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EVALUATION OF THE MICROBIOLOGICAL WATER QUALITY IN RIVERS ACROSS LEBANON BY ASSESSING DENSITIES OF FECAL INDICATORS AND ANTIBIOTIC RESISTANCE PROFILES OF *ESCHERICHIA COLI*

by
LEA AKRAM DAGHER

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Evaluating Microbiological Water Quality in Rivers Across Lebanon by Assessing Densities of Fecal Indicators and Antibiotic Resistance Profiles of *Escherichia coli*

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AN ABSTRACT OF THE THESIS OF

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Title: Evaluation of Microbiological Water Quality in Rivers across Lebanon by Assessing Densities of Fecal Indicators and Antibiotic Resistance Profiles of Escherichia coli.

Emerging microbial agents and increasing antibiotic resistance in aquatic systems pose a global concern worldwide. However, in. Lebanon, limited data on fecal pollution, bacterial indicators, and associated antibiotic resistance in rivers are available. The objective of this study was to evaluate the microbiological quality of rivers across Lebanon by assessing the densities of fecal indicators and antibiotic resistance profiles of Escherichia coli. For this purpose, 135 freshwater samples were collected from 14 major rivers in Lebanon. Results showed that 129 samples yielded E. coli colonies and 130 yielded fecal coliforms. Overall, the highest counts of fecal indicators were detected in Beirut river and Abou Ali river in the North. Three E. coli were isolated from each sample and subjected to disk diffusion assay. Therefore, ~ 388 E. coli were isolated from the 135 freshwater samples. Antimicrobial susceptibility analysis resistance showed that the E. coli isolates were resistant to penicillin (100%), ampicillin (40%), cefalexin (46%), amoxicillin-clavulanic acid (40%), cefepime (4%), cefotaxime (14%), cefixime (17%), Doripenem (0.3%), imipenem (1%), meropenem (0.3%), gentamicin (5%), kanamycin (9%), streptomycin (35%), tetracycline (34%), ciprofloxacin (11%), norfloxacin (8%), trimethoprim-sulfamethoxazole (31%), and chloramphenicol (13%). In addition, carbapenem resistance was detected in the E. coli isolated from the North of Lebanon (0.3%). 45.3% of the isolates (176 out of 388) were multi-drug resistant, and the highest numbers were in Beirut (61.1%) and Beqaa (48.4%). The high numbers obtained suggest that a proper treatment of wastewater before discharge into rivers and the control of use of antibiotics are a must to prevent the dissemination of antibiotic-resistant bacteria.

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To My
Beloved Family

CHAPTER I

INTRODUCTION

Water is a vital need for all living organisms. Whether it is *used* in households for drinking, cooking or washing, or in farms for growing crops and rearing animals, or in industries, usable water is a key for surviving and its importance can never be overemphasized. According to the WHO, half of the world's population will be living in water-stress by 2025. The increasing population growth, urbanization and industrialization increase water demand. But what if water is available, and is not safe? Water safety is a crucial point especially for children, elderly and immunosuppressed individuals, which are more prone to infections than others. The WHO estimates “about 2.1 billion people worldwide who lack access to safe drinking water, and twice that many lack adequate sanitation”. Polluted water has been affecting people causing large number of illnesses throughout the world ranging from diarrhea and fever to hepatitis A, typhoid, and polio.

Water pollution is the contamination of water bodies, such as lakes, rivers, ocean or groundwater by chemical and microbiological pollutants. Mainly rivers and groundwater provide people, animals and plants with drinking water, and it is essential for irrigation, domestic, and industrial uses. For this, the quality of freshwater and the sources of contamination have been deeply studied. First of all, agriculture which accounts for 70 percent of water abstractions, is a major source of water pollution. When it comes to agricultural practices, pesticides, herbicides and fertilizers have been extensively used as a stabilizer in soil or to kill insects affecting the crops. Some residues remain in the soil and may leach into subsurface waters, they can dissolve in runoffs or be adsorbed to sediments (Agrawal, Pandey and Sharma, 2010). A study conducted in India highlighted the presence of pesticides residues of organochlorine and organophosphorus above the permissible limits specified by European Economic Community in bottled water from 17 different brands. Most of the companies use bore-wells to pump out water from the ground. Repeated exposure to these chemicals can result in chronic effects such as kidney damage, birth deficiency and nervous system disorders (Agrawal *et al.*, 2010). Sources of water contamination include mainly agricultural runoffs, industrial waste, domestic, animals and sewage waste.

Agricultural practices which rely on *pesticides, insecticides, and fertilizers* can also affect plants and marine life. The previous study done in India revealed the presence of pesticide residues in the plants, which can be toxic to plants (Agrawal *et al.*, 2010). Another study revealed that pesticides and chemicals were responsible for more than 80% of the exceeded acute risk problems related to three major organisms: fish, invertebrates and algae. Despite the extensive regulation of pesticides use and banning of toxic herbicides and insecticides, chemical assessment revealed that harmful compounds such as organotin and polycyclic aromatic hydrocarbons have been found in water resources (Malaj *et al*., 2014).

Industrial activities are also responsible of toxic chemical discharge into rivers and can carry the excess of chemicals run off into the waterways affecting marine organisms. Moreover, the testing of samples from the largest rivers in Delhi such as Yamuna river, Ganges and Godavari, which provide drinking and irrigation water to the capital's population showed that the fresh water quality deteriorated due to discharge of untreated sewage, agricultural waste and industrial effluents mainly plasticizers, phenols, oils, pesticides and suspended solids. One of the rivers lost over 100 crocodiles in 72 days due to the release of toxins in the river. The sources of the toxin were not clarified. Another aspect of chemical water pollution source includes the olive mills water waste dumping into rivers. High quantities of organic loads and pollutants like phenols, nitrogen and trace metals (Fe, Cr, Pb, Cu…) are detected and could cause harm to aquatic life (Pavlidou *et al*., 2014).

Moreover, water pollution was evaluated in Langat River which is a major drinking water source in Malaysia. The Biochemical Oxygen Demand, the levels of nitrate and suspended total solids were studied in different point sources of pollution and sectors surrounding the river including manufacturing industries, sewage treatment plants, animal farms, non-industrial markets and food service establishment. The study revealed that the high load of ammoniacal nitrogen was mainly from sewage treatment plants (78.4%), while industries contributed to 3.5% of the total nitrogen leaching. The high concentration of this compound generally originates from landfills and open dumping sites discharging in the river. Pollution of river due to the food service establishment was high because of additive chemicals (Farid *et al*., 2016). Thus, an inadequate management of wastewater coming from urban, industrial and agricultural activities lead to the contamination of water.

Water pollutants include mainly metals, organic waste, but also *pathogenic microorganisms.* Pathogens can survive in all water sources: surface waters which are essential for humans, animals and plants; recreational water; and wastewater which includes agricultural, industrial, domestic and sewage waste. Pathogen contamination including bacteria, protozoa and viruses poses a significant risk. In fact, the EPA's National Water Quality Inventory report suggested that around 53% of the assessed rivers in the US are contaminated by pathogens (Pandey, Biswas and Singh, 2014). These pathogens can lead to serious infections in humans. A report revealed that more than 403,000 residents of the greater Milwaukee suffered from gastrointestinal illnesses such as abdominal cramps, fever, watery diarrhea and vomiting due to exposure to water from Lake Michigan contaminated with the parasite, *Cryptosporidium parvum* (Mac Kenzie *et al.*, 1994).

Rivers are also essential for *recreational* purposes, and people exposed to contaminated water can get infected. Recently, Shiga-toxin producing *E. coli* have been detected in a stream in the United States after a report of *E. coli* O157:H7 infection among children in a park. These pathogens can have serious effects on humans, including kidney failure and anemia. Water samples were collected from the stream after the outbreak, and the microbiological tests confirmed the presence of high levels of *E. coli* and enterococci, which exceeded the Environmental Protection Agency (EPA) standards for recreational water quality. The genes responsible for the production of Shiga toxin (*stx1* and *stx2*) were also detected, and the wildlife was considered as the major source of dissemination of the bacteria with the absence of anthropogenic practices in the area (Fournier *et al*., 2005). *E. coli* is not only present in polluted rivers, but can also survive in river waters for more than 260 days at 25^{o}C (Flin, 1987).

Not only pesticides and fertilizers are used in such practices and persistent in water, but also *antibiotics* which are commonly used in clinical settings, are given to animals during agriculture practices (Economou and Gousia, 2015). Antimicrobial agents have been administrated to animals including poultry, swine, and cattle for growth promotion, for prevention or treatment of bacterial infections. In fact, the farming industry is regarded as the largest consumer of antibiotics worldwide. Estimated use of antibiotics in the US only for growth supplement and for infections control in animals is about 80 percent (Peter Hughes, 2004; Aslam *et al*. 2018). In poultry and cattle production, veterinarians rely on two treatments: in case of infectious disease, the whole flock is treated with antibiotics at high doses to prevent the spread of the infection (known as metaphylaxis); whereas the prophylaxis treatment consists of the administration of low doses of antibiotics in the feed for a longer period of time as a prevention from diseases (Economou and Gousia, 2015). Moreover, antimicrobials such as penicillin and tetracycline have been successfully used as growth promoters. Therefore, bacteria that can contaminate rivers have developed resistance against antibiotics due to the heavy use of those agents.

A study conducted in a river in Pune District India in 2018, showed a rise of *E. coli* in major drinking water sources, with 28% resistance to more than six antibiotics and 12% resistance to eight antibiotics, including ampicillin, cefepime, cefotaxime, gentamicin, imipenem and piperacillin/tazobactam. The study highlighted the consequences of the malfunctioning of the Pune wastewater treatment plants on water's quality. Moreover, the heavy load of antibiotic-resistant bacteria has been related to the high prescription of the antibiotics cefixime, ciprofloxacin and tinidazole in combination and the amoxicillin–clavulanic acid in clinics in Prune city (Dhawde *et al*., 2018). Another study evaluated the antibiotics levels in a river flowing though industrial and crowded urbanized areas in China. Out of 20 antibiotics, eleven were detected at high levels including chloramphenicol, sulfonamides, and macrolides, mainly close to the sewage effluent. Consequently, freshwater can be a reservoir of antibiotic-resistant bacteria. In fact, multi-drug resistant bacteria were detected in major rivers in India, South Africa, and Malaysia, as a result of untreated sewage which is being released into the rivers (Dhawde *et al*., 2018). Therefore, the dissemination of antibiotic resistance is mainly driven by wastewater discharge, urban activities, and untreated sewage.

Moreover, the use of *wastewater in agriculture* is quite widespread, and it is mostly high in the underdeveloped countries (Tongesayi and Tongesayi, 2015). It has been reported that more than 80% of domestic and industrial wastewater is discharged untreated into the environment and used for irrigation purposes. An example includes sub-Saharan Africa, where wastewater samples from canals near agricultural fields were collected and analyzed. Bacteria belonging to *Enterobacteriaceae*, *Streptococcaceae*, *Staphylococcaceae* and *Enterococcaceae* were detected. Sixty-one different virulence factors were also identified. Therefore, untreated wastewater is a hotspot for dissemination of antibiotic-resistant bacteria, and its use for agriculture affects the crops, the environment and animals (Bougnom *et al*. 2019).

Water pollutants in rivers can be carried to *coastal waters and lakes*. In fact, 147 *Escherichia coli* and 150 enterococci were detected in recreational beaches along Lake Huron in Michigan. They were screened against commonly used antibiotics, and were resistant to penicillin, ampicillin, tetracycline, vancomycin, erythromycin and rifampin (Alm *et al*., 2014). This issue is a global concern since the dissemination of water pollutants including antibiotic-resistant bacteria does not only affect the freshwater but can affect the ocean which is shared with other countries and continents.

Due to the expansion of population in the countries of the Middle East, water demands in all sectors have been increasing rapidly while water resources have become limited (Farzaneh Roudi-Fahimi *et al*.). Compared to neighboring countries, *Lebanon* benefits from rich water resources, but they are not properly managed. In fact, Lebanon has 40 streams, 17 are perennial rivers with more than 2,000 springs feeding them. The formation of springs and dams is due to the geological structure of Lebanon which serves as a reservoir and helps in the movement of rain and snow water to spring outlets or to the sea. Interestingly, groundwater resulting from rains and snowmelt serves about half of Lebanon's water supply, with snow being the principal contribution to groundwater recharge (El-Fadel, Zeinati and Jamali, 2000). However, both surface and groundwater resources here are affected by human activities, because it is extensively used in industries, for irrigation, and domestic purposes, with also uncontrolled tapping into groundwater. Studies showed that *agriculture* is the largest consumer of water, mainly for irrigation and animal farming, which account for nearly two-thirds of the water demand in Lebanon. Nevertheless, agricultural practices such as the use of fertilizers and pesticides led to chemicals leaching such as nitrate and phosphates, causing the growth of algae and causing water pollution. A study was done on the chemical and microbiological quality of eight coastal rivers namely Qasmieh, Bared, Abu Ali, Antelias, Ibrahim and Awali rivers in Lebanon during the dry season in 2008. Results showed that the concentrations of phosphate and nitrate were the highest in Awali river and Qasmieh river, due to the increasing fertilizing activities during summer. Bacterial counts confirmed that there are significant levels of *E. coli* and total coliforms, and the highest were in Abu Ali and Antelias rivers. The presence of high levels of *E. coli* is an indication that wastewater has been released in the rivers (Houri and El Jeblawi, 2007). The pollution coming from the different rivers can affect Lebanese coasts, thereby making Lebanese beaches unfit for swimming (Houri and El Jeblawi, 2007).

A more recent study assessed the water quality of Hasbani river in South Lebanon by focusing on chemical and microbiological characteristics. A total of 60 water samples were collected during January, August, and November. Results revealed a high number of pesticides residues which were detected in the rivers. Examples include propoxur-1 which is highly toxic for freshwater invertebrates and fish; hexachlorobenzene (HCB) which has been classified as human carcinogen by the US EPA; and Diazinon which is causative of nervous system disorders. Samples were also tested for heterotrophic bacteria, total and fecal coliforms, *E. coli* and *Salmonella* and *Shigella.* The occurrence of fecal coliforms and *E. coli* has been recorded throughout the year in all the samples, with fecal coliforms exceeding 900 CFU/100mL during August and November. Also, *Salmonella* and *Shigella* numbers were the highest during November, in 65% and 50% of the samples respectively. This suggested again that some water bodies were unsafe for swimming and sewage waste has been disposed in the rivers (Badr, 2014). Still, there is not enough data about the microbiological water quality of the rivers in Lebanon, and the detection of antibiotic-resistant bacteria in waters is rarely reported.

Irrigation water accounts nearly two-thirds of the water in Lebanon. Interestingly, the first report of multi-drug resistant *Escherichia coli* in irrigation water was assessed in 27 water samples in Beqaa and South of Lebanon from April till September 2018. Resistance was observed in *Escherichia coli* isolates to penicillin (100%), ampicillin (100%), amoxicillin/ clavulanic acid (23%), cefepime (18%), cefotaxime (41%), cephalexin (73%), cefixime (32%), Doripenem (5%), meropenem (5%), gentamycin (36%), norfloxacin (45%), trimethoprim/ sulfamethoxazole (SXT) (68%) and chloramphenicol (45%) (Hmede, Kassem and Jaafar, 2019). Resistance markers can be mobile which is considered as a global threat. The irrigation water quality might also affect the surrounding environment including the Mediterranean Basin. Knowing that these waters are used to irrigate important crops in the regions, contamination spread into the soil, plants and humans causing bacterial infections.

Antibiotics: The discovery of antibiotics cured multiple common- and disease-causing bacteria in both humans and animals. The first natural antibiotic Penicillin, isolated from *Penicillium notatum*, was discovered in 1928 by Alexander Fleming. This antibiotic was useful for the treatment of Staphylococci and Streptococci. However, Fleming was unable to isolate and purify the drug to sell it for commercial use until the US Department of Agriculture (USDA) collaborated for the development of large-scale production of Penicillin, especially during World War II. At this time, Penicillin was known as the “miracle drug” due to its ability to save lives (Agrawal *et al*., 2010). Then, by the end of 1950s, new antibiotics were discovered including Streptomycin and Tetracycline, which were useful for the treatment of serious infections including bacillus tuberculosis, pneumonia, and respiratory tract infections. However, scientists also discovered the ability of bacteria to resist the effects of medication of the drugs.

The finding of drugs that can inhibit the growth of bacteria faced a big challenge, which is the rise and *rapid spread of antimicrobial-resistant bacteria*. Antimicrobial resistance is now deemed to be one of the biggest threats to human health: "in Europe, antibiotic-resistant bacteria are responsible for the deaths of more than 25,000 patients' annually and costs at least 1.5 billion euros. In the United States, 63,000 deaths occur annually due to antibiotic-resistant bacteria and costs the US health system from 21 to 34 billion dollars; from healthcare costs to loss of productivity” (Dhawde *et al*., 2018). In general, antibiotics originate from microorganisms such as bacteria or fungi, some of them are synthetically modified and others are fully synthesized like fluoroquinolones and sulphonamides. Mechanisms of defense of bacteria against those agents originated from the selective pressure of the antibiotic on the bacterial cells, which evolved several mechanisms to counteract antibiotics and survive (Röstel, 2001).

Globally, the main concern in food and water quality is *fecal contamination.* In fact, pathogenic microorganisms are associated with fecal waste, whether coming from animals or humans. Since the microbiota in water is diverse and can be composed of many different pathogens, the assessment of water quality based on the analysis of all of these pathogens is difficult. The direct testing of the pathogens is actually expensive and not feasible; thus the US Environmental Protection Agency relies on indicators of fecal contamination when assessing water and food microbiological quality (EPA, 2006). *Fecal indicators* include mainly fecal coliforms, including *Escherichia coli.* For decades, scientists have evaluated water quality by enumerating fecal coliforms and *E. coli* levels in rivers, lakes, and sea. Fecal coliform bacteria are usually found in the intestine of warm-blooded animals and are released from their feces. They can spread to the soil, aquatic environment and plants through animal and human fecal matter. As for *Escherichia coli,* it has been considered as the more accurate fecal indicator organism, and its presence in the feed, in water and the environment might suggest that other pathogens are present (Sato, 2012).

Microbiological contamination can spread to food (crops) via contaminated water, leading to wide range of illnesses: from diarrhea, fever abdominal pain to chronic health complications, even death. *Unsafe food* poses a global health concern particularly affecting children, elderly and immunosuppressed people. Although not all incidences of food poisoning are reported, the WHO estimates "almost 1 in 10 people in the world fall ill after eating contaminated food and 420 000 die every year". *Safe and readily available water* is essential for public health and food production. The CDC reported, "6,939 annual total deaths were documented for 13 diseases caused by pathogens that can be transmitted by water in the US" The major pathogens responsible of *foodborne and waterborne illnesses* are Norovirus, *Campylobacter,* and *Enterobacteriaceae,* including *Salmonella, Shigella* and *Escherichia coli.*

While studies worldwide revealed the major causes of the dissemination of chemical and microbiological pollutants in water sources including domestic, agricultural, industrial and recreational uses, the lack of data in Lebanon makes the assessment of water pollution difficult. Few are the reports which tackled the presence of pathogens in water resources and the emergence of antibiotic resistance in bacteria in these niches. Therefore, the present study examines the freshwater quality sampled from 45 locations along the rivers in Lebanon starting from the spring of each river, from middle of the river, going downstream at the outlet. The water quality is assessed by analyzing the main indicators of fecal contamination namely *Escherichia coli* and fecal coliforms, as well as the antimicrobial resistance patterns of *E. coli.*

CHAPTER II

MATERIALS AND METHODS

A. Study Area and Sample Collection

One-hundred thirty-five freshwater samples were collected from the 14 rivers in Lebanon flowing in North, South, Beqaa, Mount Lebanon and Beirut Districts between May and July 2019. The samples were collected in triplicates from upstream, the middle and downstream of the rivers and filled in 1 Liter sterile bottles. The distance between sampling points ranged from 7 to 42 km depending on the length of the river. The water samples from each location were transported to the laboratory using coolers (2 to + 5 °C) and analyzed within 16 hours of collection.

B. Sample Analysis

Water filtration was done for the samples' analysis. The samples were 10-fold serially diluted and filtered using 0.22 µm pores membranes, which were placed on Petri dishes containing agar media (Rapid *E. coli* 2) that allows the selective growth of fecal coliforms and *Escherichia coli.* These bacteria serve as indicators of fecal pollution. The plates were incubated at 44.5 °C which is the optimum temperature for the growth of fecal coliforms also known as thermotolerant coliforms. The plates were incubated for 18-24 hours and the diagnostic colonies were then counted. The number of fecal indicators were reported as colony forming unit per 100 mL of water: the purple colonies on the media represented the *E. coli* whereas the green ones were fecal coliforms. Three *E. coli* were selected (n = 388) from each positive sample and preserved in glycerol in Luria broth (LB) at -80°C for further analysis.

C. Antibiotic Susceptibility Testing

All *E. coli* isolated were subjected to antibiotic resistance analysis using the Disk Diffusion Antibiotic Sensitivity test. For this purpose, Mueller-Hinton Agar (MHA) media plates were prepared and each colony was spread on a plate to form a lawn. The optical density was adjusted at OD=0.05A using Muller Hinton broth as a diluent. Twenty commercially available antibiotic disks were then added to the plates which were incubated at 37°C for 18-24 hours. Antibiotic susceptibility was determined by measuring the diameter of the zones of inhibition around each antibiotic disk. Subsequently, the isolates were classified as resistant or sensitive to the antibiotic based on the guidelines of CLSI (formerly NCCLS) & EUCAST.

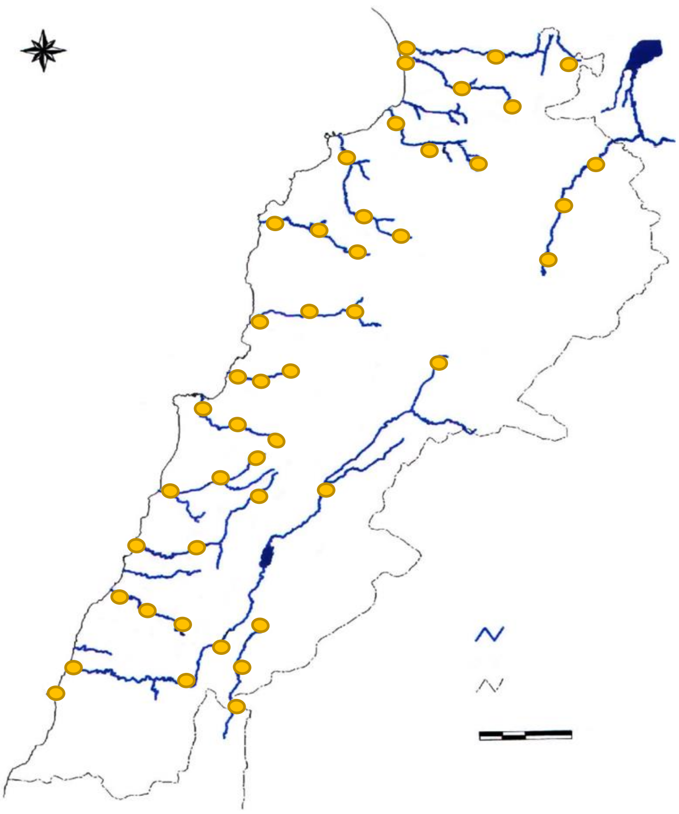

Fig. 1. Map of Lebanon with the 45 sampling locations

CHAPTER III

RESULTS

A. Bacterial Counts

1. Fecal Coliforms Count

Results showed that out of the 135 samples, 130 had fecal coliforms. The counts ranged from 1 to 70,000 fecal coliforms knowing that the numbers should not exceed 800 as per the U.S EPA standards for recreational use. The highest counts recorded were in Beirut river followed by Abou Ali river in Tripoli. The mean of fecal coliforms in Beirut river is 8791.56 fecal coliforms, and for Abou Ali river the mean of fecal coliforms is 11608.89 fecal coliforms.

In the South of Lebanon, the average fecal coliforms for Qasmieh and Jarmaq rivers were the highest, respectively 2566 and 3133 fecal coliforms. In the North, the highest counts were recorded in the middle and the source of Abou Ali river with 21,566 and 18,293 fecal coliforms respectively. In Mount Lebanon, the average fecal coliforms in El Kalb river were the highest with an average of 2866. As for Beirut, the highest counts were recorded in Beirut river with an average of 36,587 fecal coliforms in the river of Beirut. No fecal coliforms were detected in five freshwater samples from the sources of Zahrani river and Bared river. Note that all counts are per 100 mL of water.

Figure 2 illustrates that the sources in each river were less polluted than the middle and the outlet of the rivers except for Hasbani and Awali rivers. The counts were compared to the Environmental Protection Agency (EPA) standards for recreational use to check for the water quality. In Mount Lebanon only El Kalb river exceeded the EPA standards for recreational use. Also, in the Beqaa district, both El Assi River and the source of the Litani river did not exceed the EPA standards. However, in the North of Lebanon, 30 out of the 42 samples (71.4%) of the samples exceeded the standards and were deemed as unsafe. In Beirut, the samples that were retrieved from the middle also had high fecal coliforms counts which exceeded the standards.

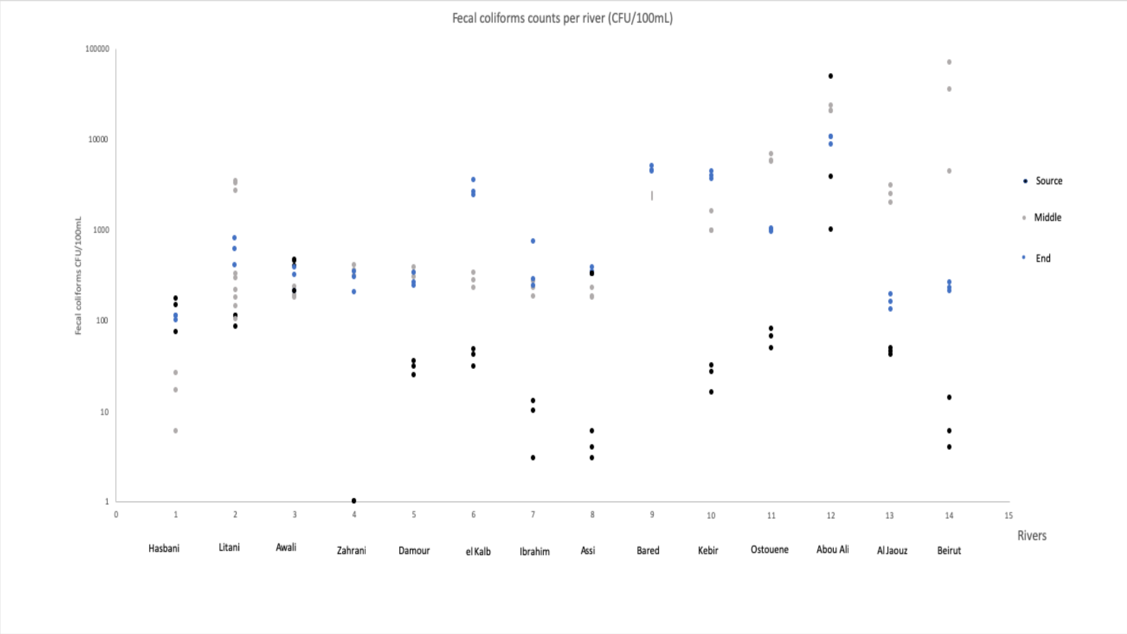

Fig. 2. Fecal coliforms' counts per river (source, middle and end)

2. Escherichia coli Counts

Of the 135 freshwater samples, 129 (95.5%) yielded *E. coli* colonies, which ranged in number between *1 and 50,000 E. coli*. The standards used for *E. coli* are 250 *E. coli*. By comparing the counts in each district, Jarmaq river in the South of Lebanon followed by Qasmieh river had the highest average of *Escherichia coli* and fecal coliforms' counts (2213 *E. coli)*. In Mount Lebanon, the highest average of *E. coli* was the highest in Nahr el Kalb 1960 *E. coli*. The outlet of Assi river in Laboueh had the highest counts of *E. coli* (191*)*. As for the North, the numbers of *E. coli* were relatively high, whereby in Zgharta, the middle of Abou Ali river recorded the highest counts of 10966 *E. coli* followed by the outlet of Abou Ali river in Tripoli with 5433 *E. coli*. The overall mean of *E. coli* number of this river is 6194.44 *E. coli*. The mean of *E. coli* counts in Beirut river was the highest with 8738.67 *E. coli*. The counts are per 100 mL of water.

Similar to the fecal coliforms', the sources in each river are less polluted than the middle and the rivers' outlet, except for Hasbani river and Awali (Figure 3).

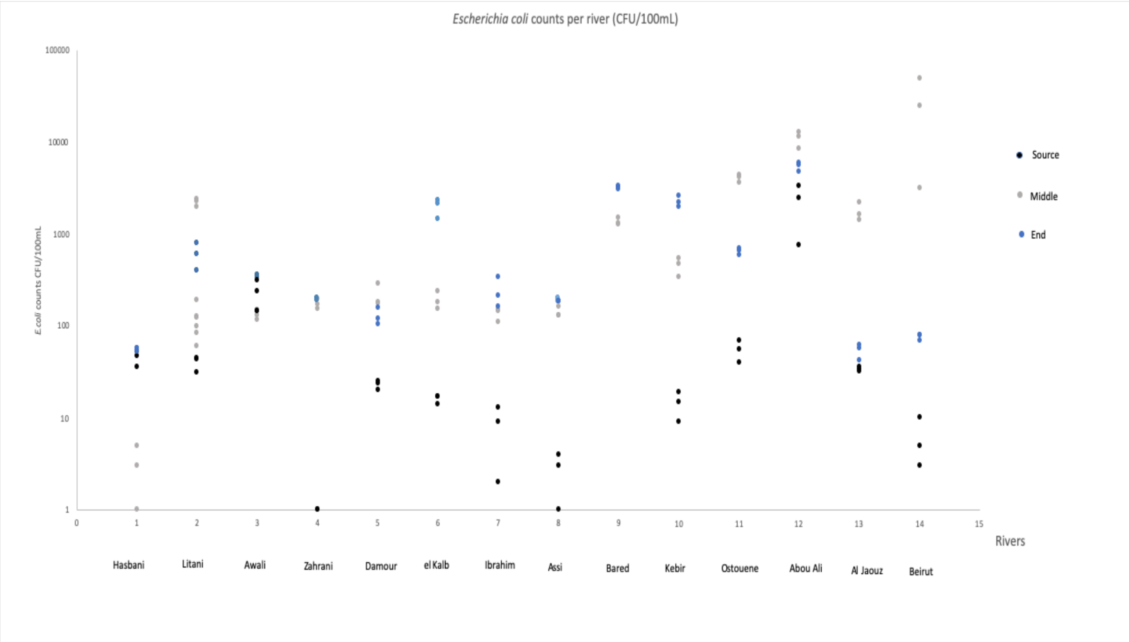

Fig. 3. *Escherichia coli* counts per river (source, middle and end of each river)

37% (49/135) of the freshwater samples exceeded the standards for the *E. coli* counts. In the North, 10 out the 14 rivers exceeded the limits for recreational use for *E. coli.* In the South, 8 out of 36 samples exceeded the standards, 8 out of 33 samples in the Mount were above the limit. However, in Beqaa none of the rivers *E. coli* counts exceeded the limit for recreational use. Beirut river is the only river in Beirut district, and the *E. coli* counts are the highest amongst all rivers in all districts.

It is noteworthy that all of the rivers except the Hasbani river had at least one sample which exceeded the standards for recreational use (Figure 3).

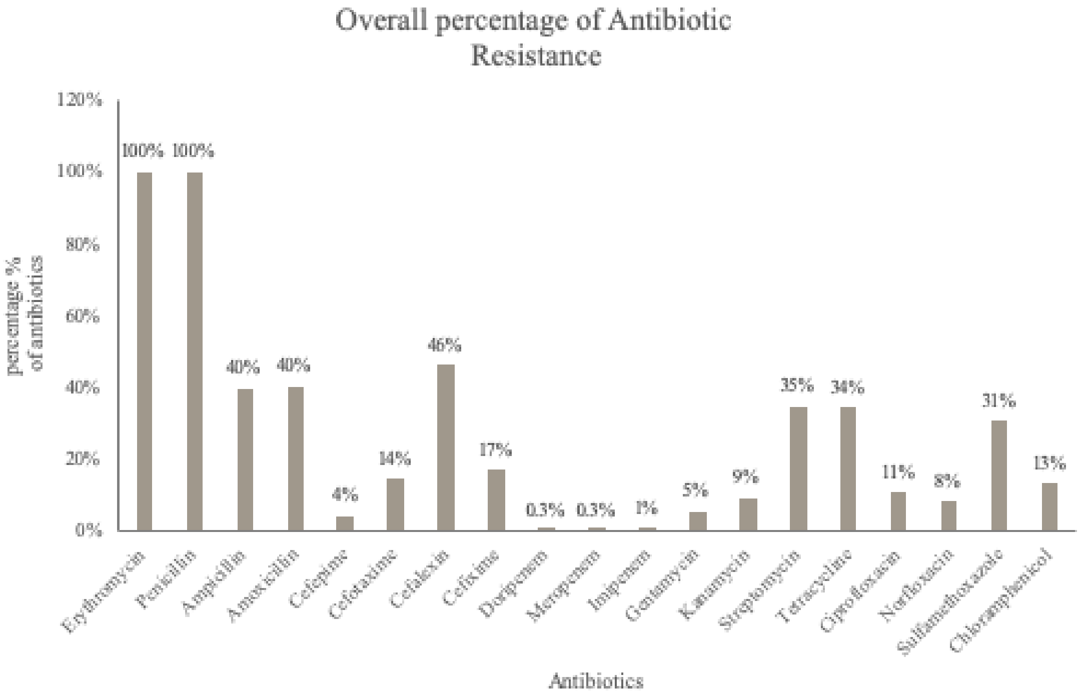

Fig. 2. Overall percentage of antibiotic resistance in the 14 rivers

B. Antibiotic Susceptibility Test

The disk diffusion assay for antibiotic susceptibility was done to screen the 388 *Escherichia coli* isolates. Overall, resistance to Cefalexin, Ampicillin, amoxicillin and clavulanic acid was the highest among the isolates, respectively 46%, 40% and 40%. Resistance was observed against cefepime (18%), cefotaxime (41%), cefixime (32%), doripenem (0.3%), meropenem (0.3%), imipenem (1%), gentamicin (36%), kanamycin (64%), streptomycin (95%), tetracycline (100%), ciprofloxacin (36%), norfloxacin (45%), trimethoprim/sulfamethoxazole (SXT) (68%) and chloramphenicol (45%). In addition, carbapenem resistance was detected in the *E. coli* isolated from the North of Lebanon (0.3%).

Furthermore, 176 out of the 388 isolates which represent almost half of the total isolates, exhibited resistance to at least three classes of antibiotics. The highest percentage of multi-drug resistant *Escherichia coli* was detected in Beirut district (61.1%), followed by the Beqaa (53.3%) then the rivers in the North (48.4%), the South (42%) and in Mount Lebanon(39.4%).

CHAPTER IV

DISCUSSION

Fecal pollution has been widely studied in water bodies. It has been assessed by the enumeration of fecal indicators mainly *E. coli* and fecal coliforms. Also, antibiotic resistance is perceived as a major global concern worldwide. Antibiotic resistance in fecal indicator bacteria, *E. coli*, has been described worldwide since the 1970s. Humans, animals and plants represent reservoirs of antibiotic-resistant bacteria; as most of the bacteria can colonize these hosts, exchange can happen between different hosts and affect the environment (Röstel, 2001). However, in Lebanon, few are the reported cases of antibiotic-resistant bacteria in waters including irrigation water, sea water and drinking water; however, none have tackled this issue in rivers in Lebanon, which highlights the importance of this study.

The highest counts of *E. coli* and fecal coliforms were detected in Abu Ali river flowing in Tripoli and in Beirut river due to the density of population in these cities, thus the extensive urban activities. Both Beirut river and Abou Ali river are walled and partially covered, and sewage outlets are discharged into the river which explains clearly the high level of fecal contamination. This could be related to a study conducted by the AUB Water Resources Center where parameters such as nitrate levels (NO_3) and BOD_5 in nine rivers were examined in order to reflect the contamination of water from different sources mainly the discharge of untreated domestic sewage, and agricultural waste including fertilizers from riverbank agriculture. For Abou Ali river, the levels of nitrate were the highest (7.3mg/L) and the BOD_5 level was 69.7 mg/L which confirm our results (El-Fadel, Zeinati & Jamali, 2000). Knowing that Beirut river ends and flows right into the Mediterranean coast, the marine waters have also demonstrated high BOD_5 and total suspended solids (TSS) levels. This is a global concern since those contaminants can spread from the ocean to other countries and cross continents.

On another note, the broadest range in the numbers of fecal indicators was seen in Beirut river, however at the last sampling point in Beirut district, the number of fecal indicators did not exceed the standards and was relatively low. This might suggest that detergents or chemicals have been thrown or that other microbial agents could compete with the *E. coli* and the bacteria did not survive (Figures 1 and 2).

In the South, the fecal indicators' numbers in Qasmieh and Jarmaq rivers which represents the Lower part of the Litani river exceeded the U.S. EPA standards for recreational water quality. This might be due to the fact that Litani carries pollutants coming from open dumps coming directly from Baalbeck, Bar Elias, Zahle, Joub Jannine and Sifri in the Upper Bassin. The river also receives industrial effluents from many industries, poultry farms and slaughterhouses. The Litani river is the largest in Lebanon (170 km) and flows from Beqaa Valley southwards arriving to the Mediterranean Sea (Koubaissy, 2014). The water quality and the pollution level change throughout the river depending on the flow of the water, which might explain why some samples collected from a point (Mazraat Tamrah) along the Litani river did not demonstrate high fecal contamination. Most importantly, the standards which were taken into account include the recreational purposes with only partial contact with the water, which means that the samples which were above the standards are not suitable for irrigation and obviously not for drinking (Figures 1 and 2).

No *E. coli* was detected in two locations: Nabaa el Tasse, the source of el Zahrani river and Nabaa el Safa in the North. This might be due to the absence of anthropogenic practices and the absence of humans during the season. It is noteworthy that the results do not mean that the water is safe and free of contaminants since they are based on fecal indicators. Actually, the season is a parameter that should be considered in some rivers. This has been demonstrated in a study of the seasonal variations of microbiological parameters in the largest river in Serbia. Fecal coliforms' numbers were much higher in autumn than in summer due to the solar irradiations and high temperatures (Kolarević *et al.*, 2012). In our case, the Wazzani river on the Southern borders had low levels of fecal indicators during May (beginning of summer season). However, in November, olive mills in Hasbaya dump their untreated wastewater in the river which may result in the production of black liquid rich in organic matter. The impacts of olive mills discharge into rivers and marine environment have been tackled in various countries. In Southern Greece, elevated amounts of phenols, ammonium and inorganic phosphorus were detected, they generate dark colored liquid wastes which also demonstrated very high toxicity for the aquatic life (Pavlidou *et al*., 2014).

In addition, antibiotic resistance was prevalent in *E. coli* recovered from river water, and 176 out of the 388 isolates were multi-drug resistant. Overall, resistance to Cefalexin was mostly common (46%) among the isolates. Cefalexin has been widely used in medicine to treat bacterial infections. Resistance to both ampicillin and amoxicillin combined with clavulanic acid was also high with 40% of the isolates being resistant to the ß-lactam antibiotics. These antibiotics have been widely subscribed in hospitals and clinics. Resistance to the ß-lactam in *E. coli* is primary due to the production of ß-lactamase enzymes. Hospital wastewater is known to carry high quantities of drug-resistant bacteria and their interaction contribute to the spread of antimicrobial resistance genes. Hence, the untreated wastewater discharge into the rivers increases the dissemination of resistance patterns in the water (Lien *et al*., 2016).

Interestingly, resistance to carbapenems was detected in three isolates from the North of Lebanon. Carbapenems possess the broadest spectrum of activity among ß-lactam antibiotics against Gram-negative bacteria, resistance is critical because carbapenem resistant bacteria require the use of last-resort antibiotics. The rivers located in the North of Lebanon include Abu Ali, Ostouene, el Kebir, and Nahr el Bared. The World Health Organization (WHO) approves that the random use of unprescribed antibiotics is major reason for the dissemination of carbapenem-resistant bacteria. These resistant genes are transmissible, and they can spread through agro-industrial practices and sewage discharge into the rivers. Moreover, El Kebir river is located on the Syrian border, and the water is shared between Lebanon and Syria. To date, three dams have been built from Syria's side, and the water is used for irrigation of up to 6,820 hectares. The quality of both surface and groundwater in this river has been facing challenges due to the uncontrolled discharge of untreated domestic sewage, solid and animal waste and unsustainability of agricultural activities (AsiaI, 2013).

High levels of antibiotic-resistant bacteria were also detected in the Upper Basin of Litani river which flows in the Beqaa Valley, and this might be due to the extension of refugee camps and domestic discharge of the displaced Syrian refugees. Antibiotic-resistant *Escherichia coli* were recently detected in water resources from drinking and sewage in the refugee camps. Not only they were abundant, but some of them carried the *mcr-1* gene which confers resistance to colistin (Sulaiman and Kassem, 2019). Moreover, the extensive agricultural practices in the Beqaa are a way of dissemination of antibiotic-resistant bacteria.

Some of the antibiotics have been also reported as carcinogenic. For instance, the use of chloramphenicol has been restricted in the United States because it causes blood abnormalities, and the National Toxicology program listed this antimicrobial agent as a human carcinogenic compound (Burnham, 2000). This compound was highly effective against gram-positive cocci and gram-negative aerobic and anaerobic bacteria especially in veterinary medicine; however, the U.S. Food and Drug Administration banned its use in food-producing animals. Chloramphenicol is being used in Lebanon and the percentage of chloramphenicol resistance of the strains isolated from the rivers is high (45%) which emphasizes on the risk posed by antibiotic-resistant bacteria to the humans and animals' health.

Almost half of the *E. coli* isolates (43.8%) exhibited resistance to at least three classes of antibiotics. The highest number of multi-drug resistant isolates was also detected in Beirut district (61.1%) and Beqaa district (55.5%). As discussed, the settlement of the refugee's camps directly next to the Litani river aggravated water quality in Beqaa due to the sewage that is released into the river. The multidrug resistance is also high in the North of Lebanon (46%) and 42% in the South. In addition, the use and prescription of antibiotics is extensively practiced in many regions including in the capital Beirut, in the North and in the South of Lebanon, where most hospitals and care centers are located. A strong correlation between population density and antibiotic consumption has been actually proved in a study done between three cities in the Netherlands, Greece and Canada. It also highlighted that population density is an important factor in the prevalence of antibiotic resistance (Bruinsma *et al*., 2003).

The present study is cross-sectional, and the data is recorded at one point in time and can be affected by many factors such as the weather, the water temperature and the proximity of the rivers to sewage outlets and agricultural areas.

CHAPTER V

CONCLUSIONS

This is the first assessment of fecal pollution along with the antibiotic resistance of fecal indicator *Escherichia coli* in the rivers in Lebanon. The rivers were highly polluted in the North of Lebanon and Beirut district. The density of population in these regions might be related to untreated domestic sewage and other waste which might be released in the rivers. Resistance to widely used antibiotics was prevalent in the *Escherichia coli* isolates especially in Beirut, in Beqaa and in the North of Lebanon. In addition, nearly half of the isolates were multi-drug resistant. This could be due to the misuse and overuse in medica and agricultural practices in Lebanon. Two samples of fresh water sources did not harbor any fecal contaminant, most probably because no anthropogenic practices are made there. Yet, this does not mean that these sources are not polluted; other parameters such as chemicals, parasites and viruses should be evaluated in order to conclude about the water quality. The season factor should be taken into consideration when assessing freshwater quality since some contaminants might not survive during heat season, and some are carried out by rainwater. Hence, the counts' patterns can change depending on the seasons.

Wastewater treatment plants, solid waste management, and control of domestic sewage discharge should be implemented in Lebanon and well managed in order to make safe and clean water available for people and animals. Moreover, the control and the regulation of antibiotics' use are urgently needed. Antibiotics should not be prescribed randomly or given without medical and veterinary prescriptions. Awareness and education should be done so that people understand the effects of random use of antibiotics as well as the effects anthropogenic practices on the environment and our health. This includes garbage dumping in rivers. Further analyses include source-tracking of the isolates to better understand the sources implicated in the freshwater pollution. Genotypic analysis can also be done to confirm the resistance patterns of the *E. coli* isolates to antibiotics and to study their pathogenicity.

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