AMERICAN UNIVERSITY OF BEIRUT

DETECTION OF PLASMID-MEDIATED COLISTIN RESISTANCE GENE, *MCR-1*, IN MULTIDRUG RESISTANT *E. COLI* AND *PROTEUS MIRABILIS* IN WATER MATRICES IN SYRIAN REFUGEE CAMPS IN LEBANON

by
ABDALLAH SAMIH ALHAJ SULAIMAN

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

Beirut, Lebanon
December 2019

AMERICAN UNIVERSITY OF BEIRUT

Detection of Plasmid-mediated Colistin Resistance Gene, *mcr-1*, in Multidrug Resistant *E. coli* and *Proteus mirabilis* in Water Matrices in Syrian Refugee Camps in Lebanon

by

ABDALLAH SAMIH ALHAJ SULAIMAN

Approved by:

Dr. Issmat Kassem, Assistant Professor Nutrition and Food Sciences	Advisor
Dr. Samer Kharroubi, Associate Professor Nutrition and Food Sciences	Member of Committee
Dr. Hadi Jaafar, Assistant Professor Agriculture	Member of Committee

Date of thesis defense: December 23, 2019

AMERICAN UNIVERSITY OF BEIRUT

THESIS, DISSERTATION, PROJECT RELEASE FORM

Student Name:	**Alhaj Sulaiman**	**Abdallah**	**Samih**
	Last	First	Middle

⊗ Master's Thesis ○ Master's Project ○ Doctoral Dissertation

☐ I authorize the American University of Beirut to: (a) reproduce hard or electronic copies of my thesis, dissertation, or project; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes.

☒ I authorize the American University of Beirut, to: (a) reproduce hard or electronic copies of it; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes after: **One -year from the date of submission of my thesis, dissertation or project.**
Two -years from the date of submission of my thesis, dissertation or project.
Three -years from the date of submission of my thesis, dissertation or project.

Signature

20.2.2020
Date

This form is signed when submitting the thesis, dissertation, or project to the University Libraries

ACKNOWLEDGMENTS

Now, this thesis has become a reality with the support of several individuals that I love and respect. I want to show my gratitude to them with a few select words.

Foremost, Praise be to Allah, the Cherisher and Sustainer of the worlds for the wisdom, patience, opportunity, strength, and peace I was provided during my master's journey.

To my **MOTHER:** Although you left the earth, you are the spark that always kindles my passion. I learned from you the definition of tender. Though you left, I still feel the consequences of your last words to me (الله يرضى عليك يا عبدالله). In every single step I take. **My MOM**, you are not on earth, but you will always be in my heart.

To my **FATHER**, thank you for supporting me in all of my pursuits. I am deeply grateful for the breadth of your emotional and financial support.

To my **SISTERS** and **BROTHERS**, thanks for the love you supplied interminably during my work. I want to dedicate the biggest part of this thank to my sister (غرودي) who made the most significant and lasting contribution to my upbringing.

To my **FRIENDS** and **LAB MATES**, thanks for all the solidarity and emotional aid you provided. I would be the first to admit; this work would not have been possible without you. Thanks to my friends **Dr. Rashad Al Abd** and **Mr. Cepan Ahmad** for accommodating me in my first few nights in Lebanon.

To my committee, **DR. ISSMAT KASSEM, DR. SAMER KHARROUBI**, and **DR. HADI JAAFAR**, thanks for the time you gave freely helping me to enhance this work.

My sincere gratitude is for **DR. ISSMAT KASSEM**, my advisor, and my big brother. I attribute the success I achieve to the help that was provided by Dr. Issmat. I learned from Dr. Kassem the dedication required to attain the heights I now target. I acquired from Dr. Issmat the researcher's spirit; he taught me how to analyze and to think correctly before acting. I will always remember his words, "If you want to take the risk, take it, but plan it well." Thank you Dr. Issmat, once more for all the effort and time you granted me. Your message that I will always carry with me like a torch is to help the region (I come from) with all that I have, and when I am successful in this, I will have shown you my gratitude.

It's my pleasure to mention my first English teacher, Miss Layla, for all the support and effort she spent to help me learn the English language. She always believed in me and gave me the spark to exert effort for learning and achieving the heights.

To **my Scholarship**: I gratefully acknowledge the funding received to do my master's degree from **Al-Ghurair Foundation for Education**. Thanks to **Miss Hiba Baalbaki**, the coordinator of the program for solving all the problems I faced. Thanks to Miss Reem Charif for her support and actions that lead me to enter the country and start the degree. I admit that I would never have this experience without their help.

Finally, I wish my country, Syria, to heal and flourish to embrace its people and me again. I hope that I will be able to contribute to passing the knowledge I acquired to others in Arab region that

"We May Have Life and Have It More Abundantly."

AN ABSTRACT OF THE THESIS OF

Abdallah Samih Alhaj Sulaiman for Master of Science
Major: Food Technology

Title: Detection of Plasmid-mediated Colistin Resistance Gene, mcr-1, in Multidrug Resistant E. coli and Proteus mirabilis in Water Matrices in Syrian Refugee Camps in Lebanon.

More than 5.6 million individuals have fled from Syria after the civil war that started in 2011, and this is considered to be the greatest humanitarian emergency after World War II. Lebanon holds the largest number (~1.5 million) of Syrian refugees per capita. Syrian refugees are distributed in random camps along the country, and the vast majority are getting exposed to unideal situations in terms of access to water, food and hygiene. Syrian refugees fall in the category of the most vulnerable population to multi-drug resistant infection due to stress and limited access to clean resources such as foods and water). Hence, the use of last-resort antibiotics such as colistin is possibly a common option that can be used to treat comex infections in this population. Although few studies assess the prevalence of multi-drug resistant bacteria, some studies reported the presence of mobile gene, *mcr-1,* that is responsible for colistin resistance in *E. coli* isolated from irrigation water and broilers in Lebanon. To assess the situation in the camps, eighteen composite samples were collected from two Syrian refugee camps in Beqaa area. Every sampling event covered three primary sources in each camp (drinking water, well water, and sewage water). All the samples were cultured on media that was previously supplemented with 4 μg/ml colistin to detect *mcr-1* positive *E. coli* and *Proteus mirabilis.* Thirty-six *E. coli* and 8 *Proteus mirabilis* isolates were harvested and analyzed. All *mcr-1* positive *E. coli* strains were multidrug-resistant and were negative for *mcr 2* to *8*; the minimum inhibitory concertation of colistin varied between 4-64 μg/ml. *E. coli* isolates (86%, 81%, 14%, 6% and 3%) carried bla_{TEM}, bla_{CTX-M}, bla_{SHV}, bla_{OXA48}, and bla_{KPC} respectively. Also, all the isolates carried class 1 integron gene and *int1* but none was positive for class 2 integron gene, int2, bla_{NDM}, and bla_{IPM}. *P. mirabilis* strains that are intrinsically resistant to colistin were detected and carried *mcr-1*, bla_{TEM} , *int1*, Class 1 Integron genes, but were negative for bla_{CTX-M}, bla_{SHV}, class 2 integron gene, and *int2*. *P. mirabilis* isolates showed the ability to swarm and to form biofilms that able to harbor *mcr-1* for more than six days. Transformation and sequencing for *mcr-1* gene were conducted and all the transformants were positive for *mcr-1*. The data suggest that the camps are at risk of exposure to *mcr-1* and that domestic water was of unacceptable quality. *P. mirabilis* can be an overlooked reservoir for the transmission of *mcr-1* gene. Besides, *P. mirabilis* is a pathogen, and the camps host children, elderly, and immunocompromised people. Furthermore, detecting *E. coli* with *mcr-1* and other resistant genes is a severe issue and can possibly threaten the treatment of infectious diseases in this vulnerable population. Hence, the aforementioned data can reflect the severity of the situation that the Syrian refugees are

suffering from in the camps. Taken together, ensuring sustainable access for safe water can secure a healthier life for Syrian refugees and the hosting community.

CONTENTS

ILLUSTRATIONS

TABLES

To
My Beloved Mom
God Bless her Soul

CHAPTER I

INTRODUCTION

A. Antibiotics: An overview

Antibiotics are chemicals that are normally produced by or derived from microorganism. Antibiotics have the ability to inactivate and/ or to damage other microorganisms [1], hence they have become important tools in the fight against infectious diseases that affect both humans and animals. The first antibiotic was found in 1928 by Sir Alexander Fleming, who received a Nobel prize in 1945 for this discovery. Fleming cultured the bacterium, *Staphylococcus aureus*, and left the plates on the bench in the laboratory for several days. He observed that a common contaminant, a mold, was able to inhibit the growth of the cultured bacteria. The mold, which is known as *Penicillium notatum*, produced penicillin, which became the first antibiotic [2].

In the summer of 1941, stakeholders from the United States of America became more interested in *Penicillium* products due to the war efforts. Two scientists, Howard Florey and Norman Heatley, devised more efficient strains of molds to produce penicillin. Also, they established procedures to extract the antibiotic, and presented initial data about the effects of penicillin on human beings. This was the spark that allowed penicillin f to be mass produced and widely used [3].

In the 1960s, more efforts were exerted by scientists to discover new compounds that can help humanity to fight against pathogenic bacteria. For example, a company worked on quinine, which was a drug used against malaria, to derive an antibacterial compound called nalidixic acid, which was able to destroy certain Gram-

negative bacteria and can be used to control urinary tract infections. Later, this led to the invention of fluoroquinolones, a group of antibiotics. As a consequence of the continuous efforts of scientists, several antibiotic classes were discovered and introduced to the market to treat bacterial infections [4].

Antibiotics are between 20 to 100 times bigger than water molecules, and they can be fairly target specific. For example, certain antibiotics (e.g. cephalosporins) block cell wall synthesis. Specifically, they inhibit the creation of the peptidoglycan layer, which is essential for bacterial cell wall formation. Given that, human cells do not produce peptidoglycan, the aforementioned antibiotics will not affect the cells of the human host [4]. This is a highly desirable property in therapeutic drugs

Antibiotics can be bacteriostatic and/or lethal against bacteria. The distinction is essential, because a bacteriostatic antibiotic will just inhibit the growth of the microorganism without killing it. The targeted microorganism might grow again when the bacteriostatic antibiotic is removed Alternatively, if the antibiotic is lethal then the targeted microorganism will be destroyed. It is important to note that some antibiotics can kill some microorganisms and inhibit others. For example, rifampicin is a deadly compound for *Mycobacterium tuberculosis*, but it is considered bacteriostatic against *Escherichia coli* at the same concentration. Antibiotics that can cause lysis in bacterial pathogens, might lead to the release of toxins that are produced inside the bacteria. The released toxin in this case might extend the duration of illness. In cases where the pathogen is not identified, broad-spectrum antibiotics can be used. However, this can affect the microflora (e.g. commensal bacteria) in the body, which may lead to undesirable effects on the patient. Therefore, if the pathogen is identified/ suspected narrow-spectrum antibiotics are preferred, especially for infections that take a long time to be cured such as tuberculosis that the latter will limit the impact on beneficial

microorganisms.

Different classes of antibiotics can target different cellular components and/or mechanisms in bacteria. This is briefly discussed below:

1. Inhibition of Protein Synthesis

Antibiotics can target and inhibit proteins synthesis in bacterial cells. For example, the antibiotic Mupirocin blocks protein synthesis, and it was used initially against the opportunistic pathogen, *Staphylococcus aureus*. Linezolid, an antibiotic that belongs to oxazolidinones, can interfere with interaction of the two ribosome subunits, and it has been widely used against Gram-positive bacteria. One of the oldest antibiotics, streptomycin, that belongs to aminoglycosides can bind to the small ribosome subunit; inhibits protein synthesis, while tetracycline can also bid to the small ribosome subunit but differently than streptomycin. Macrolides such as bind to the large ribosomal subunit; blocking protein synthesis. Similarly, clindamycin, a member of the Lincosamide group, also targets the large ribosomal subunit, and it is commonly used to against anaerobic pathogens such as *Bacteroides*.

2. Inhibition of DNA and RNA Synthesis

DNA and RNA synthesis are critical processes for the survival and growth of microorganisms, DNA topoisomerases are enzymes that can break/connect DNA and twist/untwist DNA circles and contribute to DNA synthesis. An exam of antibiotics that target DNA synthesis are fluoroquinolones that can inhibit topoisomerases activities, restricting the enzymes, and stopping DNA reproduction. An example of RNA synthesis inhibitor is rifampicin, an antibiotic that belongs to anti-mycobacterial class. Rifampicin is highly recommended against *Mycobacterium tuberculosis*, because it can destroy this

bacterium. Also, it was used against *Staphylococcus aureus.*

3. Cell Wall Synthesis Inhibitors

Cell wall integrity is essential for bacterial gr0wth and survival. A group of β-lactam antibiotics that includes penicillin, cephalosporins, carbapenems, and monobactams can target cell wall formation. However, cell wall inhibitors will not affect microorganisms that lack the cell wall. Vancomycin and daptomycin also targets cell wall synthesis. It has been used against complicated *Staphylococcus aureus* infection.

4. Folate Pathway Inhibitors

Sulfa antibiotics are examples of drugs that can inhibit the enzyme that is responsible for folate formation, which is essential for nucleic acid synthesis. Human beings' cells do not need to form folate because they acquire it from the diet. Consequently, sulfa drugs do not affect mammalians cells. The mentioned antibiotic played a significant role in world war two in treating wound infections for American soldiers [4].

5. Cell Membrane Inhibitors

An example of bacterial cell membrane inhibitors is colistin. It is known as a last-resort antibiotic, and it belongs to the polymyxins group, which are classified by letters from A to E and just polymyxin B and polymyxin E (colistin) are used for clinical purposes. The colistin mechanism of action is still not clear [5].

B. Antimicrobial Resistance

Due to the stress exerted by antibiotics, bacteria can naturally evolve and develop resistance to antibiotics via mutations, modification of cellular mechanisms, or acquisition of genes that facilitate fighting off the antibiotic. For example, fluoroquinolone is no longer active against methicillin-resistant *S. aureus* (MRSA) and *Neisseria gonorrhea* [4]. In fact, Sir Alexander Fleming stated in his Nobel Prize speech: "*It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body. The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant. Here is a hypothetical illustration. Mr. X. has a sore throat. He buys some penicillin and gives himself, not enough to kill the streptococci but enough to educate them to resist penicillin. He then infects his wife. Mrs. X gets pneumonia and is treated with penicillin. As the streptococci are now resistant to penicillin the treatment fails. Mrs. X dies. Who is primarily responsible for Mrs. X's death? Why Mr. X whose negligent use of penicillin changed the nature of the microbe. Moral: If you use penicillin, use enough*"[6]. Although Sir Fleming warned not to underuse antibiotics, it became also apparent that over use will yield to resistance. Antibiotics have been dubbed as miracle drugs which lead to their abuse and overuse in human- as well as veterinary medicine. Hence, emergence of resistance to antibiotics has been occurring rapidly, which is threatening public health and animal husbandry worldwide.

C. AMR Definition

Antimicrobial resistance (AMR) is the ability of the microorganism to survive

and reproduce with the presence of sufficient chemical compounds (e.g. antibiotics) that should restrict its activity. AMR can happen naturally or by exposing the bacterial cell to an inadequate quantity of the antibacterial agent. Under the right conditions, bacteria can develop resistance to almost any antibiotic and in many instances the AMR mechanisms can be vertically and/ or laterally transmitted to progeny or other bacteria. Since bacteria can divide fast under ideal conditions, resistance can spread rapidly.

D. The Problem and Burden of AMR

According to the World Health Organization (WHO), antimicrobial resistance has become a global concern, and it is rapidly affecting the treatment of human bacterial infections. The WHO has identified 90% of infectious diseases causative agents that are most threatening to humans. Tuberculosis is one of these diseases, and it is caused by *M. tuberculosis* [7, 8]. One-third of the Earth's population carries latent tuberculosis, and it shows up once the immune system weakened. In 2001, it was reported that the disease is responsible for 1.5 million deaths annually and is the leading reason for death for women. Unfortunately, the ability of *M. tuberculosis* to resist antibiotics is increasing rapidly worldwide. It is predicted that 50,000 lives are lost yearly in the USA and European countries as well as thousands of lives in other countries.

In both developed and developing countries, the number of patients suffering from diseases caused by resistant agents is significantly increasing and pan-resistant (no antibiotic available for treatment) pathogens can develop. In addition, the cost of treating AMR-associated diseases is high, which also highlights the economic impact of AMR.

Some experts predict that by 2030, 100 million people will lose their lives prematurely because of the AMR problem worldwide, while 300 million individuals are

estimated to die prematurely by 2050. From an economic aspect, between 2014 and 2050, the world will lose between $60 and $100 trillion as an economic output, which is equal to 1-year of global economic output. The gross domestic production is thought to decrease by 2% to 3.5% in 2050 [9].

E. AMR and Refugees

Individuals in countries with catastrophic events such as war, economic collapse, unrest and violence seek refuge and better life in more stable countries. Refugees are known to be susceptible to infectious diseases, because they represent groups of people that have been stressed, injured, and, in many cases, deprived from proper nutrition and sanitation. Therefore, antibiotics are important to maintain refugee health. However, refugees might have been exposed to AMR pathogens while in their country of origin under unideal conditions, during their travel to new countries, or while living in poorly maintained camps. Therefore, the refugee population can AMR bacteria across borders and serve as reservoirs for dissemination of resistance. Studies and information on the latter have been increasing. For example, seventeen articles reported AMR problems among refugees in different countries (Germany, Netherlands, Greece, India, Austria, Italy, Thailand, Korea, Australia, United Kingdom, Kenya, and other countries; see below).

The Middle East has witnessed several conflicts that resulted in a large number of refugees that were hosted in neighboring countries in the MENA region and Europe. AMR in Middle Eastern refugees was reported to be highly concerning in studies that were conducted in European and other hosting countries hospitals. However, AMR studies in Middle Eastern refugees remain not well characterized. A notable example of the aforementioned observations are the refugees of the Syrian conflict, which stared

in 2011 [12]. The United Nations High Committee of Refugees stated that more than 13.5 million Syrians inside Syria were in need of humanitarian aid. It was also reported in 2016 that around 6.6 million individuals were internally displaced and more than 5 million individuals left Syria to foreign places seeking safety and peace [12]. Notably, 4.8 million Syrians were registered as refugees in the MENA region and are mainly hosted in Lebanon, Jordan, Turkey, and Iraq. More than 1 million Syrian refugees have arrived to European countries in 2015 alone, and they were hosted in Germany and Serbia (62%), Sweden, Hungary, Austria, Netherlands, and Denmark (26%), and 11% in the rest of the European countries [12, 13]. Therefore, a significant number of Syrian refugees are hosted in different countries under conditions that are not always ideal. However, AMR in Syrian refugees and its impact on hosting communities remains not well characterized.

Below studies conducted to detect AMR bacteria in refugees will be discussed in relation to hosting country. The focus will be mainly on Syrian refugees:

1. Germany

Stool samples from children has arrived mainly from Syria and Afghanistan were collected and tested for the presence of resistant pathogens. Among 119 samples, 35% harbored extended-spectrum beta-lactamase (ESBL) strains. In addition, in another screening conducted during hospital admission for adults and children (mainly from Syria), 61% of the tested people carried ESBL strains, carbapenem-resistant *Enterobacteriaceae*, and multidrug-resistant *Acinetobacter baumannii* [12].

A review conducted in Germany after collecting data from several hospitals in the Rhine region between December 2015 and March 2016. Asylum seekers (n = 325) were tested for the presence of drug-resistant strains, and 9.8% were positive for

MRSA. Another study reported that 290 patients were screened for ESBL-producing bacteria, and 23.2% were positive. Also, 8.3% of the whole ESBL-producing isolates were also resistant to fluoroquinolones. Additionally, 2.1% of the tested individuals were harboring strains that were resistant to carbapenems. Around half of the patients originated from Syria and 20-30% are from Afghanistan.

Another study conducted in a German hospital (Ingolstadt) from February to August 2015. Asylum seekers (n = 96) were tested for MRSA, and 99 refugees were tested for extended-spectrum beta-lactamases and multidrug-resistant bacteria. The majority of the tested people were from Africa (58%) and some from Asia (37%). A number of individuals (4.2%) harbored MRSA, while 8.1% carried isolates resistant to most of the beta-lactam antibiotics and 6.1% were resistant to quinolones.

Samples were collected from 506 refugees in Germany in order to detect intestinal pathogens. The refugees were from Syria, Iraq, Afghanistan, Eritrea, Iran, and other countries. The isolates showed high prevalence of beta-lactamase genes such as bla_{TEM} (88.1%), $bla_{\text{CTX-M}}$ (43.6%), $bla_{\text{OXA-1}}$ (19.4%), and bla_{SHV} (35%). In addition, quinolone resistance genes were detected along with glycopeptide resistant genes. 6.3% of the total refugees harbored MRSA. However, carbapenem-resistant *Enterobacteriaceae* were not detected. The detected ESBL strains belonged to *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. The study declared that there was a high prevalence of antibiotic resistance genes among the refugees, which demand special care for asylum seekers [16].

A systematic study was conducted in Germany between June and December 2015 to screen multidrug-resistant pathogens among refugees admitted to a hospital. 47 Syrian refugees, 29 from Afghanistan, and 14 from Somalia participated in this study. 60.8% of the screened individuals carried multidrug-resistant gram-negative bacteria.

MRSA, carbapenem-resistant *Acinetobacter baumannii*, and other ESBL-producing Enterobacteriaceae were detected [17].

In Germany, 8 Libyan- and 14 Syrian refugees with war injuries were admitted to Bundeswehr hospitals. From the Libyan patients, *Klebsiella pneumoniae*, *E. coli*, *Enterobacter cloacae*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* were isolated. ESBL was expressed in most of the isolates. From Syrian patients, *E. coli*, *E. cloacae*, *Citrobacter freundii*, *Morganiella morgani*, *Proteus mirabilis*, *Roaultella planticola*, *A. baumannii*, *A. radioresistens*, *P. aeruginosa*, and *S. maltophilia* along with MRSA were detected. Also, ESBL was presented in most of the isolates. The study declared that nosocomial transmissions of pathogens could happen, and it can be prevented by strict hygiene practices [18].

A cross-sectional study between April 2016 and March 2017 was conducted in Germany in order to assess the prevalence of drug-resistant microorganisms among asylum seekers. Stool samples were collected from 1544 individuals, and 294 were colonized ESBL *Enterobacteriaceae*. The prevalence of ESBL *Enterobacteriaceae* among Syrian refugees was reported as 20.4%. No carbapenem resistance was noted among the tested samples. The study emphasized the need for continuous investigation of the newly arrived asylum seekers [19].

In 2015, Germany received the highest number of asylum seekers since World War II. 40% of the refugees that arrived in Germany came from Syria, 16.1% from Afghanistan, and 13.5% were from Iraq. 25% of the Syrians refugees were children. 325 hospitalized refugee children were screened for the presence of multidrug-resistant pathogens. 110 of those children were positive multidrug resistant organisms (MDRO). The majority were infected with multidrug-resistant gram-negative bacteria and the rest with MRSA; one patient was colonized with Vancomycin-resistant *Enterococcus*

(VRE). The study concluded with the need to enforce policies to screen MDR in pediatric refugees that reside in Germany [20].

2. The Netherlands

Between 2014 and 2015, 273 refugees admitted to the university medical center Groningen were screened for drug-resistant microorganisms. 36.5% of the refugees were from Eritrea, and 18.6% from Syria, while 12% were babies. Around half of the participants were infected with different bacteria. The study showed that tuberculosis malaria were the major diseases. Asylum seekers from Eritrea showed higher numbers of malaria. The study recommended providing asylum seekers with urgent health care, because they also carried extended-spectrum beta-lactamases-producing *Enterobacteriaceae*. However, no carbapenems-producing *Enterobacteriaceae* were detected, which is the opposite of expectations, because there is a high number of studies that reported this problem in the countries of origin of studied refugees [21].

Between 2014-2016, MDRO were screened in samples collected from 2091 refugees in the Netherlands. The targeted diseases causative agents were MRSA, and drug-resistant *Enterobacteriaceae* (MDRE). 185 asylum seekers were positive to MRSA, and 331 harbored MDRE. The most frequent detection of MRSA and MDRE was in the refugees originated from Iraq (19.1% and 43.2%) and Syria (15.8% and 39.9%), respectively. ESBL, fluoroquinolones, and aminoglycosides were detected in some of the isolates. After a median of 60 days a follow-up study was conducted in order to test the difference that occurred after spending time in the Netherlands. The carriage rate of MDRO remained high, even after spending a long term in the Netherlands [22].

In another study, 130 asylum seekers were tested in the university hospital in Groningen, Netherlands. 36.5% were from Eritrea, and 18.6% were from Syria. 40 of these refugees harbored 52 multidrug-resistant microorganisms. 7.7% of the tested people carried MRSA, 20% harbored ESBL-producing bacteria, including *E. coli, Klebsiella*, *M. morganii*, and *E. cloacae*. 10% of the total tested isolates were resistant to fluoroquinolones. However, no carbapenems resistant strains were detected [23].

An asylum seeker from Syria was admitted to the center of MDR-TB (multidrug resistant tuberculosis) in the Netherlands in September 2015. The refugee was 38 years old and was treated in Greece and advised to complete the antibiotic course provided by the physicians. However, the patient did not take the whole medicine. 5 months later, the patient developed TB symptoms again. All the antibiotics did not work with the illness. Hence, the last resort antibiotic (colistin) was used in order to treat the patient. Fortunately, the asylum seeker was cured from the disease. The study emphasized the importance of tackling the hard conditions that the refugees are facing [24].

3. Austria

Austria is one of the refugees' targets since 2015 and around 9000 asylum seekers have arrived there along with 1 million asylum passengers to other countries. In 2015, 23 Shigellosis cases (originated from Afghanistan, Syrian, and Iraq) were detected in one of Austrian medical care facilities. 11 isolates of the causative agents (*Shigella*) expressed ESBL. The minimum inhibitory concentration of azithromycin was tested for the strains, and it was less or equal to 32 µg/ml. the study suggested an initiative to educate refugees about personal hygiene practices needed in the hard conditions they were living in. The study also recommended that refugees may need

special attention [25].

4. Italy

In 2015, adults and children refugees from Syria were screened for the presence of antimicrobial-resistant strains. The results reported the detection of carbapenem-resistant *Pseudomonas* (non-aeruginosa) and MRSA [12].

In another study, 48 healthy Syrian refugees were screened for drug-resistant microorganisms; 4 of them harbored MRSA, 6 carried extended-spectrum beta-lactamases-producing *E. coli, Klebsiella*, or *Shewanella putrefaciens*, and 4 carried carbapenem resistant *Pseudomonas* [23].

Syrian refugees (n = 48) were subjected to microbiological tests. Fecal, nasal, and pharyngeal swabs were collected from refugees that arrived in Italy in 2015. MRSA, *Psuedomonaceae*, meropenem resistant pathogens, and extended-spectrum beta-lactamases species were detected. The study concluded that unusual strains were discovered, and they showed resistant profile that hasn't been reported in the hosting country. The study also recommended providing refugees with urgent medical aid in order to avoid further consequences [26].

Another case was studied in Italy that focused on a 30 years old Syrian refugee who was infected with multidrug-resistant *Klebsiella pneumonia*, including resistance to carbapenem. However, it was susceptible to colistin. The genome sequencing showed that the isolate does not cluster with any Italian strain. The study suggested establishing an effective strategy to prevent diseases among refugees [27].

5. Turkey

A study was conducted to describe the prevalence of carbapenems resistant

Acinetobacter baumannii in Turkey and Azerbaijan in 2016. The study showed a high dissemination of strains that can resist carbapenems and concluded that because of the presence of Syrian and Iraqi refugees in the mentioned areas, the resistant strains can be easily transferred to Europe. In addition, there were several studies conducted in turkey on refugees to screen their microbiota and to detect resistant pathogens. Syrian refugees were reported to carry multidrug-resistant genes. Therefore, immigration can potentially transmit whatever they carry to other spots of the world [28].

UNHCR stated that the number of Syrian refugees exceeded 3,561,707 in turkey according to data collected in December 2017. The ministry of health declared that the proportion of tuberculosis (TB) infections has increased from 1.3% in 2011 to 6.8% in 2015. In addition, 558 new TB cases in Syrian refugees were reported in October 2015. The number of Syrian refugees infected with tuberculosis has dramatically increased from 25.1% to 34.6% between 2010 and 2013[15].

6. Jordan

In Jordan (2017), the number of Syrian refugees exceeded 659,063 individuals. Before the Syrian war, 6 cases out of each 100,000 people were infected with tuberculosis. Then between March 2012 and June 2013, it was assumed that 22% of tuberculosis infection cases were among the Syrian refugee populations. Therefore, the Jordanian National TB Program enforced a strategy to regulate and control the prevalence of TB among Syrian refugees in this country in collaboration with other international entities [15].

7. Syria

The Syrian pharmaceutical sector used to be a strong before 2011. Between

1980s and 2000s, 90% of the national pharmaceutical needs. However, after 2011 the war severely affected this sector. Unfortunately, in Syria, it is easy to access antibiotics. For example, in Damascus, 87% of the pharmacies are selling antibiotics without prescriptions, and only 3% refused to sell without prescription. A cross sectional study was conducted in Kalamon area in Syria in order to understand the use of antibiotics among people. 85% took antibiotics in the past 4 weeks and 34% of the participants were not conscious of the adverse effect of antibiotics. There are no enforced regulations in Syria to control circulation of antibiotics[29].

By April 2017, 56 out of 111 public hospitals were still working in Syria, and over 70% of the health professionals have left the country. 6.4 million Syrian have been internally displaced, and over 1 million of them are living under unhygienic conditions, which makes them exposed to different diseases. Destroyed health-care infrastructure, and lack of medical supplies and medical experts have contributed to the prevalence of antibiotic resistance in Syria.

In Aleppo, between 2008 and 2011, 260 different patients were tested for the presence of multidrug-resistant bacteria. 66% harbored meropenem and imipenem resistant microorganisms. Fluoroquinolones were reported to be resisted in 81% of the isolates. The resistance to aminoglycosides was reported at 78%, and the resistance to third and fourth generations to cephalosporin was also high. *It is worth mentioning that the study declared that colistin worked with only 7% of the whole isolates*. In 2005, first-line therapy (cefotaxime and ceftriaxone) worked with 25 gram-positive isolates of *S. pneumoniae,* but 64% of the isolates resisted penicillin. Carbapenems are used as salvage therapy in case the first-line therapy failed. Hence, the resistance to the mentioned class can pose a serious risk. Six studies in Syria after 2011 showed the presence of antimicrobial-resistant pathogens. The studies reported ESBL, CRE

pathogens, and they mentioned in one of the studies that colistin did not work on 11% of 177 *Pseudomonas aeruginosa* isolated from patients in Aleppo. There are no studies conducted to evaluate the prevalence of antibiotic resistance among Syrian refugees inside Syria since 2011 because of the barriers caused by the war [30].

8. Lebanon

More than 1.5 million Syrian refugees are residing in Lebanon, which is equal to around 25% of the whole Lebanese population. There are no formal camps similar to Turkey and Jordan, but they are distributed along the country. Since 2011, a remarkable increase of tuberculosis in Lebanon has occurred, and this can be partially attributed to the massive displacement of refugees from Syria to Lebanon. The percentages of tuberculosis in Lebanon were around 33% before 2011. However, in 2015, the number reached 53%, and it is increasing gradually. The statistical analysis done by WHO showed that Lebanese people infected by tuberculosis has decreased from 66.7% to 49.4/% between 2010 to 2014. However, Syrian refugees infected by TB increased from 1.6% to 16% in the same duration. In 2016, the proportion of Syrian refugees infected by TB had reached around 21.6% in Lebanon [15].

Lebanon also hosts 450,000 Palestinian refugees. In addition, there are around 250,000 migrants, as workers, from other countries that reported to host high incidents of tuberculosis (Ethiopia, Bangladesh, Sri Lanka, and Philippines). In collaboration with the Lebanese Ministry of Public Health, a study was conducted to evaluate the prevalence of drug-resistant tuberculosis in 2016-2017. 720 suspected cases were screened, and 284 were confirmed to carry tuberculosis. However, 250 cases were further screened, and 29.6% were Syrians, 28% were Lebanese, 22.8% from Ethiopia, 5.2% from Bangladesh, 2.8% from Palestine, and 11.6% from other nations. Rifampicin

resistant tuberculosis were detected among 2.8% of the patients, and 3 patients carried extensive drug-resistant tuberculosis. The study suggested testing all positive tuberculosis cases in order to have better assessment and to enhance the diagnosis for the foreigners in Lebanon in order to suppress the prevalence of multidrug-resistant tuberculosis [31].

9. Other Countries

In occupied Palestine, some Syrian children were treated for surgical and medical conditions. 80% of the patients carried multi-drug resistant pathogens such as MRSA, Vancomycin-resistant strains of *Enterococcus* and resistant *Acinetobacter baumannii* [12].

In 2014, isolates were retrieved from 29 children and 60 adults that were treated in occupied Palestine hospitals. MRSA and carbapenem-resistant gram-negative bacteria were detected in 13.5%, and 5.6% of the tested refugees, respectively [23].

Between 2013 and 2016, some of the Syrian children were treated in a neighboring country to Syria, and microbial data were collected and analyzed. Carbapenem resistant *Enterobacter*, MRSA, multidrug resistant *A. baumannii* (MDR-AB), VRE, ESBL were detected in pathogens carried by some of the children. However, colistin was the only drug that is working on pathogens [32].

F. Importance of Colistin

The largest class of beta-lactam antibiotics is called cephalosporine. It has been divided into 1st, 2nd, 3rd, and 4th generations. The antibiotics that fall under cephalosporin umbrella are considered to be the most prescribed antibiotics. In fact, the first-generation has better activity against gram-positive bacteria but less for gram-

negative bacteria. The second generation is active against gram-negative bacteria but not gram-positive ones. The third generation is active better against gram-negative bacteria, and it has better performance against beta-lactamase than first and second generations. The fourth generation is the enhanced version of the third one in terms of stability against beta-lactamase, and its ability to fight against gram-positive bacteria[34]. Cephalosporins are considered the first-line treatment against bacterial infections[35]. However, the resistance to cephalosporin summoned the use of salvage therapy that can be conducted by carbapenems[30]. Carbapenems are also a beta-lactams class of antibiotics. They are more stable against the enzymes that inactivate beta-lactams. The combination of carbapenems with beta-lactam ring gives the carbapenems a unique structure and makes them more effective against gram-positive and negative bacteria. However, the emergence of resistance to carbapenems has resulted in the use of the last-resort antibiotic, colistin [36].

Colistin:

This antibiotic belongs to the polymyxin class and is naturally produced by *Bacillus polymyxa*. It is also known as polymyxin E, which has a similar chemical structure to polymyxin B. The first discovery of colistin dates back to 1947, and it was accepted for clinical use in 1959 [37]. Polymyxins can inhibit the cell membrane synthesis by the interaction between cationic polypeptide in their structure and the anionic lipopolysaccharides in cell membrane of gram-negative bacteria. That leads to the displacement of calcium and magnesium and increases the cell membrane permeability and leakage of cell content, which causes cell death.

Colistin was also introduced to animal husbandry in 1950s, while in humans, colistin was restricted because of its systematic toxicity. However, in the last 10 years, after the emergence of carbapenems resistant bacteria, colistin was re-introduced to

human treatment as a last resort antibiotic[38]. Some of the countries restricted the use of colistin in the animal husbandry in order to revive its role against resistant bacteria that affect people. However, in other countries, colistin has been use excessively. A study concluded that the wide use of colistin could lead to the dissemination of colistin-resistant gene (*mcr-1*).

The first detection of this gene dates back to 2015 in *E. coli* SHP45 isolated from 7a pig in China. The study showed that this gene was carried on a plasmid, and it can be transmitted horizontally. Besides, this gene is able to disseminate to other countries, threatening the control of infectious diseases globally [39].

Eleven variants of the *mcr* gene were detected in different counties around the world (*mcr-1.1* to *mcr-1.11*). This suggested the emergence of other variants under unknown selective pressure conditions in the environment, humans, or animals. Other *mcr* genes were described such as *mcr-2* that is rare and can be carried only on IncX4 replicon, and this gene is approximately 80% similar to *mcr-1*. In addition, *mcr-3*, *mcr-4*, and *mcr-5* were detected in *E. coli* and/or *Salmonella*, and they are different from *mcr-1* and *mcr-2*.

There is a strong association between the high consumption of colistin and the prevalence of *mcr-1*. China became the top user of colistin by the end of 2015, and studies showed a high prevalence of *mcr-1* in this country. However, in the US, colistin was not used in agriculture. Hence, the presence of *mcr-1* is restricted in the states. In some of the European countries like Portugal and Italy, colistin was used extensively, and the prevalence of *mcr-1* is relatively high in these countries.

Colistin functions based on the electrostatic interaction with lipid A in the bacterial cell membranes; however, *mcr-1* can reduce this interaction and lead to resistance [40].

Worldwide, *mcr-1* has been reported in more than 40 countries, including Lebanon. Very few studies in Lebanon and the MENA region showed the presence of *mcr-1*-positive *E. coli* in different sectors, such as samples collected from different locations of irrigation water, poultry, and human feces[41-43].

G. Conclusion

Syrian conflict entered its ninth year of war, and a massive number of people have been displaced from their home carrying all their microbiota and getting exposed to different dangers, including new pathogens. The bacteria that might infect refugees can evolve and lead to the emergence of new multi-drug resistance problems. Given that the number of refugees is increasing across the world, governments and organizations must prepare plans to deal with health issues of this population and give special attention to their access to clean water and food. Global health care guidelines and intervention should be established in order to develop useful health aid for refugees. After the first discovery of *mcr-1* in 2015, global health was alarmed. In Lebanon, colistin is being used extensively and is widely available. Unfortunately, in Lebanon, very few studies screened the dissemination of MDR pathogens among refugees living without access to proper hygiene, clean water, and sanitation. Hence, the work outline in this thesis has focused on the detection of *mcr-1* in Syrian refugee camps (domestic water and sewage) in Lebanon poses a serious issue and calls for urgent action in order to have a better understanding of the prevalence of resistance to a last-resort antibiotic (colistin), which might be essential for treating infections in this vulnerable population.

CHAPTER II

METHODOLOGY

Two Syrian refugee camps were selected in the Beqaa area based on two criteria: 1- clearly visible weak infrastructure, and 2- accessibility to researchers. The camps were labeled as camp 1 and camp 2.

- *Camp 1*

Consists of 7 tents and 28 individuals (seven children under ten years old and 21 persons above). The following is some information about this camp: 1- the source of drinking water is from the Lebanese domestic supply (when available). 2- well water is used in winter to wash utensils and for general use like cleaning and showering. In Summer, they use well water for drinking, because domestic supply usually stops most of the day. 3- they use a pump to transfer water from the well to a large container (around 2000 liters) inside the camp, and then they fill water in small containers to drink and clean. 4- sewage water does not contaminate well water. 5- sewage water goes to the general sewage system area after it leaves the camp.

- *Camp 2*

Consists of 42 caravans. One hundred thirty individuals live in this camp divided into 65 children under ten years and 65 above ten years old. The following is some information on this camp:1- the source of drinking water is from a Spring called (Shtora spring). Every day a truck comes to the camp and fills two large contains with drinking water (around 2000 L each). The containers have faucets so that people can fill water in small containers. Sometimes, donors pass by the camps and fill the containers from unknown sources. 2- well water is used for washing, showering, and cleaning. 3-

well water is pumped to caravans for the purposes mentioned above (number 2). 4-sometimes sewage water contaminates the well. 5-sewage water goes to the central sewage system after it leaves the camp.

A. Samples Collection

Eighteen composite samples were collected from the two camps. Every sampling event covered three sources in each camp (drinking water, well water, and sewage water).

Samples were placed in sterile bottles which were then placed on ice. The analysis conducted on the same day of sample collection.

B. Water Filtration and Culturing

The water samples were filtered using 0.22 μm Millipore® membranes, and two different volumes were used from each sample to be filtered (100 ml and 500 ml); except sewage samples that were diluted before filtration. The membranes were placed onto the selective RAPID' *E. coli* 2 Agar supplemented with 4 μg/ml colistin. The membranes of the third sampling event were also placed onto the selective XLT-4 Agar media supplemented with 4 μg/ml colistin.

The samples were incubated at 37°C for 24 h, and the suspected colonies were isolated and further purified. Then, the purified isolates were placed in 1 ml Luria-Bertani (LB) broth with 0.5 ml glycerol 80% and stored at -80°C for further analysis [41].

C. Antimicrobial Resistance (AMR)

The optical density of each strain was adjusted to 0.05 (wave-length: 600 nm)

in MH broth. Swabs were used to streak the cultures on Mueller-Hinton agar plates. Seventeen different commercially-available antibiotics disks were tested against each culture.

The antibiotics were: Penicillin (PEN), ampicillin (AMP), amoxicillin + calvulanic acid (AMC), cefepime (FEP), cefotaxime (CTX), cephalexin (LEX), cefixime (CFM), doripenem (DOR), meropenem (MEM), gentamicin (GEN), kanamycin (KAN), streptomycin (STR), tetracycline (TET), ciprofloxacin (CIP), norfloxacin (NOR), trimethoprim-sulfamethoxazole (SXT), and chloramphenicol (CHL). The antibiotics in the resistance profile were arranged according to the order of antibiotics/ classes listed in the CLSI guidelines. The diameter around each disk was measured and compared to the CLSI standards to identify whether the colony is resistant or susceptible. Erythomycin and *E. coli* DH-5-alpha were used as controls.

D. Minimum Inhibitory Concentration (MIC)

Each colony was challenged with a serial dilution of colistin in 96-well microliter. The concentrations of colistin ranged between 1 μg/ml to 640 μg/ml. The plates were incubated for 18-24 hours at 37°C, and then the plates were analyzed with an Infinite M200PRO microplate reader at λ = 600 nm. The results were compared with the reading of control wells [41].

E. Biofilm Formation

Purified colonies were incubated at 37°C in 10 ml LB broth supplemented with colistin 4 μg/ml overnight. 1/10 dilution was prepared from the cultured bacterial broth, and then 0.2 ml was distributed in each well of 96-well microliter plate. Twelve replicates were conducted for each sample. The 96-wells plates were incubated for 1, 3,

and 6 days. The optical density was noted down before and after incubation. The broth in the wells was dispensed after incubation and washed 3 times with 0.9% NaCl solution (0.2 ml). The wells were then filled with 0.2 ml crystal violet (0.1%) and incubated at room temperature for 15 min. Then, the wells were washed five times with 0.9% NaCl (0.2 ml). 0.2 ml acetic acid was added to each well and incubated for 15 min at room temperature. The absorbance of crystal violate was measured at the λ = 595 nm with the Infinite M200PRO microplate reader. The average value of the absorbance was placed on a graph with standard deviation [44]. The experiment was repeated on two separate occasions.

F. Plasmid Extraction and Transformation

Plasmid transformation was conducted by using the QIAGEN® Plasmid Mini Kit (25). Chemically-competent *E. coli* JM109 was used as recipients for the extracted plasmids. The competent cells were placed on ice for 10 min. Then 0.05 ml of the competent cells were mixed with ten µl of the purified plasmid and incubated on ice for 30 min. The mixture was placed in a water bath at 42°C for 2 min followed by 90 s on ice. 940 µl of LB broth were added to the mix and then incubated shaking at 37°C for 1hr 45 min. The mixture was centrifuged for 1 min and 0.9 ml was dispensed. The pellet was resuspended placed on RAPID' *E.coli* 2 Agar supplemented with 2 µg/ml colistin. Petri dishes were incubated at 37° C for 24 hours, and bacteria colonies (transformants) harvested and stored for further analysis [41].

G. Swarming Assay

Purified colonies were placed on LB Agar media supplemented with 4 µg/ml colistin and incubated overnight. One colony was taken and placed in 10 ml LB broth

and incubated overnight with shaking. Optical density was adjusted to 0.02 for each sample, and then five µl of the adjusted bacterial broth was inoculated at the top of an agar plate and was left to dry for 10 min. After drying, the plates were placed in the incubator at 37°C. The plates for swarming contained semi-solid LB Agar (1.5%) without colistin. Photos were taken every 12 hours.

H. Polymerase Chain Reaction (PCR)

PCR Based Replicon Typing Kit 2.0 (Diatheva) was used to identify the plasmid types as described by the manufacturer.

Gene detection was also performed using PCR analysis (Table 1). The reaction consisted of DNase free water, four µl of (5x FIREPol® Master Mix Ready to Load), primers, and the DNA to be analyzed. All the genes were tested at 38 cycles with a thermal cycler (VWR).

Table 1. A list of the tested genes and the PCR conditions

Gene and Primers	**Denaturation**	**Annealing**	**Extension**	**Size**
mcr-1 gene CLR5-F (5′-CGGTCAGTCCGTTTGTTC-3′) and CLR5-R (5′-CTTGGTCGGTCTGTA GGG-3’)	95°C for 1min	56°C for 45 sec	72°C for 1 min	309 bp
mcr-2 gene MCR-2F (GCGATGGCGGTCTATCCTGTAT) MCR-2R (TGCGATGACATGGGGTGTCAGC)	95°C for 1min	55°C for 45 sec	72°C for 1 min	378 bp
mcr-3 gene MCR-3F (TATGGGTTACTATTGCTGG) MCR-3R (CTACCCTGATGCTCATCG)	95°C for 1min	55°C for 45 sec	72°C for 1 min	814 bp
mcr-4 gene MCR-4F (GTCATAGTGGTATAAAAGTACAG) MCR-4R (CCACCGTCTATCAGAGCCAAC)	95°C for 1min	55°C for 45 sec	72°C for 1 min	669 bp
mcr-5 gene MCR-5F (GCGGTTGTCTGCATTTATCAC) MCR-5R (CTTTGAAAACCTGTCTTCGGCA)	95°C for 1min	55°C for 45 sec	72°C for 30 sec	1,042 bp
mcr-6 gene MCR-6F (GTCCGGTCAATCCCTATCTGT) MCR-6R (ATCACGGGATTGACATAGCTAC)	95°C for 1min	55°C for 45 sec	72°C for 1 min	556bp

"Table 1 - *Continued*"

Gene and Primers	Denaturation	Annealing	Extension	Size
mcr-7 gene MCR-7F (TGCTCAAGCCCTTCTTTTCGT) MCR-7R (TTCATCTGCGCCACCTCGT)	95°C for 1min	55°C for 45sec	72°C for 1 min	892 bp
mcr-8 gene MCR-8F (AACCGCCAGAGCACAGAATT) MCR-8R (TTCCCCCAGCGATTCTCCAT)	95°C for 1min	60°C for 45sec	72°C for 1 min	667 bp
16S *E. coli* gene 16S-1-f AAGAAGCTTGCTTCTTTGCTGAC-16S-1-r AGCCCGGGGATTTCACATCTGACTTA	95°C for 30 sec	50°C for 45 sec	72°C for 1 min	544 bp
*ure*C gene *ure*C F CGGAACAGAAGTTGTCGCTGGA *ure*C R GGGCTCTCCTACCGACTTGATC	94C for 1 min	60°C for 30 sec	72°C for 1 min	533 bp
bla-$_{KPC}$ gene Forward: (CATTCAAGGGCTTTCTTGCTGC) Reverse: (ACGACGGCATAGTCATTTGC)	95°C for 1min	56°C for 45 sec	68°C for 1 min	498 bp
bla-$_{IMP}$ gene Forward (TGAGCAAGTTATCTGTATTC) Reverse: (TTAGTTGCTTGGTTTTGATG)	95°C for 1min	56°C for 45 sec	68°C for 1 min	232 bp
bla-$_{OXA-48}$ Forward: (GCTTGATCGCCCTCGATT) Reverse: (GATTTGCTCCGTGGCCGAAA)	95°C for 1min	56°C for 45 sec	68°C for 1 min	238 bp
bla-$_{NDM}$ Forward: GGTTTGGCGATCTGGTTTTC Reverse: CGGAATGGCTCATCACGATC	95°C for 1min	56°C for 45 sec	68°C for 1 min	521 bp
invA gene Forward: GTGAAATTATCGCCACGTTCGGGCAA Reverse: TCATCGCACCGTCAAAGGAACC	95°C for 1min	60°C for 45 sec	72°C for 1 min	284 bp
Intl1 gene Forward: CCTCCCGCACGATGATC Reverse: TCCACGCATCGTCAGGC	95°C for 30 sec	55°C for 45 sec	65°C for 1 min	250 bp
Class 1 gene Forward: GGCATCCAAGCACAAGC Reverse: AAGCAGACTTGACTGAT	95°C for 30 sec	55°C for 45 sec	65°C for 1 min	Various
intI 2 gene forward: GTAGCAAACGAGTGACGAAATG Reverse: CACGGATATGCGACAAAAAGGT	95°C for 30 sec	55°C for 45 sec	65°C for 1 min	789 bp
Class 2 gene Hep74 CGGGATCCCGGACGGCATGCACGATTTGT A Hep51: GATGCCATCGCAAGTACGAG	95°C for 30 sec	55°C for 45 sec	65°C for 1 min	Various
bla-$_{TEM}$ gene Forward: ACCAATGCTTAATCAGTGAG Reverse: GCGGAACCCCTATTTG	95°C for 1min	56°C for 45 sec	68°C for 1 min	963 bp
bla-$_{CTX-M}$ Forward: ATGTGCAGYACCAGTAARGTKATGGC Reverse: TGGGTRAARTARGTSACCAGAAYCAGCGG	95°C for 1min	56°C for 45 sec	68°C for 1 min	529 bp
bla-$_{SHV}$ Forward: CACTCAAGGATGTATTGTG Reverse: TTAGCGTTGCCAGTGCTCG	95°C for 1min	56°C for 45 sec	68°C for 1 min	822 bp

BOX A1R PCR was used in order to type the bacterial isolates using the PCR machine. The PCR conditions were initial incubation at 94°C for 2 min then cycles start at 94°C for 30 sec followed by annealing temperature at 50°C for 1 min and 65°C for 8 min and the final elongation for 8 min at 65°C.

After conducting the PCR for all the genes, agarose gel (1.5%) was prepared and the reactions were subjected to electrophoresis at 30 min with 100 Volts. For BOX PCR the gel was 2% and electrophoresis was for 75 min. Next, the gel was placed in the imaging system, and the gels were photographed.

I. Sequencing

Commercial sequencing was performed after purifying the amplified genes using QIAquick® Gel Extraction Kit (50) and QIAquick® PCR Purification Kit (50). Then the purified product was sent to be sequenced commercially.

CHAPTER III

RESULTS

Eighteen composite water samples were collected from 2 Syrian refugee camps (Figure 1). The samples were collected from containers used to keep drinking water, wells used to get water for showering and washing, and sewage water that represents the bacteria in the camps' population. The targeted bacteria were *Enterobacteriaceae* that harbored *mcr-1*. For the first and second sampling, membranes after water filtration were placed on the RAPID' *E. coli* 2 Agar and incubated for 24 h. The count of coliforms and *E. coli* was noted down (Figure 2).

Fig. 1. Drinking, Well and Sewage Water in 2 Syrian Refugee Camps

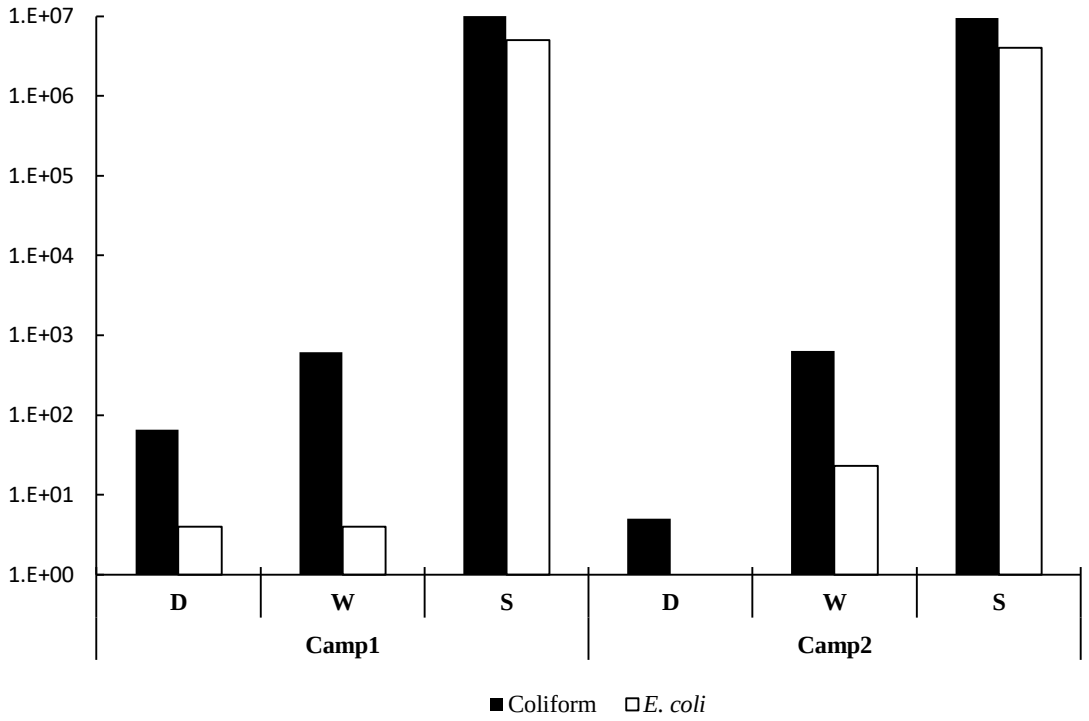

Fig. 2. Number of E. coli and coliforms that were detected on the membranes. C for Camp, D for Drinking, W for Well, S for sewage

Nine samples from the first and second sampling yielded 36 *E. coli* colonies that were further identified by detecting 16S rRNA gene-fragment that is specific to *E. coli* (Figure 3). All purple colonies were further purified and stored at -80°C for further analysis. All the colonies were tested for the presence of *mcr-1*, and they were all positive (Figure 4). *mcr-1* detected in *E. coli* colonies was commercially sequenced after purification. Sequencing data, reported in this study, were deposited in GenBank under accession numbers: GenBank MK907916-MK907920. *mcr-1* was successfully transferred to chemically competent *E. coli* JM109. All the transformants were *mcr-1* positive, and they were resistant to PEN with a colistin MIC of 4 μg/ml. Table 2 includes all the tested genes and the profile of resistance and MIC.

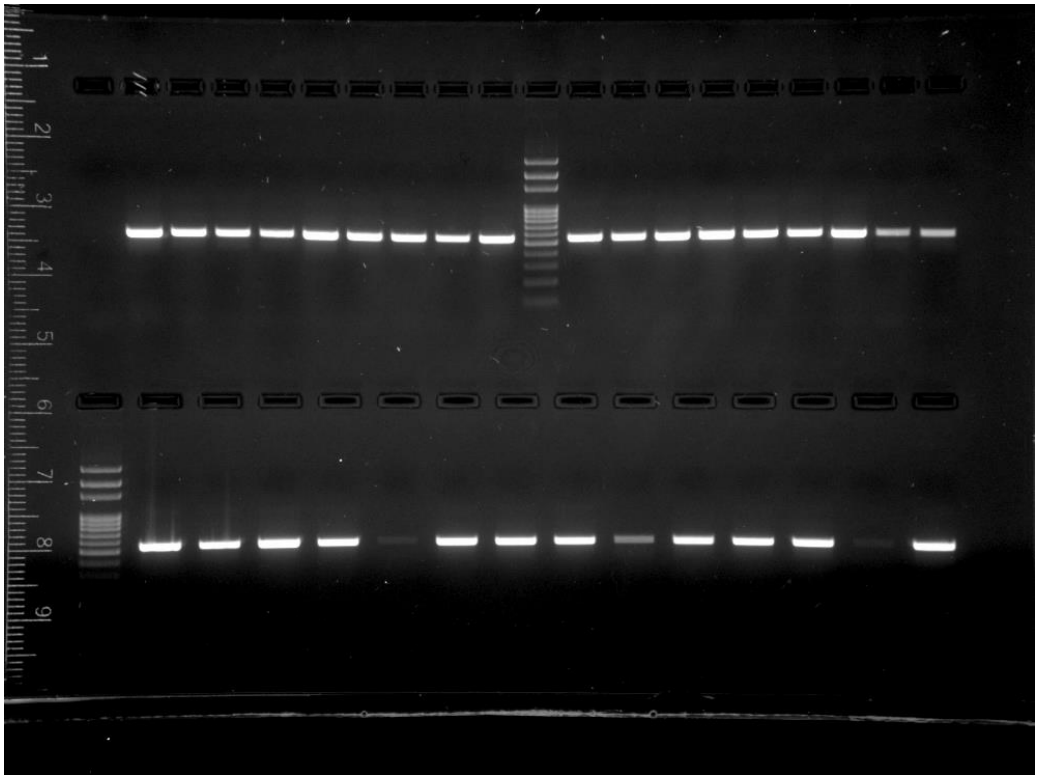

Fig. 3. PCR signals of 16 S rRNA gene

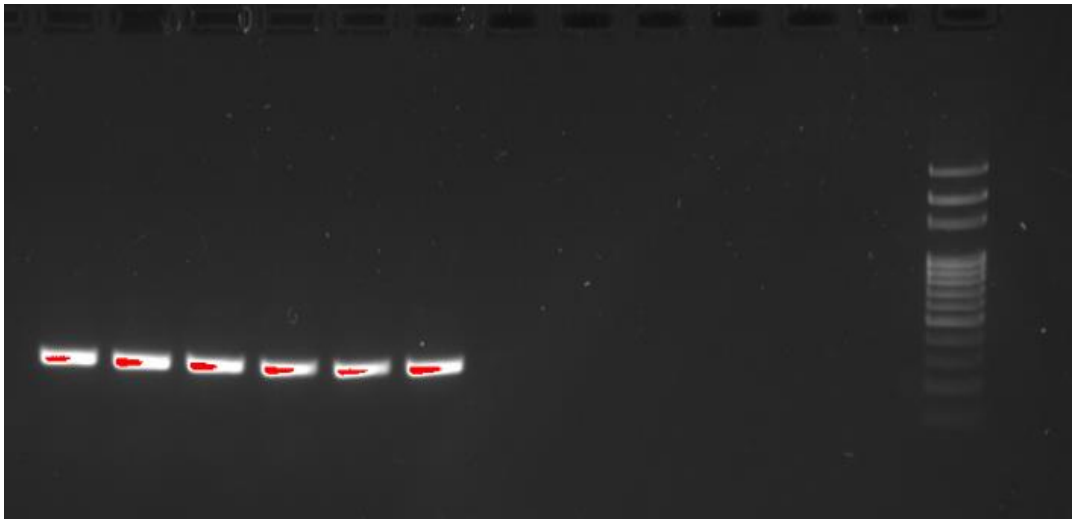

Fig. 4. PCR signals of mcr-1 gene

The plasmids were typed for 13 (36%) isolates. Table 3 shows the detected plasmids in the tested colonies.

Table 2. List of isolates and their properties

Location	Sample Identification Code	Resistance Profile	Intermediate Resistance Profile	Colistin MIC (µg/ml)	Genes
Camp 1 (First Sampling) 02/02/2019	**C1D1R1**	PEN-AMP-AMC-LEX-GEN-KAN-STR-TET-CIP-NOR-SXT-CHL	FEP	32	16S rRNA gene, *mcr-1*, *bla*$_{TEM,}$ *bla*$_{CTX-M,}$, *int1*, Class 1 Integron genes.
	C1D2R1	PEN-AMP-AMC-LEX-GEN-KAN-STR-TET-CIP-NOR-SXT-CHL	FEP-CTX	64	16S rRNA gene, *mcr-1*, *bla*$_{TEM,}$ *bla*$_{CTX-M,}$, *int1*, Class 1 Integron genes.
	C1D3R1	PEN-AMP-AMC-LEX-GEN-KAN-STR-TET-CIP-NOR-SXT-CHL	FEP-CTX	64	16S rRNA gene, *mcr-1*, *bla*$_{TEM,}$ *bla*$_{CTX-M,}$ *int1*, Class 1 Integron genes.
	C1D4R1	PEN-AMP-AMC-GEN-KAN-STR-TET-CIP-NOR-SXT-CHL	LEX	32	16S rRNA gene, *mcr-1*, *bla*$_{TEM,}$ *bla*$_{CTX-M,}$, *int1*, Class 1 Integron genes.
	C1S1R1	PEN-AMP-AMC-LEX-GEN-KAN-STR-TET-CIP-NOR-SXT-CHL	-	16	16S rRNA gene, *mcr-1*, *bla*$_{TEM,}$ *bla*$_{SHV,}$ *int1*, Class 1 Integron genes.
	C1S2R1	PEN-AMP-AMC-LEX-GEN-KAN-STR-TET-CIP-NOR-SXT-CHL	-	16	16S rRNA gene, *mcr-1*, *bla*$_{TEM,}$ *bla*$_{SHV.}$ *int1*, Class 1 Integron genes.
	C1S3R1	PEN-AMP-AMC-FEP-LEX-CFM-GEN-KAN-STR-TET-CIP-NOR-SXT-CHL	-	32	16S rRNA gene, *mcr-1*, *bla*$_{TEM,}$ *bla*$_{SHV,}$ *bla*$_{CTX-M.}$, *int1*, Class 1 Integron genes.
	C1S4R1	PEN-AMP-AMC-LEX-CFM-GEN-KAN-STR-TET-CIP-NOR-SXT-CHL	-	32	16S rRNA gene, *mcr-1*, *bla*$_{TEM,}$ *bla*$_{SHV.}$ *int1*, Class 1 Integron genes.
	C1S5R1	PEN-AMP-AMC-CTX-LEX-CFM-GEN-KAN-STR-TET-CIP-NOR-SXT-CHL	-	32	16S rRNA gene, *mcr-1*, *bla*$_{TEM,}$ *bla*$_{SHV,}$ *int1*, Class 1 Integron genes
Camp 1 (Second Sampling) 17/02/2019	**C1D1R2**	PEN-AMP-AMC-FEP-CTX-LEX-TET-CIP-NOR-SXT-CHL	CFM	32	16S rRNA gene, *mcr-1*, *bla*$_{TEM,}$ *bla*$_{CTX-M,}$ *int1*, Class 1 Integron genes.
	C1D2R2	PEN-AMP-AMC-CTX-LEX-TET-CIP-SXT-CHL	FEP-CFM-NOR	16	16S rRNA gene, *mcr-1*, *bla*$_{TEM,}$ *bla*$_{CTX-M,}$ *int1*, Class 1 Integron genes.
	C1D3R2	PEN-AMP-AMC-LEX-CFM-GEN-KAN-TET-CIP-NOR-SXT-CHL	CTX-STR	8	16S rRNA gene, *mcr-1*, *bla*$_{TEM,}$ *bla*$_{CTX-M}$, *int1*, Class 1 Integron genes.
	C1D4R2	PEN-AMP-AMC-CTX-LEX-CFM-GEN-KAN-TET-CIP-NOR-SXT-CHL	STR	8	16S rRNA gene, *mcr-1*, *bla*$_{CTX-M}$, *int1*, Class 1 Integron genes.
	C1D5R2	PEN-AMP-AMC-CTX-LEX-CFM-GEN-KAN-TET-CIP-NOR-SXT-CHL	STR	4	16S rRNA gene, *mcr-1*, *bla*$_{CTX-M}$, *int1*, Class 1 Integron genes.
	C1W1R2	PEN-AMP-LEX-CFM-GEN-KAN-TET-CIP-NOR-SXT-CHL	AMC	32	16S rRNA gene, *mcr-1*, *bla*$_{TEM,}$, *int1*, Class 1 Integron genes.

"Table 2. *Continued*"

Location	Sample Identification Code	Resistance Profile	Intermediate Resistance Profile	Colistin MIC (µg/ml)	Genes
	C1W2R2	PEN-AMP-LEX-CFM-GEN-KAN-TET-CIP-NOR-CHL	AMC-LEX-SXT	32	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ bla_{CTX-M}, *int1*, Class 1 Integron genes.
	C1W3R2	PEN-AMP-AMC-CTX-LEX-CFM-GEN-KAN-TET-CIP-NOR-SXT-CHL	STR	64	16S rRNA gene, *mcr-1*, $bla_{TEM.}$ *int1*, Class 1 Integron genes
	C1W4R2	PEN-AMP-GEN-KAN-STR-TET-CIP-NOR-SXT-CHL	AMC	32	16S rRNA gene, *mcr-1*, bla_{TEM} , *int1*, Class 1 Integron genes.
Camp 2 (First Sampling) 09/02/2019	**C2W1R1**	PEN-AMP-AMC-CTX-LEX-CFM-DOR-GEN-KAN-STR-TET-CIP-NOR-SXT-CHL	FEP	32	16S rRNA gene, *mcr-1*, $bla_{TEM,}$, $bla_{OXA-48,}$ $bla_{CTX-M,}$, *int1*, Class 1 Integron genes.
	C2W2R1	PEN-AMP-AMC-CTX-LEX-CFM-GEN-KAN-TET-CIP-NOR-SXT-CHL	STR	16	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ $bla_{CTX-M,}$, *int1*, Class 1 Integron genes.
	C2W3R1	PEN-AMP-AMC-CTX-LEX-CFM-GEN-KAN-TET-CIP-NOR-SXT-CHL	STR	16	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ bla_{CTX-M} , *int1*, Class 1 Integron genes.
	C2W4R1	PEN-AMP-AMC-CTX-LEX-CFM-GEN-KAN-TET-CIP-NOR-SXT-CHL	STR	16	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ $bla_{CTX-M,}$ *int1*, Class 1 Integron genes.
	C2W5R1	PEN-AMP-AMC-CTX-LEX-CFM-GEN-KAN-TET-CIP-NOR-SXT-CHL	STR	16	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ $bla_{CTX-M,}$ *int1*, Class 1 Integron genes.
	C2W6R1	PEN-AMP-AMC-CTX-LEX-CFM-GEN-KAN-TET-CIP-NOR-CHL	STR	16	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ $bla_{CTX-M,}$, *int1*, Class 1 Integron genes.
	C2S1R1	PEN-AMP-AMC-LEX-KAN-STR-TET-SXT-CHL	CIP	8	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ bla_{CTX-M} *int1*, Class 1 Integron genes.
	C2S2R1	PEN-AMP-AMC-LEX-KAN-STR-TET-SXT-CHL	CIP	16	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ $bla_{CTX-M.}$ *int1*, Class 1 Integron genes.
	C2S3R1	PEN-AMP-AMC-FEP-CTX-LEX-CFM-GEN-KAN-TET-CIP-SXT-CHL	STR-NOR	32	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ bla_{CTX-M}, *int1*, Class 1 Integron genes.

"Table 2. *Continued*"

Location	Sample Identification Code	Resistance Profile	Intermediate Resistance Profile	Colistin MIC (µg/ml)	Genes
	C2S4R1	PEN-AMP-KAN-STR-TET-CIP-SXT-CHL	AMC	16	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ bla_{CTX-M}, *int1*, Class 1 Integron genes.
Camp 2 (Second Sampling) 23/02/2019	**C2W1R2**	PEN-AMP-LEX-KAN-TET-CIP-SXT-CHL	-	16	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ $bla_{CTX-M,}$ *int1*, Class 1 Integron genes.
	C2W2R2	PEN-AMP-AMC-FEP-CTX-LEX-CFM-SXT	-	16	16S rRNA gene, *mcr-1*, $bla_{CTX-M.}$ *int1*, Class 1 Integron genes
	C2S1R2	PEN-AMP-AMC-CTX-LEX-CFM-DOR-KAN-STR-TET-SXT-CHL	FEP-GEN-CIP-NOR	8	16S rRNA gene, *mcr-1*, $bla_{TEM,}$, $bla_{OXA-48,}$ bla_{CTX-M} $bla_{KPC.,}$ *int1*, Class 1 Integron genes.
	C2S2R2	PEN-AMP-AMC-CTX-LEX-CFM-GEN-KAN-STR-TET-CIP-NOR-SXT-CHL	FEP	16	16S rRNA gene, *mcr-1*, $bla_{CTX-M,}$ *int1*, Class 1 Integron genes.
	C2S3R2	PEN-AMP-FEP-CTX-LEX-CFM-SXT	AMC	4	16S rRNA gene, *mcr-1*, bla_{CTX-M} *int1*, Class 1 Integron genes.
	C2S4R2	PEN-AMP-FEP-CTX-LEX-CFM-KAN-TET-CIP-NOR-SXT-CHL	-	16	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ $bla_{CTX-M.,}$ *int1*, Class 1 Integron genes.
	C2S5R2	PEN-AMP-FEP-CTX-LEX-CFM-KAN-STR-TET-CIP-NOR-SXT-CHL	-	32	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ bla_{CTX-M}, *int1*, Class 1 Integron genes.
	C2S6R2	PEN-AMP-FEP-CTX-LEX-CFM-KAN-TET-CIP-NOR-CHL	-	32	16S rRNA gene, *mcr-1*, $bla_{TEM,}$ bla_{CTX-M} *int1*, Class 1 Integron genes.

The most frequent detected plasmid replicons were IncI2 and IncX4 that are globally responsible for the dissemination of *mcr-1*. Also, IncF was detected, and it is associated with carrying antibiotic resistance and virulence genes.

Table 3. Results of plasmid typing using PBRT Kit. Isolate label includes: C: camp; D: drinking water, S: sewage; W: well water. R: indicates the round of sampling

Samples	**Detected Plasmids**
C1D1R1	IncI1α-IncHI2-IncFIB-IncFII
C1S3R1	IncI2-IncF-IncFIB-IncX4-IncFII
C1S4R1	IncI2-IncF-IncFIB-IncX4-IncFII
C1S5R1	IncI2-IncF-IncFIB-IncX4-IncFII
C1D3R2	IncI1α-IncB/O-IncX4-IncFII
C1W1R2	IncF-IncFII
C1W3R2	IncI1α-IncHI1-IncF-IncX4-IncFII
C2W1R1	IncI1α-IncHI1-IncX4
C2W5R1	IncI1α-IncHI1-IncX4
C2S3R1	IncI1α-IncHI2-IncFIIk-IncX4-IncFII
C2W2R2	IncI1α-IncHI1- IncFIB-IncX4- IncFII
C2S2R2	IncFIB-IncFII
C2S5R2	IncHI2-IncI2-IncF-IncFIB- IncFII

All the detected isolates were multidrug-resistant, because they resisted ≥3 antibiotic classes. In addition to being colistin-resistant, resistance was observed against penicillin, ampicillin, amoxicillin + clavulanic acid, cefepime, cefotaxime, cephalexin, cefixime, doripenem, gentamicin, kanamycin, streptomycin, tetracycline, ciprofloxacin, norfloxacin, trimethoprim sulfamethoxazole, and chloramphenicol (Figure 5). No resistance was detected against Meropenem and Imipenem in the tested isolates. All the isolates harbored Class-1 Integron genes and *int1* (encoding integrase). However, the strains did not harbor Class-2 Integron genes and *int2*. bla_{TEM} was detected in 86% of the isolates while $bla_{\text{CTX-M}}$ and bla_{SHV} were detected in 81% and 14%, respectively. The doripenem resistant strains (C2W1R1 and C2S1R2) were found to be $bla_{\text{OXA-48}}$ positive. bla_{KPC} was just found in the C2S1R2 *E. coli* isolate (Figure 6).

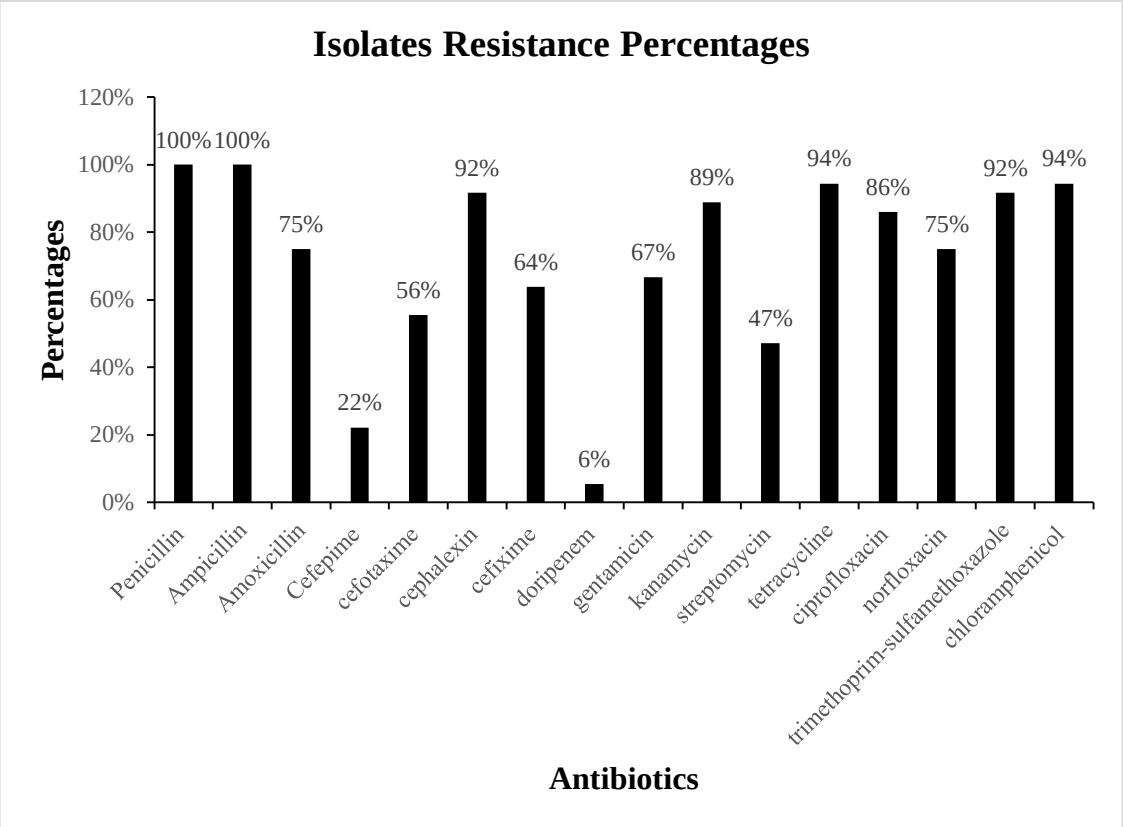

Fig. 5. Number of isolates (percent) resistance to each antibiotic tested

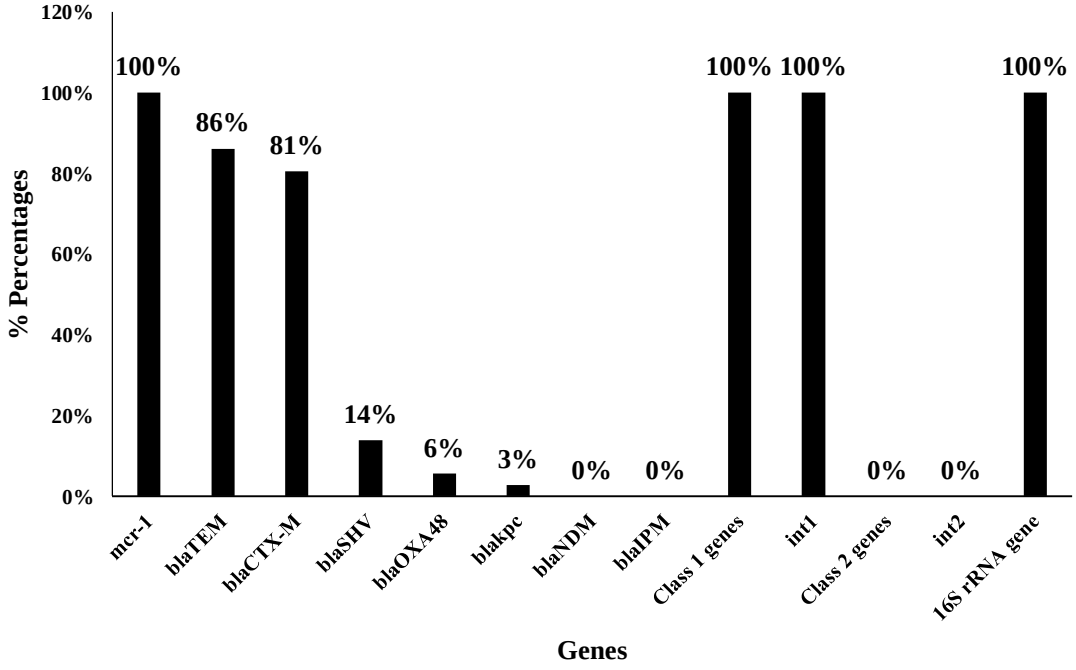

Fig. 6. The number of isolates (percent) harboring the resistance genes

BOX PCR fingerprinting analysis showed that the 36 *E. coli* isolates belonged to 25 different genotypes, and *E. coli* (C2W1R2) isolated from well water was 96.9% similar to C2S2R1 and 92.9% identical to C2S1R1 in Camp 2. Also, *E. coli* isolated from drinking water (C1D3R1) was 84.7% similar to those isolated from sewage (C1S3R1, C1S4R1 and C1S5R1).

All the isolates were negative for *mcr* 2 to 8, and the colistin minimum inhibitory concertation varied between 4-64 µg/ml (Figure 7).

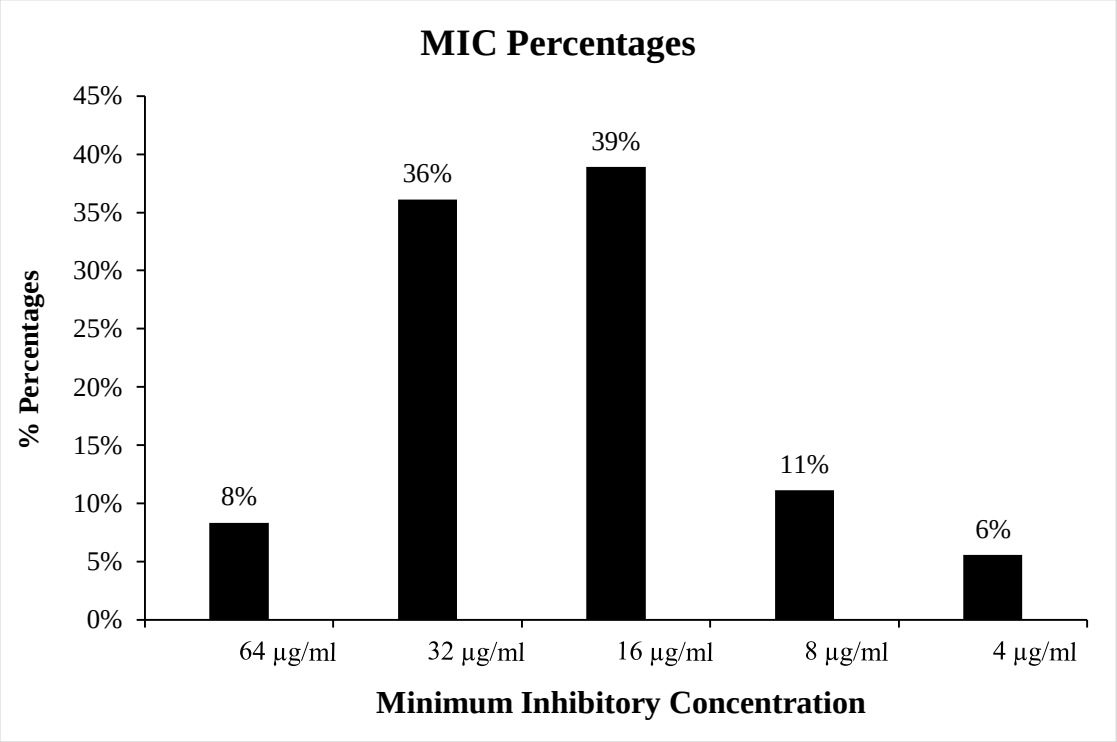

Fig. 7. Number of isolates (percent) that was inhibited on each colistin concertation

Six samples were filtered and placed on Eosin methylene blue (EMB), Xlt-4, and Rapid' Salmonella agar supplemented with 4 µg/ml colistin. Eight colonies (one to three per positive sample) were isolated from 5 (84%) samples, and they were phenotypically different from *E. coli*. Then, the colonies were plated on MacConkey

agar, and all the isolates grew whitish. Upon further observation, the strains appeared to be irregular and unable to ferment lactose. The isolates emitted a fishy odor and swarmed in a typical concentric-circles-motif on LB agar. All the isolates were tested for the presence of species-specific gene (*UreC*) *Proteus mirabilis,* and they were all positive (Figure 8). The gene was sequenced for all the isolates commercially. Although *P. mirabilis* is intrinsically colistin-resistant, the isolates carried *mcr-1* gene, which was detected by PCR and sequenced commercially. The colistin MIC was more than 640 μg/ml. The plasmid from all the isolates was extracted and introduced to chemically-competent *E. coli* JM109 by heat shock. The transformants were colistin-resistant and carried *mcr-1* with MIC≥8 μg/ml.

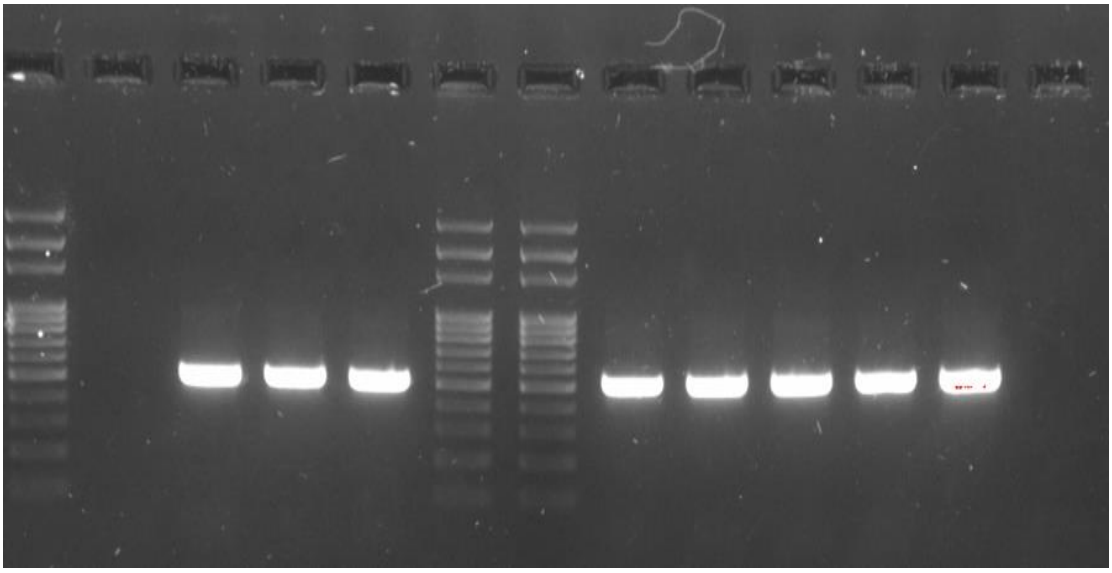

Fig. 8. PCR signals of ureC gene

All the *P. mirabilis* isolates resisted more than three classes of antibiotics, which marked them as multi-drug resistant bacteria. The isolates exhibited resistance to gentamicin, kanamycin, streptomycin, trimethoprim sulfamethoxazole, and

chloramphenicol and intermediate resistance to imipenem and meropenem (Table 4). *P. mirabilis* isolates were also resistant to penicillin, erythromycin, and tetracycline which is an intrinsic property for the tested strains.

All the isolates were positive for bla_{TEM}, class 1 integron gene, and *int1* gene. However, the isolates were negative for bla_{CTX-M}, bla_{SHV}, class 2 integron gene, and *int2*.

All the isolates had 100% the same genotype except C2S1 strain, which was different based on BOX PCR analysis. Besides, all the isolates were biofilm formers after testing for 1, 3, and 6 days. All the isolates retrieved from all biofilm on all tested days were *mcr-1* positive. Figure 9 shows the OD at 595 for each strain after incubating for the mentioned days.

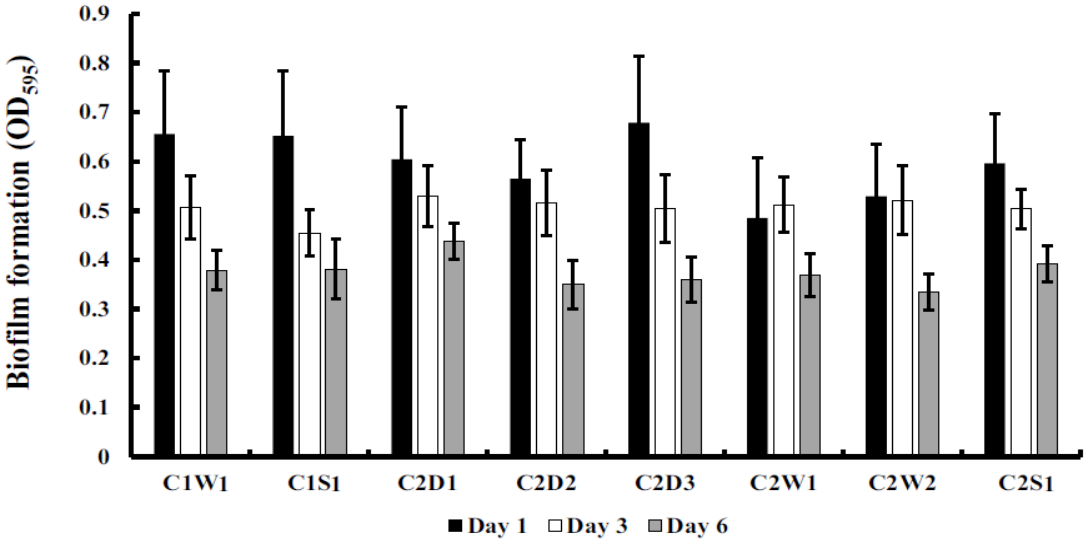

Fig. 9. Biofilm formation by mcr-1 positive Proteus mirabilis

Proteus mirabilis isolates were able to swarm on LB Agar (1.5%) after the incubation for 24 hours (Figure 10).

Table 4. List of the *Proteus mirabilis* isolates and their properties

Location	**Sample Identification Code**	**Resistance Profile**	**Intermediate Resistance Profile**	**Sensible Resistance Profile**	**Colistin MIC (µg/ml)**	**Genes**
Camp 1 Sampling Date 24/06/2019	**C1W**	PEN-GEN-KAN-STR-TET-SXT-CHL	IPM-MEM	AMP-AMC-FEP-CTX-CFM-DOR-CIP-NOR	> 640	*ureC*, invA, *mcr-1*, bla_{TEM}, *int1*, Class 1 Integron genes.
	C1S	PEN-GEN-KAN-STR-TET-SXT-CHL	IPM-MEM	AMP-AMC-FEP-CTX-CFM-DOR-CIP-NOR	> 640	*ureC*, invA, *mcr-1*, bla_{TEM}, *int1*, Class 1 Integron genes.
Camp 2 Sampling Date 24/06/2019	**C2D1**	PEN-GEN-KAN-STR-TET-SXT-CHL	FEP-IPM-MEM	AMP-AMC-CTX-CFM-DOR-CIP-NOR	> 640	*ureC*, invA, *mcr-1*, bla_{TEM}, *int1*, Class 1 Integron genes.
	C2D2	PEN-GEN-KAN-STR-TET-SXT-CHL	IPM-MEM	AMP-AMC-FEP-CTX-CFM-DOR-CIP-NOR	> 640	*ureC*, invA, *mcr-1*, bla_{TEM}, *int1*, Class 1 Integron genes.
	C2D3	PEN-GEN-KAN-STR-TET-SXT-CHL	IPM-MEM	AMP-AMC-FEP-CTX-CFM-DOR-CIP-NOR	> 640	*ureC*, invA, *mcr-1*, bla_{TEM}, *int1*, Class 1 Integron genes.
	C2W1	PEN-GEN-KAN-STR-TET-SXT-CHL	IPM-MEM	AMP-AMC-FEP-CTX-CFM-DOR-CIP-NOR	> 640	*ureC*, invA, *mcr-1*, bla_{TEM}, *int1*, Class 1 Integron genes.
	C2W2	PEN-GEN-KAN-STR-TET-SXT-CHL	IPM-MEM	AMP-AMC-FEP-CTX-CFM-DOR-CIP-NOR	> 640	*ureC*, invA, *mcr-1*, bla_{TEM}, *int1*, Class 1 Integron genes.
	C2S	PEN-GEN-KAN-STR-TET-SXT-CHL	IPM-MEM	AMP-AMC-FEP-CTX-CFM-DOR-CIP-NOR	> 640	*ureC*, invA, *mcr-1*, bla_{TEM}, *int1*, Class 1 Integron genes.

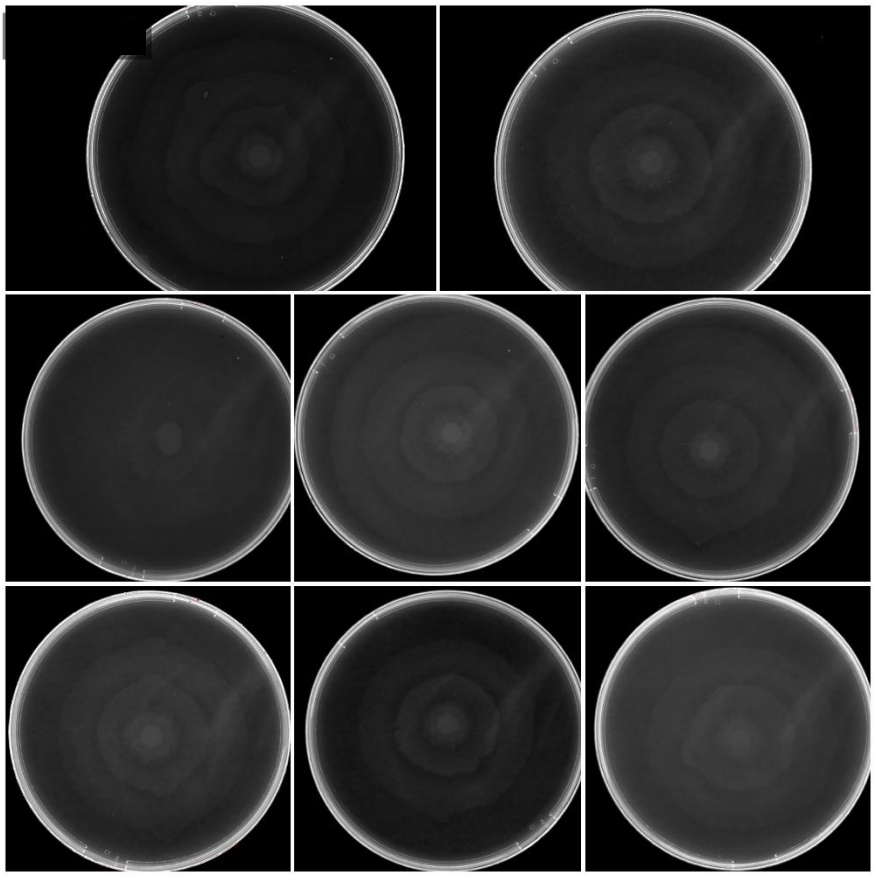

Fig. 10. Swarming phenotype of *mcr-1* positive *Proteus mirabilis* isolates

CHAPTER IV

DISCUSSION

Coliforms and *E. coli* are always tested to assess the water quality, and they are good indicators for fecal contamination. Mainly, *E. coli* is a member of fecal coliforms group, and it is a more specific indicator of fecal contamination of water. It is known that safe water is crucial for life. In fact, 1.1 billion people still do not have access to clean water, and children under five years old die worldwide because of contaminated water. The illnesses caused by contaminated water are a public health concern and many efforts are being exerted to control all the diseases transmitted by water in the world.

E. coli is a rod shape Gram-negative bacteria that belong to *Enterobacteriaceae*. *E. coli* commonly inhabit the lower intestines of mammalians. Poor infrastructure, lack of sanitization, and poor water storage conditions can help *E. coli* to spread and pollute important resources. Besides, *E. coli* can indicate the presence of other microorganisms that can be pathogenic bacteria [45].

Lebanon has challenges in the infrastructure and clean water resources. Implicitly, Syrian refugees in Lebanon do not have access to right environmental conditions and clean water. Multidrug-resistant bacteria were reported to be present in Syrian refugees worldwide. The counts of *E. coli* in the drinking water in the camps were higher than the standards determined by US EPA (the count should be 0). This can predict the presence of fecal contamination and other pathogens and indicates that the refugees are exposed to severe risk daily. The reported numbers in this study were after adding colistin to the media, so the susceptible *E. coli* for colistin were not assessed in this study. Therefore, the *E. coli* numbers are projected to be higher.

Disk diffusion assay was used to determine the antimicrobial-resistant profile which showed that *E. coli* isolates resisted more than three antibiotic classes. The mentioned statement suggests that Syrian refugees are in direct exposure to multidrug-resistant bacteria. Some of the Syrian refugees are children, and they are considered to be one of the most susceptible populations for infections. Therefore, infections in these vulnerable populations might be difficult to treat because of resistance.

The plasmid that carries *mcr-1* was successfully transformed into other bacteria, which can have indicated that *mcr-1* might be transmitted to many bacteria that affect the refugees. Additionally, *mcr-1* is harbored in bacteria that can be amplified in the refugees and then introduced again to the environment. bla_{TEM}, $bla_{\text{CTX-M,}}$ and bla_{SHV} were detected in 86%, 80%, and 13.8% of the isolates, respectively. The first detection of bla_{TEM} and bla_{SHV} dates back to 1983. These genes can confer different levels of resistance to broad-spectrum of cephalosporins and monobactams. In 1989, the $bla_{\text{CTX-M}}$ gene was identified and named cefotaximase (CTX-M); this gene confers a high ability to resist cefotaxime. The mentioned genes can also be carried on plasmids and transmitted between *Enterobacteriaceae*. In addition, plasmids can carry other genes that can resist aminoglycosides, tetracycline, chloramphenicol, trimethoprim, and sulfonamides. The transferable plasmids that carry resistant genes pose a serious risk to public health by disseminating resistance to antibiotics. The results in this study show that refugees might be high risk of exposure to these plasmids harbored by bacteria, which can be amplified and shed to the environment. It is worth mentioning that those genes are active and were expressed by bacteria to resist antibiotics with high percentages especially for cephalosporins [46].

bla_{KPC} and bla_{OXA48} were first reported in 2001 and 2004, respectively. The mentioned genes encode carbapenem-hydrolyzing class D β-lactamase. Although the

genes were detected in 6% and 3% of the isolates for *bla*$_{OXA48}$ and *bla*$_{KPC}$, respectively, they can pose a severe risk to the Syrian refugee, when exposure to cephalosporin, carbapenems, and colistin-resistant genes is taken together [47, 48]. All the isolates were *mcr-1* positive, which means all the people in the camps (children, adults, pregnant women, and elderly) are exposed to *mcr-1* positive bacteria via drinking and wash water.

Integrons are highly associated with the dissemination of antimicrobial resistance problems globally. The data in this study showed that all the *E. coli* and *Proteus mirabilis* isolates harbored class 1 integron genes, which emphasizes the presence of other resistant genes in the isolates that are in direct contact with the Syrian refugees in Lebanon[49].

BOX PCR analysis showed that *E. coli* from drinking water (C1D3R1) was 84.7% similar to the strains from sewage (C1S3R1, C1S4R1, and C1S5R1). In camp2, people knew that sewage water contaminates well water but not the drinking water, and the analysis showed that some isolates from sewage and well water were genotypically compatible with high percentages (above 90%). This can suggest a cross-contamination is happening between sewage and domestic waters in camps. Also, this can reflect the low sanitary conditions in the camps.

Proteus mirabilis is Gram-negative bacteria that is well known to its urease production and ability to swarm in a striking bulls' eye shape. *P. mirabilis* can cause urinary tract infections including cystitis and pyelonephritis. *P. mirabilis* can also form stones in the urinary tract. In 2006, the reason behind 11 million physician visits in the United States was urinary tract infections which cost $3.5 billion [50].

The stones that caused by *Proteus mirabilis* can block urine flow and damage the tissues. The stones can be more than (> 1 cm^2) in terms of size. Interaction can occur

between the precipitated minerals and the biofilm forming bacteria, which can block urine flow through catheters. *Proteus mirabilis* different from other urease producing bacteria is its definite association with catheter obstruction[50].

To expand the analysis, a third sampling was conducted, and *Proteus mirabilis* was detected in 5 samples out of 6. The isolates were *mcr-1* positive which emphasized that refugees in the camps are exposed to pathogens harboring resistant genes. Intrinsically, *P. mirabilis* is colistin, penicillin, erythromycin, and tetracycline-resistant. The isolates carried bla_{TEM} (encoding β-lactamase). Besides, all the isolates harbored Class-1 Integron genes and *int1* (encoding integrase). $bla_{\text{CTX-M}}$ was tested for all the isolates but was not detected.

Seven of the isolates were 100% genotypically similar, which can suggest that the strains are geographically unconstrained. Furthermore, the isolates were biofilm formers, and all the biofilm isolates were positive for *mcr-1* after 1, 3, and 6 days. This observation can suggest that *mcr-1* can persist in the camps in forms that can resist normal cleaning.

These data on *E. coli* and *Proteus mirabilis* are the first report of *mcr-1* in Syrian refugee camps worldwide. In addition, it shed light on new epidemiological discovery, which is the presence of *mcr-1* in unreported bacterium, *P. mirabilis*.

CHAPTER V

CONCLUSION

Proteus mirabilis can cause severe outbreaks such as urinary, respiratory, and eye infections. Besides, it can cause bacteremia and be life-threatening to immune-compromised human beings. This work shows the first detection of *mcr-1* in *P. mirabilis*. This detection suggests that *P. mirabilis* can be an overlooked reservoir for the transmission of *mcr-1* gene. Besides, *P. mirabilis* is an opportunistic pathogen, and the camps host children, elderly, and immunocompromised people. Hence, the statement above can reflect the severity of the situation that the Syrian refugees are suffering from in the camps. Furthermore, detecting *E. coli* that harbored *mcr-1*, and other resistant genes in the drinking and domestic waters is a severe issue for an already vulnerable population.

Antimicrobial resistance, in general, is a problem and is considered one of the severe challenges in the modern era. In the Arab world, there are not enough studies to assess the prevalence of antibiotic resistance problem, which can pose a burden on humanity control of infectious diseases worldwide. Lebanon is suffering from a lack of water resources, contaminated rivers, and shortage of studies that assess microbial quality of different sectors. Lebanon is a small country that hosts large density of population. Hence, it is urgent to conduct more studies related to microbiology to control potential outbreaks. It is highly essential to enforce regulations that can control the random use of colistin and other antibiotics. Taken together, there is a severe need to ensure sustainable access for safe water and hygienic infrastructure for Syrian refugees, because contamination with multi-drug resistant bacteria and transmissible genetic

elements can pose a severe health problem to the camp residents as well as to the hosting communities.

Finally, the problem is serious, and more consequences can occur in the future if actions is not taken. Studies, regulations, and awareness are the only available solutions nowadays to stop the antibiotic resistance problem; with the hope of preserving the role of antibiotics for the next coming generations.

REFERENCES

1. Etebu, E. and Arikekpar, I. (2016). “Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives”. *Int. J. Appl. Microbiol. Biotechnol. Res* 4: 90-101.

2. Fleming, A. (1929). “On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of *B. influenzæ*”. *British journal of experimental pathology* 10(3): 226-236.

3. Quinn, R. (2013). “Rethinking antibiotic research and development: World War II and the penicillin collaborative”. *American journal of public health* 103(3): 426-434.

4. Drlica, K.S. and Perlin, D.S. (2011). *Antibiotics: An Overview*. Pearson Education.

5. MacLaren, G. and Spelman, F.D. “Polymyxins: An overview”.

6. Fleming, S.A.. (1945). “Sir Alexander Fleming Nobel Lecture”. NobelPrize.org.

7. van Helden, P.D. *et al.* (2001). “Isolation of DNA from Mycobacterium tubercolosis”. In: *Mycobacterium tuberculosis Protocols*, T. Parish and N.G. Stoker, Editors. Totowa, NJ: Humana Press, 19-30.

8. Acar, J. and Rostel, B. (2001). “Antimicrobial resistance: an overview”. *Revue Scientifique et Technique-Office International des Epizooties* 20(3): 797-810.

9. O’neill, J. (2014). “Antimicrobial resistance: tackling a crisis for the health and wealth of nations”. *Rev. Antimicrob. Resist* 20: 1-16.

10. Mc Dermott, P.F., Walker, R.D. and White, D.G. (2003). “Antimicrobials: modes of action and mechanisms of resistance”. *International journal of toxicology* 22(2): 135-143.

11. Aslam, B. *et al*. (2018). “Antibiotic resistance: a rundown of a global crisis”. *Infection and drug resistance* 11: 1645.

12. Maltezou, H.C., Elhadad, D. and Glikman, D. (2017). “Monitoring and managing antibiotic resistance in refugee children. Expert review of anti-infective therapy.” 15(11): 1015-1025.

13. Denaro, C. (2016). “Agency, resistance and (forced) mobilities. The case of Syrian refugees in transit through Italy”. *REMHU: Revista Interdisciplinar da Mobilidade Humana* 24(47): 77-96.

14. de Smalen, A.W. *et al*. (2017). “Refugees and antimicrobial resistance: A systematic review”. *Travel Medicine and Infectious Disease* 15: 23-28.

15. Ismail, M.B. *et al*. (2018). "Tuberculosis, war, and refugees: spotlight on the Syrian humanitarian crisis". *PLoS pathogens* 14(6): e1007014.

16. Häsler, R. *et al*. (2018). "The antibiotic resistome and microbiota landscape of refugees from Syria, Iraq and Afghanistan in Germany". *Microbiome* 6(1): 37.

17. Reinheimer, C. *et al*. (2016). "Multidrug-resistant organisms detected in refugee patients admitted to a University Hospital, Germany June–December 2015". *Eurosurveillance* 21(2): 30110.

18. Frickmann, H. *et al*. (2018). "Molecular epidemiology of multidrug-resistant bacteria isolated from Libyan and Syrian patients with war injuries in two Bundeswehr hospitals in Germany". *European Journal of Microbiology and Immunology* 8(1): 1-11.

19. Ehlkes, L. *et al*. (2019). "No evidence of carbapenemase-producing Enterobacteriaceae in stool samples of 1,544 asylum seekers arriving in Rhineland-Palatinate, Germany, April 2016 to March, 2017". *Eurosurveillance* 24(8).

20. Tenenbaum, T. *et al*. (2016). "Prevalence of multidrug-resistant organisms in hospitalized pediatric refugees in an University Children's Hospital in Germany 2015–2016". *Infection control & hospital epidemiology* 37(11): 1310-1314.

21. Ravensbergen, S.J. *et al*. (2016). "High prevalence of infectious diseases and drug-resistant microorganisms in asylum seekers admitted to hospital; no carbapenemase producing Enterobacteriaceae until September 2015". *Plos one* 11(5): e0154791.

22. Ravensbergen, S.J. *et al*. (2019). "Proportion of asylum seekers carrying multi-drug resistant microorganisms is persistently increased after arrival in the Netherlands". *Antimicrobial Resistance & Infection Control* 8(1): 6.

23. Heudorf, U. *et al*. (2016). "Multidrug-resistant organisms in refugees: prevalences and impact on infection control in hospitals". *GMS hygiene and infection control* 11.

24. Ravensbergen, S.J. *et al*. (2018). "Case Report: Carbapenemase-Producing Enterobacteriaceae in an Asylum Seeker with Multidrug–Resistant Tuberculosis". *The American journal of tropical medicine and hygiene* 98(2): 376-378.

25. Lederer, I. *et al*. (2015). "Shigellosis in refugees, Austria, July to November 2015". *Eurosurveillance* 20(48).

26. Angeletti, S. *et al*. (2016). "Unusual microorganisms and antimicrobial resistances in a group of Syrian migrants: Sentinel surveillance data from an asylum seekers centre in Italy". *Travel medicine and infectious disease* 14(2): 115-122.

27. Angeletti, S. *et al*. (2017). "Whole-genome sequencing of Klebsiella pneumoniae MDR strain isolated in a Syrian refugee". *Pathogens and global health* 111(4): 212-215.

28. Ahmed, S.S. *et al*. (2016). "Spread of carbapenem-resistant international clones of Acinetobacter baumannii in Turkey and Azerbaijan: a collaborative study". *European Journal of Clinical Microbiology & Infectious Diseases* 35(9): 1463-1468.

29. Jakovljevic, M. *et al*. (2018). "Antibiotic Resistance in Syria: A Local Problem Turns Into a Global Threat". *Frontiers in public health* 6: 212-212.

30. Abbara, A. *et al*. (2018). "A summary and appraisal of existing evidence of antimicrobial resistance in the Syrian conflict". *International Journal of Infectious Diseases* 75: 26-33.

31. El Achkar, S. *et al*. (2019). "Drug-Resistant Tuberculosis, Lebanon, 2016–2017". *Emerging infectious diseases* 25(3): 564.

32. Kassem, D.F. *et al*. (2017). "Multidrug-resistant pathogens in hospitalized Syrian children". *Emerging infectious diseases* 23(1): 166.

33. Kamenshchikova, A. *et al*. (2018). "Complex narratives of health, stigma and control: Antimicrobial resistance screening among non-hospitalized refugees". *Social Science & Medicine* 212: 43-49.

34. Scholar, E. (2007). "Cephalosporins." In: *x Pharm: The Comprehensive Pharmacology Reference*." Enna, S.J. and D.B. Bylund, Editors. New York: Elsevier, 1-4.

35. Novovic, S. *et al*. (2012). "First-line treatment with cephalosporins in spontaneous bacterial peritonitis provides poor antibiotic coverage". *Scandinavian journal of gastroenterology* 47(2): 212-216.

36. Meletis, G. (2015). "Carbapenem resistance: overview of the problem and future perspectives". *Therapeutic Advances in Infectious Disease* 3(1): 15-21.

37. Sauberan, J.B. and Bradley, J.S. (2018). "292 - Antimicrobial Agents." In: *Principles and Practice of Pediatric Infectious Diseases* (Fifth Edition), Long, S.S., Prober, C.G. and Fischer, M. Editors. Elsevier, 1499-1531.e3.

38. EMA. (2016). "Updated advice on the use of colistin products in animals 3 within the European Union: development of resistance 4 and possible impact on human and animal health". *European Medicines Agency*.

39. Liu, Y.-Y. *et al*. (2016). "Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study". *The Lancet Infectious Diseases* 16(2): 161-168.

40. Sun, J. *et al*. (2018). "Towards Understanding MCR-like Colistin Resistance". *Trends in Microbiology* 26(9): 794-808.

41. Hmede, Z. *et al*. (2019). “Emergence of plasmid-borne colistin resistance gene mcr-1 in multidrug-resistant Escherichia coli isolated from irrigation water in Lebanon”. *International Journal of Antimicrobial Agents* 54(1): 102-104.

42. Al-Mir, H. *et al*. (2019). “Emergence of clinical mcr-1-positive *Escherichia coli* in Lebanon”. *Journal of Global Antimicrobial Resistance* 19: 83-84.

43. Hmede, Z. and Kassem, I.I. (2018). “The Colistin Resistance Gene, mcr-1, is Prevalent in Commensal E. coli Isolated from Lebanese Pre-harvest Poultry”. *Antimicrobial agents and chemotherapy* AAC. 01304-18.

44. Czerwonka, G. *et al*. (2016). “The role of Proteus mirabilis cell wall features in biofilm formation”. *Archives of microbiology* 198(9): 877-884.

45. Odonkor, S.T. and Ampofo, J.K. (2013). “Escherichia coli as an indicator of bacteriological quality of water: an overview”. *Microbiology research* 4(1): e2-e2.

46. Coque, T.M. *et al*. (2002). “Genes encoding TEM-4, SHV-2, and CTX-M-10 extended-spectrum β-lactamases are carried by multiple Klebsiella pneumoniae clones in a single hospital (Madrid, 1989 to 2000)”. *Antimicrobial agents and chemotherapy* 46(2): 500-510.

47. Cuzon, G. *et al*. (2010). “Worldwide diversity of Klebsiella pneumoniae that produce β-lactamase blaKPC-2 gene”. *Emerging infectious diseases* 16(9): 1349.

48. Potron, A. *et al*. (2011). “Occurrence of the carbapenem-hydrolyzing β-lactamase gene blaOXA-48 in the environment in Morocco”. *Antimicrobial agents and chemotherapy* 55(11): 5413-5414.

49. Fluit, A.C. and Schmitz, F.J. (1999). “Class 1 Integrons, Gene Cassettes, Mobility, and Epidemiology”. *European Journal of Clinical Microbiology and Infectious Diseases* 18(11): 761-770.

50. Schaffer, J.N. and Pearson, M.M. (2015). “Proteus mirabilis and Urinary Tract Infections”. *Microbiology spectrum* 3(5): 10.1128.