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PREVALENCE AND LOADS OF FECAL POLLUTION INDICATORS AND ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI* IN RAW MINCED BEEF IN LEBANON

by
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AN ABSTRACT OF THE THESIS OF

<u>Joanna Pierre Salibi</u> for <u>Master of Science</u>
<u>Major</u>: Food Technology

Title: <u>Prevalence and Loads of Fecal Pollution Indicators and Antibiotic Resistance of *Escherichia coli* in Raw Minced Beef in Lebanon</u>.

Meat is an important source of high biological value proteins as well as many vitamins and minerals. Meat production has been increasing rapidly over the years. In Lebanon, beef meat is the most consumed out of the meat products. In 2014, the average consumption in Lebanon was 39.63 kg/capita. Contaminated beef meat can be an important source of bacterial pathogens, which cause severe foodborne illnesses. However, data on the contamination of beef meat is scarce in Lebanon. This is important, because beef meat is also consumed raw in Lebanon. Raw minced beef meat was selected for this study, because mincing can increase the risk of contamination and this matrix is used in a variety of Lebanese recipes.

The overall aim of this research was to provide an assessment of microbiological quality of raw minced beef in Lebanon. The specific objectives of this study were to determine and quantify the prevalence and loads of fecal coliforms and *Escherichia coli* in raw minced beef in Lebanon. In addition, the antimicrobial resistance profiles of *E. coli* isolated from the raw minced beef were also determined.

A total of 50 raw minced beef samples were obtained from butcheries and grocery stores in Beirut city. For each sample, 25 grams of raw minced beef was placed in a stomacher bag with 225 ml of sterile buffered peptone water. The bag was homogenized for up to 60 seconds in a stomacher. Serial 10-fold dilutions were prepared (10-1, 10-2, 10-4) using sterile peptone buffered water. The dilutions were spread onto RAPID' *E .coli* 2 Agar plates, which were then incubated for 18 to 24 hours under aerobic conditions. After incubation, colonies that matched diagnostic phenotypes for fecal coliforms and *E. coli* were counted per gram of raw minced beef. The fecal coliforms counts were compared to LIBNOR standards, while the *E. coli* counts were compared to standards from other countries.

From the samples, 120 *E. coli* isolates were retrieved and analyzed for antimicrobial susceptibility using the Kirby Bauer disk diffusion method. Eighteen different clinically and agriculturally important antibiotics were tested. The inhibition zone was measured and *E. coli* isolates were classified as sensitive or resistant based on CLSI (The Clinical and Laboratory Standards Institute) standards.

The results showed that 98% of the samples were positive for fecal coliforms, while 78% harbored *E. coli*. Notably, 98% and 76% of the samples exceeded the acceptance loads of fecal coliforms and *E. coli*, respectively. Concerning antibiotic resistance, 100% of the *E. coli* isolates were resistant to at least one antibiotic, while 65% were resistant to up to 2 classes of antibiotics. Notably, 35% of the *E. coli* isolates were classified as multidrug resistant (MDR), which means that the isolates were resistant to 3 or more classes of antibiotics.

The findings in this study are concerning, because beef is popular and is eaten raw on

some occasions in Lebanon. There was also a high resistance to many important antibiotics. Therefore, it appears that Lebanon is in need of updated and strict monitoring and surveillance programs to improve the microbiological quality of important foods such as beef meat. Strong regulations should also be issued and implemented to control the spread of antibiotic resistance in food systems. These are essential needs in order to control the safety of food and protect consumers in Lebanon.

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To My
Beloved Family

CHAPTER I

INTRODUCTION: MEAT, FOODBORNE DISEASES AND ANTIBIOTIC RESISTANCE

A. Introduction

Meat is an important source of many essential nutrients such as high biological value proteins, vitamins, and minerals. Over the years meat production has increased significantly. Worldwide, cattle meat production has more than doubled between 1961 and 2014. In Lebanon, beef meat is the most consumed out of all types of meat products. In 2014, the average consumption in Lebanon was 39.63 kg/capita. However, beef meat can be contaminated and has been shown to be an important source of foodborne pathogenic bacteria, which can cause severe diseases in humans. Minced beef meat is considered to be of particular concern in terms of food safety. This is because of the added processing steps that can result in embedding potential contamination inside the product. In addition, minced beef meat in Lebanon can go into preparation of high-risk foods (that contain raw meat) in Lebanon. However, the microbiological safety and acceptability of minced beef remains not extensively studied in Lebanon.

Illnesses caused by contaminated foods affect 600 million people per year globally. In addition pathogenic bacteria can also acquire antibiotic resistance (AMR) that results in infections that are recalcitrant to treatment. It is predicted by certain models that antibiotic resistance, if left unchecked, might cost the global economy approximately 100 trillion dollars by 2050. More importantly, AMR infections are also predicted to result in severe mortality and morbidity in the human population. Given that animal food production practices have been associated with the rise of AMR foodborne bacterial pathogen, resistance to antibiotics is now considered as an emerging food safety issue worldwide.

Antibiotics have been around since the 1940's and have been essential for treating and controlling infectious diseases in humans and animals. However, the over-use and misuse of antibiotics in farm animals and food production. The latter is poorly studied in Lebanon, and data on the role of food in the dissemination of AMR bacteria is scant. One major approach to assess antimicrobial resistance is to use *Escherichia coli* as an indicator. *E. coli* is a Gram-negative bacterium and is a type of fecal coliform. Therefore, *E. coli* has been suggested as an indicator of fecal pollution as well as antimicrobial resistance in Gram-negative bacteria in a variety of matrices, including retail meat.

In this work, potential microbiological safety/ acceptability of minced beef meat in Lebanon was assessed using indicators of fecal pollution (fecal coliforms and *E. coli*). Additionally, *E. coli* isolates from the minced beef samples were profiled for their antimicrobial resistance phenotypes. The overall objectives were to provide base-line data on the microbiological safety/acceptability of minced beef meat in Lebanon and investigate the role of this matrix as a potential source of AMR *E. coli*.

B. Nutritional Importance of Beef

Red meat is an important component in human diet, because it is a good source of high quality proteins in addition to beneficial fatty acids, vitamins and minerals that are necessary for good health (Wyness, 2015). Red meat contains an average of 20-24 grams of protein per 100 g of raw meat (Wyness, 2015). The proteins are of high biological value, containing the eight essential amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, histidine and arginine) that are necessary for adults (Wyness, 2015). Red meat also contains polyunsaturated fatty acids, including the essential linoleic (omega-6) and linolenic (omega-3) fatty acids (Wyness, 2015). Beef meat is one of the richest sources of iron and zinc (Williams, 2007) and consumption of 100 g of meat provides a minimum of the quarter of the daily recommendations for adults (Williams, 2007). Iron found in red meat is in the heme iron form, which is more readily absorbed (at ~ 20-30%) than the non-heme iron (5-15%) (Wyness, 2015; Williams, 2007). In addition, red meat is also a great source of vitamin B12 and the consumption of 100 g of meat covers more than two thirds of the daily requirements for adults (Williams, 2007). Riboflavin, niacin, vitamin B6 and pathothenic acid are provided at up to 25% of the required intake in 100 g of red meat (Williams, 2007).

C. Meat Production and Consumption

Meat production has markedly increased across the years (Ritchie & Roser, 2017). From 1961 to 2014, meat production more than doubled; from 28 million tons to 68 million tons per year (Ritchie & Roser, 2017). In the United States of America, cattle production is one of the most important industries (USDA, 2016). In 2015, cattle production in USA reached up to 11.45 million tons, which was a 3% increase from 2014 (USDA, 2016; Ritchie & Roser, 2017). Furthermore, this production accounted for 78.2 billion dollars in cash receipts, which represents 21% of the Economic Research Service's total cash receipts of 377 billion dollars from agriculture merchandises in the USA (USDA, 2016). In Lebanon, meat production more than doubled since 1990, reaching almost 200,000 tons in 2012 (IDA of Lebanon, 2015). In 2014, beef meat production alone reached up to 47,484 tons (Ritchie & Roser, 2017). In 2013, beef meat consumption in Lebanon was estimated at approximately 39.63

kg/capita (Ritchie & Roser, 2017).

D. Foodborne Diseases

There are foods that pose a higher risk of foodborne diseases than others (CDC, 2018a). For example, raw foods that come from animals have a higher chance of being contaminated (CDC, 2018a). The latter includes raw or undercooked beef and poultry meat, raw or lightly cooked eggs, unpasteurized milk or juices as well as fruits or vegetables that were exposed to animal manure (CDC, 2018a).

Unsafe foods that contain pathogenic bacteria or other contaminants such as viruses, parasites or chemicals cause over 200 diseases that vary from diarrhea to cancer (WHO, 2017a). It is estimated that 600 million people per year worldwide become sick after consuming contaminated food, and it is estimated that ~ 420,000 die (WHO, 2017a). Children under the age of 5 years old account for 40% of the casualties associated with foodborne disease (WHO, 2017a). Globally, diarrhea is one of the most common illnesses that result from contaminated food; it affects 550 million and causes 230,000 deaths every year (WHO, 2017a). In the USA, 48 million people get sick, 128,000 are hospitalized and 3000 die yearly because of foodborne diseases (CDC, 2018a). It is estimated that foodborne illnesses can result in $15.5 billion in losses yearly (Hoffman, 2015). Developing countries are most likely at higher risks of foodborne illnesses, because many have under-developed infrastructures and food safety programs and very little surveillance and control strategies (Todd, 2017). The Middle East is considered a region with the highest burden of foodborne diseases per population (Todd, 2017). However, it isn’t easy to precisely determine this burden because very little is published on the issue as well as the complexity in tracing foodborne diseases and their ramifications (Todd, 2017).

In Lebanon, a food safety law has been developed and adopted officially by the government in 2015 in order to enhance the safety of Lebanese foods. However, the implementation of the law has been facing many challenges, while data on the safety of Lebanese foods remain scant and not readily available. Therefore, the status of food safety in Lebanon is at best unclear. However, pathogenic bacteria were detected in Lebanese foods and food poisoning outbreaks were reported (El-Jardali *et al*., 2014). For example, a report by the Ministry of Public Health showed that from 2011 to 2012, 665 cases and 84 episodes of food poisoning occurred in Lebanon, which is a conservative estimate given that the poisoning is under-reported (Saleh *et al*., 2012). Stool and food samples were tested and detection of *Salmonella* spp. was the most frequent followed by *Escherichia coli*, *Shigella* and others (Saleh *et al*., 2012). Notably, the most frequent food product that resulted in food poisoning was raw meat (Saleh *et al*., 2012). This is not surprising, given that popular Lebanese meals include raw beef meat. However, to date, there has not been an extensive study on the microbiological safety/acceptability of minced beef in Lebanon.

Although many foodborne diseases may not require treatment or hospitalization, severe infections can result in death and require medical interventions (CDC, 2015). Antibiotics are main drugs that are used to control infectious diseases. However, the rise in antibiotic resistance have jeopardizes the ability of treating simple infections, including those that are foodborne, and this has been posing a serious threat to public health (CDC, 2015). Therefore, it is important to understand the role of antibiotics the associated resistance mechanisms in the persistence, dissemination, and control of foodborne diseases.

E. History of Antibiotics

Antibiotics are drugs that either kill or suppress growth of bacteria, allowing patients to recover from infections and/ or the immune system to get rid of the pathogenic bacteria (Zaman *et al*., 2017). In general, antibiotics have a wide range of effect on bacteria, and may work by inhibiting the synthesis of bacterial cells, proteins, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or by other actions (Zaman *et al*., 2017). Due to their efficacy in fighting infectious diseases, antibiotics were called the "wonder drugs" in the middle of the 20th century (Zaman *et al*., 2017). They saved millions of people by fighting off bacterial infections (Zaman *et al*., 2017). Throughout the years, many different classes of antibiotics have been used for therapeutic purposes (Zaman *et al*., 2017). At one point, it was believed that antibiotics were magic bullets that would terminate transmissible diseases (Zaman *et al*., 2017). However, Alexander Fleming who discovered penicillin, the first antibiotic (1928), warned about the "resistance" that may be acquired by bacteria if the drug is used in inappropriately (Zaman *et al*., 2017). This warning has proved to be valid as humanity is predicted to witness an unprecedented increase in antibiotic resistant infections that may result in a global crisis. In fact, antimicrobial resistance has been dubbed as one of the major challenges that are facing humanity.

The first antibiotic, penicillin, was prescribed to treat serious infections around 1940 (Ventola, 2015). Penicillin was massively produced and used to treat soldiers suffering from infections in World War II (Ventola, 2015). Not so long after the discovery of penicillin, bacterial strains started to exhibit resistance to the drug (Ventola, 2015). By the 1950's, most of the medical advances of previous years became threatened by increased resistance to penicillin (Ventola, 2015). Soon after, other beta-lactams and classes of antibiotics were discovered; however, relatively rapid resistance to these antibiotics was also noted (Ventola, 2015). Today, reports show that pathogens can acquire resistance to multiple classes of antibiotics; giving rise to multi-drug and pan resistant pathogens. The latter cannot be treated by any class of available antibiotics

Over the last 60 years, new classes of antibiotics have been produced in millions of metric tons, indicating the reliance on these drugs in human medicine (Zaman *et al*., 2017). The production of new antibiotics used to be directly proportionate to the emergence of resistant strains (Zaman *et al*., 2017). Nowadays, the main focus is on modifying present antibiotics so they are able to fight against existing resistant strains around the world (Zaman *et al*., 2017). However, in addition to their clinical applications, antibiotics are heavily used in non-medical purposes, mainly in food-animal production (Zaman *et al*., 2017). The increase in demand on antibiotics for agricultural and medical purposes resulted in cheaper drugs. This, in turn, rendered the drugs highly accessible and coveted and led to their overuse and misuse (Zaman *et al*., 2017). Therefore, it is now accepted that antibiotics are being used more frequently than they should and for the wrong purposes (Zaman *et al*., 2017) such as treatment of viral infections and self-medication in the human population and animal growth promotion in agriculture.

F. Classes of Antibiotics and Their Mechanisms of Action

Antibiotics can be classified in many ways; however, the most common classification is based on their molecular structures, mode of action, and spectrum of activity (Etebu & Arikekpar, 2016). Table 1 shows the different classes of available antibiotics, including their mode of action.

The mechanisms of action of antibiotics include (Etebu & Arikekpar, 2016):

- Disrupting cell wall synthesis

- Damaging cell membrane structure or function
- Inhibition of the structure and function of nucleic acids
- Disruption of protein synthesis
- Inhibition of key metabolic pathways

Table 1. Classes of Antibiotics and their Mode of Action

Antibiotic Class (Reference)	**Mode of Action**	**Examples**
Beta-lactams (Etebu & Arikekpar, 2016)	-Interfere with proteins necessary for synthesis of the bacterial cell wall -Penicillin-binding proteins (PBP) are enzymes involved in the synthesis of peptidoglycan. Antibiotics in this class can bind to PBPs, which results in disrupting peptidoglycan synthesis and causing lysis and cell death	-Penicillin Penicillin Amoxicillin -Cephalosporins Cephalexin Cefuroxime Cefixime Cefepime -Monobactams Tigemonam -Carbapenems Imipenem Meropenem
Macrolides (Etebu & Arikekpar, 2016)	-Inhibit protein synthesis. -By binding to the ribosome, they prevent amino acids of binding to polypeptide chains during protein synthesis	Erythromycin, Azithromycin, and Clarithromycin
Tetracyclines (Etebu & Arikekpar, 2016)	-Disrupt the addition of amino acids to the polypeptide chain during protein synthesis	Tetracycline, Chlortetracycline, and Doxycycline.
Quinolones (Etebu & Arikekpar, 2016)	-Disrupt DNA replication and transcription	Cinoxacin, Norfloxacin, and Ciprofloxacin
Aminoglycosides (Etebu & Arikekpar, 2016)	-Inhibit protein synthesis by binding to ribosomal subunits	Streptomycin, Gentamicin, and Neomycin
Sulphonamides (Tacic *et al*., 2017)	-Inhibit folic acid synthesis	Sulfamethoxazole-trimethoprim and Sulfasalazine
Glycopeptides (Kang & Park, 2015)	-Inhibit cell wall synthesis	Vancomycin and Telavancin
Oxazolidinones (Etebu & Arikekpar, 2016)	-Inhibit protein synthesis by binding to ribosomal subunit 50S	Linezolid

G. Antibiotic Resistance

The World Health Organization (WHO) states that antibiotic resistance is one of the biggest threats to global health and food security (WHO, 2018). Antibiotic resistance is when bacteria become able to tolerate or withstand the drug that is intended to kill them. When the latter happens, the antibiotics will reduce its efficacy and the bacteria will continue to survive and even multiply (CDC, 2018b). In the United States, 2 million people get antibiotic resistant infections per year and 23,000 people die (CDC, 2013b). In addition to these deaths, studies have shown that antibiotic resistance causes $20 billion a year in health care costs (CDC, 2013b). If productivity losses are included, the cost increases to around $35 billion per year (CDC, 2013b). Estimates suggest that antibiotic resistance could cost $100 trillion and millions of mortalities by the year 2050 (WEF, 2018). Therefore, antibiotic resistance needs global attention especially, because it is predicted to increase.

Lebanon also has high levels of antibiotic resistance (Salameh *et al*., 2017). Antibiotics in Lebanon are available without a medical prescription. A study showed that in over 50% of cases antibiotics were prescribed inadequately. For example, antibiotics were prescribed for viral diseases in 40% of the cases. In addition, there are limited regulations regarding the use of antibiotics in agriculture and data on the amount of antibiotics used for these purposes are scant in Lebanon.

H. Causes of Antibiotic Resistance

In this section, the main drivers in the rise and spread of antibiotic resistance are discussed:

1. The Overuse and Inappropriate Prescription of Antibiotics

Regardless of the warnings concerning overuse, antibiotics are overprescribed worldwide; whether for medical or agricultural purposes (Ventola, 2015). Many studies have shown a direct link between the consumption of antibiotics and the emergence of resistance (Ventola, 2015). In many developing countries, antibiotics are unregulated and are available without a prescription of a qualified health professional (Ventola, 2015). This lack of policy promotes antibiotic overuse and misuse, because the drugs are easily accessible and are relatively cheap (Ventola, 2015).

According to previous studies, 30% to 50% of cases show incorrect treatment indication, choice of agent or duration of antibiotic therapy (Ventola, 2015). Additionally, 30% to 60% of antibiotics prescribed in intensive care units (ICUs) have been found to be unnecessary and/ or inappropriate (Ventola, 2015). Other common practices that are linked to inappropriate antibiotic use include the failure to complete the full treatment or self-medication and misdiagnosis of the etiologic agent (Ventola, 2015). In developed countries, the excess of prescriptions of antibiotics is also a factor that contributes to the resistance of bacteria (Ventola, 2015).

A study in the U.S showed that only 7.6% of 17,435 patients suffering from community acquired pneumonia had a defined pathogen for the disease (Ventola, 2015). The latter will result in erroneously prescribing antibiotics to treat non-bacterial infections. Additionally, the therapeutic effect of antibiotics that are not properly prescribed is uncertain and patients that are being exposed to these drugs may also suffer from complications (Swistock & Sharpe, 2016; Ventola, 2015). Notably, subinhibitory and subtherapeutic concentrations stimulate the development of antibiotic resistance (Ventola, 2015).

2. The Widespread Use of Antibiotics in Agriculture

Antibiotics are used in livestock for disease treatment or prevention and for growth promotion (Ventola, 2015). Animals fed antibiotics are believed to have improved overall health, produce larger yields and better quality products with less feed intake (Ventola, 2015). However, if the antibiotics are improperly used in these operations, humans can ingest the antibiotics used in livestock when they consume food (Ventola, 2015). Furthermore, the antibiotics that are ingested by the animals kill or suppress susceptible bacteria and allow resistant bacteria to persist (Ventola, 2015). These resistant bacteria are then transmitted to humans through the food supply (Ventola, 2015). These bacteria can cause infections in humans and lead to many complicated health consequences (Ventola, 2015).

Up to 90% of the antibiotics administered to animals are excreted in urine and stool (Swistock & Sharpe, 2016). These antibiotics are then dispersed through fertilizers and groundwater (Ventola, 2015). In addition, antibiotics are used on plants to prevent plant disease (Ventola, 2015). This is a concern, because the antibiotic use may increase the antibiotic resistant genes in bacteria living on plant surfaces (Ventola, 2015). The resistance genes might be transferred to clinically important bacteria that can infect humans (Ventola, 2015).

3. Shortage the Discovery and Development of New Antibiotics

In the past, increase in antibiotic resistance was addressed by the production of new and different antibiotics (Ventola, 2015). However, nowadays, economic and regulatory barriers have limited the discovery of new antibiotics (Ventola, 2015). Out of 18 large pharmaceutical companies, 15 switched their focus to fields other than antibiotics. Antibiotics are not considered profitable or a viable investment as compared to other drugs that treat chronic conditions like diabetes, asthma and gastroesophageal reflux, because antibiotics are usually used for a short period of time (Ventola, 2015). Furthermore, the rapid emergence of resistance to antibiotics does not justify large investments and multiyear efforts required to identify, clinically test, and commercialize a new antibiotic. A cost analysis by the Office of Health Economics in London revealed that the net present value of a new antibiotic is $50 million as compared to $1 billion for a drug to treat neuromuscular disease (Ventola, 2015). In addition, new antibiotics are more judiciously used nowadays (Ventola, 2015). This is due to the fear of developing resistance to the new antibiotics, which would exacerbate the problem of antibiotic resistance further (Ventola, 2015). Therefore, this decreases the demand on the new antibiotics (Ventola, 2015). For the companies that are interested and would like to discover new antibiotics are limited, getting approval for clinical trials and to commercialize the new drug is not an easy process. Between 1983 and 2007, a large reduction rate was observed for new antibiotic approval (Ventola, 2015). Changes that have been made by the U.S Food and Drug Administration (FDA) have also complicated clinical trials (Swistock & Sharpe, 2016). These issues discourage manufacturers from investing large amounts of money in antibiotic discovery (Ventola, 2015).

I. Acquisition and Development of Antibiotic Resistance

Bacteria have the ability to respond to environmental threats around them in order to prolong their survival, such as when antibiotic molecules are present (Munita & Arias, 2016). There are two main strategies that bacteria develop to acquire resistance and to counteract the effect of antibiotics (Munita & Arias, 2016). These are:

- *Mutagenesis*: this is when antibiotic susceptible bacteria acquire resistance by mutations in their genes (Munita & Arias, 2016). Mutations can lead to antibiotic resistance through several different mechanisms, such as modifying the antibiotic target, decreasing the drug uptake and activating efflux mechanisms to eject the antibiotic molecule (Munita & Arias, 2016).

- *Horizontal Gene Transfer (HGT)*: this process is often associated with the transfer of antibiotic resistance genes between bacteria of the same or different species, increasing the dissemination of resistance in bacteria (Munita & Arias, 2016). It is known that many antibiotics originate from microorganisms that are present in the environment such as the soil. It has also been known that bacteria that share the same environment harbor resistance genes that allow them to survive and compete with the antibiotic-producing microorganisms (Munita & Arias, 2016). Under antibiotic stress, the genes can be transmitted between bacterial cells bestowing resistance on previously susceptible strains.

Bacteria can obtain external genetic material via 3 processes: transformation, transduction, and conjugation (Munita & Arias, 2016). In transformation, the bacteria which will become resistant, takes up extracellular DNA from the environment (Holmes & Jobling, 1996). In transduction, DNA is transferred by bacteriophages (Holmes & Jobling, 1996). Finally, conjugation is when a donor bacterium transfers the DNA to the recipient by mating (Holmes & Jobling, 1996). Transformation is known as the easiest type of HGT; however; not all bacterial species are capable of naturally incorporating naked DNA to acquire resistance (Zaman *et al*., 2017). Conjugation is usually the method is which bacteria develop resistance the clinical settings (Zaman *et al.,* 2017). It is likely to occur between bacteria residing in humans undergoing antibiotic treatment (Zaman *et al*., 2017).

J. Mechanisms of Antibiotic Resistance

Bacteria can fight off antibiotics by stopping the antibiotic from reaching high concentrations inside the cell or by modifying the cellular target that the antibiotics act upon (ReAct, n.d.).

Bacteria can pump antibiotics out of the cell. They do so by creating a pump that resides in the membrane or cell wall (ReAct, n.d.). These pumps are called efflux pumps and are usually used to transport compounds such as nutrients (ReAct, n.d.). Some of these pumps are also used to transport antibiotics out from the bacterium, therefore lowering its concentration inside the cell (ReAct, n.d.). Some mutations can cause the bacteria to produce more pumps, so resistance is increased and sped up (ReAct, n.d.). Another way that bacteria can reduce the concentration of antibiotics is by changing the permeability of their membrane making it more difficult for the drug to pass into the cell (ReAct, n.d.).

Bacteria can also destroy the antibiotic (ReAct, n.d.). Bacteria can produce enzymes that inactivate the drug. For example, beta-lactamases destroy the beta-lactam ring (the active component) of penicillins (ReAct, n.d.). Bacteria also can produce extended-spectrum beta-lactamases (ESBL), which have become a major problem, because they can breakdown a large variety of beta-lactam antibiotics (ReAct, n.d.). These drugs are sometimes used as a last resort options for treating infections (ReAct, n.d.). Bacteria can also modify antibiotics (ReAct, n.d.). This is done when the bacteria produce enzymes that can add chemical groups to the drug (ReAct, n.d.). Therefore, the antibiotic can no longer bind to its target in the bacterial cell (ReAct, n.d.).

Bacteria have also developed strategies to modify or protect the cellular target that the antibiotics act upon (ReAct, n.d.). One way is by camouflaging the target of the antibiotic. Mutations in the DNA might change the composition or structure of the target, and this can stop the antibiotic from identifying and interacting with it (ReAct, n.d.). The target can also be modified by adding a chemical group to its structure, which provides protection against the antibiotic (ReAct, n.d.). Bacteria can produce proteins that bind to the antibiotic and block them from interacting with the target of the antibiotic (ReAct, n.d.). For example, *Staphylococcus aureus* harbor *mecA* which encodes a variant penicillin-binding protein (ReAct, n.d.). This protein has low affinity to the drug so the bacterium survives the antibiotic treatment (ReAct, n.d.).

Taken together, the discussion above highlights the remarkable ability of bacteria to develop diverse mechanisms and strategies to counteract the impact of an antibiotic. This further emphasizes the need to use these valuable drugs with care; given that resistance is primed to develop in stressed bacterial populations.

K. Antibiotic Resistance and *Escherichia coli*

The WHO states that the increasing resistance in Gram-negative enteric bacilli is worrying (Collignon *et al*., 2016). *Escherichia coli* is type of fecal coliform bacteria, and they are used as indicators of fecal pollution and antibiotic resistance in Gram-negative bacteria (Navarro-Gonzalez *et al*., 2013). *E. coli* is an inhabitant of the intestines of humans and animals (New Zealand Government, 2015). *E. coli* plays an important role in the synthesis of essential nutrients from foods like vitamin K, which is essential for blood clotting and in the metabolism of bile acids and other sterols (New Zealand Government, 2015). In addition, *E. coli* also stimulates the secretion of mucins that protect the intestines and allows the greatest absorption of nutrients as well as maintaining the proper development of the human immune system (New Zealand Government, 2015). Furthermore, many strains of *E. coli* are highly pathogenic to humans and animals. Some *E. coli* can cause bloodstream and urinary tract infections and have been associated with severe and life-threatening foodborne infections. Antibiotic resistance in *E. coli* is increasing quickly (Collignon, 2009). For example, in India, large numbers of *E. coli* that cause urinary tract infections were found to be resistant to carbapenems and other drugs (Collignon *et al*., 2016). Another study in Nigeria showed that *E. coli* that cause urinary tract infections were highly resistant to 10 out of 11 antibiotics tested. The latter belonged to different classes such as quinolones, aminoglycosides, penicillins and sulfonamides (Olorunmola *et al*., 2013). Recently, there has been a spread of bacteria carrying metallo-betalactamase genes that are resistant to carbapenems and other beta-lactam antibiotics (WHO, 2011). One of the most worrying strains that are spreading is a multi-resistant strain of *E. coli* that is carrying the New Delhi metallo-betalactamase (NDM) (WHO, 2011). The gene has spread to many other bacteria such as *Klebsiella* and *Vibrio* (WHO, 2011).

The World Health Organization (WHO) has compiled a list of priority pathogens in terms of their antibiotic resistance properties (WHO, 2017b). The list focuses in general on Gram-negative bacteria, which have been causing recalcitrant infections in humans (WHO, 2017b). Enterobacteriaceae a family of Gram-negative bacteria that includes *Escherichia coli* is included among the most critical group, because bacteria in this family are becoming resistant to even the best available antibiotics such as carbapenems and third generation cephalosporins (WHO, 2017b).

The WHO has recently ranked antibiotic according to their importance in human and veterinary medicine (Collignon *et al*., 2016) based on the following criteria:

- The antibiotic class is the only or one of the only therapies available of treating serious bacterial infections in humans (Collignon *et al*., 2016).
- The antibiotic class is used to treat infections that are caused by bacteria that are transmitted to humans from nonhuman sources (e.g. animals/zoonotic bacterial

pathogens) or bacteria that may acquire resistance in nonhuman sources (Collignon *et al*., 2016).

If an antibiotic class meets both criteria, it is considered as critically important. If an antibiotic meets one of the criteria then it is considered as highly important (Collignon *et al*., 2016). Antibiotics that were classified as critically important include aminoglycosides, carbapenems, quinolones, third and fourth generation cephalosporins, macrolides, penicillins, and glycopeptides (Table 2) (Collignon *et al*., 2016). While highly important antibiotics consist of first and second generation cephalosporins, sulfonamides and tetracyclines (Collignon *et al*., 2016).

Table 2. Critically Important Antibiotics in Human Medicine

Antibiotic Class	**Example**
Aminoglycosides	Gentamicin, Kanamycin
Carbapenems	Doripenem, Meropenem
Cephalosporins (third and fourth generation)	Cefepime, Cefixime
Glycopeptides	Vancomycin
Macrolides	Erythromycin
Oxazolidinones	Linezolid
Penicillins	Ampicillin, Amoxicillin + clavulanic acid
Quinolones	Ciproflaxin, Norfloxacin

L. Fecal coliforms and *Escherichia coli*

Coliforms are a large group of different types of bacteria that are wide-spread in different environments (Swistock & Sharpe, 2016). They are commonly found in the soil and in water (Swistock & Sharpe, 2016). A great number of some types of coliform bacteria can be found in human and animal waste (Swistock & Sharpe, 2016). Fecal coliform bacteria, a subgroup of total coliforms, can be used as indicators, because they indicate the presence of fecal contamination and the potential existence of pathogens

(CDC, 2017). Normally, fecal coliforms are present in the intestinal tract of animals and can also be pathogenic (CDC, 2017). *Escherichia coli* is a type of fecal coliform bacteria, which is also commonly, used an indicator when testing for fecal contamination in water and food.

E. coli has been shown to contaminate undercooked ground beef, unpasteurized milk and juice, cheeses made from raw milk, and raw fruits and vegetables (USDHHS, 2018; New Zealand Government, 2015). Studies have shown that resistant *E. coli* has emerged in food animal production and the food chain (Collignon, 2009). In the Netherlands, the same ESBL genes in *E. coli* have been found in food animals and in people with serious infections (WHO, 2011). Retail meat is suggested as an important carrier for antimicrobial resistant *E. coli* (Pittout, 2017). Contaminated water, animals and their environment, and humans are all important sources of *E. coli* (USDHHS, 2018; New Zealand Government, 2015).

M. The Problem in Lebanon

As mentioned earlier, there is an increase in meat consumption and production worldwide. In parallel, there are foodborne pathogens that can be acquired from the consumption of contaminated meat. These pathogens are becoming increasingly antibiotic resistant. Therefore, preventive measures should be taken to produce and consume safe meat. Food safety programs that ensure the appropriate measures are being implemented in order to keep meat within the acceptable levels in terms of microbiological quality and to decrease the number of food safety illnesses. Furthermore, policies that control the use antibiotics in humans and animals have been formulated and implemented. Taken together, these efforts are facilitated by monitoring and surveillance programs. However, these programs are lagging in Lebanon due to many reasons such as a weak infrastructure and depleted resources. This resulted in lack of data on the safety status of essential food systems in Lebanon; most notably poultry and beef meat products. Some of the most famous Lebanese dishes include raw beef meat in the recipe, which increases the infection risk associated with contaminated beef meat. Therefore, the objective of this thesis was to assess the microbiological contamination and the occurrence of antibiotic resistant *E. coli* on raw minced beef products.

CHAPTER II

PREVALENCE AND LOADS OF FECAL POLLUTION INDICATORS AND ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI* IN RAW MINCED BEEF IN LEBANON

A. Abstract

Meat is an important source of high biological value proteins as well as many vitamins and minerals. In Lebanon, beef meat such as minced beef cuts, is the most consumed out of the meat products. However, minced beef meat can be an important source food borne illnesses. This is of a major concern, because food safety in Lebanon suffers from well-documented challenges. For this purpose, the prevalence and loads of fecal coliforms and *E. coli* were determined in raw minced beef. The antibiotic resistant phenotypes of *E. coli* isolated from the meat were also determined. A total of 50 meat samples and 120 *E. coli* isolates were analyzed. Results showed that 98% of meat samples harbored fecal coliforms, while 78% harbored also *E. coli*. Regarding the loads, 98% of the fecal coliforms and 76 % of *E. coli* were above the microbiological acceptance level. Concerning antibiotic resistance, 100% of isolates were resistant to at least one antibiotic, while 65% of *E. coli* isolates were resistant to up to 2 classes of antibiotics. These results strongly suggest that Lebanon is in need of updated food safety systems to track and reduce the levels of potential contamination in important foods.

B. Introduction

Red meat is a very important source of high biological value proteins. In addition, it is a great source of vitamin B12, niacin, vitamin B6, iron, zinc and phosphorous (Williams, 2007). Meat production has increased rapidly over the past 50 years worldwide (Ritchie & Roser, 2017). Since the 1960's, total production has grown 4-5 folds (Ritchie & Roser, 2017). Cattle meat production has more than doubled since the; increasing from 28 million tons in 1961 to 68 million tons in 2014 (Ritchie & Roser, 2017). The United States of America is the largest beef meat producer. In 2014, approximately 11 million tons were produced in the USA (Ritchie & Roser, 2017). The consumption of beef in the same year in the USA was 115.13 kg/capita (Ritchie & Roser, 2017). In comparison, a food consumption pattern study in Beirut, Lebanon, showed that beef products were also the most consumed out of all meats (Nasreddine *et al*., 2006). Lebanon produced 47,484 tons of beef meat in 2014 and the average consumption during that year was 39.63 kg/capita (Ritchie & Roser, 2017). Notably, contaminated beef meat can be contaminated by different pathogens, and it has been implicated in foodborne outbreaks and severe illnesses in humans. Foodborne diseases are a public health concern globally (WHO, 2017a). The WHO states that almost 1 in 10 people (600 million) fall ill and 420,000 people die annually because of eating contaminated food (WHO, 2017a). Therefore the safety of the meat along with its nutritional importance is of paramount importance.

Fecal coliforms and *Escherichia coli*, a species of coliform bacteria, have been regularly used as indicators of fecal contamination in foods and other matrices (New Zealand Government, 2015; Swistock, 2018). The presence of these bacteria at certain levels is considered a high risk, because they can be associated with the occurrence of serious pathogens. Therefore, food inspection agencies worldwide use these bacteria to assess the microbiological acceptability of food. For example, the Lebanese Standards Institution (LIBNOR), a public institute associated to the Ministry of Industry (LIBNOR, 2004), has a pre-determined acceptance level for fecal coliforms in minced beef. However, in recent years, there has been a direction to focus on *E. coli* loads, because they are widely accepted as better indicators of contamination in comparison to fecal coliforms (Swistock, 2018).

Retail meat can be an important carrier of antimicrobial-resistant *E. coli* (Nekouei *et al*., 2018), which can also serve as indicator of antimicrobial resistance in Gram-negative bacteria. This is important, because the emergence and spread of antimicrobial resistance in foodborne bacteria is a food safety issue worldwide (WHO, 2018). Antibiotic use in livestock is one important factor for increasing this widespread resistance (Nekouei *et al*., 2018). In Lebanon, antibiotic use policies need to be updated and implemented (Gelband & Delay, 2014). Taken together, this highlights the need to monitor both the acceptability of minced beef meat and the associated antimicrobial resistance properties of *E. coli* in Lebanon.

To our knowledge, there is there is no recent data on the microbiological acceptability and antimicrobial resistant *E. coli* in raw minced beef samples in Lebanon. Therefore, the overall objective of this study was to assess the acceptability of minced beef meat in Lebanon. For this purpose, prevalence and loads of fecal coliforms and *E. coli* in raw minced beef in Lebanon were quantified. In addition, the antimicrobial resistance profiles of minced-meat associated *E. coli* isolates were characterized.

C. Materials and Methods

Sample Collection: A total of 50 samples of raw minced beef were collected from 50 different butcheries and grocery stores in Beirut city. The samples collected were from this area since Beirut is Lebanon’s capital, and it is the largest city in the country as well at the most populated (SEV Hellenic Federation of Enterprises, 2018). The samples were placed in a cooler containing ice and transported to laboratory for testing immediately.

Prevalence and loads of fecal coliforms and *E. coli*: To determine the prevalence and loads, 25 grams of minced beef meat were added to 225 ml of sterile buffered peptone water in a stomacher bag under aseptic conditions (Park *et al*., 2010). Each sample was homogenized for up to 1 minute. Then 10-fold serial dilutions were prepared (10^{-1} up to 10^{-4}) using 9 ml sterile buffered peptone water (Park *et al*., 2010). 100µl from three dilutions (10^{-1}, 10^{-2}, 10^{-4}) were plated on RAPID' *E.coli* 2 Agar (Bio-Rad Laboratories, Hemel Hempstead, UK) (Park *et al*., 2010). The plates were then incubated for 18-24 hours under aerobic conditions (Park *et al*., 2010). Colonies that matched diagnostic phenotypes were counted and loads were determined per gram of raw minced beef (Park *et al*., 2010). Fecal coliform counts were compared to LIBNOR Standards (2003), while *E. coli* counts were compared to numbers reported in the literature; the maximal acceptance level was 50 CFU/g (LIBNOR, 2004; Goepfert, 1976). The latter is referred to as the OSU standard (in reference to the location where the standard was developed) and is used here as a proxy and to compare to the LIBNOR standard.

Antimicrobial resistance: Antimicrobial susceptibility tests were performed on Mueller-Hinton Agar (Oxoid, Hampshire, England) using the Kirby Bauer disk diffusion method (Kibret & Abera, 2011). There were 120 isolates of *E. coli* retrieved from the 50 samples that were tested. The following antibiotics (Bio-Rad, France): kanamycin (30 µg), ampicillin (10 µg), imipenem (10 µg), ciprofloxacin (5 µg), penicillin (6 µg), chloramphenicol (30 µg), streptomycin (10 µg), meropenem (10 µg), doripenem (10 µg), norfloxacin (10 µg), cefixime (6 µg), cephalexin (30 µg), cefotaxime (30 µg), cefepime (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), amoxicillin + clavulanic acid (20/10 µg), gentamicin (10 µg), tetracycline (30 µg) were tested. Isolates were classified as susceptible or resistant based on the zone of inhibition following the criteria of Clinical Laboratory Standards Institute (CLSI, 2017).

D. Results and Discussion

1. Prevalence and Loads of Fecal Coliforms and E. coli

The results from the microbiological analysis of raw minced beef showed that fecal coliforms were observed in 49 samples (98%). As for the acceptability, all 49 samples that showed positive results for fecal coliforms were above the acceptance level of 100 CFU/g that is adopted by LIBNOR (Figure 1). The lowest count (disregarding the sample that had 0 CFU/g) was at 63,000 CFU/g, while the highest reached up to 12,721,500 CFU/g (Figure 2).

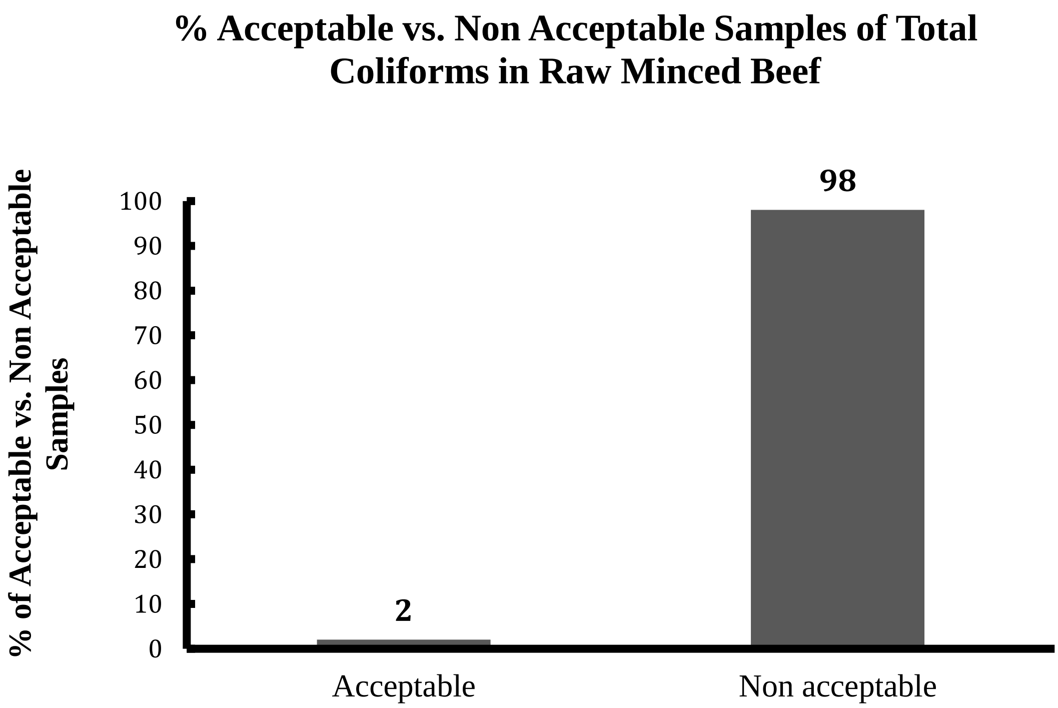

Fig. 1. Percentage of Acceptable Raw Minced Beef Samples vs. Non-acceptable Samples According to LIBNOR's standard for Fecal Coliforms Counts

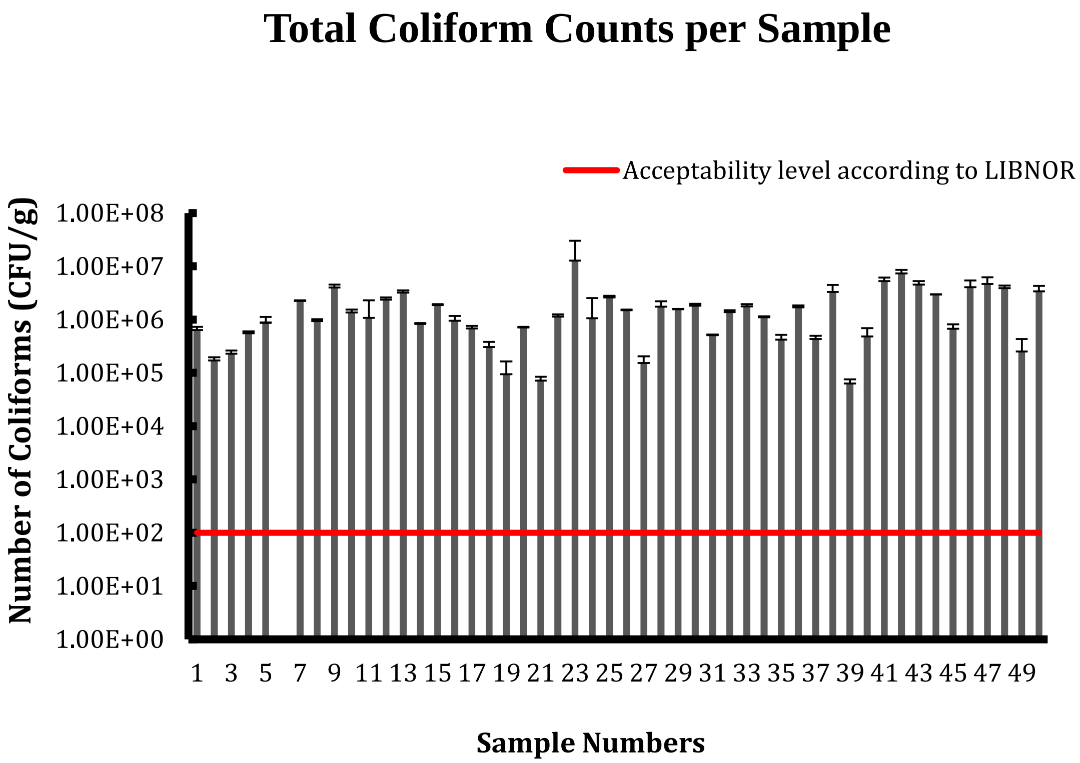

Fig. 2. Fecal Coliform Counts per Sample of Raw Minced Beef. Numbers of the x-axis represent meat samples that were collected from different locations. The number of coliforms was determined by plating on a selective medium as described in the material and methods

The microbiological analysis showed that 39 samples (78%) of minced meat were positive for *E. coli*. In terms of acceptability, 38 samples (76%) were above the maximal acceptance level of 50 CFU/g according to OSU standard (Figure 3). All samples classified as acceptable had negative *E. coli* results. The lowest count for positive samples was 4500 CFU/g, while the highest reached 3,478,500 CFU/g (Figure 4).

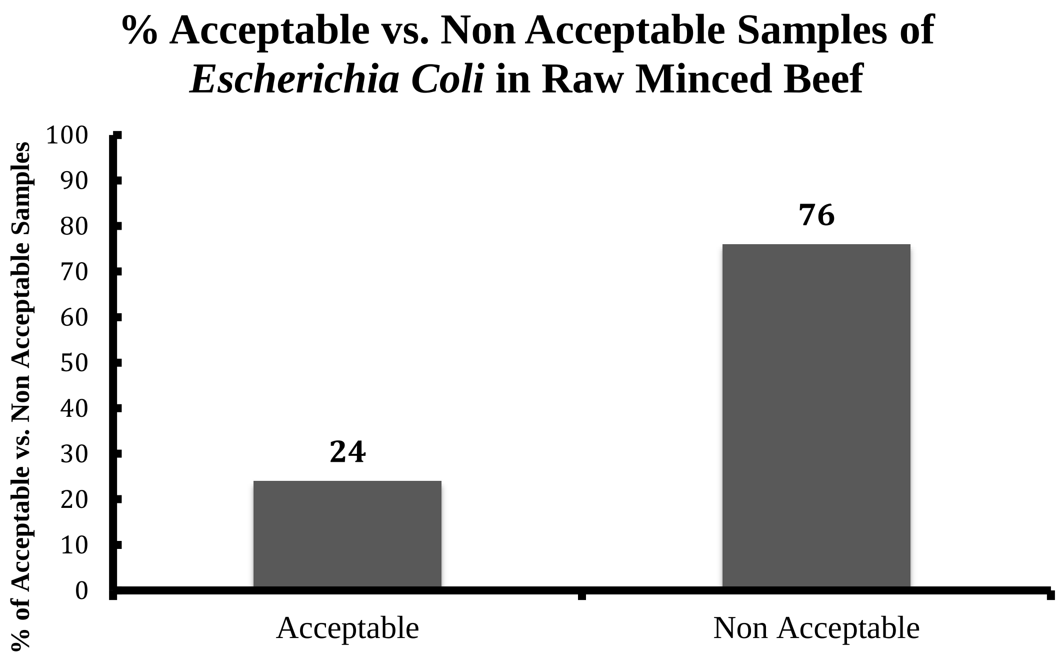

Fig. 3. Percentage of Acceptable Raw Minced Beef Samples vs. Non-acceptable Samples According to OSU's standard for *E. coli* Counts

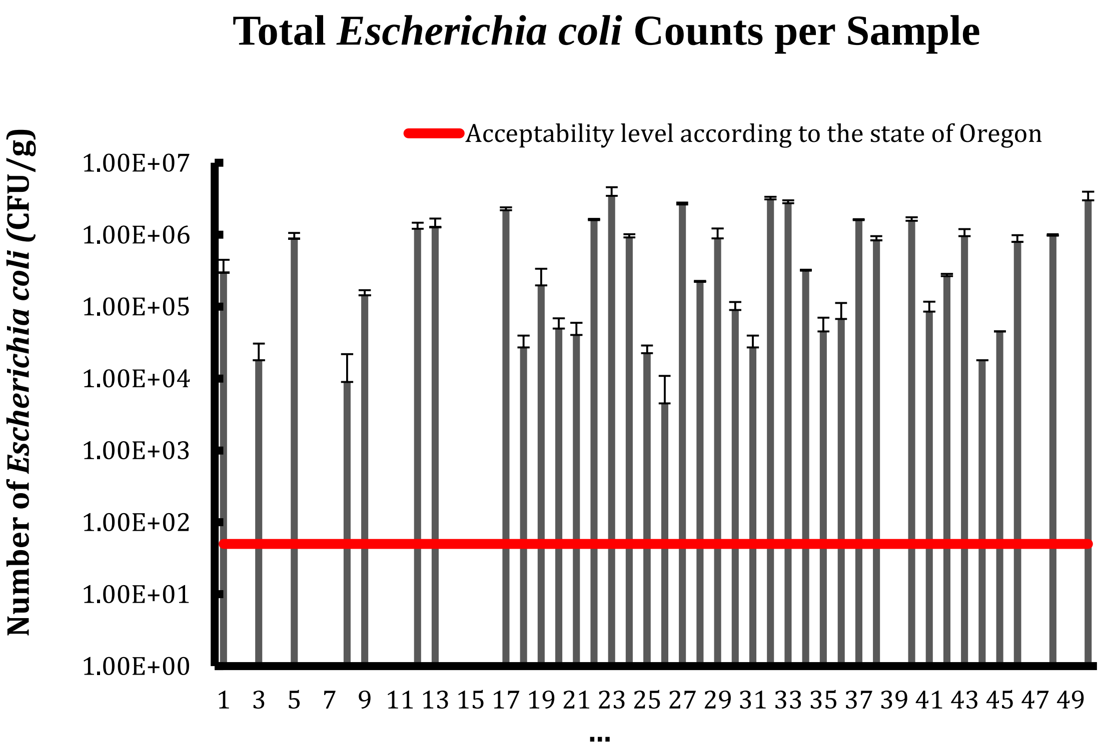

Fig. 4. *E. coli* Counts per Sample of Raw Minced Beef. Numbers of the x-axis represent meat samples that were collected from different locations. The number of *E. coli* was determined by plating on a selective medium as described in the material and methods

Comparing the acceptability and unacceptability levels between LIBNOR and the OSU standards, 38 samples (76%) were labeled as unacceptable for both standards, 1 sample (2%) was labeled acceptable in both standards, while 11 samples (22%) were labeled differently between standards.

Rejection rates were higher according to the LIBNOR standards at 98% as compared to the OSU standard, which was at 76%. Referring to these rejection rates, it might be safer to choose LIBNOR standards as a reference, because it is stricter. However, the drawbacks of LIBNOR might be an overestimation of rejection rates, which can be a major problem in production and can result in great economic losses. Although it resulted in lower rejection percentage, the OSU standard might be more specific, because it targets a better indicator; *E. coli*. However, the latter requires further analysis to ensure the safety of the samples that are deemed acceptable by the OSU standard.

In other countries *E. coli* in minced meat samples are present at lower distribution in comparison to Lebanon. For examples, a study in Turkey showed prevalence of 48.2% (Gencan & Arslan, 2012). Another study conducted in Washington DC, USA showed a prevalence of 19% in tested samples (Zhao *et al*., 2001). . In Ethiopia, a study showed that only 5.5% of tested samples contained *E. coli* (Messele *et al*., 2017). While prevalence does differ between countries depending on different factors, it is clear that the loads and prevalence in Lebanon are on the higher end.

In terms of acceptability for fecal coliforms, Lebanon has a standard of 100 CFU/g for raw minced beef, anything above this number is considered unacceptable, though no maximal acceptance levels exist. Similarly, other countries such as Iran have a set standard of 100 CFU/g (Kheyri *et al*., 2014), while New Zealand has a standard varying from of 100 to 1000 CFU/g (New Zealand Government, 1995). It should be noted that Lebanon's minced beef standards are nearly 15 years old and might benefit from updates that are specific to the country. The latter should be based on scientific studies that take into account the country's properties such as the climate Lebanon and the value and accuracy of fecal coliforms as indicators of pathogens in minced beef.

As for *E. coli*, no standards exist in Lebanon in relation to minced beef meat. Therefore, the OSU standard of 50 CFU/g was adopted here. *E. coli* standards also differ between countries. Canada has an acceptance level of 100 CFU *E. coli* /g (Government of Canada, Canadian Food Inspection Agency, Domestic Food Safety Systems & Meat Hygiene Directorate, 2017). The European Union has a higher range from 50 to 500 CFU/g (The Food Safety Authority of Ireland, 2005). In Iran and Japan no acceptable limit of *E. coli* exists for raw meat (Kheyri *et al*., 2014; Hye-Jin *et al*., 2018). However, it should be noted that all of these standards are concerned with meat that will be cooked and not be consumed raw. Regardless of what standards were used in this case, results for acceptability using *E. coli* did not differ, because all the positive samples harbored more than 1000 CFU *E. coli*/ g. The lowest *E. coli* count in Lebanese samples was 4500 CFU/g. Therefore, Lebanon has very high prevalence and loads of *E. coli* in raw minced beef meat, which calls for urgent action to address the problem. This is especially needed when the meat is consumed raw.

2. Antimicrobial Resistant E. coli in Minced Beef Meat

Overall, 18 antibiotics were tested on 120 *Escherichia coli* isolates retrieved from the 50 samples of minced meat.

Figure 5 shows the different types of antibiotics tested and the percentage of resistant isolates. All 120 isolates (100%) were resistant to penicillin. The highest rates of resistance (>30%) were found against streptomycin, cephalexin, and tetracycline.

None of the isolates showed resistance to imipenem, meropenem, cefixime, and amoxicillin + clavulanic acid.

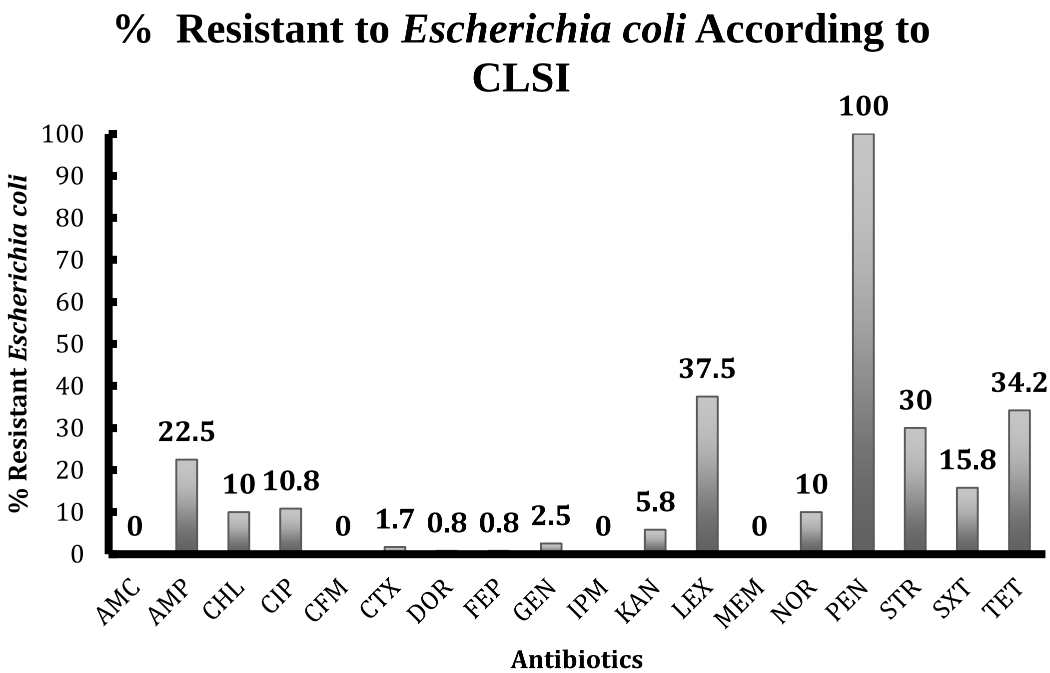

Fig. 5. Percentage of Resistance Against Each Antibiotic. The antibiotics tested were the following: kanamycin (KAN), ampicillin (AMP), imipenem (IPM), ciprofloxacin (CIP), penicillin (PEN), chloramphenicol (CHL), streptomycin (STR), meropenem (MEM), doripenem (DOR), norfloxacin (NOR), cefixime (CFM), cephalexin (LEX), cefotaxime (CTX), cefepime (FEP), trimethoprim-sulfamethoxazole (SXT), amoxicillin + clavulanic acid (AMC), gentamicin (GEN), tetracycline (TET)

Table 3 shows the different antibiotic resistance profile for each isolate. All 120 isolates (100%) were resistant to at least one antibiotic; however, none were resistant to all 18. Whereas 78 isolates (65%) were resistant to 1-2 classes of antibiotics, 39 isolates (32.5%) were resistant to 3-5 classes and 3 isolates (2.5%) were resistant to 6 classes. In total 35% of the *E. coli* isolates can be classified as multidrug resistant (MDR), which means that the isolates were resistant to 3 or more classes of antibiotics.

Table 3. Antibiotic Resistance Profile of the *E. coli* Isolates

Antibiotic Resistance Profile	**Number of Isolates**	**Number of Classes**
PEN	35	1
PEN-AMP	2	1
PEN-DOR	1	2
PEN-LEX	29	2
PEN-STR	5	2
PEN-TET	4	2
PEN-CTX-FEP	1	2
PEN-CTX-LEX	1	2
PEN-KAN-LEX	1	3
PEN-LEX-NOR	1	3
PEN-LEX-STR	1	3
PEN-LEX-TET	5	3
PEN-STR-TET	1	3
PEN-AMP-STR-TET	4	3
PEN-AMP--SXT-TET	1	3
PEN-CHL-LEX-TET	1	4
PEN-LEX-STR-TET	1	4
PEN-AMP-CHL-STR-TET	1	4
PEN-AMP-CIP-NOR-STR	1	3
PEN-AMP-STR-SXT-TET	7	4
PEN-CHL-STR-SXT-TET	2	5
PEN-CIP-STR-SXT-TET	1	5
PEN-AMP-CHL-CIP-NOR-SXT	1	4
PEN-AMP-CHL-STR-SXT-TET	1	5
PEN-AMP-KAN-LEX-STR-TET	1	4
PEN-CHL-CIP-NOR-SXT-TET	1	5
PEN-CIP-NOR-STR-SXT-TET	1	5
PEN-AMP-CIP-LEX-NOR-STR-TET	2	5
PEN-AMP-CHL-CIP-KAN-NOR-STR-TET	2	5
PEN-AMP-CHL-CIP-KAN-LEX-STR-TET	1	6
PEN-AMP-CIP-NOR-STR-SXT-TET	1	5
PEN-CHL-GEN-KAN-STR-SXT-TET	1	5
PEN-AMP-CIP-GEN-LEX-NOR-STR-SXT-TET	1	6
PEN-AMP-CHL-CIP-GEN-KAN-NOR-STR-SXT-TET	1	6

In this study the highest resistant rates were to penicillin (100%), cephalexin (37.5%), tetracycline (34.5%), streptomycin (30%), ampicillin (22.5%) and trimethoprim-sulfamethoxazole (15.8%). A similar study in another country showed that *E. coli* isolates were most resistant to tetracycline (27%), trimethoprim-sulfamethoxazole (26%) and cephalothin (17%)(Schroeder *et al*., 2002). Another study also showed that *E. coli* was most resistant to ampicillin (13.3%), tetracycline (12.6%) and streptomycin (8%) (WHO, 2018). A study in Ethiopia also found that the isolated *E. coli* was highly resistant to streptomycin, cephalothin, tetracycline and ampicillin (Messele *et al*., 2017). The CDC states that Gram-negative bacterial pathogens are specifically worrying, because they are becoming resistant to nearly all drugs (CDC, 2013a). This matter is of great concern, especially for a country like Lebanon who has quite high levels of infectious diseases that require the use of antibiotics (Salameh *et al*., 2017).

E. Conclusions

Results from this study showed that there was a high prevalence and loads for both coliforms and *Escherichia coli* in raw minced beef. The raw minced beef samples were also contaminated with MDR *E. coli*. These high numbers are worrying, especially because beef meat is very popular amongst the Lebanese. Contaminated meat leads to infection, especially in the most susceptible populations such as to children, the elderly and the immunocompromised. This is especially applicable when the meat is consumed raw. The results support that there should be updated and strict safety programs in Lebanon to monitor the microbiological quality of beef meat. Guidelines and regulations to control antibiotic use are also necessary in order to control the spread in resistance.

CHAPTER III

CONCLUSION

Meat production and consumption are growing worldwide. Unfortunately, meat is an important cause of many foodborne illnesses in developing and developed countries. This affects millions of people around the world, particularly the immunocompromised, the elderly and children under the age of five years old. Additionally, foodborne pathogens are developing antibiotic resistance. This makes treating infections more difficult and leads to failure of treatment or to longer hospital stays and increased medical costs.

In Lebanon there are food safety laws that were recently updated in 2015; however, these laws are not properly implemented. Food poisoning outbreaks and the presence of pathogenic bacteria have been reported by governmental agencies. Yet, there are no baseline studies on important foods such as meat in Lebanon. Antibiotic resistance is also an issue reported in Lebanon and policies for antibiotic use in humans or livestock as well as studies on the emergence of resistance in food systems are urgently needed.

In this study, a high percentage of raw minced beef were deemed microbiologically unacceptable. Even more concerning was that many of the *E. coli* isolates were also found to be resistant to different clinically important antibiotic classes.

Foodborne diseases and antibiotic resistance are two emerging problems globally. It is important for Lebanon to develop a set of updated and strict programs to monitor the microbiological quality of meat and associated emergence of antimicrobial resistance in order to ensure the safety of the public. Regarding antibiotic control in livestock, it is important to only give antibiotics under the supervision of a licensed veterinarian (Hye-Jin *et al*., 2018). Using the drugs for growth promotion should be banned (Hye-Jin *et al*., 2018). Improving biosecurity and preventing infections on farms, by enhancing hygiene and animal wellbeing is also an important factor in controlling and preventing the proliferation of disease resistance (Hye-Jin *et al*., 2018).

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