# PREVALENCE OF ANTIMICROBIAL RESISTANT STRAINS *SALMONELLA SPP*. AND *E. COLI* IN MEAT PRODUCTS IN LEBANON

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HALA SAEED AL MOURAWED

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Faculty of Nursing and Health Sciences

Department of Nursing and Health Sciences

We hereby approve the thesis of

**Hala Saeed Al Mourawed**

Candidate for the degree of Master in Food Safety and Quality Management

| | |
|---|---|
| Dr. Christelle Bou Mitri | 6 May 2019<br>Co-Supervisor, Chair |
| Dr. Lara Youssef | 21 May 2019<br>Co-Supervisor |
| Dr. Pauline Aad | 14 May 2019<br>Reader |
| Dr. Elie Bou Yazbeck | 17/5/2019<br>Readers |

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## ABSTRACT

Antibiotic resistance is one of the biggest threats to global health and food security. The aim of this study is to assess the prevalence of the pathogenic bacteria *Salmonella spp*. and *E. coli* in fresh and frozen red meat and meat organs collected from different supermarkets and butcher shops across Mount Lebanon and further evaluate their antimicrobial resistance. A total of 80 beef meat samples were collected from butchers and supermarkets in Mount Lebanon between February 2017 and December 2018. Bacterial isolation and biochemical identification were conducted using the API method. Using the disk diffusion method, the resistance of isolated strains to certain antimicrobial drugs was evaluated. The results showed that among the collected samples 57.7% were contaminated with *Salmonella spp.* and 72.5% with *E. Coli*. The prevalence of *Salmonella spp*. in meat samples collected from supermarkets (76.9%) was significantly higher compared to that from butcher shops (47.0%) (p=0.04). The fresh ground beef samples were significantly more contaminated with *E. coli* and 78.9% and *Salmonella spp* as compared to the frozen samples. The isolated *Salmonella spp*. and *E. coli* from the samples were 100% resistant to Oxacillin, Clindamycin, Erythromycin, Teicoplanin, and Vancomycin. Of the *Salmonella spp*. isolated, 50% showed resistance to Ampicillin and 30% to Cefuroxime. Among the isolated *E. coli*, 58% showed resistance to Ampicillin and 30% to Cefuroxime. The overall results revealed the importance of controlling the use of antibiotics to limit the emergence of multidrug resistant bacteria and emphasized the need to implement more stringent protective measures on the application of food safety laws to reduce the risk of contamination in meat production.

Keywords: *Salmonella spp*., *E. coli,* Meat, Antimicrobial resistance, Lebanon.

CHAPTER 1

## 1.1 Introduction

Antimicrobial resistance is a major global threat of increasing concern to human and animal health, besides its implications in both food safety and food security and the economic wellbeing of millions of farming households (FAO, 2018). Antibiotic resistance occurs when bacteria develop the ability to defeat the drugs designed to kill them (CDC, 2018). As a result of antimicrobial resistance, antibiotic medications that were once effective treatment for infectious diseases are becoming less effective or even useless, leading to a reduced ability to treat infections, increased mortality, prolonged illnesses, production losses in agriculture, and reduced livelihoods and food security (FAO, 2018). The CDC (Centers for Diseases Control and Prevention) estimates that antibiotic resistance caused over 2 million illnesses and 23,000 deaths nationally in 2013 (Donovan *et al*., 2015). It is also predicted that antibiotic resistance will lead to 300 million premature deaths by the year 2050, if no action is taken, which will exceed the predicted combined mortality of cancer and diabetes (Donovan *et al*., 2015). Moreover, Hilal *et al*., (2015) has shown that the treatment of diseases caused by antibiotic resistant bacteria can lead to increased mortality, morbidity, higher expenses, and prolonged hospital stay (Hilal *et al*., 2015). In 2016 the world health organization reported that 490,000 people developed multi-drug resistant and that drug resistance is starting to complicate the fight against HIV and malaria (WHO, 2016). Half of the deaths from clinical infections in Europe are associated with multi drug-resistant bacteria (Watson *et al*., 2008). Antibiotics use plays a major role in the emerging public health crisis of antibiotic resistance. In addition, this resistance is caused by the excessive and misuse of antimicrobials by humans in veterinary medicine and in animal feed (Alwan *et al*.,

2010). In animal agriculture, antimicrobials are added in low concentrations to animal feed in order to stimulate growth (FAO, 2019). The misuse of antibiotics in the agricultural fields aids in spreading antimicrobial resistance and compromises the veterinary medicines effectiveness (FAO, 2019). Antimicrobial resistant bacteria can develop in our food chains and move from animals to humans by direct exposure, consumption, or through the environment. All this makes antimicrobial resistance a global health issue which requires a coordinated response (FAO, 2019). Therefore, it is important to study antimicrobial resistance in order to detect changes in the antimicrobial susceptibility patterns that will help in implementing control measures on the use of antimicrobial drugs and will limit the spread of antimicrobial resistant strains of bacteria.

## 1.2. LITERATURE REVIEW

### 1.2.1. Antibiotic resistance

Antibiotic resistance is defined as "the ability of a microorganism to resist the antibiotic pressure and survive" (Kumar *et al*., 2017). Some antibiotics induce resistance readily, for example, rifampicin (Lambert *et al*., 2005), while others, such as those that target the cell membrane, may do so more slowly (Zhanel *et al*., 2008). The key mechanisms of genetic resistance are summarized by the following:

(i) bacteria can inactivate the antibiotic by producing, for example, β-lactamase which degrades the β-lactam ring which is a key part of penicillin and cephalosporins;

(ii) reduce membrane permeability to the antibiotic;

(iii) increase the efflux of antibiotic from the cell;

(iv) overproduce the target enzyme;

(v) bypass the inhibited step; and

(vi) alter the site of action of the antibiotic.

Some antibiotics, notably fluoroquinolones, induce the SOS response, which increases the error rate of DNA replication and speeds the development of resistance (Da Re *et al*., 2009). In addition, before the onset of genetic resistance, bacteria can survive antibiotic treatment by entering into a slow or non-multiplying state (Coates *et al*., 2002). Commensal bacteria that naturally live on the skin, in the mouth, nose and intestines contain large numbers of antibiotic-resistant organisms, and these may be a source of antibiotic-resistance markers for pathogenic bacteria (Gillings *et al*., 2008). Besides, about half of all antibiotics that are used each year in the world are consumed by animals. It is debatable that this might be a source of antibiotic resistance in humans (Soulsby *et al*., 2008). Meanwhile, multi-drug-resistant bacteria emerges throughout the world (Levy*et al*., 2004) and is associated with half of the deaths from clinical infection in Europe (Watson *et al*., 2008). The Director-General of the World Health Organization (WHO) fears that this global rise in antibiotic-resistant is threatening and can "send the world back to a pre-antibiotic age." According to CDC's antibiotic-resistance experts, it takes 17 years to develop an antibiotic but only few minutes for a bacterium to develop resistance (Watson *et al*., 2008).

### 1.2.2. Antibiotic Regulations

On a world scale, antibiotics use as animal growth promoters differs dramatically. While Sweden bans antibiotics use for growth promotion purposes, the USA uses a wide range of antibiotics in food producing animals (FAO, 2015) including some that are considered "medically important"

according to the Joint Expert Advisory Committee on Antibiotic Resistance (JETACAR, 1999). The use of these growth promoters is more limited in the European Community (FAO, 2015). In 1998, the EU banned the use of antibiotics in farm animals for non-treatment purposes. Then in 2001, it launched a plan to fight antibiotic resistance that calls to stop the non-medical antibiotic usage in animals and to increase awareness. This was followed by the creation of an independent body called the European Food Safety Authority (EFSA) by the EU in 2002, which assesses food and feed safety risks (Donovan *et al*., 2015). Moreover, in 2008 the ESFA established the European Antibiotic Awareness Day, stated that food may transmit antibiotic-resistant bacteria to humans and released recommendations about its prevention. In addition, the EU founded also an Intergovernmental Task Force on Antimicrobial Resistance, in which the member countries coordinate in research and management (Donovan *et al*., 2015).

In 2013, FDA implemented a voluntary plan to figure out the usage of antibiotics in food production. The aim was to develop a system in which antibiotics were used on farm animals only for treating illnesses, and not as growth promoters (Donovan *et al*., 2015). In 2014, the Natural Resources Defense Council examined FDA documents showing that FDA recently studied thirty antibiotics that were approved decades ago and found that half of the antibiotics had a high risk of exposing humans to antibiotic-resistant pathogens through the food supply. The group found that only one of the antibiotics that FDA approved met FDA's safety standards at the time of approval (Donovan *et al*., 2015). Moreover, President Obama in September 2014 issued an executive order to create an annual "Get Smart About Antibiotics Week" to raise awareness about antibiotic abuse and overuse. The executive order mandates that the U.S. Department of Agriculture (USDA), United States Environmental Protection Agency (EPA), and

Food and Drug Administration FDA work together, coordinating research and surveillance specifically in the context of food-producing animals. USDA regulations state that cows raised for meat should only be slaughtered after a 'withdrawal' period from the time antibiotics are administered, so residues can exit the animal's system. USDA randomly tests cattle at slaughter for antibiotic residues and establish voluntary milk quality assurance programs for educational purposes about the importance of good quality milk and meat free of adulterants and residues (Donovan *et al*., 2015).

In recognition of the growing problem of antimicrobial resistance (AMR), the WHO presented to its Health Assembly a global action plan draft on antimicrobial resistance. In May 2015, the World Health Assembly called for strengthened collaboration between the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE) and the World Health Organization (WHO) to address (AMR) in the context of "One Health" (FAO, 2015). Moreover, in 2016, the UN general assembly arranged a High-Level Meeting on Antimicrobial Resistance to combat antimicrobial resistance worldwide (FAO, 2016)

**1.2.3. Antibiotic use in animal agriculture**

Antimicrobials have been widely used in the agricultural field. In 2010, the total consumption of antimicrobials in the livestock sector was 63,151 tons. It is predicted that by 2030 the global use will rise by 67% reaching 105,596 tons (FAO, 2018). "Antibiotic growth promoter" term is used to describe any medicine that destroys or inhibits bacteria overgrowth and is administered at a low sub-therapeutic dose (FAO, 2018). The Animal Health Institute of America (AHI, 1998) estimated that without the use of growth promoting antibiotics the USA would require an additional 452 million chickens, 23 million more cattle and 12 million more pigs to reach the levels of production attained by the current practices (FAO, 2018). When antibiotics are given to animals for growth promotion bacteria become exposed to low doses

of these drugs over a long period of time, which leads to the development of resistant bacteria (CDC, 2017).

### 1.2.4. Potential risks to human health

The extensive use and misuse of antibiotics are associated with various harmful effects on human health (Bouki *et al*., 2013; Gao *et al*., 2012; Jakobsson *et al*., 2010). Multidrug-resistant bacteria are recently considered as an emerging global disease and a major public health problem (Roca *et al*., 2015). Humans are at risk of exposure to new resistant pathogens from animals through direct contact, ingestion of contaminated meat or water, and through the contact with infected humans (Chang *et al*., 2015). It has been reported that long term as well as short term exposures to low concentrations of antibiotics can lead to the development of allergic reactions, disturbance of natural intestinal micro-flora, obesity (Ajslev *et al*., 2011; Bailey *et al*., 2014; Thuny *et al*., 2010), type 2 diabetes with glucose homeostasis disturbances (Chou *et al*., 2013), multidrug resistance and increase the prevalence of antibiotic resistance genes (Wright *et al*., 2007; Castres *et al*., 2014).

Many studies have reported the potential risks of antimicrobial resistant strains; however the current situation is more critical as the present antibiotic-resistance crisis is different from the ones that have occurred in the past (Rather *et al*., 2017). Recent studies reveal the availability of medicines to treat new infections by resistant pathogenic strains but there have not been many new discoveries of antibiotics to combat the antibiotic resistant pathogens (Rather *et al*., 2017).

### 1.2.5. Antibiotic resistant bacteria

Antibiotic resistant bacteria could result from transferable drug resistance between pathogenic organisms, between organisms of different species, such as *Eschereca. coli*, *Salmonella* and *Shigella*; and also, between pathogenic and non-pathogenic organisms. Fecal contamination of meat during slaughter may result in the transfer of antibiotic resistant *E. coli* to the meat (Okolo *et al*., 1986).

WHO's first release of surveillance data on antibiotic resistance reveals high levels of resistance to a number of serious bacterial infections in both high- and low-income countries (WHO, 2018). WHO's new Global Antimicrobial Surveillance System (GLASS) reveals widespread occurrence of antibiotic resistance among 500,000 people with suspected bacterial infections across 22 countries (WHO, 2018). The most commonly reported resistant bacteria were *Escherichia coli*, *Salmonella spp.*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. (WHO, 2018).

### 1.2.6. *Salmonella spp.*

*Salmonella spp*. are gram negative motile and one of the leading causes of food borne illness in human (Dahama *et al*., 2011). There are many types of *Salmonella spp.* that can be divided into two groups, *typhoidal Salmonella* (TS), such as *S. enterica* and *S. paratyphoid.* This group only colonize humans and are usually acquired by the consumption of food or water contaminated with human fecal material (Kadhiravan *et al*., 2005). The second group with broad spectrum is the *non-typhoidal Salmonella* (NTS) that results from improperly handled food and that has been contaminated by animal or human fecal material. Salmonellosis ranges from self-limiting

gastroenteritis caused by NTS that usually lasts for 4 to 7 days to a more complicated life-threatening typhoid fever (Kadhiravan *et al*., 2005).

### 1.2.7. *Escherichia coli*

*E. coli* are gram negative rods of the family *Enterobaceriaceae* (Dahama *et al*., 2011). Among the bacterial pathogens, diarrheagenic *E. coli* (DEC) is one of the important etiological agents of diarrhea (Moyo *et al*., 2007; Blanco *et al*., 2005). Diarrheal disease is still a major health problem, especially in developing countries (Abbasi *et al*., 2014). *E. coli* is considered the main cause of morbidity and mortality especially in children (Jafari *et al*., 2012). Pathogenic strains of *E. coli* are divided into two groups; The intestinal *E. coli* pathogens that cause diarrhea, and the extra intestinal *E. coli* that causes urinary tract infections (UTI), meningitis and septicemia (Jafari *et al*., 2012). DEC strains are divided into six categories based on epidemiological and clinical features, and specific virulence determinants (Blanco *et al*., 2006). These includes *Enteropathogenic E. coli* (EPEC), *Enterotoxigenic E. coli* (ETEC), *Enteroinvasive E. coli* (EIEC), *Enterohaemorrhagic E. coli* (EHEC) or *Shiga-toxin producing E. coli* (STEC), *Enteroaggregative E. coli* (EAEC), and *Diffusely Adherent E. coli* (DAEC) (Majowicz *et al*., 2014). *Shiga toxin-producing E. coli* is a heterogeneous group of organisms (Abbasi *et al*., 2014). Shiga toxin-producing *E. coli* (STEC) can cause severe foodborne disease. The primary sources of STEC outbreaks are raw or undercooked meat products, raw milk, and fecal contamination of vegetables (WHO, 2018). In most cases, the illness caused by STEC is self-limiting, but it may lead to a life-threatening disease especially in young children and the elderly (WHO, 2018).

### 1.2.8. Antimicrobials

Antimicrobials are drugs that are used to kill or prevent the growth of microorganisms (bacteria, fungi, and viruses) (Ramalingam *et al*., 2015). Antibiotics are substances produced by one type of bacteria that can favor in killing or preventing the growth of another microorganism (Ramalingam *et al*., 2015). The invention of antimicrobials such as penicillin and tetracycline saved and improved the health for millions around the world. However, the future effectiveness of such antimicrobial therapy is somehow in doubt because the bacteria are becoming resistance to antimicrobial agents (Ramalingam *et al*., 2015).

### 1.2.9. The main classes of antibiotics

More than 20 novel classes of antibiotics were produced worldwide between years 1930 and 1962 (Coates *et al*., 2002; Powers *et al*., 2004). Penicillin was discovered by Alexander Fleming through observing the antibacterial effect of the Penicillium fungus (Fleming *et al*., 1929), which led to the discovery of many analogues (Table 1.1). There are several ways of antibiotic classification but the most common schemes are based on their molecular structures, mode of action and spectrum of activity (Calderon *et al*., 2007). Antibiotics that have the same structural class will show similar pattern of effectiveness, toxicity and potential allergic side effects (Etebu *et al*., 2016). Antibiotic classes based on chemical or molecular structures include Beta-lactams, Macrolides, Tetracyclines, Quinolones, Aminoglycosides, Sulphonamides, Glycopeptides and Oxazolidinones (van Hoek *et al*., 2011; Frank *et al*., 2012; Adzitey *et al*., 2015). Beta-lactams class contains a 3-carbon and 1-nitrogen ring that is highly reactive. The most prominent

representatives of the beta-lactam class include Penicillins, Cephalosporins, Monobactams and Carbapenems (Etebu*et al*., 2016). Macrolides class includes Erythromycin, Azithromycin and Clarithromycin (Hamilton-Miller *et al*., 1973). They are characterized by large lactone ring attached to one or more sugars. Tetracyclines class first member is chlortetracycline (Aureomycin) (Etebu*et al*., 2016). Members of this class have four (4) hydrocarbon rings and they are known by name with the suffix "–cycline". These members are grouped into generations according to their synthesis method. First generation are those obtained by biosynthesis, including Tetracycline, Chlortetecycline, Oxytetracycline and Demeclocycline. Second generation are those obtained by semi-synthesis including, Doxycycline, Lymecycline, Meclo cycline, Methacycline, Minocycline, and Rolitetracycline. Those obtained from total synthesis such as Tigecycline are considered Third generation (Etebu *et al*., 2016).

Quinolones structure generally consists of two rings but recent generations of quinolones possess an added ring structure that enables them to extend their spectrum of antimicrobial activity to some bacteria, particularly anaerobic bacteria. Two major groups of compounds have been developed from the basic molecule: quinolones and naphthyridones that include cinoxacin, norfloxacin, ofloxacin, ciproxacin, temafloxacin, sparfloxacin, nalidixic acid, enoxacin (Etebu*et al*., 2016).

Aminoglycosides are compounds of usually 3-amino sugars connected by glycosidic bonds, they are effective against aerobic Gram-negative rods and certain Gram-positive bacteria (Etebu *et al*., 2016). This class includes Streptomycin, Gentamicin, Neomycin, Tobramycin and Amikacin generation (Etebu*et al*., 2016).

Sulphonamides inhibit both Gram-positive and Gram-negative bacteria such as *E. coli* and S*almonella spp.* They contain the sulphonamide group generation (Etebu*et al*., 2016).

Glycopeptides are made of a cyclic peptide of 7 amino acids to which are bound 2 sugars, hence the name glycopeptides (Kang*et al*., 2015).

Oxazolidinones are a group of synthetic antibiotics in which Linezolid represents the first member to be synthesized and it was approved for clinical application only in the year 2000 (Etebu*et al*., 2016).

The most prolific number of analogues has been made using the cephalosporin and penicillin cores (Table.1), but the quinolone and aminoglycoside cores have also been used extensively (Coates *et al*., 2011). Resistance to all these compounds has unfortunately arisen and thus analogue development merely ‘buys time’ until the discovery of the next novel class (Coates *et al*., 2011).

Table 1.1. Main classes of antibiotics (Coates *et al*., 2011).

| Class name | Example of Antibiotics |
|---|---|
| Beta- Lactams | Penicillin (ampicillin, amoxicillin)<br>Cephalosporins (cefazolin, cephalexin, Cefotaxime, Cefpirome)<br>Carbapenems: Imipenem<br>Monobactams: tazobactam |
| Tetracyclines | Tetracycline, chlortetracycline, demeclocycline, minocycline, oxytetracycline, methacycline, doxycycline, tigecycline |
| Rifamycin | Rifampicin (also called rifampin), rifapentine, rifabutin, bezoxazinorifamycin, rifaximin |
| Macrolides | Erythromycin, azithromycin, clarithromycin |
| Ketolides | Telithromycin |
| Lincosamides | Lincomycin, clindamycin |
| Glycopeptides | Vancomycin, teicoplanin, telavancin |
| Lipopeptides | Daptomycin |
| Streptogramins | Quinupristin, dalfopristin, pristinamycin |
| Sulphonamides | Sulphanilamide, *para*-aminobenzoic acid, sulfadiazine, sulfisoxazole, sulfamethoxazole, sulfathalidine |
| Oxazolidinones | Linezolid |
| Quinolones | Nalidixic acid, oxolinic acid, norfloxacin, pefloxacin, enoxacin, ofloxacin/levofloxacin, ciprofloxacin, temafloxacin, lomefloxacin, fleroxacin, grepafloxacin, sparfloxacin, trovafloxacin, clinafloxacin, gatifloxacin, moxifloxacin, sitafloxacin |

Table 1.2. The four classical groups of Penicillin antimicrobials (Beta-Lactams) (Coates *et al*., 2011).

| Group Name | Antibiotic Names |
|---|---|
| Penicillins | penicillin V, methicillin, oxacillin, cloxacillin, dicloxacillin, nafcillin, ampicillin, amoxicillin, carbenicillin, ticarcillin, mezlocillin, piperacillin, azlocillin, temocillin |
| Cephalosporins | *First generation:* Cephalothin, cephapirin, cephradine, cephaloridine, cefazolin<br>*Second generation:* Cefamandole, cefuroxime, cephalexin, cefprozil, cefaclor, loracarbef, cefoxitin, cefmetazole<br>*Third generation* Cefotaxime, ceftizoxime, ceftriaxone, cefoperazone, ceftazidime, cefixime, cefpodoxime, ceftibuten, cefdinir<br>*Fourth generation:* Cefpirome, cefepime<br>*Fifth generation:* Ceftaroline Ceftobiprole |
| Carbapenems | Imipenem, meropenem, doripenem |
| Monobactams | Aztreonam b-Lactamase inhibitors Clavulanate, sulbactam, tazobactam |

### 1.2.10. Prevalence of *Salmonella spp*. and *E. coli* pathogenic isolates worldwide

Bacterial contamination in meat products was studied in various countries worldwide. In northern Egypt, *Salmonella spp.* and *E. coli* were recovered from raw beef meat and out of 180 investigated samples, Salmonella spp. was present in 8.3% of the samples and *E. coli* was detected in 11.7% of the samples. Zhang *et al* carried out a study assessing 559 retail meat samples collected from cities in China and their results showed that 39.2% of the tested meat samples were contaminated with *E. coli* and 6.4% of them were contaminated with ETEC (Zhang *et al*., 2016). In Mexico, the prevalence of *Salmonella spp.* and *E. coli* was assessed in 1154 samples of retail, whole and ground beef where *Salmonella spp.* was recovered from 4.4%

of whole beef samples and 7.3% of the ground beef samples (Pond *et al*., 2016). Moreover, in Tanzania researchers looked for the prevalence of *E. coli* in 124 raw goat meat samples. They found that *E. coli* isolates were present in 98.6% of the tested samples (Mwanyika *et al*., 2016). In Ethiopia, among 440 beef samples, *E. coli* was present in 4.5% of carcass swabs and 3.6 % of cutting board swabs (Abdissa *et al*., 2017).

In Tunisia, the prevalence of isolated *Salmonella* was 48.3% in chicken, 29.8% in beef, 10.7% in minced meat and 6% in lamb (Abbassi-Ghozzi *et al*., 2012).

Nevertheless, in Jeddah Saudi Arabia, retail meat was found to be contaminated by *E. coli* and *Salmonella spp.*. A higher rate of both pathogens' incidence was found in open butcher shops compared to hypermarkets and groceries. While the incidence of *E. coli* was 65% in open butcher shops, its incidence was 40% in groceries and 20% in hypermarkets. With respect to *Salmonella spp*, the incidence was 45% in samples obtained from butcher shops, 25% from groceries, and 5% from hypermarkets (Iyer *et al*., 2013). In Lebanon, the prevalence of *Salmonella spp*. and *E. coli* was studied in meat-based fast food products showing that up to 55% of the collected samples were contaminated with *E. coli* and 47.5% of them contained *Salmonella spp*. (Harakeh *et al*., 2005).

Moreover**,** a study that was conducted in Alberta Canada showed that *Salmonella spp.* was detected in 40% of the chicken samples, 27% of the turkey samples and 2% of the pork samples. However, all ground beef samples were negative for *Salmonella spp* (Aslam *et al*., 2012). Another study conducted in Jordan on Mediterranean Ready-to-Eat Meat Products (RTE) showed that the overall prevalence of Salmonella spp. and *E. coli* was low. *Salmonella spp.* prevalence was 0.5% while *E. coli* was not isolated from any of the 1,028 tested samples.

However, *E. coli* strains were only prevalent in 2% of beef kubba samples and 1% of beef pastry samples (Osaili *et al*., 2014).

Findings from these studies (Table 1.3) showed high prevalence of meat and meat products contamination around the world. *Salmonella spp.* and *E. coli* being the highest detected bacteria in all meat products with highest prevalence found in raw meat including beef (Zhang *et al*., 2016; Abbassi-Ghozzi *et al*., 2012; Abdissa *et al*., 2017). Higher rates of incidence of both pathogens were found in meat obtained from open butcher shops compared to those obtained from hypermarkets and groceries (Pond *et al*., 2016; Iyer *et al*., 2013). The presence of these pathogens can be due to contamination during meat processing at the slaughterhouses, where hygienic practices are not strictly followed and where antibiotic resistant bacteria are spread as a result of the wide application of antibiotics (Harakeh *et al*., 2005).

### 1.2.11. Prevalence of *Salmonella spp*. and *E. coli* among retail channels

The prevalence of pathogenic *Salmonella spp*. and *E. coli* among retail channels (butcher shops and supermarkets) was studied worldwide (table 1.4). In Mexico and Saudi Arabia, samples obtained from open butcher shops were found to have higher prevalence of *Salmonella spp*. and *E. coli* as compared to supermarkets (Pond *et al*., 2016; Iyer *et al*., 2013). In Mexico, *Salmonella spp*. prevalence was 9.1% in whole beef and 4.2% in ground beef ($p < 0.01$) (Pond *et al*., 2016). In Jeddah Saudi Arabia, a significant difference was associated with the presence of *Salmonella spp.* and *E. coli* in meat taken from butcher shops compared to that from supermarkets ($p < 0.05$). *Salmonella spp*. prevalence was 45% in meat samples from butcher shops compared to 5% in

Table 1.3. Prevalence of pathogenic isolates worldwide

| Country | Bacteria assessed | Food Items (n=) | Results *Salmonella spp.* | Results *E. coli* | Author nameStudy |
|---|---|---|---|---|---|
| Malaysia | *Salmonella* | Raw retail beef meat (n=312) | (15.4%) | | Shafini *et al* ., 2017 |
| China | *E. coli* | Retail meat (n=559%) | | *E. Coli* (39.2% ) ETEC (6.4% ) | Zhang *et al*. 2016 |
| Mexico | *Salmonella* *E. coli* | Retail, whole and ground beef (n=1154) | Whole beef (4.4 %) Ground beef (7.3%) | Not detected | Pond *et al*., 2016 |
| Alberta Canada | *Salmonella* | Raw retail meat (n= 564) | Beef (0%) Chicken (40%) Turkey (27%) Pork (2%) | | Aslam *et al* .,2012 |
| northern Northern Egypt | *Salmonella* *E. coli* | Raw Beef & meat organs (90) (n=180) | *15 S. enterica (8.3%)* | *21 E. Coli (11.7%)* | Moawad *et al*., 2017 |
| Jordan | *Salmonella* *E. coli* | RTE Shawarma beef pastry beef kubba beef kabab (n=1028) | *Salmonella (*0.5%) | Beef kubba ( 2%) Beef pastry (1%) | Osaili *et al*., 2014 |
| Tunisia | *Salmonella* | Raw meat(n=315) | Beef (29.8%) Mincemeat (10.7%) Lamb (6%) | | Abbassi-Ghozzi *et al*., 2012 |
| Ethiopia | *E. Coli* | Beef (n=440) | | carcass swabs (4.5%) Cutting board swabs (3.6%) | Abdissa *et al*., 2017 |

Table 1.4. Prevalence of pathogenic isolates among different retail channels

| Country | Bacteria assessed | Food Items (n) | Results | Study |
|---|---|---|---|---|
| Jeddah, Saudi Arabia | *E. coli*<br>*Salmonella* | retail meat (n=60) | *E. coli*<br>Open butcher shops (65%)<br>Groceries (40%)<br>Hypermarkets (20%)<br>*Salmonella*<br>Open butcher shops (45%)<br>groceries(25%),<br>Hypermarkets(5%) | Lyer *et al*., 2013 |
| Mexico | *Salmonella* | Retail , whole and ground beef & pork (n=1154) | *Salmonella :*<br>retail channels, supermarkets 1.3%<br>Butcher shops 8.4%<br>vendors 13.6%<br>city markets22.3% | Pond *et al*., 2016 |

those collected from supermarkets. *E. coli* was prevalent in 65% of meat samples from butcher shops and 20% in meat samples from supermarkets.

### 1.2.12. Antibiotic Susceptibility

Multidrug resistant strains among foodborne pathogens and their resistance to clinically important antibiotics are currently growing global public health concerns (Osaili *et al*., 2014). Many studies conducted worldwide aimed to assess antimicrobial susceptibility in meat products (Table 1.5). Many findings showed that meat isolates were resistant to at least one of the tested antimicrobials with high resistance to $\beta$-Lactams particularly ampicillin and amoxicillin, to streptomycin, Oxacillin, Teicoplanin, Clindamycin, Erythromycin, Vancomycin, Cefuroxime,

Gentamicin and Trimethoprim/sulfamethoxazole and with low resistance to Cefotaxime (Ren *et al*., 2016; Zhang *et al*., 2014; Domnech *et al*., 2015; Abbassi-Ghozzi *et al*., 2012).

In China, antimicrobial resistance of Salmonella spp. isolates was tested in broiler supply chain and the results indicated that it was frequently resistant to streptomycin (38.2%), tetracycline (36.3%), sulfisoxazole (35.3%) and gentamicin (34.3%). Moreover, 31.4% of the isolates were multidrug resistant (Ren *et al*., 2016). In addition, *E. coli* isolates from retail meats were resistant to sulfamethoxazole (61.6%), tetracycline (61.2%), ampicillin (48.2%), and to a lesser extent cefalotin (29.8%), kanamycin (22.4%), streptomycin (21.2%), ciprofloxacin (14.5%), and gentamicin (11.4%). The isolates also showed 2.8–6.7% resistance to ceftazidime, ceftriaxone, and ampicillin/sulbactam (hang *et al*., 2014).

In addition, *Salmonella spp.* isolates from retail meat in Alberta, Canada were found to be resistant to several antibiotics. 29% (32/110) of the isolates were susceptible to all tested antimicrobials. Amoxicilline clavulanic acid (AMC), ceftiofur (TIO), cetriaxone (CRO), and cefoxitin (FOX) were concurrently present in 21% (23/110) of the isolates. On the other hand, resistance to ciprofloxacin (CIP), amikacin (AMK), and nalidixic acid (NAL) was not found in any *Salmonella* isolates (Aslam *et al*., 2012).

In Tunisia, studies on antimicrobial resistance showed that 20% of the isolated *Salmonella spp*. from raw meat was resistant to at least one antimicrobial agent. While 13 isolates (16.2%) were resistant to ampicillin, five isolates (6.2%) were resistant to streptomycin, four isolates (5%) were resistant to amoxicillin-clavulanic acid, two isolates (2.5%) were resistant to cefoperazone and furazolidone and one isolate (1.2%) was resistant to each of nalidixic acid, tetracycline, sulphamethox- azole trimethoprim, ceftazidine and sulphonamides compound (Abbassi-Ghozzi

*et al*., 2012). Nevertheless, in Jordan, *Salmonella spp. and E.coli* isolated from Mediterranean RTE meat products were resistant to more than two antibiotics. Two isolates of *Salmonella spp.* from shawirma were resistant to ampicillin, ampicillin-sulbactam, and gentamicin, and one Salmonella Typhi isolate was resistant to ampicillin and ampicillin-sulbactam, especially the isolates from chicken shawirma (Osaili *et al*., 2014).

Table 1.5. Prevalence of pathogenic isolates and antibiotic susceptibility assessed worldwide

| **Country** | **Bacteria assessed** | **Prevalence of antibiotic resistance** | **Study** |
|---|---|---|---|
| China | *E.coli* (Retail Meat) | Multidrug resistance | Zhang *et al*., 2016 |
| Eastern Spain | *Salmonella* (Cooked ham) | Multidrug resistance | Domnech *et al*., 2015 |
| Canada (Alberta) | *Salmonella* (Retail meat) | 29% Salmonella isolates were susceptible to all tested antimicrobials.<br>No Resistance to CIP, AMK, and NAL<br>21%AMC, TIO, CRO, and FOX. | Aslam *et al*., 2012 |
| Jordan | *Salmonella*<br>*E.coli (RTE)* | multidrug resistance in all the tested isolated | Osaili *et al*., 2014 |
| Tunisia | *Salmonella* (Raw meat) | 80 isolates (20.0%) showed resistance to at least one antimicrobial<br>No resistance in lamb meat or minced meat | Abbassi-Ghozzi *et al*., 2012 |
| Egypt | *Salmonella*<br>*E. Coli* (Beef) | multidrug resistant | Moawad *et al*., 2017 |

### 1.2.13. Antimicrobial susceptibility of bacteria isolates from food products in Lebanon

Studies in Lebanon assessed different types of bacteria isolated from Lebanese dairy products and tested them for their susceptibility to different antibiotics (Table 1.6). In 2009, 164 samples including kishk (83), baladi cheese (45) and shankleesh (36) were assessed. 15.6% of the kishek, 46.6% of the baladi cheese, and 8.3% of the shanklish presented pathogenic *E-coli*. Among the *E. coli* isolates, 84% were resistant to tetracycline, 72% were resistant to ampicillin, 64% were resistant to cefuroxime, 59% were resistant to nalidixic acid, 45% to ofloxacin, 32% to ciprofloxacin and 31% to cefotaxime (Saleh *et al*., 2009). Moreover, 26.6% of the baladi cheese samples, 13.89% of shankleesh samples, and 7.23% of kishk samples were contaminated with *L. monocytogenes*. Tested *L. monocytogenes* were found to be resistant to gentamicin (93.34%), *oxacillin* (93.33%), penicillin (90%) trimethoprim-sulfamethoxazole (83.33%), tetracycline (80%), erythromycin (73.34%), ampicillin (60%) and vancomycin (26.66%) (Harakeh *et al*., 2009). Staphylococcus species were also isolated from these dairy-based product samples in which 29 isolates were confirmed. Of the baladi cheese samples, 42.2% were positive for *S. aureus* and 6.7% were contaminated with *S. saprophyticus*. Of the shankleesh sample, 5.6% were tested positive for *S. aureus* and 25% were positive for *S. saprophyticus*. Moreover, of the 83 kishk samples, 89.6% were contaminated with *S. aureus* and 6% had S. *saprophyticus*. Isolates were tested for antimicrobial resistance and showed resistance to oxacillin (93.5%), clindamycin (93.5%), methicillin (84.8%), teicoplanin (76.1%), vancomycin (71.7%) and gentamicin (67.4%) (Zouhairi *et al*., 2010).

In 2016, Banna & Nawas studied the prevalence of *Salmonella spp*. in ready to eat chicken shawarma sandwiches from 10 restaurants in Ras Beirut rejoin. Three out of the ten samples (30.0%) confirmed the growth of S*almonella* colonies (Banna & Nawas).

Table 1.6. Antimicrobial susceptibility of bacteria isolates from food products in Lebanon

| **Bacteria assessed** | **Results** | **Prevalence of antibiotic resistance** | **Study** |
|---|---|---|---|
| *E.coli*<br>diary products<br>(n=164) | baladi cheese (46.6%)<br>kishek (15.6%)<br>shanklish (8.3%) | 84%tetracycline<br>32% ciprofloxacin<br>72% ampicillin<br>64% cefuroxime<br>31%cefotaxime<br>32% ciprofloxacin<br>59%nalidixic acid<br>45%ofloxacin | (Saleh *et al*., 2009) |
| *L. monocytogenes*<br>dairy-based<br>food(n=164) | 26.67%baladi cheese<br>13.89% shankleesh<br>7.23% kishk | 93.33%oxacillin<br>90% penicillin<br>60%ampicillin<br>93.34%gentamicin<br>83.33% trimethoprim-sulfamethoxazole<br>80% tetracycline<br>73.84% erythromycin<br>26.66% vancomycin | (Harakeh *et al*., 2009) |
| *Staphylococcus*<br>dairy-based products<br>(n=164) | *S. aureus* :<br>Cheese 42.2%<br>Shankleesh 5.6%<br>Kishk 9.6%<br>S. *saprophyticus:*<br>Cheese 6.3%<br>Shankleesh 25.0%<br>Kishk 6.0% | 3.5%oxacillin<br>93.5% clindamycin<br>84.8%methicillin<br>76.1% teicoplanin<br>71.7% vancomycin<br>67.4% gentamicin | (Zouhairi *et al*., 2010) |
| *Brucella abortus*<br>(n=164) dairy-based<br>food | Shankleesh16.7%<br>Baladi cheese13.3%<br>Kishk 4.8% | High resistance :Streptomycin and Ciprofloxacin,<br>3 out of 6 isolates showed resistance to Gentamicin.<br>Lower resistance: Rifampicin, Tetracycline and Trimethoprim-sulfamethoxazole. High susceptibility :Ceftriaxone and Doxycycline | (Alwan *et al*., 2010) |

One study in Lebanon regarding bacterial isolation and antimicrobial resistance testing in meat products was reported (Table 1.7). In 2005, *Salmonella spp*. and *E. coli* were isolates from meat-based fast food shawarma and lahm bi ajeen (Harakeh *et al*., 2005). Up to 55% of the samples were contaminated with *E. coli*, whereas 47.5% of the samples were contaminated with *Salmonella Spp.* Antimicrobial susceptibility testing showed that the detected isolates were resistant to at least one of the tested antimicrobials. Moreover, 100% of the tested *Salmonella spp*. were resistant to Oxacillin, teicoplanin, clindamycin, erythromycin, and vancomycin. While 86% showed resistance to trimethoprim /sulfamethoxazole, 25.9% were resistant to cefotaxime and with moderate susceptibility against both cefuroxime and gentamycin (57.1%). As for *E. coli,* 69.1% were resistant to at least one of the tested antibiotics. 100% resistance was documented for teicoplanin, and 88.9% resistance was reported for Oxacillin, Clindamycin, vancomycin and erythromycin (Harakeh *et al*., 2005).

Table 1.7. Prevalence and Antimicrobial susceptibility of bacteria isolates from food products in Lebanon.

| Country | Food items (n=) | Bacteria assessed | Results | Antimicrobial resistance | Study |
|---|---|---|---|---|---|
| Lebanon | meat based fast food products (n=95) | *E. coli* *Salmonella* | *E. coli (55%)* *Salmonella (47.5)* | 100 % to Oxacillin, teicoplanin, clindamycin, erythromycin, and vancomycin<br>86% resistant to trimethoprim /sulfamethoxazole<br>25.9% to cefotaxime<br>57.1%cefuroxime and gentamycin<br>*E. coli*<br>69.1% resistant to at least one of the tested antibiotics.<br>100% teicoplanin<br>88.9% Oxacillin, Clindamycin, vancomycin and erythromycin | Harakeh *et al*., 2005 |

### 1.3. Rational

Based on literature review, meat products are considered an important vehicle for the transmission of antibacterial resistant strains, and many studies confirmed the presence of *Salmonella spp*. and *E-coli* pathogens in meat products and their resistance to several antibiotics. Moreover, studies showed that meat from open butchers are more contaminated with pathogens compared to closed ones. In Lebanon, there is only one recent study that confirmed the prevalence of *Salmonella spp.* in chicken shawarma and another one that confirmed the presence of Salmonella spp. and E-coli in a Lebanese meat-based fast food and assessed their antibiotic resistance. According to the Central Administration of Statistics (CAS), in 2007 the governorate of mount Lebanon account for the largest share of the population. LMOPH (Lebanese Ministry Of Public Health) records showed 12203 hospital cases of Intestinal Infectious Diseases (LMOPH statistics 2015) and 611 cases of *Salmonella* infection per year were reported over the last 13 years (range 398–891). These reported numbers and the high emergence of antibiotic resistance worldwide have highlighted the need to implement control measures in food products and to study the microbiological quality in food, especially in meat.

### 1.4. Objectives

The aim of our study is to assess the prevalence of pathogenic bacteria *Salmonella spp*. and *E. coli* in fresh and frozen red meat and meat cuts collected from different supermarket and butchers' shops across Mount Lebanon. The study also aims to assess the presence of antibiotic resistant bacterial strains in these samples.

# CHAPTER 2

## 2.1. Introduction

The use of antibiotics has highly increased in the last decades. Their excessive use has generated antibiotic resistant bacteria (CDC, 2018). Resistance occurs when certain bacteria become able to withstand the drugs intended to kill them, leading to bacteria's endurance and overgrowth (CDC, 2018). The use of antibiotics is the most important single factor leading to antibiotic resistance around the world (CDC, 2018).

Antimicrobial resistance is one of the biggest public health challenges of our time and a major global threat of increasing concern to human and animal health (CDC, 2018; FAO, 2018; Ha *et al*., 2017). Each year in the United States (U.S.), at least 2 million people get an antibiotic-resistant infection, causing more than 23,000 death cases (CDC, 2017). The treatments of infectious diseases caused by antibiotic resistant bacteria are becoming less effective. Thus, they are leading to prolonged hospital stays, higher medical expenses, and increased mortality (Hilal *et al.,* 2015). Drug resistance is also starting to complicate the fight against Human Immunodeficiency Virus (HIV) and malaria, where half of the deaths from clinical infection in Europe were associated with multi drug-resistant bacteria (WHO, 2016; Watson *et al*., 2008). It is forecasted that antibiotic resistance will lead to 300 million premature deaths by the year 2050 if no action is taken, which will exceed the predicted combined mortality of cancer and diabetes (Donovan, 2015).

Resistant bacteria are more present where antibiotics are used frequently, in healthcare settings, community, and food animal production (CDC, 2018). Antimicrobial resistance is caused by the

use, and misuse of antibiotics by humans and animals. Antimicrobials have been used in animal feed for about 70 years, not only to treat diseases, but also to promote growth, improve feed utilization and decrease mortality and thus to increase productivity (FAO, 2019). When food animals are slaughtered and processed, resistant bacteria can contaminate the meat and may also penetrate into the environment and spread through fruits and vegetables (CDC, 2018). *Salmonella spp.* and *E. coli* are the most commonly reported resistant bacteria in food (WHO, 2018). In the US, the Centers for Disease Control and Prevention (CDC) estimates that food is the source for about 1 million illnesses, 19 000 hospitalizations, and 380 deaths related to Salmonella spp. infections, and most of these infections are linked to ground beef (CDC, 2018). Moreover, raw or undercooked meat products were the main sources of *E. coli* outbreaks (Abbasi *et al*., 2014). Many studies conducted worldwide showed high contamination of *Salmonella spp*. and *E. coli* in beef meat (Shafini *et al*., 2017; Pond *et al*., 2016; Aslam *et al*.,2012; Zhang *et al*. 2016; Moawad *et al*., 2017; Osaili *et al*., 2014; Abbassi-Ghozzi *et al*., 2012; Abdissa *et al*., 2017). *Salmonella spp.* contamination in beef meat varied between countries, ranging between 0% in Canada and 47.5% in Lebanon.*E. coli* showed the highest prevalence in Lebanon 55% and the lowest in Jordan 1%. Bacterial contamination also varied between different meat cuts among various countries in which

up to 29.8% of whole beef showed to be contaminated with *Salmonella spp*. (Abbassi-Ghozzi *et al*., 2012; Shafini *et al*., 2017; Moawad *et al*., 2017; Pond *et al*., 2016; Aslam *et al*.,2012) and up to 10.7% *Salmonella spp.* contamination was recorded in ground beef contaminated (Abbassi-Ghozzi *et al*., 2012; Pond *et al*., 2016). Among recent studies *E. coli* contamination in beef was the highest in China (up to 39.2%), while in Mexico it was not detected neither in whole nor in

ground beef (Zhang *et al*. 2016; Pond *et al*., 2016)). In addition, some studies highlighted on the prevalence of pathogenic isolates among different retail channels (butchers, supermarkets and street vendors) where meat samples taken from butchers and street vendors showed to have higher prevalence of *Salmonella spp*. and *E. coli* contamination compared to those from supermarkets (Iyer *et al*., 2013, Pond *et al*., 2016).

Antimicrobial resistance of *Salmonella spp.* and *E. coli* was assessed worldwide and most studies reported their multidrug resistance, with high degree of resistance to a huge number of antimicrobials that are also used for human treatment (Aslam et., al 2012, Domenech et., al 2015, Zhang *et al*., 2016, Moawad *et al*., 2017, Osaili *et al*., 2014, Abbassi-Ghozzi *et al*., 2012).

Many regulations worldwide were developed to combat the use of antibiotics for non-treatment purposes (EU, 1998; FDA, 2013; UN, 2016; WHO, 2016; FAO, 2016; OIE, 2016). In 1998, the European Union (EU) banned the use of antibiotics in farm animal for non-therapeutic purposes (Donovan *et al*., 2015). In 2001, it launched a plan to fight antibiotic resistance, and in 2002 it created the European Food Standards Agency (EFSA) that asses food and feed safety risks (Donovan *et al*., 2015). In 2013, the Food and Drug Administration (FDA) implemented a plan to resolve the use of antibiotics as growth promoters (Donovan *et al*., 2015). Moreover, in 2016 the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Organization for Animal Health (OIE) developed a tripartite partnership "One Health" a global action plan against antimicrobial resistance (FAO, 2016). Furthermore, in 2016 the United Nations (UN) arranged a high-level meeting in the US to combat the use of antibiotics worldwide (UN, 2016). In Lebanon, the Ministry of Agriculture prohibited the import of certain veterinary drugs (Ministry of Agriculture Decisions No. 1/275 of 20 June 1997 and No. 1/94 of

20 May 1998). The National Antibiotic Resistance Committee at the Lebanese Ministry of Public Health has been working for more than a decade to develop an action plan to combat the emergence of antibiotic resistance. However, the taken measures remain ineffective in controlling antibiotic resistance in Lebanon (Salameh *et al*., 2017). This study aims to assess the prevalence of pathogenic bacterial isolates *Salmonella spp*. and *E. coli* in fresh and frozen red meat as well as meat organs collected from different supermarkets and butcher shops across Mount Lebanon and further evaluate their antimicrobial resistance.**.**

## 2.2. Materials and methods

### 2.2.1. Materials

The media including MacConkey agar, sorbitol MacConkey agar, Salmonella-Shigella agar (SS) agar, the Mueller Hinton and the brain heart infusion agar (BHIA) were all purchased from HIMEDIA laboratories. All the antibiotics including oxacillin 1 µg, teicoplanin 30 µg, sulpha / trimethoprim 25µg, gentamicin 50 µg, clindamycin 2 µg, cefotaxime 30 µg, cefuroxime 30 µg, erythromycin 15 µg**,** vancomycin 30 µg, and ampicillin 10 µg were also obtained from HIMEDIA laboratories. Ethanol was obtained from Merk & Co company, and deionized water was produced using the deionizer (LABCONCO).

### 2.2.2. Sample collection

A total of 80 beef meat samples, including 19 from fresh ground beef, 61 from frozen meat with 23 being ground, 26 steak samples, 9 liver and 3 kidneys samples were collected between February 2017 and December 2018. Samples were collected from butchers and supermarkets in

Mount Lebanon regions in collaboration with the Lebanese Ministry of Economy and Trade. All the samples were transferred in a cooler. The frozen meat samples were stored at -20°C for further analysis while fresh samples were analyzed the same day.

### 2.2.3. Bacterial isolation

Bacterial isolation procedure was conducted according to American Public Health Association (APHA, 2001). A portion of 25 g meat was weighed in a sterile stomacher bags diluted with 225 ml sterilized peptone water (0.1% w/v), and macerated for 3 min, using a stomacher (MiniMix, Interscience, France). Each sample was then diluted by adding 1 ml of the homogenate by using sterile stomacher pipets to 9 ml sterilized peptone water (1:10 dilution factor). For isolation, 0.1 ml of each dilution was plated on agar plate for bacteriological analysis in antiseptic condition. The plates were named according to the bacteria being studied, the date of isolation, and media used in each plate, in order to make sure no mistake is allowed. Then the plates were incubated at 37ºC for 48 h, (Sorbitol MacConkey agar MCA for *E-coli* detection, Bismuth sulfate agar and SS agar for *Salmonella spp*. detection). Purple colonies on sorbitol MacConkey agar (MCA) were identified as *E. coli*. white colonies on SS were identified as *Salmonella spp*., and brown to black colonies were identified visually as *Salmonella. Typhi* (APHA, 2001).

### 2.2.4. Biochemical identification

Freshly isolated strains *Salmonella spp*. and *E. coli* were biochemically identified using the API method by using BioMérieux's API kits (France). Incubation tray and lid were prepared ,5ml of sterile distilled was placed into the tray to create a humid chamber, where the API 20E strips

were placed. Single colony of each bacteria was carried by a sterile loop from the culture and was diluted in 5ml API suspension and then the diluted bacterial suspension was inoculated in each well by using a sterile pipet. Mineral oil was added to the wells ADH, LDC, ODC, $H_2S$, and URE to create anaerobic medium. The trays were covered by the lids, named, and incubated for 24 hours. Results were interpreted using the API reading scale (color chart). Specific reagents were added to TDA, VP, and IND wells before reading. Results were recorded on the report sheets provided by the kit. In the report sheet, the tests are separated into groups of three and numbers 1, 2 or 4 is marked for each test corresponding to the positive result. Group, a7- digit profile number is obtained for 20 tests of the API 20E strip. The 7- digit profile is then compared to the numerical profile in the API 20 E analytical profile index book to obtain the organism identification.

**2.2.5. Antimicrobial susceptibility testing**

Isolated strains were tested for their antimicrobial susceptibility to 10 antimicrobials using the disk diffusion method as set by the National Committee for Clinical Laboratory standards (NCCLS, 1997). The organisms were grown in a shaking water bath at 37ºC until 0.5 McFarland turbidity standard was obtained. Of the culture, 0.1 ml was spread over BHIA plates. Antimicrobial disks impregnated with either of the following antimicrobials, oxacillin 1 µg, teicoplanin 30 µg, sulpha / trimethoprim 25µg, gentamicin 50 µg, clindamycin 2 µg, cefotaxime 30 µg, cefuroxime 30 µg, erythromycin 15 µg**,** vancomycin 30 µg, and ampicillin 10 µg were placed on the surface of inoculated agar plates. The zones of inhibition around each microbial disk were measured after incubation period of 24 h at 37ºC. Each organism was classified as

susceptible, sensitive, intermediate or resistant to the antimicrobials, based on NCCLs guidelines. Intermediate-resistant and resistant strains were pooled together.

### 2.2.6. Data analysis

IBM SPSS Statistics version 22 was used to determine the prevalence of bacterial distribution between different meat types and retail channels. Descriptive statistics were used to assess the prevalence of bacteriological contamination of *Salmonella spp.* and *E. coli* in different meat types. Chi square test was used to compare bacteriological distribution of *Salmonella spp.* and *E. coli* between fresh and frozen ground meat, and between the samples collected from different butchers and supermarkets across Mount Lebanon.

## 2.3. Results and Discussion

### 2.3.1. Bacterial Contamination of Salmonella spp. and E. coli in different meat types

The assessment of the contaminated meat samples was conducted (Table 2.1). The results showed that among the collected samples 57.7% were contaminated with *Salmonella spp.* and 72.5% with *E. Coli*.

Previous study conducted in Lebanon were somehow similar in which 55% of the tested Lahm b-ajeen (meat pie) and shawarma (shredded and cooked meat) samples (n=95) were contaminated with *E. coli* and 47.4% of them were contaminated with *Salmonella spp.* (Harakeh *et al*., 2005). Worldwide studies showed different results related to *Salmonella spp*. prevalence in beef meat. The prevalence of *Salmonella spp.* in beef meats in the most recent studies ranged between 4.4% and 28.9% (Abbassi Ghozzi *et al*., 2012; Moawad *et al*., 2017; Shafini *et al*., 2017; Aslam *et*

*al*.,2012; Pond *et al*., 2016). Moreover, many recent studies conducted worldwide reported the prevalence of *E. coli* in beef meat ranged between 4.5% and 39.2% (Zhang *et al*., 2016; Abdissa *et al*., 2017; Moawad *et al*., 2017). In contrast to our findings, *Salmonella spp.* and *E. coli* were not detected in raw beef samples in Alberta Canada (Aslam *et al*., 2012). In Jordan as well, meat samples had only (1% to 2%) *E. coli* and 0.5% *Salmonella spp*. contamination (Osaili *et al*., 2014). This low contamination might be related to following the principles of meat hygiene applied to primary production set by the Codex Alimentarius stating that primary production should be managed in a way that reduces the likelihood of introduction of hazards and appropriately contributes to meat being safe and suitable for human consumption. In addition, Good hygienic practice (GHP) at the level of primary production that involves the health and hygiene of animals, treatments records, feed and feed ingredients and relevant environmental factors, and should include application of HACCP principles to the greatest extent practicable (CAC/RCP 1-1969). We suggest that bacterial contamination of red meat with *Salmonella spp.* and *E. coli* might be related to handling issues during slaughtering and processing and to the absence of Good Hygiene Practices (GHP) that also may lead to the transfer of bacteria to clean meat by cross contamination. The meat samples in this study were more contaminated with the studied bacteria as compared to previous study conducted also in Lebanon because the previously assessed meat based fast food were studied after being cooked. We suggest that this might be related to cooking that decreases the bacterial contamination by killing them. Although cooking processes at 60.0°C or higher inactivate *Salmonella spp*. and *E. coli*, (McMinn *et al*., 2018), Harakeh *et al*. (2005) highlighted a very important issue related to Lebanese food. Even though the Lebanese food are cooked some bacteria may survive, stressing on the importance of

managing meat production to reduce the likelihood of biological hazard and come up with safe meat product, suitable for human consumption.

### 2.3.2. Salmonella spp. and E. coli in Fresh and Frozen meat samples

Table 2.1. aslo showed that all the fresh minced meat samples (100%) were contaminated with *E. coli* and 78.9% with *Salmonella spp.* On the other hand, fresh minced meat was significantly more contaminated with E. coli (100%) and Salmonella (78.9%) as compared to the frozen ones (65.2 and 60.8% with respective p values of 0.021 and 0.026). The Ad hoc analysis also showed that the prevalence of contamination with *E. coli* was similar in all the frozen samples types. In addition, the analysis showed that the prevalence of contamination of organs with *Salmonella spp*. were significantly lower than the other meat types.

Similar results were reported in Egypt, fresh meat samples showed higher prevalence of *Salmonella spp*. (11.6%) and *E. coli* (6.6%) as compared to frozen meat where 3.3% were contaminated with *Salmonella spp*. and 1.3% with *E. coli* (Mouawad *et al*, 2017). Fresh samples higher contamination might be related to their storage conditions in unappropriated temperature (danger zone) favoring bacterial multiplication. Low temperature might affect bacterial growth and results in the reduction of healthy pathogens *Salmonella spp*. and *E. coli* at (− 22 °C) frozen storage (Manios *et al*., 2015). Temperatures below 5°C (41ºF) can retard bacterial growth and multiplication (FAO, 2017).

Contamination in different meat cuts was assessed (Table 2.1). In which frozen liver meat samples showed the highest prevalence of *E. coli* (66.6%) and *Salmonella spp.* (22.2%), while the kidneys samples showed the lowest prevalence of *Salmonella spp*. (33%) and *E. coli* (33.3%)

contamination. *Salmonella spp*. and *E. coli* prevalence in frozen ground beef was (60.8%) and (65.2%) respectively and in frozen steak *Salmonella spp*. prevalence was (53.8%) and *E. coli* (65.3%).

In Egypt, in contrast to our findings, the prevalence of *E. coli* (4%) and *Salmonella spp*. (4%) was low in frozen liver. These results show that there is no fecal contamination of the carcasses and their organs in slaughter houses, which is the main source of *Salmonella spp.* and *E. coli* contamination. Indicating a proper sanitary environment and good hygienic practices under which the animals are slaughtered, in addition to good handling and product preparation during sale to consumers (Kirrella *et al*.,2017).All these factors help lessen the contamination. In Korea, different results were recorded. In edible cattle offal (liver, kidneys, and heart), *Salmonella spp*. was present in 7.1% of the samples while *E. coli* was not detected (Im *et al*., 2016). Different results were reported in Tunisia, where the prevalence of *Salmonella spp*. was 10.7% in ground beef (n=56) and 28.9% in whole beef (n=144) (Abbassi-Ghozzi *et al*., 2012). In Mexico, *Salmonella spp*. was 7.3% in ground beef and 4.4% in whole beef (Pond *et al*., 2016). The results in this study indicated high prevalence of *Salmonella spp*. and *E. coli* in Liver and ground beef compared to the other meat cuts. These findings could be due to the meat exposure to more microorganisms due to poor hygienic practices during slaughtering such as skinning, scalding, evisceration, and carcass that are common contamination points (FAO, 2019), caused by food handlers during processing and preparation, and also through instruments such as cutting boards, cutting and grinding machines, and all other related materials used for preparation until it reaches the consumer (Kirrella *et al*., 2017). In addition, meat spoilage bacteria will grow if

temperatures are not kept in the cooling (-1°C to +4°C) or freezing (below -1°C) range (FAO, 2019).

Table 2.1 Bacterial Contamination of Salmonella spp. and E. coli in different meat types [a]

| | Meat type | Total | E. coli | | Salmonella spp | |
|---|---|---|---|---|---|---|
| | | N | n | % | N | % |
| Fresh | Minced | 19 | 19 | 100b | 15 | 78.9b |
| Frozen | Minced | 23 | 15 | 65.2a | 14 | 60.8a |
| | Steak | 26 | 17 | 65.3a | 14 | 53.8a |
| | Organs | 12 | 7 | 58.3a | 3 | 42.9b |
| | Total | 80 | 58 | 72.5 | 46 | 57.7 |

Bacteriological analysis was done according to American Public Health Association (APHA, 2001)

**The letters a, and b refer to the results obtained by the ad hoc analysis.

### 2.3.3. Salmonella spp. and E. coli in red meat collected from butchers and supermarkets in Mount Lebanon

Table 2.2. showed the prevalence of bacterial contamination of samples collected from butchers and supermarkets. The results showed that the samples collected from supermarkets were statistically more contaminated with *Salmonella spp*. as compared to those obtained from butchers with prevalence of 76.9% and 47.0% respectively (p=0.041). Meat samples collected from supermarkets in Mount Lebanon showed high prevalence of *E. coli* (80.7 %) compared to butcher shops (66.6%) but these results are not statistically significant (p value 0.235).

In contrast to these findings, in Mexico and Saudi Arabia, samples obtained from open butcher shops were found to have higher prevalence of *Salmonella spp*. and *E. coli* as compared to

supermarkets (Pond *et al*., 2016; Iyer *et al*., 2013). In Mexico, *Salmonella spp*. prevalence in whole beef was 9.1% and in ground beef 4.2% ($p < 0.01$) (Pond *et al*., 2016). In Jeddah Saudi Arabia significant difference was found associated with the presence of *Salmonella spp.* and *E. coli* in meats taken from butcher shops compared to that from supermarkets ($p < 0.05$). *Salmonella spp*. prevalence was 45% in meat samples from butcher shops compared to 5% in those collected from supermarkets. *E. coli* was prevalent in 65% of meat samples from butcher shops and 20% in meat samples from supermarkets. The different results can be attributed to poor hygienic practices in the Lebanese supermarkets and improper sanitation during meat processing and packaging. This might also be related to the storage conditions that contribute to bacterial growth, which suggests improving safety and sanitation practices and more training hours for the workers in order to reduce the levels of contamination.

Table 2.2. Bacterial contamination of Salmonella spp. and E. coli in red meat collected from butcher shops and supermarkets in Mount Lebanon

| Meat source | Total | E. coli | | Salmonella spp | |
|---|---|---|---|---|---|
| | n | n | % | N | % |
| Butcher | 52 | 34 | 66.6 | 24 | 47.0 |
| Supermarket | 28 | 21 | 80.7 | 20 | 76.9 |
| Total | 80 | 55 | 71.4 | 44 | 57.1 |

[a] Bacteriological analysis were done according to American Public Health Association (APHA, 2001)

Table 2.3. Bacterial contamination of Salmonella spp. and E. coli of Fresh and Frozen meat samples [a]

| Meat type | Total | Total Positive | | E. coli | | Salmonella spp | |
|---|---|---|---|---|---|---|---|
| | n | n | % | N | % | N | % |
| Fresh minced | 19 | 19 | 100 | 19 | 100 | 15 | 78.9 |
| Frozen minced | 23 | 15 | 65.2 | 15 | 65.2 | 14 | 60.8 |
| Total | 42 | 34 | 80.9 | 34 | 80.9 | 29 | 69.0 |

[a] Bacteriological analysis were done according to American Public Health Association (APHA,2001)

### 2.3.4. Biochemical identification

Isolated pathogens were randomly subjected to biochemical tests by using the API method from (BioMérieux's - France) for further identification. The results indicated that all of the 20 tested isolates were oxidase negative. According to the API software the identified organisms were *Salmonella spp., and E. coli*. *Serratia marcescens*, *Serratia odorifera*, *Serratia liquefaciens*. These results confirm that the tested isolates are *Salmonella spp*. and *E. coli.* However, we suggest that the identification of other pathogens could be related to the time delay between the isolation and the identification procedure.

### 2.3.5. Antimicrobial susceptibility testing

In the current study antimicrobial susceptibility of 46 *Salmonella spp*. and 58 *E. coli* isolates was assessed using the agar disk diffusion method tables (Table 2.5, 2.6). Both *Salmonella spp*. and *E. coli* isolates were 100% resistant to Oxacillin, Clindamycin, Erythromycin, Teicoplanin, and Vancomycin. Of the *Salmonella spp*. isolates, 50% showed resistance to Ampicillin and 30% to Cefuroxime. On the other hand, 75% were susceptible to Gentamicin, 91 % to Co-Trimaxazole and 56% to cefotaxime. Of the *E. coli* isolates 58% showed resistance to Ampicillin and 30% to Cefuroxime, 90% were susceptible to Co-Trimaxazole, 70% were susceptible to Gentamicin, and 50% to Cefotaxime.

Similar to our results, multidrug resistant bacteria were recorded in Spain, China, Canada, Jordan, Egypt, Morocco, South Italy and Lebanon in previous studies (Domenech *et al*., 2015; Zhang *et al*., 2016; Aslam et. Al., 2012; Osaili *et al*., 2014; Moawad *et al*., 2017; Amajoud *et al*., 2017; Nobili *et al*., 2017; Harakeh *et al*., 2005). In Korea, 50 % of *E. coli* isolates showed high resistance to tetracycline (Park *et al*., 2015). In South Italy, *E. coli* resistance was most frequently observed to amoxicillin-clavulanic acid (100%), ampicillin (100%) and tetracyclin (80%) (Nobili *et al*., 2017). Moreover, in Egypt, *E. coli* strains were found to be highly resistant to ampicillin (71.4%) (Moawad *et al*., 2017), while in China, moderate resistance to ampicillin (48.2%) were recorded (Zhang *et al*., 2016; Zhu *et al*., 2016). In contrast to our findings in China, Egypt and Eastern Spain, *E. coli* isolated from beef meat showed to have high resistance towards Gentamicin and Trimaxazole (sulpha/Trimethoprime) (Zhang, *et al*., 2016, Moawad *et al*., 2017, Domnech *et al*., 2015).

Not far from our results, in Egypt, and Jordan, *Salmonella spp*. showed high resistance towards ampicillin (77.1%, 60%, respectively) (Moawad *et al*., 2017; Osaili *et al*., 2014). In contrast to our findings, in Tunisia, *Salmonella spp*. strains showed low resistance against ampicillin 16% (Abbassi-Ghozzi *et al*., 2012). Moreover, in China, Egypt and Eastern Spain, *Salmonella spp.* showed high resistance towards Gentamicin and Trimaxazole (sulpha/Trimethoprime) (Zhang, *et al*., 2016; Zhu *et al*., 2016; Moawad *et al*., 2017Domnech *et al*., 2015).

Different results were recorded in Canada (29%) and in Morocco (60.4%) of Salmonella *spp.* isolates were susceptible to all tested antimicrobials, while in Tunisia no resistance of *Salmonella spp.* strains in lamb meat or ground meat was detected (Aslam *et al*., 2012; Amajoud *et al*., 2017; Abbasi-Ghozzi *et al*., 2012). This might be related to the strict compliance to the laws set by the Codex Alimentarius and the application of the principles of meat hygiene to primary production, which reduces the likelihood of introduction of hazards and appropriately contributes to meat being safe and suitable for human consumption, in addition to the application of HACCP principles to the greatest extent practicable (CAC/RCP 1-1969). As well as the application of hygiene of the feed and feed ingredients principle, stating that animals should not be given feeds containing chemical substances, (e.g. veterinary drugs) or contaminants that could result in residues in meat at levels that make the product unsafe for human consumption (CAC/GL 71-2009).

Similar results were also reported in a previous study conducted in Lebanon, in which *Salmonella spp*. was (100%) resistant to Oxacillin, Clindamycin, Erythromycin, Vancomycin, and Teicoplanin. In contrast to our findings high resistance was reported towards Co-Trimaxazole (86%), followed by Cefotaxime (25%) and moderate susceptibility was recorded

towards Gentamicin and Cefuroxime (57.1%) (Harakeh *et al*., 2005). We suggest that this could be related to decreasing the usage of Gentamicin, Co-Trimaxazole and Cefotaxime in the agricultural field leading to increase in susceptibility.

Similar to our findings, *E. coli* isolates in meat-based food in Lebanon showed (100%) resistance against Teicoplanin. Not far from our results (88.9%) of the isolates were found to be resistant to Oxacillin, Clindamycin, Erythromycin and Vancomycin but less resistant compared to our findings that showed 100% to these antibiotics. In contrast to our findings where 30% of *E. coli* isolates showed resistance towards Cefuroxime, 55.6% of *E. coli* isolates were susceptible towards it in a previous study. Of the *E. coli* isolates 77.8% were susceptible to Cefotaxime, according to our findings this susceptibility decreased to 50% same for Gentamicin susceptibility that decreased from 100% to 90%. We suggest that there is a change in the resistance patterns of *E. coli* due to transferable genes in the environment making it more resistant to some antibiotics. We recommend the development of national action plans on anti-microbial resistance to raise the awareness to combat the use of antibiotics in the agricultural filed for non-therapeutic purposes.

Table. 2.4. Antimicrobial resistance pattern of Salmonella spp.

| | | | Resistance of *Salmonella spp.* to antibiotic [a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | Susceptible | Sensitive | Intermediate | Resistant | Total |
| Antibiotic used | Ampicillin 10 mcg | Count | 17 | 6 | 0 | 23 | 46 |
| | | %within antibiotic used | 37% | 13% | 0 | 50% | 100% |
| | Oxacillin 1mcg | Count | 0 | 0 | 0 | 45 | 45 |
| | | %within antibiotic used | 0% | 0% | 0% | 100% | 100% |
| | Cefotaxime 30mcg | Count | 26 | 0 | 14 | 6 | 46 |
| | | %within antibiotic used | 56% | 0% | 30% | 13% | 100% |
| | CoTrimaxazole(sulpha/Trimethoprime) 25mcg | Count | 42 | 0 | 0 | 4 | 46 |
| | | %within antibiotic used | 91% | 0% | 0% | 9% | 100% |
| | Cefuroxime 30mcg | Count | 13 | 5 | 14 | 14 | 46 |
| | | %within antibiotic used | 28% | 11% | 30% | 30% | 100% |
| | Clindamycin 2mcg | Count | 0 | 0 | 0 | 46 | 46 |
| | | %within antibiotic used | 0% | 0% | 0% | 100% | 100% |
| | Erythromycin 15mcg | Count | 0 | 0 | 0 | 46 | 46 |
| | | %within antibiotic used | 0% | 0% | 0% | 100% | 100% |
| | Gentamicin 50mcg | Count | 33 | 0 | 0 | 11 | 44 |
| | | %within antibiotic used | 75% | 0% | 0% | 25% | 100% |
| | Teicoplanin 30mcg | Count | 0 | 0 | 0 | 46 | 46 |
| | | %within antibiotic used | 0% | 0% | 0% | 100% | 100% |
| | Vancomycin 30mcg | Count | 0 | 0 | 0 | 46 | 46 |
| | | %within antibiotic used | 0% | 0% | 0% | 100% | 100% |
| Total | | Count | 131 | 11 | 28 | 287 | 501 |
| | | %within antibiotic used | 26% | 2% | 5.5% | 57.2% | 100% |

[a] *Salmonella spp. isolates* were tested for their susceptibility to nine antimicrobials, using the disk diffusion method as set by the NCCL guidelines.

Table 2.5 Antimicrobial resistance pattern of *E. coli.*

| | | | Resistance of E. coli to antibiotic (%) [a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | Susceptible | Sensitive | Intermediate | Resistant | Total |
| Antibiotic used | Ampicillin 10 mcg | Count | 19 | 1 | 3 | 32 | 55 |
| | | %within antibiotic used | 34.5% | 2% | 5% | 58% | 100% |
| | Oxacillin 1mcg | Count | 0 | 0 | 0 | 55 | 55 |
| | | %within antibiotic used | 0% | 0% | 0% | 100% | 100% |
| | Cefotaxime 30mcg | Count | 28 | 1 | 17 | 9 | 55 |
| | | %within antibiotic used | 50% | 2% | 31% | 16.3% | 100% |
| | Co-Trimaxazole(sulpha/Trimethoprime) 25mcg | Count | 39 | 1 | 0 | 15 | 55 |
| | | %within antibiotic used | 70% | 2% | 0% | 27% | 100% |
| | Cefuroxime 30mcg | Count | 9 | 1 | 34 | 11 | 55 |
| | | %within antibiotic used | 16% | 2% | 62% | 20% | 100% |
| | Clindamycin 2mcg | Count | 0 | 0 | 0 | 55 | 55 |
| | | %within antibiotic used | 0% | 0% | 0% | 100% | 100% |
| | Erythromycin 15mcg | Count | 0 | 0 | 0 | 55 | 55 |
| | | %within antibiotic used | 0% | 0% | 0% | 100% | 100% |
| | Gentamicin 50mcg | Count | 5 | 0 | 0 | 5 | 55 |
| | | %within antibiotic used | 90% | 0% | 0% | 10% | 100% |
| | Teicoplanin 30mcg | Count | 0 | 0 | 0 | 55 | 55 |
| | | %within antibiotic used | 0% | 0% | 0% | 100% | 100% |
| | Vancomycin 30mcg | Count | 0 | 0 | 0 | 55 | 55 |
| | | %within antibiotic used | 0% | 0% | 0% | 100% | 100% |
| Total | | Count | 100 | 4 | 54 | 292 | 550 |
| | | %within antibiotic used | 18% | 1% | 10% | 53% | 100% |

[a] *E. coli* isolates were tested for their susceptibility to nine antimicrobials, using the disk diffusion method as set by the NCCL guidelines.

## 2.4. Limitations

The author is aware of the limitations in this study. We highlighted the high prevalence of pathogenic resistant strains *Salmonella spp.* and *E. coli* in meat beef. However, biochemical identification showed the prevalence of additional pathogens and this could be related to the time delay between the isolation and identification procedure. Thus, further molecular identification of bacterial strains is to be studied in addition to antimicrobial susceptibility to more antibiotics that are widely used in the medical and agricultural field. Moreover, this study was conducted in Mount Lebanon that might limit its generalization. Further research that covers Lebanon is needed. In addition, we didn't study bacterial count due to lag in time between meat collection and analysis. In this study we used antibiotics that are used for gram positive bacterial treatment in contrast to the studied bacteria treatment and this is because we followed what previous studies did.

## 2.5. Conclusion

Several antibiotics that were used worldwide as growth promoters have been banned. In Lebanon the use of these antimicrobials is not well controlled. In this study, we discussed the resistance patterns of *E. coli* and *Salmonella spp.* strains in beef meat products. The high and multi drug resistant of these bacterial strains and their increasing resistance to some antibiotics reveals the importance of controlling the use of antibiotics to limit the emergence of multidrug resistant bacteria. The results of this study showed high prevalence of *Salmonella spp*. and *E. coli* as food borne pathogens isolated from fresh and frozen beef meat and meat cuts in butcher shops and supermarkets in Mount Lebanon. Antimicrobial resistance of *Salmonella spp*. and *E. coli* of

public concern in Lebanon and high resistance was detected towards Oxacillin, Clindamycin, Erythromycin, Teicoplanin, Vancomycin, ampicillin and Cefuroxime. Our results highlight the obligatory need for the implementation of strict food safety regulations that should be monitored by regulatory agencies to assure food safety from farm-to-fork and the application of safety assurance programs such as HACCP in preparation and processing of food that reduce the risk of infection. In addition, creating public health awareness is highly needed in order to combat the misuse and over use of antibiotics including incorrect diagnosis, and incorrect consumption. It is also recommended to cook meat properly especially when it is prepared for vulnerable people such as the elderly, pregnant women, and the kids.

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