in the USA show that bacteria with plasmid-mediated resistant genes of cephalosporins and β-lactams were present in water samples (Ash et al., 2001). Using water treatments to purify wastewater was shown to reduce the number of bacteria especially a 0.5 to 3 log reduction in *Escherichia coli*, however treatments could not eliminate entirely the resistant genes or bacteria (Da Costa et al., 2008).

2.4.6 The Effects of Resistant Bacteria in Clinical Settings

Antimicrobial resistance occurs in both Gram-positive and Gram negative bacteria. However, resistance in Gram-negative bacteria serve as the most pressing and important issue in terms of human infections (Kuenzli, 2016). Resistance in Gram-negative bacteria such as *Escherichia coli* from food producing animals is widely present and is a challenge to clinicians, because it limits the ability to control critical infections caused by important strains such as carbapenam-resistant *Enterobacteriacae* and extended spectrum cephalosporin-resistant *E. coli* (Vasoo et al., 2015). This is further complicated by the emergence of multi-drug resistant (MDR) bacteria (Szmolka and Nagy, 2013).

MDR bacteria are characterized by having complex mechanisms that allow them to be resistant to a wide range of antimicrobial classes (> 3 or more classes) (Szmolka and Nagy, 2013). The simultaneous resistance is due to the interaction of several mechanisms of resistance including, efflux pumps, target protection by modification, and enzymatic inactivation (Szmolka and Nagy, 2013). MDR bacterial pathogens can be resistant to several critically important antimicrobial agents that are used in human medicine as listed by the World Health Organization in Table 2 (WHO, 2017; Szmolka and Nagy, 2013). Nowadays, MDR bacterial infections increase the rate of morbidity and mortality worldwide and have a major effect in developing countries where the health systems are arguably overly dependent on antibiotic interventions due to the low cost and wide accessibility to these drugs (Mead, 2004).

Table 2. Classification of antimicrobial classes (WHO, 2017)

Classification in Relation to Human Medicine	**Antimicrobial Class**
Critically Important Antimicrobials	Aminoglycosides
	Ansamycins
	Carbapenems and other penems
	(3rd, 4th and 5th generation) Cephalosporins
	Glycopeptides
	Glycylcyclines
	Lipopeptides
	Macrolides and ketolides
	Monobactams
	Oxazolidinones
	Penicillins (natural, aminopenicillins, and antipseudomonal)
	Phosphonic acid derivatives
	Polymyxins
	Quinolones
	Drugs used solely to treat tuberculosis or other mycobacterial diseases
Highly Important Antimicrobials	Amidinopenicillins
	Amphenicols
	Cephalosporins (1st and 2nd generation) and cephamycins
	Lincosamides
	Penicillins (anti-staphylococcal)
	Pseudomonic acids
	Riminofenazines
	Steroid antibacterials
	Streptogramins
	Sulfonamides, dihydrofolate reductase inhibitors and combinations
	Sulfones
	Tetracyclines
Important Antimicrobials	Aminocyclitols
	Cyclic polypeptides
	Nitrofurantoins
	Nitroimidazoles
	Pleuromutilins

2.5 Using *Escherichia coli* as a Target for Resistance Surveillance

As mentioned previously, poultry meat can be contaminated with food-borne pathogens (Zhao et al. 2001). Monitoring of each pathogen in the laboratory would be

time-consuming, difficult and expensive (CSIRO 2002). Therefore, testing for indicator organisms such as *E. coli* serve as an indirect evidence of possible presence of pathogens (CSIRO 2002). *E. coli* in poultry feces and poultry products makes a good surveillance target due to its high recovery rate of 99% in comparison to other bacteria such as *Campylobacter* and *Salmonella* with recovery rates of 20 and 59%, respectively (Government of Canada, 2015).

Resistance surveillance programs such as NARMS in the United States of America and CIPARS in Canada have been using *E. coli* as an indicator organism for antimicrobial resistance in Gram-negative bacteria (Franklin et al., 2001). Testing resistant *Escherichia coli* as an indicator for the resistance of Gram-negative bacteria in poultry is considered an acceptable practice (Franklin et al., 2001). Given that many *E. coli* strains are pathogenic or can be reservoirs for the dissemination of resistance genes, antimicrobial resistant *E. coli*, have a direct and serious impact on the health of human beings (Bergeron et al., 2012).

2.6 Food Safety in Lebanon

Food safety plays an important role in the health of the population as well as the economic prosperity of a country (Kamleh et al., 2012). The food industry makes up the majority (18.2%) of the agro-industries in Lebanon and involves 25% of the workforce (Bissat, 2014). Despite the decrease in Lebanon's economy and its political unrest, food has been the number one export since 2009 (El Jardali et al., 2014). Although the food sector is of great importance in the economy of Lebanon, food safety still remains a major issue and concern (El Jardali et al., 2014).

Due to evolving and high international food safety standards, exports of food products from developing countries can be hindered (Jongwanich, 2009). In Lebanon, pathogenic microorganisms are being detected in food products such as poultry meat and food poisoning outbreaks have been routinely reported (El Jardali et al., 2014). There are evidence that pathogens such as *Escherichia coli*, *Salmonella* and *Listeria monocytogenes* are being detected in extremely high levels that exceed the limits set by international standards (Saleh et al., 2009; Kassaify et al., 2010, Harakeh et al., 2005). In 2014, the Minister of Public Health provided to the public a list of food retails and restaurants that do not meet food safety standards (El Jardali et al., 2014). Most of these were due to improper hygienic practices from food handlers and contaminated warehouses and storage areas (El Jardali et al., 2014). Limited testing also showed the presence of bacterial contamination, sewage water and human sweat (El Jardali et al., 2014).

Practices in the food industry do not ensure the safety of consumers, because these practices do not necessarily comply with international standards (El Jardali et al., 2014). The food safety standard for fresh poultry in Lebanon is found in the Lebanese Standards institution (LIBNOR), which is linked to the Ministry of Industry (Fresh Poultry, 2006). The most updated version is that of 2006-2008 where a maximal acceptance level is set at 5000 colony-forming units/g for fecal coliforms (Fresh Poultry, 2006). In comparison to Lebanon, countries such as the United States of America and Europe have specialized agencies, surveillance and monitoring programs to ensure the quality and safety of their products (El Jardali et al., 2014). In addition, monitoring and quantifying microorganisms such as *Escherichia coli* is also recommended in order to better assess the level and possibility of contamination.

To our knowledge, Lebanon faces a lot of problems when dealing with the safety of its food. This is due to lack of resources and associated weakness of surveillance and control of bacterial contamination that can happen along the processing and distribution lines of different foods, including broiler meat. The environment of poultry production is complex and dynamic and has numerous reservoirs of microorganisms that can spread to consumable chicken products (Kassem et al., 2016). Furthermore, beyond food safety, the proliferation of pathogenic microorganisms can result in affecting the health of broilers, jeopardizing biosecurity and hygiene control efforts, causing financial losses and burdens (Kassem et al., 2016). Given that the poultry industry is more developed than other local animal farming in Lebanon and is a good source of economic gain (Mikhael and Daou, 2016), monitoring and ensuring the Lebanese poultry products microbiological quality and safety are a priority. The latter is further emphasized by scant academic research on the quality of Lebanese poultry meat and the very few primary publications on critical poultry associated pathogens and indicator organisms such as *Escherichia coli*.

Antimicrobial use in animal farming is receiving a lot of scrutiny because of the link between its use and the rise of antimicrobial resistant bacterial pathogens. In the USA and Europe, agencies have regulated the use of antimicrobial agents and recommended programs to remove or phase out the non-therapeutic or non-preventive use of antimicrobial agents in poultry production. In Lebanon, the use of antimicrobial agents in poultry production is unclearly regulated and untightly monitored. Data on antimicrobial resistant bacteria such as *Escherichia coli* in poultry meat is lacking. Therefore, there is a need to establish monitoring systems and regulations to ensure the safety and antimicrobial use in Lebanese poultry meat.

2.7 This Study: Objectives

The main objective of this study in Lebanon is to conduct analysis of pre-harvest and post-harvest poultry; mainly focusing on broiler chickens. In order to assess the acceptability of poultry products in the Lebanese market, the first objective was to analyze the prevalence and loads of fecal coliforms and *E. coli* and antimicrobial resistance of *E. coli* in Lebanese skinless chicken breast samples. In order to assess the impact of poultry production on the emergence of antimicrobial resistance, the second objective was to analyze the antimicrobial resistance profiles of *Escherichia coli* in fecal matter. In order to assess antimicrobial resistance in the poultry production chain, the third objective was to compare the antimicrobial resistance profiles of *Escherichia coli* between the skinless chicken breast and fecal samples.

Taken together, the overall goal was to contribute to a better understanding of the acceptability and antimicrobial resistance in Lebanese poultry. This can serve public health and provide a better assessment of the economic risks that are associated with food safety in major food production sector in Lebanon.

CHAPTER 3

METHODOLOGY

3.1 Sample Collection

To investigate the prevalence and antimicrobial susceptibilities of *Escherichia coli*, this study was conducted from February 2017 till July 2018 in Lebanon. Samples were collected from major cities as an approximation for different Lebanese territories in order to generate a more representative data (Hamamy et al., 2008). For post-harvest, a total of 151 skinless chicken breast samples were purchased from 52 shops and retail outlets that sell the products of major producers. For pre-harvest, a total of 183 fecal samples were obtained from 2 farms in the North, 2 farms in the Beqaa Valley and 2 farms in the south. The samples were then transported to the laboratory using cool boxes with ice packs and processed within a maximum of 12-24 hours after collection.

3.2 Microbiological Analysis

To enumerate fecal coliforms and *Escherichia coli*, 25 grams of skinless chicken breasts were added to 225 ml of sterile buffered peptone water (BPW; Bio-Rad) in a sterile stomacher bag under aseptic conditions. The sample was then homogenized for 60 seconds in a stomacher and then serial dilutions (10-fold) were prepared using 9 ml of sterile BPW as a diluent. From the dilutions (10^{-1}, 10^{-2}, 10^{-4}), 100µl were plated in duplicates on RAPID' *E.coli* 2 Agar (Bio-Rad) and incubated at 37°C for 24 hours under aerobic conditions (Park et al., 2011). Colonies exhibiting diagnostic phenotypes were counted and loads were determined per gram of skinless chicken breasts. Results were interpreted based on LIBNOR and FSIS Standards for fecal coliforms and

Escherichia coli, respectively. The most updated version of LIBNOR is that of 2006-2008 where a maximal acceptance level is set at 5000 colony-forming units/g for fecal coliforms (Fresh Poultry, 2006). The Food Safety and Inspection Services (FSIS) conducted studies in federally inspected facilities to determine the maximal acceptance level of *Escherichia coli* which was proposed 100 colony-forming units/g, while a marginal acceptance level was set at 1000 colony-forming units/g (USDA).

To isolate *Escherichia coli* from the fecal samples, 2 grams of feces were diluted in 2 ml of buffered peptone water (BPW; Bio-Rad). Direct inoculation was performed by streaking a loopful of the solution onto RAPID' *E.coli* 2 Agar (Bio-Rad) which was incubated at 37°C for 24 hours under aerobic conditions. Then 3 colonies exhibiting diagnostic phenotypes were selected from each sample.

The diagnostic colonies of *Escherichia coli* from fecal and skinless chicken breast samples were further purified on RAPID' *E.coli* 2 Agar. Using an inoculated loop, each colony was added to a tube containing 3 ml Luria Bertani Broth (LB; Oxoid). The tubes were then placed in a shaker at 37°C overnight under aerobic conditions to allow the *Escherichia coli* to multiply. Then, 1 ml of each tube was transferred to cryogenic vials containing 0.5ml of 80% sterile glycerol for long term storage at a temperature of -80°C until further testing.

3.3 Antimicrobial Susceptibility Testing

To determine the antimicrobial susceptibility of *Escherichia coli*, the Kirby-Bauer disc diffusion method was performed according to the conditions defined by the Clinical and Laboratory Standards Institute (CLSI). Testing was performed on 549 *E. coli* isolates from fecal samples and 120 out of 122 isolates (2 isolates could not be

retrieved from glycerol stocks) from skinless chicken breast samples. Using a sterile loop, colonies recovered from frozen stocks on RAPID' *E.coli* 2 Agar (Bio-Rad) were transferred to tubes containing 3 ml of Mueller-Hinton Broth and incubated in an orbital shaker (MH; Oxoid). The absorbance of each solution was measured using an optical density spectrophotometer to insure that all solutions have the same concentration of *Escherichia coli*. Solutions were adjusted to reach an OD_{600} of 0.05 (the McFarland equivalent in our protocol) then a sterile cotton swab was dipped into the solution and spread on MH agar plates. *Escherichia coli* isolates were tested for 13 antibiotics of specific disc contents: tetracycline (30µg), ampicillin (10µg), ciprofloxacin (5µg), gentamicin (10µg), kanamycin (30 µg), cefepime (30µg), cefexime (5µg), cefotaxime (30µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), amoxicillin- clavulanate (20/10 µg), and erythromycin(15µg). Erythromycin was used as a quality control to insure that the bacteria tested is *Escherichia coli*, because it is intrinsically resistant to erythromycin (Nguyen *et al.* 2009). Antimicrobial susceptibilities of the isolates were interpreted based on breakpoints available in CLSI, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the British Society for Antimicrobial Chemotherapy (BSAC) Standards.

CHAPTER 4

RESULTS

4.1 Microbiological Analysis

Results from the microbiological analysis of fecal samples showed that *Escherichia coli* were detected in 549 (100%) out of 549 samples. The skinless chicken breast samples showed that fecal coliforms were detected in 151 (100%) out of 151 samples, while *Escherichia coli* were detected in 122 (80.79%) out of 151 samples. The distribution of contamination level of fecal coliforms and *E. coli* are shown in Table 3.

Table 3. Distribution of the contamination levels of fecal coliforms and *Escherichia coli* in 151 skinless chicken breast samples

Samples	Fecal Coliforms			*Escherichia coli*		
	Cell Count (CFU/g)	No. of samples (n=151)	Percentage (%)	Cell Count (CFU/g)	Number of samples (n=151)	Percentage (%)
Skinless Chicken Breasts	<1000	8	5.30	≤100	51	33.77
	1000 - 5000	14	9.27	101-1000	39	25.83
	5001-100000	59	39.07	>1000	61	40.40
	≥ 100001	70	46.36			

For fecal coliforms, 22 (14.57%) out of 151 samples were considered acceptable since they were below the maximal acceptance level of 5000 CFU/g, while 129 (85.43%) out of 151 samples were considered unacceptable. The lowest fecal coliform cell count was 150 CFU/g while the highest was 388500000 CFU/g. According to the LIBNOR Standard, the percentage of rejection rate from 151 samples

was 85.43%. Using *Escherichia coli*, 51 (33.77%) out of 151 samples were labeled acceptable, because they were below the maximal acceptance level of 100 CFU/g, 39 (25.83%) out of 151 samples were in the marginal range of 101-1000 CFU/g, and 61 (40.40%) out of 151 samples were unacceptable, because they were higher than the maximal acceptance level. The lowest *E. coli* cell count was below detection limit, while the highest was 2190000 CFU/g. The percentage rejection rate for 151 samples using *E. coli* was 40.40%.

Comparing the acceptability of the skinless chicken breast samples between LIBNOR and FSIS Standards, 13.24% (20 out of 151) samples were labeled acceptable by both standards, 40.40% (61 out of 151) samples were labeled unacceptable by both standards, while 46.36% (70 out of 151) samples were labeled differently by each standard. As mentioned above, samples were purchased from 52 outlets which was repeated thrice across time. Figure 4 shows that 31 samples from the same locations were rejected by LIBNOR 3 times, 15 samples were rejected twice and accepted once, 2 of the samples were rejected once and accepted twice, and 1 sample was accepted three times.

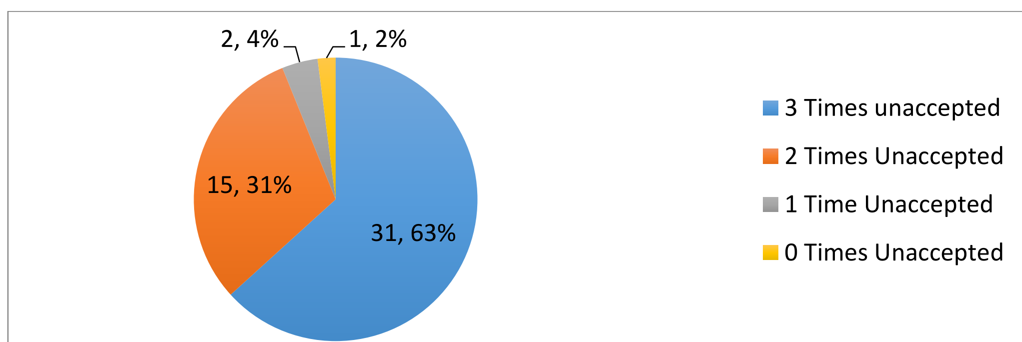

Fig. 4. Frequency of samples from the same outlets that were unaccepted by LIBNOR.

4.2 Antimicrobial Susceptibility Testing

A total of 120 out of 122 *E. coli* isolates (2 isolates could not be retrieved from glycerol stocks) from skinless chicken breast samples and 549 *E. coli* isolates from fecal samples were tested for their potential to resist 13 antimicrobial agents by the Kirby-Bauer disc diffusion method. All isolates tested from the skinless chicken breast and fecal samples showed resistance to erythromycin since it was used as control.

As shown in Figure 5, *E. coli* isolated from skinless chicken breast samples were mostly resistant to ampicillin (69.17%), tetracycline (67.50%), and ciprofloxacin (59.17%). Some isolates showed resistance to gentamicin (34.17%), kanamycin (38.33%), chloramphenicol (51.67%) and trimethoprim-sulfamethoxazole (45%). Resistance to the class of cephalosporin, including cefepime (15.83%), cefexime (22.50%), cefotaxime (19.17%), and cephalexin (25.83%) were notable. Also, few isolates of *Escherichia coli* were resistant to amoxicillin-clavulanate (23.33%) from the β-lactam class. As shown in Table 4, 14 (11.67%) out of 120 isolates were pan-susceptible to all antimicrobials, while 106 (88.33%) out of 120 were at least resistant to one or more antimicrobial agents. Nine (7.5%) isolates showed resistance to a single antimicrobial agent, twelve (10%) isolates showed resistance to two antimicrobial agents, while eighty-five (70.83%) showed resistant to three or more antimicrobial agents. Some isolates that showed resistance to only one antimicrobial agent; AMP (3.33%), CHL (0.83%), CIP (0.83%), GEN (1.67%) and TET (0. 83%).Eighty-four (70%) of 120 isolates tested were MDR (resistant to 3 or more classes of antibiotics). The most common MDR pattern observed among isolates were resistant to AMP-CHL-CIP-(GEN-KMN)-SXT-TET (5.83%).

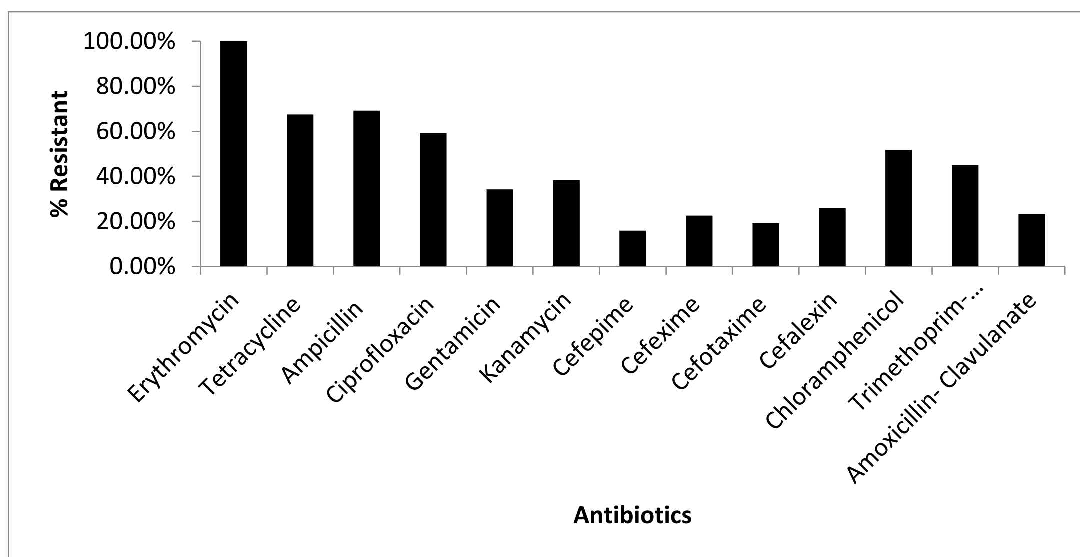

Fig. 5. Percentage of antimicrobial resistant *Escherichia coli* isolates from skinless chicken breast samples.

Table 4. Resistance Profiles of *Escherichia coli* isolated from skinless chicken breasts in Lebanon.

Isolates	Resistance Profile	No. of Classes resistant	No. of resistant isolates (%)
E 24, E 27, E 32, E 41, E 43, E 52, E 60, E 98, E 14, E 15, E 16, E 17, E 19, E 47	Pan-susceptible	0	14 (11.67)
E 25, E 36, E 106, E 111	AMP	1	4 (3.33)
E 97	CHL	1	1 (0.83)
E 21	CIP	1	1 (0.83)
E 22, E 82	GEN	1	2 (1.67)
E 81	TET	1	1 (0.83)
E 8, E 42, E 94, E 126	CIP-TET	2	4 (3.33)
E 11	GEN-SXT	2	1 (0.83)
E 20	AMC-CIP	2	1 (0.83)
E 103	AMC-SXT	2	1 (0.83)
E 30, E 34, E 88	AMP-CIP	2	3 (2.5)
E 151	AMP-SXT	2	1 (0.83)
E 39	AMP-TET	2	1 (0.83)
E 92	CIP-SXT-TET	3	1 (0.83)
E 17	AMP-CIP-FEP	3	1 (0.83)

E 4, E 148	AMP-CHL-CIP	3	2 (1.67)
E 55	AMP-CIP-TET	3	1 (0.83)
E 14, E 128	AMP-CHL-TET	3	2 (1.67)
E 9, E 93	AMC-AMP-CIP	3	2 (1.67)
E 47	AMP-(CTX-LEX)	2	1 (0.83)
E 132	CHL-CIP-TET	3	1 (0.83)
E 78	CIP-CFM-TET	3	1 (0.83)
E 5, E 59	CHL-CIP-SXT-TET	4	2 (1.67)
E 130, E 139	AMP-CHL-CIP-TET	4	2 (1.67)
E 74	AMP-CHL-CIP-SXT	4	1 (0.83)
E 38	AMP-CHL-CIP-GEN	4	1 (0.83)
E 89	(GEN-KMN)-SXT-TET	3	1 (0.83)
E 12	AMC-AMP-LEX-TET	4	1 (0.83)
E 101	AMP-CHL-KMN-TET	4	1 (0.83)
E 90	CHL-CIP-GEN-SXT-TET	5	1 (0.83)
E 63, E 70	AMP-CHL-CIP-SXT-TET	5	2 (1.67)
E 146	CIP-FEP-KMN-SXT-TET	5	1 (0.83)
E 86	AMP-CHL-GEN-SXT-TET	5	1 (0.83)
E 28, E 40	AMP-CHL-CIP-GEN-TET	5	2 (1.67)
E 124	AMP-CHL-CIP-LEX-TET	5	1 (0.83)
E 31	AMP-CIP-KMN-SXT-TET	5	1 (0.83)
E 95	AMP-CHL-CIP-KMN-TET	5	1 (0.83)
E 140	AMP-(CFM-CTX-LEX)-SXT	3	1 (0.83)
E 18	AMC-(CFM-CTX-LEX)-TET	3	1 (0.83)
E 72, E 102	AMP-CHL-(GEN-KMN)-TET	4	2 (1.67)
E 96	CHL-CIP-LEX-GEN-SXT-TET	6	1 (0.83)
E 125	AMP-CHL-CIP-FEP-KMN-TET	6	1 (0.83)
E 57, E 91, E 110, E 133	AMP-CHL-CIP-KMN-SXT-TET	6	4 (3.33)
E 65	AMC-AMP-CIP-CFM-SXT-TET	6	1 (0.83)
E 19	CHL-CIP-(GEN-KMN)-SXT-TET	5	1 (0.83)
E 143	AMC-AMP-CHL-KMN-SXT-TET	6	1 (0.83)
E 75	AMC-AMP-CIP-(FEP-CFM-CTX-LEX)	4	1 (0.83)
E 29, E 99, E 150	AMP-CHL-(GEN-KMN)-SXT-TET	5	3 (2.5)
E 6	AMP-CHL-CIP-(GEN-KMN)-TET	5	1 (0.83)
E 69	AMP-CHL-CIP-LEX-KMN-SXT-TET	7	1 (0.83)
E 7	AMC-AMP-CHL-CIP-KMN-SXT-TET	7	1 (0.83)
E 1, E 48, E 77, E 83, E 100, E 121, E 149	AMP-CHL-CIP-(GEN-KMN)-SXT-TET	6	7 (5.83)
E 37, E 58	AMP-(FEP-CFM-CTX-LEX)-SXT-TET	4	2 (1.67)
E 120	AMC-AMP-(CFM-CTX-LEX)-SXT-TET	5	1 (0.83)
E 85	AMC-AMP-(FEP-CFM-CTX-LEX)-TET	4	1 (0.83)

E 33	AMC-AMP-CIP-LEX-(GEN-KMN)-TET	6	1 (0.83)
E 76	AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-TET	5	1 (0.83)
E 109	AMP-(FEP-CFM-CTX-LEX)-GEN-SXT-TET	5	1 (0.83)
E 104	AMC-AMP-CIP-(FEP-CFM-CTX-LEX)-TET	5	1 (0.83)
E 87	AMC-AMP-CHL-CIP-LEX-GEN-SXT-TET	8	1 (0.83)
E 131	AMC-AMP-CHL-CIP-FEP- KMN-SXT-TET	8	1 (0.83)
E 45	AMC-AMP-CHL-CIP-(GEN-KMN)-SXT-TET	7	1 (0.83)
E 15	AMC-AMP-CHL-CIP-LEX-(GEN-KMN)-TET	7	1 (0.83)
E 137	AMP-CIP-(FEP-CFM)-(GEN-KMN)-SXT-TET	6	1 (0.83)
E 105	AMP-(FEP-CFM-CTX-LEX)-(GEN-KMN)-TET	4	1 (0.83)
E 68	AMC-AMP-CHL-CIP-CFM-(GEN-KMN)-SXT-TET	8	1 (0.83)
E 71	AMP-CHL-CIP-(CFM-CTX-LEX)-(GEN-KMN)-TET	6	1 (0.83)
E 127	AMC-AMP-CHL-(FEP-CFM-CTX-LEX)-KMN- SXT-TET	7	1 (0.83)
E 3	AMC-AMP-CHL-CIP-(CFM-CTX- LEX)-KMN-SXT-TET	8	1 (0.83)
E 67	AMP-CHL-(FEP-CFM-CTX-LEX)-(GEN-KMN)- SXT-TET	6	1 (0.83)
E 107	AMC-AMP-CHL-CIP-(CFM-LEX)-(GEN-KMN)-SXT-TET	8	1 (0.83)
E 49	AMC-AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-KMN- SXT-TET	8	1 (0.83)
E 112	AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-(GEN-KMN)- SXT-TET	7	1 (0.83)
E 44, E 129, E 134	AMC-AMP-CHL-CIP-(CFM-CTX-LEX)-(GEN-KMN)-SXT-TET	8	3 (2.5)
E 84, E 136	AMC-AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-(GEN-KMN)- SXT-TET	8	2 (1.67)

*Pan-susceptible, susceptible to all tested antimicrobials; AMC, amoxicillin-clavulanate; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; FEP, cefepime; CFM, cefexime; CTX, cefotaxime; LEX, cephalexin; GEN, gentamicin; KMN, kanamycin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline.

As shown in Figure 6, analysis from the fecal samples showed that *E. coli* isolates were mostly resistant to ampicillin (95.44%), tetracycline (89.07%) and chloramphenicol (83.42%). The isolates were also resistant to trimethoprim-sulfamethoxazole (75.59%), ciprofloxacin (71.40%), cephalexin (58.83%), gentamicin (56.65%) and kanamycin (55.56%). A notable number of isolates were resistant to cefotaxime (37.70%), cefexime (33.33%) and cefepime (21.49%). As shown in Table 5, 549 (100%) out of 549 were at least resistant to one or more antimicrobial agents. Only, 3 (0.55%) isolates showed resistance to a single antimicrobial agent, while 546 (99.45%) showed resistance to three or more antimicrobial agents. Eight (1.46%) out of

549 isolates were resistant to all 12 antimicrobial agents tested. Out of the 549 isolates tested, 540 (98.36%) were MDR. Isolates that showed resistance to only one antimicrobial agent had a MDR pattern of TET (0.18%) and CFM (0.36%). Some (15 out of 549) of the isolates showed a MDR pattern of AMP-CHL-CIP-KMN-SXT-TET (2.73%), while the most common MDR pattern observed among isolates (28 out of 549) was AMP-CHL-CIP-(GEN-KMN)-SXT-TET (5.1%).

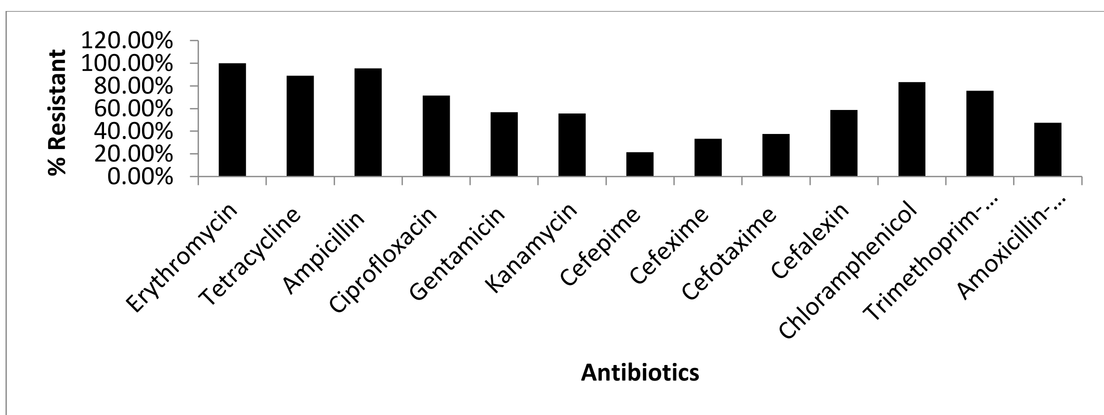

Fig. 6. Percentage of antibiotic resistant *Escherichia coli* isolates from fecal samples tested by Kirby-Bauer disc diffusion method.

Table 5. Resistance Profiles of *Escherichia coli* isolated from Fecal Samples in Lebanon

Isolates	Resistance Profile	No. of Classes resistant	No. of resistant isolates (%)
E1	TET	1	1 (0.18%)
E2, E3	CFM	1	2 (0.36%)
E4	AMP-CHL-CIP	3	1 (0.18%)
E5	AMP-CIP-LEX	3	1 (0.18%)
E6, E7, E8	AMP-CHL-TET	3	3 (0.55%)
E9	CHL-GEN-TET	3	1 (0.18%)
E10	AMC-AMP-SXT	3	1 (0.18%)
E11	AMP-KMN-TET	3	1 (0.18%)

E12	CHL-CIP-SXT-TET	4	1 (0.18%)
E13, E14, E15, E16, E17	AMP-CIP-SXT-TET	4	5 (0.91%)
E18, E19, E20	AMP-CHL-CIP-SXT	4	3 (0.55%)
E21, E22, E23, E24, E25	AMP-CHL-CIP-TET	4	5 (0.91%)
E26, E27, E28, E29, E30, E31, E32, E33, E34, E35, E36	AMP-CHL-SXT-TET	4	11 (2%)
E37	AMP-CHL-LEX-TET	4	1 (0.18%)
E38	AMP-CHL-CTX-TET	4	1 (0.18%)
E39	AMC-CHL-CTX-TET	4	1 (0.18%)
E40, E41, E42, E43, E44, E45, E46, E47, E48, E49, E50, E51, E52	AMP-CHL-GEN-TET	4	13 (2.37%)
E53	AMC-CHL-GEN-TET	4	1 (0.18%)
E54	AMC-AMP-LEX-TET	4	1 (0.18%)
E55, E56	AMP-CHL-KMN-TET	4	2 (0.36%)
E57, E58, E59, E60, E61	AMP-(FEP-CTX-LEX)	2	5 (0.91%)
E62	AMP-(CTX-LEX)-GEN	3	1 (0.18%)
E63, E64	AMP-(GEN-KMN)-TET	3	2 (0.36%)
E65, E66, E67, E68	CHL-CIP-GEN-SXT-TET	5	4 (0.73%)
E69, E70, E71, E72, E73, E74, E75, E76, E77, E78, E79, E80	AMP-CHL-CIP-SXT-TET	5	12 (2.19%)
E81, E82, E83, E84	AMP-CHL-CIP-GEN-TET	5	4 (0.73%)
E85, E86, E87	AMP-CIP-GEN-SXT-TET	5	3 (0.55%)
E88	AMP-CIP-LEX-GEN-TET	5	1 (0.18%)
E89	CHL-CIP-CFM-GEN-SXT	5	1 (0.18%)
E90	AMP-CHL-CTX-SXT-TET	5	1 (0.18%)
E91, E92, E93, E94	AMP-CHL-GEN-SXT-TET	5	4 (0.73%)
E95, E96, E97	AMC-AMP-CIP-SXT-TET	5	3 (0.55%)
E98, E99, E100, E101	AMP-CHL-CIP-KMN-TET	5	4 (0.73%)
E102	AMC-AMP-CHL-CIP-LEX	5	1 (0.18%)
E103, E104, E105	AMP-CIP-KMN-SXT-TET	5	3 (0.55%)
E106, E107, E108, E109	AMP-CHL-KMN-SXT-TET	5	4 (0.73%)
E110	AMP-CHL-CFM-GEN-TET	5	1 (0.18%)

E111	AMP-LEX-KMN-SXT-TET	5	1 (0.18%)
E112	AMP-(FEP-CTX-LEX)-SXT	3	1 (0.18%)
E113	AMC-AMP-CHL-SXT-TET	5	1 (0.18%)
E114	AMP-(FEP-CTX-LEX)-TET	3	1 (0.18%)
E115	AMP-CHL-(FEP-CTX-LEX)	3	1 (0.18%)
E116	AMP-(FEP-CTX-LEX)-GEN	3	1 (0.18%)
E117	AMP-(FEP-CFM-CTX-LEX)	2	1 (0.18%)
E118	AMP-(FEP-CTX-LEX)-KMN	3	1 (0.18%)
E119	AMP-CHL-(CTX-LEX)-TET	4	1 (0.18%)
E120, E121	AMP-(CTX-LEX)-KMN-TET	4	2 (0.36%)
E122	AMP-(GEN-KMN)-SXT-TET	4	1 (0.18%)
E123, E124, E125, E126	AMP-CHL-(GEN-KMN)-TET	4	4 (0.73%)
E127, E128, E129, E130, E131, E132	AMP-CHL-CIP-LEX-SXT-TET	6	6 (1.09%)
E133, E134	CHL -CIP-LEX-GEN-SXT-TET	6	2 (0.36%)
E135, E136	AMP-CIP-LEX-GEN-SXT-TET	6	2 (0.36%)
E137, E138, E139, E140, E141, E142, E143, E144	AMP-CHL-CIP-GEN-SXT-TET	6	8 (1.46%)
E145, E146, E147, E148, E149, E150, E151, E152, E153, E154, E155, E156, E157, E158, E159	AMP-CHL-CIP-KMN-SXT-TET	6	15 (2.73%)
E160, E161, E162	AMC-AMP-CIP-LEX-SXT-TET	6	3 (0.55%)
E163, E164, E165	AMC-AMP-CHL-CIP-SXT-TET	6	3 (0.55%)
E166	AMP-CHL-LEX-GEN-SXT-TET	6	1 (0.18%)
E167	AMC-AMP-CIP-CTX-SXT-TET	6	1 (0.18%)
E168	AMC-AMP-CIP-GEN-SXT-TET	6	1 (0.18%)
E169	AMP-CIP-(FEP-CTX)-SXT-TET	5	1 (0.18%)
E170, E171	AMC-AMP-CHL-CIP-GEN-TET	6	2 (0.36%)
E172	AMC-AMP-CIP-KMN-SXT-TET	6	1 (0.18%)
E173	AMP-CHL-CIP-(CTX-LEX)-TET	5	1 (0.18%)
E174	AMC-AMP-CHL-LEX-GEN-TET	6	1 (0.18%)
E175, E176	AMC-AMP-CHL-CIP-KMN-TET	6	2 (0.36%)
E177	AMC-AMP-CHL-GEN-SXT-TET	6	1 (0.18%)

E178	CIP-(FEP-CFM-CTX-LEX)-KMN	3	1 (0.18%)
E179	AMC-CIP-(CFM-CTX)-SXT-TET	5	1 (0.18%)
E180	CHL-CIP-LEX-(GEN-KMN)-TET	5	1 (0.18%)
E181	AMC-CHL-(FEP-CTX)-GEN-SXT	5	1 (0.18%)
E182	AMC-AMP-CHL-KMN-SXT-TET	6	1 (0.18%)
E183	AMP-CHL-(CFM-CTX-LEX)-TET	4	1 (0.18%)
E184	AMC-AMP-CHL-CIP-(CFM-LEX)	5	1 (0.18%)
E185	AMP-CIP-(GEN-KMN)-SXT-TET	5	1 (0.18%)
E186, E187, E188	AMP-CHL-CIP-(GEN-KMN)-TET	5	3 (0.55%)
E189, E190	AMP-(FEP-CTX-LEX)-KMN -TET	4	2 (0.36%)
E191	AMP-CHL-(GEN-KMN)-SXT-TET	5	1 (0.18%)
E192, E193, E194, E195, E196, E197	AMC-AMP-(CFM-CTX-LEX)-TET	4	6 (1.09%)
E198, E199, E200	AMC-AMP-CHL-(CFM-CTX-LEX)	4	3 (0.55%)
E201	AMC-CIP-(FEP-CTX)-(GEN-KMN)	4	1 (0.18%)
E202	AMC-AMP-CHL-(CFM-LEX)-KMN	5	1 (0.18%)
E203, E204, E205, E206, E207, E208, E209, E210, E211, E212, E213	AMP-CHL-CIP-LEX-GEN-SXT-TET	7	11 (2%)
E214, E215	AMC-CHL-CIP-FEP-GEN-SXT-TET	7	2 (0.36%)
E216	AMP-CHL-CIP-FEP-KMN-SXT-TET	7	1 (0.18%)
E217, E218, E219, E220	AMC-AMP-CHL-CIP-LEX-SXT-TET	7	4 (0.73%)
E221, E222, E223, E224, E225	AMP-CHL-CIP-LEX-KMN-SXT-TET	7	5 (0.91%)
E226, E227	AMP-CHL-(CTX-LEX)-(GEN-KMN)	4	2 (0.36%)
E228	AMP-CHL-CIP-CTX-KMN-SXT-TET	7	1 (0.18%)
E229	AMP-(CFM-CTX-LEX)-(GEN-KMN)	3	1 (0.18%)
E230	AMC-AMP-CIP-LEX-GEN-SXT-TET	7	1 (0.18%)
E231	AMC-AMP-CHL-CIP-GEN-SXT-TET	7	1 (0.18%)
E232	AMP-CHL-CIP-(CTX-LEX)-SXT-TET	6	1 (0.18%)
E233	AMC-AMP-CHL-CIP-LEX-GEN-TET	7	1 (0.18%)
E234	AMC-AMP-CHL-CIP-CFM-SXT-TET	7	1 (0.18%)
E235, E236	AMC-AMP-CHL-CIP-GEN-SXT-TET	7	2 (0.36%)
E237, E238, E239, E240, E241, E242, E243, E244, E245	AMC-AMP-CHL-CIP-KMN-SXT-TET	7	9 (1.64%)

E246, E247	CHL-CIP-LEX-(GEN-KMN)-SXT-TET	6	2 (0.36%)
E248	AMC-CHL-CIP-(FEP-CTX)-GEN-SXT	6	1 (0.18%)
E249	AMP-CIP-(CFM-LEX)-GEN-SXT-TET	6	1 (0.18%)
E250	AMC-AMP-(FEP-CTX-LEX)-SXT-TET	5	1 (0.18%)
E251	AMP-CHL-(FEP-CFM-CTX-LEX)-SXT	4	1 (0.18%)
E252, E253, E254	AMP-CHL-(FEP-LEX)-KMN-SXT-TET	6	3 (0.55%)
E255	AMC-CHL-CIP-LEX-(GEN-KMN)-SXT	6	1 (0.18%)
E256	AMP-CHL-CIP-LEX-(GEN-KMN)-SXT	6	1 (0.18%)
E257, E258, E259, E260, E261, E262, E263, E264, E265, E266, E267, E268, E269, E270, E271, E272 E273, E274, E275, E276, E277, E278, E279, E280, E281, E282, E283, E284	AMP-CHL-CIP-(GEN-KMN)-SXT-TET	6	28 (5.1%)
E285	AMC-AMP-CHL-CIP-(FEP-CTX)-TET	6	1 (0.18%)
E286, E287, E288	AMP-CHL-CIP-LEX-(GEN-KMN)-TET	6	3 (0.55%)
E289	AMC-AMP-CHL-(CTX-LEX)-SXT-TET	6	1 (0.18%)
E290	AMP-CHL-LEX-(GEN-KMN)-SXT-TET	6	1 (0.18%)
E291	AMC-AMP-CHL-CIP-(CFM-CTX-LEX)	5	1 (0.18%)
E292	AMC-AMP-CHL-CIP-(CFM-CTX)-TET	6	1 (0.18%)
E293, E294	AMP-CHL-CIP-CFM-(GEN-KMN)-TET	6	2 (0.36%)
E295	AMP-CHL-CIP-CFM-(GEN-KMN)-SXT	6	1 (0.18%)
E296	AMC-AMP-CHL-(FEP-LEX)-KMN-TET	6	1 (0.18%)
E297, E298	AMC-CHL-(CFM-CTX-LEX)-KMN-TET	5	2 (0.36%)
E299	AMP-CIP-(CTX-LEX)-(GEN-KMN)-TET	5	1 (0.18%)
E300	AMC-AMP-CHL-(GEN-KMN)-SXT-TET	6	1 (0.18%)
E301	AMC- AMP-CHL-CIP-(GEN-KMN)-TET	6	1 (0.18%)
E302	AMP-(CTX-LEX)-(GEN-KMN)-SXT-TET	5	1 (0.18%)
E303	AMC-AMP-(CFM-CTX-LEX)-KMN-TET	5	1 (0.18%)
E304	AMP-(FEP-CTX-LEX)-(GEN-KMN)-TET	4	1 (0.18%)
E305	AMC-AMP-CHL-(CFM-LEX)-KMN-TET	6	1 (0.18%)
E306	AMC-AMP-CHL-CIP-FEP-GEN-SXT-TET	8	1 (0.18%)

E307, E308, E309, E310, E311, E312, E313, E314, E315, E316, E317, E318	AMC-AMP-CHL-CIP-LEX-GEN-SXT-TET	8	12 (2.19%)
E319	AMP-CHL-CIP-(FEP-LEX)-GEN-SXT-TET	7	1 (0.18%)
E320	AMC-AMP-CHL-CIP-CTX-GEN-SXT-TET	8	1 (0.18%)
E321	AMC-AMP-CHL-CIP-CFM-GEN-SXT-TET	8	1 (0.18%)
E322	AMC-AMP-CHL-CIP-FEP-KMN-SXT-TET	8	1 (0.18%)
E323, E324, E325, E326, E327, E328, E329	AMC-AMP-CHL-CIP-LEX-KMN-SXT-TET	8	7 (1.28%)
E330	AMP-CHL-CIP-(CFM-CTX-LEX)-SXT-TET	6	1 (0.18%)
E331, E332	AMP-CHL-CIP-(CTX-LEX)-GEN-SXT-TET	7	2 (0.36%)
E333	AMC-AMP-CHL-CIP-(CTX-LEX)-SXT-TET	7	1 (0.18%)
E334	AMP-CHL-CIP-(CFM-LEX)-GEN-SXT-TET	7	1 (0.18%)
E335, E336	AMC-AMP-CHL-CIP-(CTX-LEX)-GEN-TET	7	2 (0.36%)
E337	AMP-CHL-CIP-(CFM-LEX)-KMN-SXT-TET	7	1 (0.18%)
E338	AMP-CHL-(CFM-CTX-LEX)-GEN-SXT-TET	7	1 (0.18%)
E339, E340	AMC-AMP-CHL-CIP-(CFM-CTX-LEX)-SXT	6	2 (0.36%)
E341 E342, E343, E344, E345, E346, E347	AMP-CHL-CIP-LEX-(GEN-KMN)-SXT-TET	7	7 (1.28%)
E348	AMP-CHL-CIP-CTX-(GEN-KMN)-SXT-TET	7	1 (0.18%)
E349	AMP-CHL-CIP-FEP-(GEN-KMN)-SXT-TET	7	1 (0.18%)
E350	AMC-AMP-CIP-(CTX-LEX)-GEN-SXT-TET	7	1 (0.18%)
E351	AMP-CHL-CIP-CFM-(GEN-KMN)-SXT-TET	7	1 (0.18%)
E352	AMC-AMP-CIP-(FEP-CFM-CTX-LEX)-GEN	5	1 (0.18%)
E353, E354, E355, E356	AMC-AMP-CHL-CIP-(CFM-CTX-LEX)-TET	6	4 (0.73%)
E357	AMC-AMP-CHL-CIP-(CTX-LEX)-KMN-TET	7	1 (0.18%)
E358	AMP-CHL-(CFM-CTX-LEX)-KMN-SXT-TET	6	1 (0.18%)
E359	AMC-AMP-CHL-(FEP-CFM-CTX-LEX)-TET	5	1 (0.18%)
E360, E361	AMC-AMP-(CFM-CTX-LEX)-KMN-SXT-TET	6	2 (0.36%)
E362	AMC-AMP-CHL-CIP-LEX-(GEN-KMN)-SXT	7	1 (0.18%)
E363, E364, E365, E366, E367, E368, E369, E370, E371, E372, E373, E374	AMC-AMP-CHL-CIP-(GEN-KMN)-SXT-TET	7	12 (2.19%)
E375	AMC-AMP-CIP-LEX-(GEN-KMN)-SXT-TET	7	1 (0.18%)

E376	AMP-(FEP-CTX-LEX)-(GEN-KMN)-SXT-TET	5	1 (0.18%)
E377, E378, E379, E380	AMC-AMP-CHL-(CFM-LEX)-KMN-SXT-TET	7	4 (0.73%)
E381	AMP-CIP-(FEP-CFM-CTX-LEX)-(GEN-KMN)	4	1 (0.18%)
E382	AMC-AMP-CHL-CIP-CFM-(GEN-KMN)-TET	7	1 (0.18%)
E383	AMC-AMP-CHL-(CFM-CTX-LEX)-KMN-TET	6	1 (0.18%)
E384, E385	AMP-CHL-(CTX-LEX)-(GEN-KMN)-SXT-TET	6	2 (0.36%)
E386	AMC-AMP-(CTX-LEX)-(GEN-KMN)-SXT-TET	6	1 (0.18%)
E387, E388, E389, E390	AMC-AMP-CHL-(CFM-LEX)-(GEN-KMN)-SXT	6	4 (0.73%)
E391	AMP-CHL-CIP-(FEP-CTX-LEX)-KMN-SXT-TET	7	1 (0.18%)
E392	AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-GEN-TET	6	1 (0.18%)
E393	AMP-CHL-(FEP-CFM-CTX-LEX)-GEN-SXT-TET	6	1 (0.18%)
E394	AMC-AMP-CHL-CIP-(CFM-CTX-LEX)-GEN-TET	7	1 (0.18%)
E395	AMC-AMP-CHL-CIP-CTX-(GEN-KMN)-SXT-TET	8	1 (0.18%)
E396, E397, E398, E399, E400, E401, E402, E403, E404, E405, E406, E407, E408, E409, E410, E411 E412, E413, E414, E415, E416	AMC-AMP-CHL-CIP-LEX-(GEN-KMN)-SXT-TET	8	21 (3.83%)
E417, E418, E419, E420	AMC-AMP-CHL-CIP-(CFM-LEX)-KMN-SXT-TET	8	4 (0.73%)
E421	AMC-AMP-(FEP-CFM-CTX-LEX)-KMN-SXT-TET	6	1 (0.18%)
E422	AMC-AMP-CHL-CIP-(CFM-CTX)-KMN-SXT-TET	8	1 (0.18%)
E423	AMC-AMP-CHL-CIP-(CFM-CTX-LEX)-KMN-TET	7	1 (0.18%)
E424, E425, E426, E427, E428	AMC-AMP-CHL-(CFM-CTX-LEX)-KMN-SXT-TET	7	5 (0.91%)
E429, E430	AMP-CHL-(FEP-CTX-LEX)-(GEN-KMN)-SXT-TET	6	2 (0.36%)
E431	AMC-AMP-CHL-(FEP-CFM-CTX-LEX)-KMN-TET	6	1 (0.18%)
E432, E433	AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-(GEN-KMN)	5	2 (0.36%)
E434, E435, E436, E437	AMP-(FEP-CFM-CTX-LEX)-(GEN-KMN)-SXT-TET	5	4 (0.73%)
E438, E439, E440	AMC-AMP-(FEP-CTX-LEX)-(GEN-KMN)-SXT-TET	6	3 (0.55%)
E441	AMC-AMP-CHL-CIP-(FEP-CTX)-(GEN-KMN)-TET	7	1 (0.18%)
E442	AMC-AMP-CHL-CIP-(CFM-LEX)-(GEN-KMN)-TET	7	1 (0.18%)
E443	AMC-AMP-CHL-CIP-(CTX-LEX)-(GEN-KMN)-SXT	7	1 (0.18%)

E444, E445, E446	AMC-AMP-CHL-(CFM-CTX-LEX)-(GEN-KMN)-SXT	6	3 (0.55%)
E447	AMC-AMP-CHL-(CFM-LEX)-(GEN-KMN)-SXT-TET	7	1 (0.18%)
E448, E449, E450, E451, E452, E453	AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-GEN-SXT-TET	7	6 (1.09%)
E454, E455	AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-KMN-SXT-TET	7	2 (0.36%)
E456	AMC-AMP-CHL-CIP-(FEP-CTX-LEX)-KMN-SXT-TET	8	1 (0.18%)
E457, E458, E459, E460, E461, E462, E463, E464, E465, E466	AMC-AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-SXT-TET	7	10 (1.82%)
E467, E468, E469, E470, E471, E472, E473	AMC-AMP-CHL-CIP-(CFM-CTX-LEX)-GEN-SXT-TET	8	7 (1.28%)
E474	AMC-AMP-CHL-CIP-(FEP-CFM-LEX)-GEN-SXT-TET	8	1 (0.18%)
E475, E476	AMC-AMP-CIP-(FEP-CFM-CTX-LEX)-KMN-SXT-TET	7	2 (0.36%)
E477	AMC-AMP-CHL-(FEP-CFM-CTX-LEX)-GEN-SXT-TET	7	1 (0.18%)
E478, E479, E480, E481, E482, E483	AMC-AMP-CHL-CIP-(CFM-CTX-LEX)-KMN-SXT-TET	8	6 (1.09%)
E484	AMC-AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-KMN-TET	7	1 (0.18%)
E485, E486	AMP-CHL-CIP-(FEP-CTX-LEX)-(GEN-KMN)-SXT-TET	7	2 (0.36%)
E487	AMC-AMP-CHL-CIP-(FEP-LEX)-(GEN-KMN)-SXT-TET	8	1 (0.18%)
E488	AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-(GEN-KMN)-SXT	6	1 (0.18%)
E489, E490	AMC-AMP-CHL-CIP-(CTX-LEX)-(GEN-KMN)-SXT-TET	8	2 (0.36%)
E491, E492, E493	AMP-CHL-CIP-(CFM-CTX-LEX)-(GEN-KMN)-SXT-TET	7	3 (0.55%)
E494	AMC-AMP-CIP-(FEP-CFM-LEX)-(GEN-KMN)-SXT-TET	7	1 (0.18%)
E495	AMC-AMP-CIP-(FEP-CFM-CTX-LEX)-(GEN-KMN)-SXT	6	1 (0.18%)
E496	AMC-AMP-CHL-CIP-(CFM-CTX-LEX)-(GEN-KMN)-SXT	7	1 (0.18%)
E497, E498, E499, E500, E501	AMC-AMP-CHL-CIP-(CFM-LEX)-(GEN-KMN)-SXT-TET	8	5 (0.91%)
E502, E503	AMC-AMP-(FEP-CFM-CTX-LEX)-(GEN-KMN)-SXT-TET	6	2 (0.36%)
E504	AMC-AMP-CHL-CIP-(CFM-CTX)-(GEN-KMN)-SXT-TET	8	1 (0.18%)
E505	AMC-AMP-CHL-CIP-(CFM-CTX-LEX)-(GEN-KMN)-TET	7	1 (0.18%)
E506, E507, E508, E509	AMC-AMP-CHL-(CFM-CTX-LEX)-(GEN-KMN)-SXT-TET	7	4 (0.73%)
E510, E511, E512, E513	AMC-AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-GEN-SXT-TET	8	4 (0.73%)
E514, E515, E516, E517, E518	AMC-AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-KMN-SXT-TET	8	5 (0.91%)
E519, E520, E521, E522, E523, E524, E525, E526, E527, E528	AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-(GEN-KMN)-SXT-TET	7	10 (1.82%)
E529	AMC-AMP-CIP-(FEP-CFM-CTX-LEX)-(GEN-KMN)-SXT-TET	7	1 (0.18%)

E530, E531, E532, E533	AMC-AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-(GEN-KMN)-SXT	7	4 (0.73%)
E534, E535, E536, E537, E538, E539, E540, E541	AMC-AMP-CHL-CIP-(CFM-CTX-LEX)-(GEN-KMN)-SXT-TET	8	8 (1.46%)
E542. E543, E544, E545, E546, E547, E548, E549	AMC-AMP-CHL-CIP-(FEP-CFM-CTX-LEX)-(GEN-KMN)-SXT-TET	8	8 (1.46%)

*Pan-susceptible, susceptible to all tested antimicrobials; AMC, amoxicillin-clavulanate; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; FEP, cefepime; CFM, cefexime; CTX, cefotaxime; LEX, cephalexin; GEN, gentamicin; KMN, kanamycin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline.

CHAPTER 5

DISCUSSION

5.1 Microbiological Analysis

This study demonstrated high (80.79%) prevalence and loads of *Escherichia coli* isolated from skinless chicken breasts in Lebanon. The percentage rejection rate using the LIBNOR Standard was 85.43%, while that of the FSIS Standard was 40.40%. When comparing the rates, it might be argued that it is safe to choose the LIBNOR Standard because it rejects more samples. However, excessive rejection may not be favorable economically. This is important because the LIBNOR Standard was adopted and not necessarily tested on or modified for Lebanese products. Also, *E. coli* is considered a better indicator of fecal pollution as compared to coliforms; however, the LIBNOR Standard does not specify *E. coli* loads. Although the FSIS Standard analyzes the drippings from poultry carcasses, it was used as a proxy when assessing the acceptability of the samples. The *E. coli* based FSIS standard was more conservative in rejecting the poultry meat in comparison to the LIBNOR Standard.

5.2 Antimicrobial Susceptibility Testing

In Lebanon, despite scant studies, clinicians have concerns regarding the levels of antimicrobial resistance that is affecting public health (Salameh et al., 2017). In addition, few data are available regarding the antimicrobial resistance of *Escherichia coli* from food animals that may have a role in transmitting antimicrobial resistance to

humans in Lebanon. In this study the antimicrobial resistant profiles and MDR patterns among isolates were determined.

All of the isolates from the fecal samples (100%) and most of the isolates from the skinless chicken breast samples (88.33%) were resistant to one or more antimicrobial agent. These high numbers are alarming and are an indicator of overuse/ misuse and unregulated use of antimicrobial agents in the Lebanese poultry industry. This is very important given that many of the antibiotics tested were of great importance in human medicine.

β-lactam antimicrobials are useful in treating Gram-negative bacterial infections in humans (Frye and Jackson, 2013). In this study the percentage of resistance of *E. coli* to ampicillin was the highest among isolates from fecal and skinless chicken breast samples. This is a major issue since increasing resistance to ampicillin and its potential transmission may render the drug unavailable for treatment of human infections in the future (Frye and Jackson, 2013). Other antimicrobial agents such as tetracycline are important in treating infections in food animals. Resistance to tetracycline worldwide corroborate the high resistance observed in this study (89.07% in fecal samples and 67.50% in skinless chicken breast samples) (Adenipekun et al., 2015). Aminoglycosides such as gentamicin are used to prevent and control *Escherichia coli* infections in poultry. Aminoglycosides uses in food animals is limited in the United States of America, because it is toxic in nature and drug residues accumulate through time (Frye and Jackson, 2013). Regardless, results from this study show resistance to aminoglycosides such as gentamicin and kanamycin, which indicate that these antibiotics are used in poultry farming.

Table 6 shows the percentages of antimicrobial resistance of *Escherichia coli* in chicken meat in Finland, France, Thailand, and Egypt (Akbar et al., 2014; Moawad et al., 2017; EFSA, 2018). In developed countries such as Finland and France, the rate of resistance is lower than that of Lebanon. For example, gentamicin showed 47.4% resistance in Thailand and 34.17% in Lebanon, whereas it showed low resistance in France and Finland with 3.2% and 0%, respectively. Developed countries have lower resistance due partially to monitoring programs and strict regulations that limit and regulate the use of antimicrobial agents in food production. The resistance in Lebanon is alarming and can be correlated with the lack of control on antibiotic use and/ or improper use. Lebanon is in great need to develop and enforce regulations and limitations concerning the use of antimicrobial agents in animal farming. Furthermore, national programs should be established to monitor the use of and resistance to antibiotic in food.

Table 6. Resistant *Escherichia coli* isolates in Finland, France, Thailand, and Egypt in comparison with results from this study in Lebanon

	Ampicillin	**Ciprofloxacin**	**Chloramphenicol**	**Cefotaxime**	**Gentamicin**	**Tetracycline**
Finland	8.7	3.8	0	0.5	0	9.8
France	55.9	35.6	7.4	3.7	3.2	62.2
Thailand	92.1	63.2	39.5	-	47.4	92.1
Egypt	80	26.7	20	40	-	80
Lebanon	69.17	59.17	51.67	19.17	34.17	67.5

In this study, 96.36% of the isolates from the fecal samples and 70% of the isolates from the skinless chicken breast samples were MDR. Results of MDR from the European Union and United States of America show 55% and 62% MDR *Escherichia* coli, respectively (Founou et al., 2016). Lebanon, a much smaller country, shows MDR *Escherichia coli* in higher percentages. Lebanon also surpasses other developing countries, including Egypt that had 57.4% MDR *Escherichia coli* (Founou et al., 2016). MDR can translate to failure in treatment of human infections even with the use of different antimicrobial agents. In this study, 210 resistant patterns from the fecal samples and 74 resistant patterns from the skinless chicken breast samples were present (excluding pan-susceptible) which shows that a variety of antimicrobials are being used in poultry in Lebanon. Expectations of this study were to compare the resistant patterns from the fecal samples as an indication of possible resistance found in chicken meat purchased in Lebanon. However, the data in figure 7 show that the resistant patterns of the pre-harvest and post-harvest poultry were not always compatible. This partially indicates that some of the sources of chicken meat in supermarkets or butchery shops might not be local (imported) or that interventions during processing have changed the resistance patterns. Regardless, antimicrobial resistance appears to be widely spread and poses a significant problem in Lebanon.

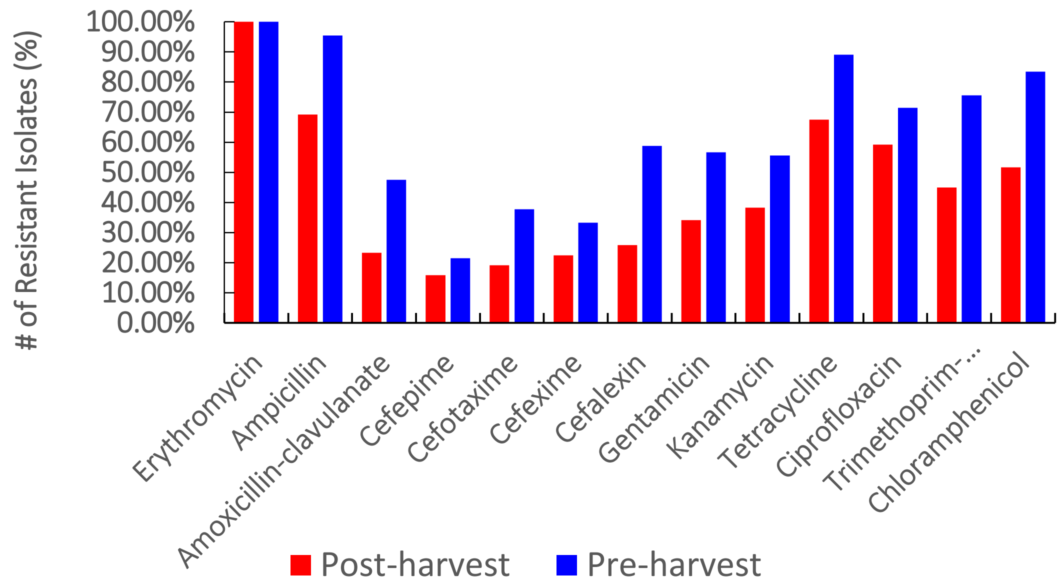

Fig. 7. The resistant patterns of *E. coli* from fecal (pre-harvest) and skinless chicken (post-harvest) breast samples

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Results obtained from testing the microbiological acceptability of skinless chicken breasts and antimicrobial susceptibility of *Escherichia coli* associated with Lebanese poultry are important, because there is a lack of data in Lebanon regarding this subject. The results show high prevalence and loads of fecal coliforms and *Escherichia coli* in skinless chicken breasts. The results assert the conclusion that there is a need of better quality control systems and monitoring programs that can cover all the steps of poultry production; from farm to fork. This can be achieved when all stakeholders are well aware of the practices needed to improve food safety. A risk analysis plan should be conducted in order to prioritize and tackle the major problem that are leading to the contamination of the chicken breasts. Stakeholders need to be educated on hygienic production and handling techniques, including producing and handling raw and cooked poultry meat. There is also a strong need for governmental actions and enforcements, while Lebanese standards might need to be updated or further scientifically reviewed to be able to address the public health and economy in Lebanon, which might be country specific.

Results in this study show a high prevalence of antimicrobial resistant *Escherichia coli* in poultry meat and fecal matter. A more crucial concern was the prevalence of multidrug resistant *Escherichia coli*. Poultry farming plays a major role in the spread of antimicrobial resistance from poultry to humans by direct or indirect pathways, which include consumption of poultry meat. This might tremendously affect

the human health in the clinical settings, since treating bacterial infections will become harder and more expensive. Therefore, there is a critical need for several recommendations to solve this issue.

The first of many recommendations include the establishment of monitoring programs to ensure controlling the use of all clinically important antimicrobial agents in animal (poultry) farming. Farmers, producers and veterinarians should be well aware and trained on how to correctly use antimicrobial agents and on the consequences of their misuse. There is also a need to limit the use of antimicrobial agents in poultry farming and to restrict the use of sub-therapeutic doses of antimicrobial agents that are used in growth promotion. Furthermore, microbiological testing of the accumulation of antimicrobial residues in consumable poultry meat products should be done. Lebanon should investigate using antimicrobial independent methods to enhance poultry production.

Antimicrobial independent methods are available and can be used in poultry farming for growth promotion and control of infections. Some applications include the use of prebiotic and probiotics, bacteriophages, phytochemicals, vaccines, biosecurity, correct hygienic practices and organic farming (Sahin et al., 2015). Those applications can be used as tools for reducing the dependence on antimicrobial agents. The aforementioned actions have shown to decrease the risk of developing antimicrobial resistant bacteria.

Finally, Lebanon should consider rapid and comprehensive actions and recommendations regarding the control of antimicrobial resistance. This study highlighted the presence of a serious problem regarding antimicrobial resistance in Lebanon. The introduction of antimicrobial agents was a revolutionary achievement in

human medicine, because they decreased the rates of human morbidity and mortality associated with bacterial infections. Therefore, serious efforts should be established to conserve the efficiency of antimicrobials because their abuse and misuse might lead to jeopardizing the public health in Lebanon as well as globally. The latter is notable, because antimicrobial resistance cannot be restricted to one location and can be easily transmitted to other countries.

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