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Polymerase chain reaction for detection of waterborne bacterial pathogens in potable water in Tubas district-Palestine

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Dedication

I dedicate my thesis to science, enlightening us

I would like to dedicate my thesis to my parents whom I owe everything since I was born. There is no doubt in my mind that without their continued support and counsel I couldn't have completed this process.

To my wonderful husband, thank you for your unconditional support with my studies, who had given me the dreams to look forward. Thank you for giving me a chance to prove and improve myself.

To my kids; Seedra and Kareem, you have made me stronger, better and more fulfilled than I could have ever imagined who were supported me in their childhood innocent and their patience has at times. I love you to the moon and back.

Acknowledgements

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الإقرار

أنا الموقع أدناه، مقدم الرسالة التي تحمل العنوان:

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Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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List of Abbreviations

Abbreviation	Term
AT	Annealing Temperature
CFU	Colony Forming Unit
CUT	Citrate Utilization Test
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribonucleotide Triphosphates
EAEC	Enteroaggregative E. coli
EDTA	Ethelendiaminetetraacetic Acid
EHEC	Enterohemorrhagic E. coli
EIEC	Enteroinvasive E. coli
EMB	Eosin Methylene Blue
EPA	Environmental Protection Agency
EPEC	Enteropathogenic E. coli
ETEC	Enterotoxigenic E. coli
FC	Fecal Coliform
GapA	Glyceraldehyde 3- phosphate dehydrogenase Gene
HUS	Hemolytic Uremic Syndrome
IMS	Immune Magnetic Separation
IMViC	Indole, Methyle Red, Vogus Proskauer and Citrate Biochemical Tests
IND	Indole Test
IpaB	Invasion Plasmid Antigene B Gene
JMP	Joint Monitoring Program
KOH	Potassium hydroxide
LF	Lactose Fermentation
M.R.V.P	Methyle Red and Vogus Proskauer
MCL	Maximum Contamination Level
Mdh	Malate Dehydrognase Gene
MF	Membrane Filtration method
MgCl2	Magnesium Chloride
M-PCR	Multiplex-Polymerase Chain Reaction
MR	Methyl Red
NLF	Non Lactose Fermentation
PCR	Polymerase Chain Reaction
PSI	Palestinian Standard International
PWA	Palestinian Water Authority
RFLP	Restriction Fragment Length

	Polymorphisms
SB	Selenite Broth
SIM	Sulfide Indole Motility
SS Agar	Salmonella-Shigella Agar
Taq	Thermus aquaticus
TC	*Total Coliform*
THMs	Trihalomethanes
TSI	Triple Suger Iron
VP	Vogus Proskauer
WHO	World Health Organization

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Abstract

In developing countries, sanitation and hygiene are associated with water quality and contaminations with water borne pathogens. This study was conducted to determine the microbiological quality of potable water in Tubas Governorate. The prevalence of *Total Coliform* (*TC*), *Feacal Coliform* (*FC*), *E.coli, Klebsiella* and *Salmonella* contamination were assessed in 60 water samples collected from 6 groundwater wells, 15 network distribution systems, 9 tankers, 15 tanks (homes, school and restaurants) and 15 rain fed cisterns in Tubas Governorate, by using polymerase chain reaction (PCR) technique. The results showed that 40% and 31.6% of water samples were generally exceeding the Palestinian and WHO standard for *TC* which is (3 CFU/100ml) and *FC* which is (0 CFU/100ml) for potable water. Results showed that the prevalence of *E. coli, Klebsiella* and *Salmonella* was 31.67%, 18.33% and 10% respectively. It was found that the water samples collected from rain fed cisterns had the maximal proportion of risk compared with other water resources, as they constitutes 15% for *TC* and 13.33% for *FC*. Specifically collected water samples from rain fed cisterns contained 53.3% of *E. coli*, 40% of

Klebsiella and 20% of *Salmonella* contaminants; meanwhile the samples taken from the groundwater wells and networks almost free from *FC*. On the other hand, these samples that were taken from groundwater wells and networks were found slightly contaminated with *TC* (1.67%) making them less risky referring to Palestinian and WHO guideline.

The study revealed poor bacteriological quality of drinking water sources, which may be due to several reasons such as leakage from pits for rain fed cisterns or transporting tankers. The study recommended increasing the health surveillance on the rain fed cisterns and transporting tankers in Tubas Governorate and monitoring the quantity of chlorine used for disinfection (0.8-1 mg/L). In addition to that, increase the education regarding waterborne diseases in collaboration with the Ministry of Health and the Palestinian Water Authority (PWA). The study also recommended the application of the Polymerase Chain Reaction (PCR) technique for routine test in Palestinian water laboratories due to its accuracy for detection of the pathogens with high sensitivity and efficiency.

Key word: water borne diseases, *Total Coliform* (*TC*), *Feacal Coliform* (*FC*), polymerase chain reaction (PCR)

Chapter One

Introduction

1.1 General background

Water is a main nutrient of all organisms and it's a basic unit to human life. Water is important to all living organisms, agricultural production, industrial processes and domestic use for humans (Anake *et al*., 2013). Water is intricately connected to life, so must be given the good attention at all times because without safe drinking water there is no life (Adetunde and Glover, 2010).

Water is vital to the existence of all living organisms. Shortage of water resources and deterioration in water quality related to many reasons such as increase in human population growth by the time and demand of water for domestic purpose, In addition to agricultural production, mining, economic activities, power generation, industrial production, forestry practices and impact of living organisms and their interacting to aquatic ecosystem. The management of aquatic ecosystem requires an understanding of the important linkages between environment properties and human activities that had an effect on aquatic ecosystem to sustain healthy and functional environment (Carr and Nerry, 2008).

World Health Organization (WHO) set a specific strategy to manage water quality and provide safe water for drinking, food production and domestic purpose. Safe drinking water support public health and promote socioeconomic development, while water of poor quality can cause disease outbreaks and it can contribute to background rates of disease manifesting themselves on different time scales (WHO, 2013).

Potable water should be safety water for drinking, cooking and washing for consumer. Potable water must meet the physical, chemical and microbiological parameter when supplied from approved resources to consumer through protected distribution system in good quality and palatability (Dezuane, 1997).

Water quality includes the physical, chemical and biological characteristics of water. Water quality assessment against set of standards reference guideline is usually used to assess water quality related to safe drinking water to human and for the health of ecosystems. Water quality is a very complex subject, because water is a complex medium intrinsically tied to the ecology of the earth. The vast majority of surface water on the earth is nor toxic neither potable (Ertuð and Mirza, 2010).

Human activities are the main cause of water contamination. Humans produce wastes that enter water resources such as industries discharge contain heavy metals, oils nutrients, organic toxins and solids. Many of these substances are toxic or carcinogenic. These wastes also increase the concentration of suspended solids (turbidity), bacteria and virus growth leading to produce water borne diseases (Aqeel et al., 2010).

Water quality and quantity is an important strategic issue related to health, sanitation and economic development which receive little attention in Palestine (Kouttab, 2013). Palestine is among the countries with the shortage renewable water resources due to both natural and artificial constraints, which provide only 100 cubic meters per each capita annually and this amount is less than the available water in other countries in the

Middle East and the World (150 cubic meters per each capita). Elevation in population growth, improvement of human health and development of industrial and agriculture increase the gap between the water supply and water demand which effect on availability of water (Jayyousi *et al*., 2004). Groundwater in Palestine is considered the main source for water supply. Water from Jordan River which tributary upstream to Israel National carrier, was deteriorated to levels where it cannot be used for domestic or irrigation purposes and its flow was dropped significantly. Palestine was considered one from areas where their water supply is dependening on direct recharge from rainfall. The gap between water supply and water demand was increase due to climate changes. The main impacts of climate change are noticed as high temperature, decreased precipitation and drought that lead to decrease water supply and increased water demand (Froukh, 2010).

1.2 Study area

1.2.1 Geographical location

Tubas Governorate is located in the northeastern part of the West Bank. It is bordered by Nablus and Jericho Governorates to the west and south, Jenin Governorate and Armistice Line (1948 borders) to the north, and Jordan valley to the east. It is located south of Bissan plain and west of Jordan River. The total area is about 440 km² which forms 8% of the West Bank area, distributed over 23 communities as in Figure 1 (Tubas, Tammun, 'Aqqaba, El Far'a Camp, Tayasir, Wadi al Far'a, Bardala, 'Ein el-

Beida, Khirbet 'Atuf, Ras al Far'a, Khirbet Ras al Ahmar, Khirbet Kishda, Al 'Aqaba, Al-Thaghra, Al-Farisiya, Al-Malih, Al-Hadidiya, Kardala, Khirbit Ebziq, Khirbet Humsa, Khirbet Tell el-Himma, Khirbet Yarza and Khirbet Salhab) (Applied Research Institute, 2006).
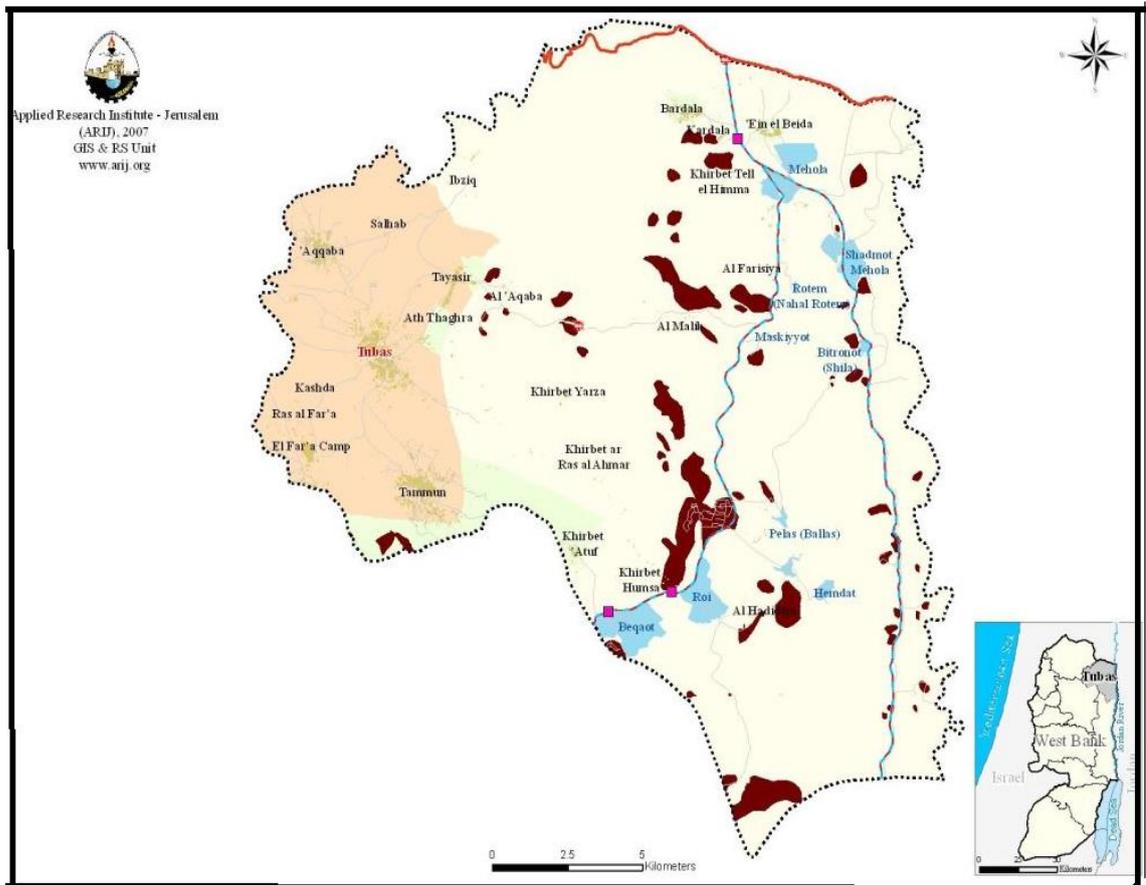
Figure 1: Geographic location of Tubas Governorate

Tubas city is the largest locality by area in Tubas Governorate, which extends over 295123 dunums. El Far'a Camp is the smallest locality by area which extends over 225 dunums. Tubas Governorate is recognized as an agricultural area. Tubas Governorate is a moderate elevated area, where its lowest elevation at Khirbet Tell el Himma reaches to 182 m below the Sea level, and the highest elevation at Aqqaba reaches up to 495 m above the Sea level (Applied Research Institute, 2006).

Tubas Governorate is recognized as an agricultural area. Agriculture drainage is contributing one of many important factors of water contaminant and health hazard. Fertilizers with high nitrogen rates may increase the potential of groundwater pollution; when the nitrogen from fertilizers, animal manure and human excrement combine with organic chemicals like ammonia which leads to nitrate formation, and increased in heavy metals and organic compound especially in Al-Fara'a Stream. Any of these compound discharge in ground water may causes threat of human health, such as respiratory and reproductive system illness, cancers, and blue baby syndrome. Numerous studies in different places in Palestine such as, Tulkarm, Selfiet, Nablus, Al-Fara'a Catchment, Tubas and Gasa Strip were showed the relationship between elevated in nitrate concentration and contamination of drinking water. In addition, other studies in China, United State, UK and Khairpur Sindh had shown elevated in the percentage of nitrate in ground water exceeds the allowable limit for drinking water (50 mg/l) (Zhang *et al*. 1996; Reida *et al*. 2003; Menawee, 2004; Shar *et al*. 2008; HWE, 2008; Al-Khatib and Arafat, 2009; Al-Khatib and Abu-Hejleh, 2011; Ghanem and Samhan, 2012; Abu Hijleh, 2014).

1.2.2 Temperature and annual rainfall

The geographic location of Tubas Governorate gives it moderately temperature ranged approximately (21˚C). It is dry and warm in summer, rainy in winter. Tubas Governorate annual rainfall is 329 mm (varies between 180 mm in the east to 440 in the west) (Applied Research Institute, 2006).

1.2.3 Population

The total population of Tubas Governorate was 56642 people (PCBS, 2010), the population forms about 3 % of the total population of Palestine. Tubas Governorate divided in to three areas in terms of the population life style, urban areas (one locality, Tubas city) constitute up to 33.4 % of total population, whereas rural areas (21 localities) constitute 54.6 % of the population and refugee camp (one locality El Far'a Camp) constitute 11.9 % of population (Applied Research Institute, 2006).

1.2.4 Water resources

Tubas governorate water resources are ground water wells, springs and additional mounts were supplied mainly through Mekorot. Tubas governorate has only two main water supplying wells: the old well of Tubas municipality with a capacity declined to less than 15 cubic meters per hour, and Tammoun well that was recently drilled by the Palestinian Water Authority (PWA) that has a production capacity of 130 cubic meters per hour, and is used to supply the town of Tubas with about 1400 cubic meters of water per day (PWA, 2012).

In Tubas Governorate two main Projects for water supply had been completed by (PWA), international donors and nongovernmental organization in 2011. The first one was construction of water lines (Farďa refugee camp water line) and the other one was construction of water wells (Tammoun well, tanks, poster, electronic panel and vertical well pump) (PWA, 2011).

Chapter Two

Litreture Review

2.1 Water quality

Potable water quality is important for human health and hazards related to change in environmental condition due to human activities. Also, it is a basic unit for protecting the environment and sustainable development. Physical, chemical and microbiological standards are designed to ensure that the water is safe for drinking and are free from microbial diseases and /or chemical substances that deleterious to health (Okonko *et al*., 2008).

Most of the drinking waters sources in developing countries are often dirty and have aquatic microbes. If some of these microbes enter the human body they could be dangerous. Conformation with physiochemical and microbiological standards is a special aim for decision makers to provide safe and to reduce the possibility of water borne diseases spreading to the minimum degree (Ezel, 2012).

2.2 Physicochemical characteristics of potable water

Physicochemical characteristics of potable water are vital for water quality and monitoring parameters which affect the quality of a water resource and necessary to consider water suitability to drink. Temperature, taste and odor are originated from various types of organic chemicals, inorganic chemicals and biological sources or process. Taste and odor in potable water may be indicated of some contamination or malfunction during storage and distribution result from synthetic chemicals, corrosions, water disinfection process as chlorination and presence of potential harmful substances. If any change in taste or odor were detected, it might indicate a water quality problem that requires further investigation. Color, cloudiness,

particulate matter and visible organisms should be noticed by consumers through visual observation that may create concerns about the quality and acceptability of a drinking-water supply. The presence of iron, manganese, industrial waste and human material causes high intensity in color of water resources (Dezuane, 1997).

Conductivity is a measurement of the ability of water to pass an electrical current using conductivity meter (µs/cm). It indicates the presence of inorganic dissolved solids in water such as major anions or major cations. Organic compounds like oil, alcohol, and sugar have a low conductivity because they don't conduct electrical current very well (WHO, 2008).

Turbidity is a measurement of the clarity of water using turbidity meter (NTU) related to suspended materials include soil particles (clay, silt, and sand), algae, microbes, and other substances which decreases the passage of light through the water (Dezuane, 1997; WHO, 2008).

Chemical substances in potable water have different health concerns from microbial contaminant due to prolonged period of exposure and the ability of some chemical constituents to cause health problems from single exposure. Therefore the priority was given to both monitoring and remedial action for chemical contaminants in drinking water to ensure little or no health concern. An exposure to high levels of fluoride for example, can lead to mottling of teeth and crippling skeletal fluorosis. Other chemicals including excess exposure to arsenic in drinking-water may increase significant risk of cancer and skin lesions (WHO, 2004). Some potentially chronic effects may occur due to overuse of agrochemicals such as

pesticides and fertilizers which leads to increase the levels of nitrate and nitrite in water resources or may result from leaching of wastewater or organic wastes into surface and ground water (WHO, 2011).

2.3 Microbiological characteristics of potable water

The world in 20th century was suffering from the greatest development failure in providing the basic safe drinking water service and adequate sanitation services for all people, which causes high rate of mortality among children less than 5 years due to water-related diseases. This failure refers to many water problems faces nations and individuals around the world as water shortage, contamination of water resources, unsustainable use of groundwater, ecological degradation, and the threat of the climate change. The most serious consequence of this failure is widespread water-related disease and death (peter, 2002).

Burden of water could be related to diarrheal diseases which spread in recent decades (Pr€uss-Ust€un *et al*., 2008). Water, sanitation and hygiene are considered the most common risk factors related to burden of water disease. In 1990, the researchers examined these risk factors in terms of diarrheal disease and selected parasitic diseases; it was found that the worldwide risk factor accounted for 5.3% of all deaths based on the partial attribution of their disease burden to the water, sanitation and hygiene. In study of Prüss *et al*., 2002, the estimated diarrheal disease related to water hygiene and sanitation causes 2,213,000 deaths especially in children younger than 5 years old. This represents 4% of all deaths, and the disease

burden can be up to 240 times higher in developing regions if compared to developed regions (Prüss *et al*., 2002).

Diarrheal disease is caused mainly by the ingestion of pathogens especially in unsafe drinking water or from unclean hands or from contaminated food. Unsafe drinking water, inadequate sanitation and insufficient hygiene promote the transmission of these pathogens. Eighty eight percent of cases of diarrheal worldwide are attributable to unsafe water, inadequate sanitation or insufficient hygiene. These cases result in 1.5 million deaths each year, most being the deaths of children younger than 5 years. The category diarrhea includes some more severe diseases, such as cholera, typhoid and dysentery. All of which are pathogenic indicator (Prüss-Üstün, 2008).

Inadequate drinking water, sanitation and hygiene are important risk factors especially in developing countries. In 2011, WHO/UNICEF Joint Monitoring Program for Water and Sanitation (JMP) estimated 768 million people relied on unimproved water supplies due to high levels of pathogen contamination, and by the end of 2011 there were 2.5 billion people who lacked access to an improved sanitation facility which did not meet the minimum standards of hygiene (WHO & UNICEF, 2013).

In study of Abu-Hejleh (2004) in Tubas Governorate, it was found that (63.7%) of the households didn't test the drinking water from their rain-fed cisterns, which increases the risk of potable water contamination and water borne diseases. It was then related to the many factors such as breeding

animals near the water cisterns and the close distance between the sewage pits and cisterns (Abu-Hejleh, 2004).

In other study of Al-Khatib and Abu-Hejleh (2011), investigating the relationship between drinking water and human health in Tubas Governorate, it was found that (14.8%) of population had high incidence of water related diseases (mainly diarrheal and skin diseases). This result was referred to many factors such as lack of water quality and hygiene, in addition to (63.7%) of the population had bad management practices in cleaning of their storage resources of water in their homes. The study was explained the relationship between the water resources and their surrounding environment. The results were showed that (86.3%) of the population uses pits in the discharge of wastewater, and (70%) of rain-fed water cistern with close distance to the sanitation facilities and cesspits in homes (less than 16 meter). Other factor related to (46.8%) of the population in Tubas Governorate were breeding animals and pet birds which there feces were contributed in home rooftops contamination, especially since some of the tanks are fed in winter home arenas, which causes drinking water contamination (Al-Khatib and Abu-Hejleh, 2011).

2.4 Water-related illnesses

Water-related illnesses fall into four major categories:

First: Water-based diseases which are spread by hosts that develop in water or require water for part of their life cycle, and then become human parasites. They are usually passed to humans by contaminated water or use it for washing, and by eating insufficiently cooked fish. The most

widespread examples in this category are schistosomiasis which currently infect 200 million people in 70 countries (Peter, 2002).

Second: Water-washed diseases caused by poor personal hygiene and when contaminated water contact with skin or eye. These include trachoma, scabies, and diarrheal diseases (Peter, 2002 and Griffiths, 2008).

Third: Water-related insect vectors, includes diseases spread by insects breed in or near contaminated water such as mosquitoes, including dengue and malaria diseases. These diseases are not associated with lack of access to safe drinking water or sanitation services and they are not estimates of water-related deaths, but their spread create conditions favorable to their hosts (Peter, 2002 and Carr and Nerry, 2007).

Forth: Water-borne diseases which are all enteric and opportunistic pathogens that are transmissible by the fecal-oral route, caused by drinking water contaminated by pathogenic microorganisms from human or animal faeces (Ford, 1999).

2.5 Water borne diseases

Waterborne disease outbreak is defined as an outbreak in which epidemiologic evidence points to a drinking water source from which two or more persons become ill at similar times present a serious global health threat (Curriero *et al*., 2001). Bacteria, viruses and protozoa pathogens are responsible of water borne infections. The potential for these pathogens can be predicted by many factors that contributed in water borne infection spread by pathogenic agents such as pathogens survival, ability for multiplication in water, latency (the period between pathogens excretion

and acquisition of actual infectious power) and infective dose required for initiation infection in individuals. Water borne pathogens are called new emerging pathogens; because they contribute in water borne diseases and they are one of the biggest human tragedies which kill more than 5 million people each year. In addition to that, 2.3 billion people are suffering from water related diseases while 60 % of all infant mortality in the world is related to water borne diseases (Cloete *et al*., 2004).

2.5.1 Bacteria

Bacteria are the most widely distributed forms of life. The primary sources of pathogenic bacteria are human and animal wastes. The most vulnerable to bacterial contamination are the potable water from older network distribution systems, dug wells, spring-fed systems and cistern-type systems. Additional sources include discharge from septic tanks and sewage treatment facilities which can enter open wells. The approximately range of pathogenic bacteria length is from 0.4 to 14 μm and 0.2 to 1.2 μm in width. Many bacterial pathogens are responsible for waterborne disease including *E. coli, Legionella, S. typhi, Shigella, Klebsiella* and *Vibrio cholera* (Gleeson and Gray, 2002 and EPA, 2014).

Bacteria in potable water have the unique ability to persist in the environment in association with biofilms or in dormant stages that improve their chances of survival, even though they are to be more susceptible to disinfectants. Unlike viruses, bacteria are well adapted to grow outside of their host and may greatly increase in a given number for appropriate growth conditions. Re-growth of bacteria in hot water heaters (i.e.,

Legionella) and distribution systems (i.e., *Pseudomonas*) has led to adverse health outcomes and poor water quality (Reynolds, 2012).

2.5.1.1 *Escherichia coli (E. coli)*

E. coli is a member of the coliform bacterial group and part of the *Enterobacteria* family. It is gram negative, facultative anaerobic rod shaped bacterium found in high numbers in the gut of warm-blooded animals, capable to produce *β-glucuronidase* enzyme which is facilitates their detection and identification. It has been used as an indicator of water safety globally and waterborne disease risk and to detect faecal contamination in water resources (Brown *et al.*, 2010 and Health Canada, 2012).

Pathogenic forms of *E. coli* can cause a variety of diarrheal diseases in hosts due to the presence of specific virulence factors and pathogenicity associated genes which are generally not present in other *E. coli*. It had the ability to causes intestinal disease. These bacteria include strains of *Enterohemorrhagic E. coli* (EHEC) also called *Shiga toxin-producing E.coli* (STEC), *Enteropathogenic E. coli* (EPEC), *Enteroaggregative E. coli* (EAEC), *Enterotoxigenic E. coli* (ETEC), *Diffuse adherent E. coli* (DAEC), and *Enteroinvasive E. coli* (EIEC) (Bugarel *et al.*, 2011).

E. coli O157:H7 is one of hundreds strains of the *E. coli,* a bacterium that is belonged to the *Enterobacteriaceae* family. It was recognized in 1982 as a human pathogen. *E. coli O157:H7* is gram negative, facultative anaerobic, rod shaped bacterium and have the ability of adaptation for long time and survival in disinfection process. Most *E. coli* strains are harmless and can be lived in the intestines of healthy humans and animals. But *E.*

coli O157:H7 strain can cause severe illness due to toxin production and outbreak associated bloody diarrhea. The cell wall contains the "O" antigen, and the "H" represents flagellar antigen. It is most common as a causative agent of Hemolytic Uremic Syndrome (HUS) (Riley *et al.*, 1983). The prevalence of *E. coli* ranged between (7%) to (65%) in different water resources (Baeumner *et al.,* 2003; Juhna *et al*., 2007; Okonko *et al*., 2008; Kinge *et al.,* 2012; Allevi *et al*., 2013; Benjamina *et al.*, 2013; Momtaz *et al*., 2013; Nwachukwu and Ume, 2013; Alqahtani *et al.,* 2015; Tryland *et al*., 2015).

2.5.1.2 *Salmonella* spp.

Salmonella spp. are ubiquitous enteric bacteria belong to the *Enterobacteriaceae* family. They are Gram negative bacilli, facultative anaerobic, oxidase negative and non-lactose fermented but most are produce hydrogen sulfide or gas from carbohydrate fermentation. *Salmonella enterica, Salmonella bongori* and *Salmonella typhi* are examples of salmonella species. *Salmonella* serotypes are ubiquitous enteric bacteria and etiological agents of water-borne gastroenteritis (salmonellosis), causing typhoid and paratyphoid fevers. WHO was estimated the burden of typhoid fever caused by *S. typhi* serotype to more than 2 million illnesses and 200 thousands death during 2000. The burden of typhoid fever disease in developing countries is elevated by the spread of multiple drug resistance *S. typhi* (Crump *et al*., 2004; Lynch *et al*., 2009; Kinge *et al*., 2012). Faecal contamination from wild animals, bird faeces and sewage discharges are the main causes of entry of *Salmonella* spp. to

the water systems and transmission to the rainwater supply (WHO, 2004). The majority of *Salmonella* cause food poisoning, waterborne diseases and gastroenteritis in humans all over the world. But one species *S. typhi*, frequently disseminates into the blood and causes a severe form of salmonellosis called typhoid fever (Herikstad *et al.*, 2002).

The prevalence of *Salmonella* in different water resources ranged between (1%) to (58.8%) in Pakistan, South Africa, Canada, United States, India, Nigeria, Taiwan, Thailand, California and Ireland (Johnson *et al*., 2003; Kinge *et al*., 2012; Benjamina *et al*., 2013; Momtaz *et al*., 2013; Vereen *et al*., 2013; Banmairuroy *et al*., 2014; Samanta *et al*., 2014; Yhiler *et al*., 2015).

2.5.1.3 *Klebsiella* spp.

Klebsiella spp. are belonging to the family *Enterobacteriaceae*. They are Gram-negative, non-motile bacilli. *Klebsiella* is ubiquitous in nature and is a commensal organism of the gastrointestinal tract causes gastroenteritis and urinary tract infection. The genus *Klebsiella* are consist many species, including *K. pneumoniae,* pathogenic *K. oxytoca, K. planticola* and *K. terrigena*. 60–80% of all *Klebsiella* spp. are *K. pneumoniae* (Thermotolerant coliform) which are detected as coliform in potable water and the main cause of respiratory and urinary tract infection. The growth of *Klebsiella* spp. within distribution systems can be minimized by maintenance of disinfectant residuals and restriction of the residence time of water in distribution systems (WHO, 2004).

The prevalence of *Klebsiella* ranged between (4%) to (58.6%) in different water resources (Podschun *et al.,* 2001; Siri *et al*., 2011; Kinge *et al.,* 2012; Amin *et al*., 2014; Ichor *et al*., 2014).

2.5.2 Viruses

Human enteric viruses enter the water environment through the faecal contamination from the faeces of patients suffering from gastroenteritis or hepatitis and discharge of sewage to water resources causes wide variety of diseases include hepatitis, gastroenteritis, meningitis and fever. In 1979 it was estimated the mortality between 5 and 18 million people every year from gastroenteritis. Rotaviruses are responsible for mortality over 1 million children from diarrhea. In addition to that hepatitis A was endemic in Mediterranean region (Bosch, 1998). The viral quality of water is hard to assess because viruses persist longer than enteric bacteria and they are more resistance to disinfection process as chlorination (Bosch, 1998).

2.5.3 Protozoa

Protozoan parasites have been recorded to cause a significant percentage of the reported cases of waterborne disease and diarrhea, especially with cryptosporidiosis and giardiasis. *Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica* and *Entamoeba dispar* are the most common causes of water borne diseases in the world (Faghiri and Widmer, 2011). Cryptosporidiosis and giardiasis which occur with prevalence of 2 to 5 percent in developed countries, while 20% to 30% in developing countries. They are threats to water supplies because they are resistance to

chlorine disinfection mainly in cold temperature and high pH (Alam *et al.*, 2014)**.**

Studies by Jarroll *et al.* (1981) remind that the chlorine doesn't always result in 100% inactivation of Giardia cysts. The inactivation of cysts by chlorine is less effective at higher pH values and lower water temperatures (Jarroll, 1981). Oocytes of Cryptosporidium are an environmentally resistant. They are causes disease with low infectious dose and can remain infectious for more than a year in aquatic environments.

In Palestine the first study use the molecular technology in diagnosis of cryptosporidiosis in Al-Qaryoon- Nablus, suggested that Cryptosporidium was the causative agent of the diarrhea and vomiting outbreak that occurred in Nablus region due to the sewage discharge to the surface water (Hussein, 2011).

2.6 Microbial indicator of water quality

2.6.1 Indicator organisms (Coliform)

Indicator organisms are a fundamental monitoring tool used to measure both changes in water quality and the potential presence of hard to detect target pathogenic organisms. Indicator organisms should be easily detected using simple laboratory tests; because many pathogens are not easily detected are usually harmless, more plentiful and provides evidence of the presence or absence of a pathogenic organism surviving under similar physical, chemical, and nutrient conditions (Dezuane, 1997).

General characteristics of indicator organism for health risk prediction:

1. Fecal origin of pathogenic organism in contaminated water sources.

2. The concentration of indicator organism should be more than the associated pathogen.

3. Resistant to environmental stresses and persistence for a long time.

A positive result for the indicator organism means that the indicator is present in the water body, that doesn't necessarly mean waterborne pathogens are also present or capable of causing disease and if the source of the contamination is humans or other animals (EPA, 2000).

The ability of indicators to predict potential risks to human health are variable. Bacteria are found in the intestines of warm-blooded animals uses as indicators of the presence of dangerous pathogens that can cause human diseases. Faecal indicator organisms remain at the forefront of water and wastewater microbiology. They are representing our first technique for detecting and quantifying aquatic contamination. Viruses and protozoans cannot use as indicators because they are difficult in isolation and detection of their presence in water samples (Mara and Horan, 2003).

Faecal bacteria are used as indicator of faecal contamination of drinking water and the potential presence of diseases causing microorganisms. Faecal contamination causes numerous disease outbreaks therefore it is important to understand the potential and limitation of those indicators before implementation guideline and regulation the disinfection concentration in our water resources (Tallon *et al*., 2005).

2.6.2 *Total Coliform (TC)*

Coliforms bacteria are always present in the digestive tracts of animals, including humans, and are found in their wastes. They are relatively easy to identify. They are come from the same sources as pathogenic organisms and usually present in larger numbers than more dangerous pathogens. Testing for coliform bacteria can be a reasonable indication of whether pathogenic bacteria are present in water samples instead test for pathogens in every water sample collected (Dezuane, 1997).

(*TC*) bacteria are described in Standard Methods for the Examination of Water and Wastewater, 19th Edition, as gram negative, rod-shaped, facultative anaerobes, oxidase negative, non-pathogenic, lactose fermentation, producing gas and acid within 48 hours when cultured at 35°C. The non-spore formation makes them more susceptible to destruction by environmental conditions (Eaton *et al.*, 1995).

(*TC*) have been used as standard for sanitary quality of water since 1914 by US public health service. Now many states have stopped using *TC* as indicator to faecal contamination of water because some coliform occur naturally in soil and aquatic environment including networks of water distribution systems and because diseases outbreak have occurred even when coliform are not present (Nollet, 1997). (*TC*) are not useful as an index of faecal pathogens, but they can be used as an indicator of treatment effectiveness and to assess the cleanliness and integrity of distribution systems and the potential presence of biofilms (WHO, 2004).

E. coli and thermotolerant coliforms are a subset of the *TC* group that can ferment lactose at higher temperatures. (*TC*) detection is based on the presence of *B-galactosidase,* an enzyme that catalyzes the breakdown of lactose in to galactose and glucose (Gray, 2008). (*TC*) include species of genera *Citrobactor, Enterobacter, Klebsiella,* and *Escherichia. TC* group is more heterogeneous and includes a wider range of genera, such as Serratia and Hafnia.

Coliforms can be classified into faecal or non-faecal origin. The faecal coliform group (*FC*) is referred to as organisms that grow in the gastrointestinal tract of humans and of the warm blooded animals, while the non-faecal ones contain environmental species (WHO, 2004).

Environmental Protection Agency (EPA) set Maximum Contaminant Level (MCL) based on the presence or absence concepts for coliform (Gray, 2008). (*TC*) are generally measured in 100-ml samples of water under guidelines established by the WHO. Water that used for human consumption should contain no microbiological agents that are pathogenic to humans, with 3 CFU/100 ml for *(TC)* as MCL (WHO, 2004).

2.6.3 *Faecal Coliform (FC)*

(*FC*) is a group of thermotolerant coliforms that produce acid and gas from lactose at 44.5± 0.2°C within 24±2h. They are found in feces. (*FC)* is a subset of a larger group of organisms known as coliform bacteria. (*FC*) is found in the intestinal tract of warm-blooded animals. They are short-lived outside of a warm-blooded host compared to the coliform bacteria that are free-living not associated with the digestive tract of warm blooded animals.

The fecal coliform types divided to both pathogenic (disease-causing) and nonpathogenic bacteria. The potential presence of enteric pathogens in the digestive system and faecal contamination can be detected by presence of *FC* in aquatic environment (WHO, 2001).

(*FC*) is a reliable indicator of the survival of most bacterial pathogens, but less reliable as an indicator for the presence of viruses and parasites because they are unable to reproduce without a warm-blooded host (EPA, 2014).

E. coli is considered the most suitable index of faecal contamination. *E. coli* is thermotolerant coliforms. It is the first organism of choice in monitoring of drinking water quality. It is based on the detection of *B-glucuronidase* activity or by the ability to produce indole from tryptophan. Thermotolerant coliforms including *E. coli* are generally measured in 100-ml samples of water. Based on WHO guidelines for water quality (*FC*) should not be detected in water samples (0 CFU/100ml) (WHO, 2004).

Both (*TC*) and (*FC*) are counted in different potable water resources from Palestine, Saudi Arabia, Ethiopia, Nigeria, Brazil, UK and Pakistan, ranges from (6%) to (77.1%) for (*TC*) and from (2%) to (45.7%) for (*FC*) (Nogueira *et al*., 2003; Reida *et al*., 2003; Menawee, 2004; Okonko *et al*., 2008; Al-Khatib and Arafat, 2009; Milkiyas *et al*., 2011; Alqahtani *et al*., 2015).

In Nablus district, the researchers studied the microbial water quality in the year 1997, 2000. 2001 and 2003, and the results showed that the (*TC*) and (*FC*) percentage are higher than the recommended in safe drinking water

by Palestinian and WHO guideline. Whereas the highest percentage of (*FC*) contamination was in the year 1997 (43.1%), while the lowest percentage of (*FC*) contamination was in the year 2001 (10.4%) (Menawee, 2004).

In Gaza Strip, Al-Khatib (2009) studied the physiochemical and microbiological quality of the water in rain-fed cisterns, network distribution system and desalinated water, that showed high percentage of nitrate in water samples from all sources which were exceeded the WHO and the Palestinian limit (50mg/l). Beside that the (*TC*) and (*FC*) tests for rain fed cistern samples, recorded reported (8.6%) and (3.9%) respectively; meanwhile (15.5%) and (7.1%) from network distribution system and (15.2%) and (7%) from desalinated water samples exceeding the WHO limits for (*TC*) and (*FC*), respectively. This might be related to insufficient disinfection dose, excessive use of fertilizer and infiltration of waste water to water resources, since 36.6% of the households in the Gaza Strip are not connected to the sewage network and use pits for waste water discharge (Al-Khatib and Arafat, 2009). Other study in Wadi-Al Qilt, showed that (47%) of water samples from springs resources were contaminated with (*FC*) (Daghrah, 2010).

In Brazil, The enumeration of (*TC*) and (*FC*) contamination in the water samples collected from reservoirs were (17%) and (8%), while from taps water samples located along the distribution system were (6%) and (2%), respectively (Nogueira *et al*., 2003). In UK, (41%) and (30%) of water samples from ground water and tap water resources were contaminated with (*TC*) and (*FC*), respectively. That contamination caused by the action

of high nitrate ratio in water resources (Reida *et al,.* 2003). In Nigeria, the (*TC*) contamination ranged between (9.3%) to (44%) and the (*FC*) counts were ranged between (5%) and (48%). These results were exceeding the standard limit for (*TC)* and (*FC*) in drinking water samples (Okonko *et al.*, 2008). In Pakistan, the range of (*TC*) and (*FC*) were exceeded the WHO (MCL). The contamination referred to many reasons such as, human activities, old pipes in distribution system and infiltration of sewage to water resources, in addition to nitrogenous fertilizers and toxic substances are responsible for ground water contamination in industrial and agricultural studied areas (Shar *et al.*, 2008). In Ethiopia, (77.1%) and (45.7%) of the household water samples were contaminated with (*TC*) and (*FC*), respectively (Milkiyas *et al.*, 2011). In Saudi Arabia, (*TC*) were detected in the water samples from wells, tankers and roof tanks in ranged between (20%) to (68.8), and *E. coli* was found contaminating (25%) of different water resources (Alqahtani *et al.*, 2015).

2.6.4 Other Less Commonly Used Bacterial Indicators.

2.6.4.1 Intestinal Enterococci

Intestinal Enterococci are a subgroup of faecal Streptococci group, comprising species of two genera; *Enterococcus* and *Streptococcus,* which do not multiply in the environment. These bacteria are Gram-positive and facultative anaerobic. *Intestinal Enterococci* occur in intestine and are excreted in the faeces of warm blooded animals and humans. They are present in sewage, polluted water and some members of the group were detected in soil free from faecal contamination. They occur in much higher

number compared with other (*FC)* and viral pathogen (Mara and Horan, 2003).

The Intestinal Enterococci group can be used as an indicator of faecal contamination; because they are resistant to drying and chlorination. They are also be used to test water quality after distribution systems repairs or after new mains have been laid; because they tend to survive longer in water environments than *E. coli* and other Thermotolerant Coliforms (WHO, 2004).

2.6.4.2 Sulfite-Reducing Clostridia

Clostridium spp. is Gram-positive, anaerobic, sulfite-reducing bacilli and spore forming bacteria. *C. perfringens* is a species of this genus which present in animal and human feces but in lower number than *(FC)* and *Faecal Enterococci*, which does not multiply in water environments. They are normal intestinal flora of 13–35% of humans and other warm blooded animals and that are resistant to water environments condition, including UV irradiation, temperature, pH extremes, and disinfection such as chlorination (WHO, 2004).

C. perfringens is a highly specific indicator of faecal contamination especially when (*FC*) and *Faecal Enterococci* cannot be detected or when suspended matter or toxic substances from industrial are presented. Its energy sources are obtained from oxidizing the industrial organic compounds by reducing sulfur to hydrogen sulfide (Mara and Horan, 2003).

2.7 Water disinfection by chlorination

Microorganisms are found in raw water from all resources (surface water and groundwater). Most microorganisms are harmful to human health, but some are pathogenic transmitted through distribution system causing waterborne disease. Different disinfection methods are used to inactivate waterborne pathogens including those that can cause typhoid fever, dysentery, cholera and Legionnaires disease. Along with other water treatment processes such as coagulation, sedimentation, and filtration, chlorination creates drinking water that is safer for public health.

Chlorination is a chemical disinfection and the most popular method of disinfection and water treatment all over the world. It is cost-effective method of water disinfection and effective against bacteria and viruses. However, it cannot inactivate all microbes. Some protozoan cysts are resistant to the effects of chlorine. This method was first discovered in Sweden in 1744 and is still used today. Chlorination method uses various types of chlorine or chlorine-containing substances for the oxidation and disinfection of potable water source to control biological growth and efficiently kill microorganisms during water treatment. In addition to that, chlorination method has the ability to remove the taste, odors, iron and manganese. In this case it will improve the water quality from the treatment plant to the consumer's tap (Calomiris and Christman, 1998).

Water pH and pathogens type affect the efficacy of chlorine disinfection against pathogens. Bacteria and viruses are easy targeted by chlorination over a wide range of pH, even though protozoa are more resistance to

chlorine at high pH. This is because when chlorine added to water two kinds of chemicals will be formed, known together as "free chlorine." These compounds are hypochlorous acid (HOCl, electrically neutral) and hypochlorite ion (OCl-, electrically negative). Hypochlorous acid is considered as stronger disinfectant, oxidant and more reactive than the hypochlorite ion. The ratio of hypochlorous acid to hypochlorite ion in water is determined by water pH. At low pH, hypochlorous acid dominates, while at high pH hypochlorite ion dominates.

Quantity of chlorine which added to drinking water was dependent on the impurities and contamination of the water that needs to be treated. The amount of chlorine that is required to satisfy all the contaminants and impurities is termed the chlorine demand. The difference between the quantity of chlorine added and the chlorine demand is termed as residual chlorine. Residual chlorine can react with organic materials in the water to form byproducts, such as Trihalomethanes (THMs), carcinogenic products and haloacetic acids, which may pose health risks and increase risk of bladder cancer (WHO, 2008).

People who use water containing chlorine well in excess of the maximum residual disinfectant level especially children could experience irritating effects to their eyes, esophagus, nose, stomach discomfort, burning sensation in mouth or throat and spontaneous vomiting (WHO, 1996; EPA, 2013).

In study of quantitative and qualitative properties of drinking water in the Tulkarm district of Palestine in 1999, five hundred water samples were

collected from different water resources and examined for their free chlorine residual concentration, (*TC*) and (*FC*). It was found that (60.6%) of the samples had concentrations of free chlorine residual less than the minimum concentration which recommended by WHO (0.2 ppm) and unacceptable according to the Palestinian and WHO standards, beside (34%) and (9.2%) were contaminated with (*TC*) and (*FC*), respectively (Al–Khatib, 2003).

2.8 Biochemical tests for identification waterborne bacteria

In the biochemical test, the acronym IMViC stands for Indole Test (IND), Methyl Red (MR), Voges-Proskauer (VP) and Citrate Utilization Test (CUT); which referring to the tests needed to detect fecal contamination of food and water (Koneman, *et al*., 1996).

(MR) test determines whether the microorganism performs mixed acids fermentation or butylene glycol fermentation pathway when supplied glucose, resulting in a ratio of CO_2 to H_2 gas produced by fermentation. Some organisms such as *E. coli* and *Salmonella* ferment sugars by the mixed acid pathway which produces acidic end products such as lactic, acetic, and formic acid. These acidic end products are stable and will remain acidic, resulting in a low ratio of CO_2 to H_2 gas. High acids quantity produced causes decrease in the pH (at or below 4.4) of the culture medium and turn the culture color to red by addition methyl red indicator after 24 hours incubation of culture medium at 37°C (The culture has a positive result for the MR test). In contrast, other microorganisms such as *Klebsiella*, ferment sugars by the butylene glycol pathway, which produces

neutral end products, including acetoin and 2, 3-butanediol, resulting in high ratio of CO_2 to H_2 gas. Low acids degree produced causes increase in pH (at or high 6.6) and yellow color of culture medium by addition of methyl red indicator after 24 hours incubation of culture medium at 37°C (the culture has a negative result for the MR test) (Koneman, *et al*., 1996 and Engelkirk, 2008).

(VP) test are based on the conversion of acetyl methyl carbinol to diacetyl (acetoin) from glucose metabolism through the action of Barritt's reagent. Positive result which appeared with deep rose color after the addition of Barritt's reagent to the culture media after 24 hour's incubation at 37°C, as in *Klebsiella*. The absence of rose color is representing the negative result, as in *E. coli* and *Salmonella* (Koneman, *et al*., 1996).

(SIM) medium was used for the identification of microorganisms on the basis of hydrogen sulfide production, indole production and motility. Indole test is determine if the microorganisms possesses *tryptophanase* enzyme to convert tryptophane to indole by observing the development of red color by addition of 0.5 ml of Kovac's reagent after 24 hours' incubation at 37°C, as in *E. coli*. Ferric ammonium citrate and sodium thiosulfate in SIM media components used to detect H_2S production, which appeared with black color on the culture media, as in *Salmonella.* And the motility of microorganisms was detected by observing the growth extending outward from the original line of colonies inoculation due to the semisolid nature of SIM media (Koneman, *et al*., 1996).

(CUT) determines the ability of bacteria to utilize citrate as its sole carbon and energy source. The slant portion of citrate agar tube was inoculated with the organism to be tested, then incubation for 24 hours at 37°C. A positive result is demonstrated by the color change of a pH indicator to blue, due to the generation of alkaline and increase in the pH of the citrate medium, as in *Klebsiella* and *Salmonella*. Absence of growth was negative test result, as in *E. coli* (Engelkirk, 2008).

(TSI) test is used for the differentiation of microorganisms on the basis of dextrose, lactose, and sucrose fermentation and (H_2S) production. When the carbohydrates are fermented, acid production is detected by the Phenol Red pH indicator. Ferric Ammonium Citrate is the (H_2S) indicator. The test was performed by inoculating TSI media with an inoculating needle by stabbing the butt and streaking the slant then incubation for 24 hours at 37°C (Parija, 2009).

An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose, lactose and/or sucrose. An alkaline slant-alkaline butt (red/red) indicates dextrose or lactose was not fermented. Cracks, splits, or bubbles in medium indicate gas production. A black precipitate in butt indicates (H_2S) production, as in *Salmonella* (Koneman *et al.,* 1996).

2.9 Molecular analysis of water borne pathogens

Different methods can be used for identification, differentiation and detection of bacterial characteristic's in water ecology. Traditional methods for the detection of water borne pathogens depends on the selective

cultivation of target microorganisms which use broths enrichment followed by isolation of colonies on selective media, and identification by standard biochemical methods, immunofluorescence or staining. Traditional methods are complex and time consuming. In addition, some enteric pathogens are not growing.

Molecular techniques are easy, rapid, and sensitive methods, with high accuracy and capacity to detect small amounts of target gene in a sample and more specify method for detection water borne pathogens. Also, it can be easily detect the presence or absence of pathogens in the environment. Molecular methods are based on hybridization with fluorescence probes or specific amplification of RNA or DNA for their target genes of the microorganism. Most applied molecular techniques are based on nucleic acid amplification protocols; and most commonly used one is Polymerase Chain Reaction (PCR) (Kazner *et al*., 2012 and Kinge *et al*. 2012).

Nucleic acid amplification techniques have an enormous range of application and have become an indispensible tool in molecular biology and powerful rapid screening method in the detection of waterborne and foodborne pathogens by targeting and amplifying DNA sequences with sensitivities down to a single target copy per reaction, and in many cases quantifying the results. PCR is a revolutionary molecular tool, developed in the 1980s by Kary Mullis, that allows Taq DNA polymerase for the amplification of target DNA strand by specific primers in a chain of thermocycles. This tool is used for detection, enumeration the indicator microorganisms in environmental samples and investigation of food and

water borne pathogens with high sensitivity without requirement for additional conformational tests. In addition, the precision of achievement of test results would be within a few hours in automatic procedure (Gleeson, 1997).

PCR is a method for the amplification of single or double strands DNA sequences *in vitro*. The reaction proceeds in response to specific thermal condition driven steps of double stranded DNA denaturation, DNA polymerase extension and specific primer to complementary single strand target DNA sequences. The primer defines the 5' ends of the discrete products that are subsequently formed. Three steps PCR use three individual temperature steps for denaturation, annealing and extension (Dongyou, 2009).

Detection and quantification for some enteric viruses and parasite need combination technique between cell culture; nucleic acid hybridization and PCR amplification. The samples are inoculated into susceptible host cell culture then incubated to allow the viruses and protozoa to infect the cells and proliferate. After an incubation period sufficient to produce enough nucleic acid for direct detection or further amplification, the nucleic acid is denatured and fixed after extraction. Then the target gene is detected after amplification by PCR. These methods facilitate the detection and rapidly amplified the small amount of target nucleic acid produced in culture by PCR. Recovery of *cryptosporidium parvum* and *Giardia lamblia* in environmental water samples need centrifugation and immunomagnetic separation method of oocyst and cyst before amplification of target gene,

because direct amplification of both by PCR are difficult, due to the low quantity of it in the environmental sources (GBNRC, 1999).

Molecular methods offer not only the advantage of high sensitivity detection, but also the ability to discriminate genotypes, which determine the prevalence of *C. parvum* and *G. lamblia* in different water sources using molecular technique. PCR remains the gold standard for the identification of many waterborne pathogens since it delivers specificity and sensitivity in detection. PCR used for the simultaneous detection of *C. parvum* and *G. lamblia* using primers amplifying 256 bp and 163 bp products from the 18S rRNA gene of *C. parvum* and the heat shock protein gene of *G. lamblia*. The results demonstrate the potential utility of PCR for the detection of pathogenic protozoa in water. It has successfully been applied for the detection and identification of pathogenic bacteria in clinical and environmental samples, as well as for the investigation of food and waterborne disease outbreaks (Rochelle *et al*., 1997; Alam *et al*., 2014).

In Nablus region of Palestine, the target gene of *cryptosporidium parvum* parasite was detected by using IMS kit and PCR-RFLP, and the results showed that *cryptosporidium parvum* was the causative agent of vomiting and diarrhea outbreak in this region (Hussein, 2011). In other studies, *cryptosporidium parvum* and *Giardia lamblia* were detected with high specificity and sensitivity in environmental water samples from Choptank River, Severn River, Miles River and drinking water from different places in Pakistan, by IMS method by using magnetic beads coated with an

anticryptosporidial monoclonal antibody, integrated with PCR technique (Xiao *et al*., 2001 and Alam *et al.,* 2014).

Molecular epidemiology has become a particularly powerful tool for investigation of foodborne and waterborne outbreaks for the diseases. The availability of large and growing databases of Hepatitis Virus sequences has allowed the identification of the source of viruses' contamination and may help to accelerate the public health concern and immediate response to the outbreaks (Dongyou, 2010).

PCR analysis has been reported in several studies to be more sensitive and accurate for identification of *IpaH* and *GapA* genes in *Shigella* and *Klebsiella* species, respectively, in different water samples. An enrichment step is usually required prior to performing a PCR. In addition, the rapid and simultaneous detection of pathogenic *E. coli, S. typhimurium* and *Vibrio cholera* in drinking water samples (Faruque *et al*., 2002; Momba *et al*., 2006; Sharma *et al.,* 2010 and Siri *et al.,* 2011).

Although single round PCR is the most familiar molecular assay for detection and genotyping of pathogens in clinical microbiology, the Multiplex PCR (M-PCR) assay is also getting much attention and has been developed and widely used to serve these purpose in several viruses and bacteria. M-PCR is a molecular technique involves the simultaneous detection of several pathogens by introducing up to ten difference primers to amplify DNA regions coding for specific targeted genes of each bacterial strain in the same reaction mix (Verkuil *et al.,* 2008). Examples of M-PCR technique for the rapid and simultaneous detection of water borne

pathogens include M-PCR assay for detection of *Mdh, IpaB, IpaH* and *GapA* genes in *E. coli, Salmonella, Shigella* and *Klebsiella,* respectively, and stimulus detection of *Yersinia enterocolitica, Vibrio cholera* and *Campylobacter* spp. (Gomez-Duarte *et al*., 2009 and Kinge *et al*., 2012). The use of PCR for detecting and identifying enteric pathogens is rapid and easy, while the conventional identification methods are not only time consuming, but also require an experienced laboratory technician to isolate and identify bacterial colonies accurately. Studies in several parts of the world had shown that the sensitivity and specificity of a direct PCR method for the detection of enteric pathogens in environmental samples was quite high when compared with conventional methods. In spite of PCR and M-PCR technique advantages, detection of microorganisms using molecular technique can be complicated, expensive and requiring skilled workers to carry out the tests. However, molecular protocols, unlike traditional culture-based methods, do not distinguish between viable and non-viable organisms hence the need for more information before replacing the current conventional methods by molecular ones.

2.10 Justification

Regarding to PWA regulation, the water resources in Tubas well; which was fed from Tammoun well and distributed to Tubas Governorate across a single network, had an elevated nitrate exceeding what the limits allowed by WHO for drinking water (50 mg/l). This could make water more susceptible to contamination with (*TC*) and (*FC*), in addition to other enteric bacterial pathogens.

Tubas Governorate is recognized as an agricultural area where fertilizer and pesticides are drained to Tubas water resources ground water leading to water contamination, especially with health hazards, nitrate, heavy metals and organic compound. Lack of water networks in some villages in Tubas governorate such as, Bziq, Salhab, Kherbit Atof , Al-Aqaba and Al-Maleh, made them mainly depending on tankers to supply their home cisterns with drinking water. The absence of village councils and/or municipalities control on tankers and household cisterns, made them more susceptible to contamination with (*TC*), (*FC*) and other enteric pathogens.

Water safety is a global health issue where waterborne diseases had taken a major crisis on health. *Salmonella* spp*, E. coli,* and *Klebsiella* are considered as major waterborne pathogens that represent a permanent challenge to the potable water. These bacterial species are considered dangerous pathogens with their ability to cause diseases to humans and animals. Detection and identification of microbial pathogens in potable water is the first to the prevention and recognition of problems related to health and safety.

2.11 Aims of study

Since the presence of microbial contaminants in potable water is a vital health issue, then the sanitary assessment of water quality in different locations of Tubas Governorate has been targeted in this study in order to:

- Identify the prevalence of *TC, FC*, *E. coli, Klebsiella* and *Salmonella* in drinking water samples collected from rain-fed cisterns, tap water

(homes, school and restaurants), groundwater wells, network distribution systems and tankers.

- Molecular detection of water borne pathogens (*E. coli, Klebsiella* and *Salmonella*) using PCR

- Comparison study between traditional and molecular method of microbial detection

Chapter Three

Material and Methods

3.1 Collection of samples

Sixty potable water samples [(15) samples from network distribution systems; (15) tap water samples from tanks (houses, restaurants and school); (15) samples from rain fed cisterns (houses, restaurants and school), (9) samples from tankers and (6) samples from groundwater wells] were collected randomly during dry season (September-November) 2014, from 14 locations in Tubas governorate as shown in (Figure 1). These samples were placed in sterilized glass bottles (1L) with 1 ml/L Sodium Thiosulfate to neutralize any presence of chlorine, and kept under aseptic condition in ice-bag containers and transported to the Microbiological Laboratory at An-Najah National University, at the same day for microbiological analysis.
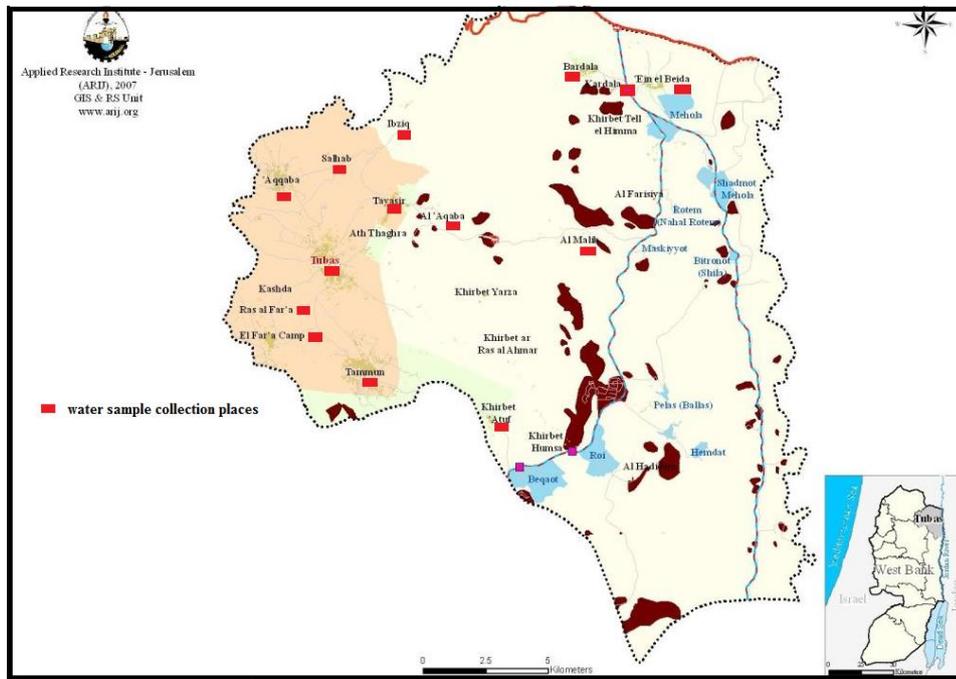
Figure 2. Location and distribution of collected samples in Tubas governorate.

3.2 Media preparation

3.2.1 MacConkey Agar

MacConkey agar (HIMEDIA) was prepared according to the manufacturer's instructions labeled on the bottle. A two liter flask containing 1 liter deionized water and 49 g MacConkey agar was heated and stirred until the agar dissolved. The solution was allowed to boil for 1min, and then was autoclaved at 121°C for 15 min. After that it was allowed to cool, and the agar was poured into sterile Petri dishes to have 20 ml that was covered and left overnight. The following day the Petri dishes were turned upside down and stored at 4°C (Atlas, 2010).

3.2.2 Salmonella- Shigella (SS) Agar

SS Agar (HIMEDIA) was prepared according to the manufacturer's instructions labeled on the bottle. A 2L flask containing 1L deionized water and 59 g (SS) Agar was heated and stirred until the agar dissolved. The solution was allowed to boil for 1min, without being autoclaved. After that it was allowed to cool, and the agar was poured into sterile Petri dishes to have 20 ml, then they were covered and left overnight. The following day the Petri dishes were turned upside down and stored at 4°C (Atlas, 2010).

3.2.3 Selenite Broth (SB) Enrichment Media

(SB) Enrichment Media (HIMEDIA) was prepared according to the manufacturer's instructions labeled on the bottle. A 2L flask containing 1L deionized water and 19 g Selenite broth and 4 g Sodium Biselenite was heated and stirred until the broth dissolved. The solution was allowed to

boil for 1min, without being autoclaved. After that it was allowed to cool, and the broth was poured into sterile tubes that was covered and left overnight. The following day tubes were stored at 4°C (Atlas, 2010).

3.2.4 M-Endo Agar

M-Endo Agar (HIMEDIA) was prepared according to the manufacturer's instructions labeled on the bottle. A 2L flask containing 1L deionized water and 45 g M-Endo Agar was heated and stirred until the agar dissolved then added 20 ml of 10% Basic Fuchsion supplement. The solution was allowed to boil for 1min, without being autoclaved. After that it was allowed to cool, and the agar was poured into sterile Petri dishes in darks to have 6 ml that was covered and left overnight. The following day the Petri dishes were turned upside down and stored at 4°C (Clesceri and Greenberg, 1998).

3.2.5 M-Fc Agar

M-Fc Agar (HIMEDIA) was prepared according to the manufacturer's instructions labeled on the bottle. A 2L flask containing 1L deionized water and 50 g M-Fc Agar was heated and stirred until the agar dissolved then added 10 ml of 1% Rosolic Acid supplement. The solution was allowed to boil for 1min, without being autoclaved. After that it was allowed to cool, and the agar was poured into sterile Petri dishes in darks to have 6 ml that was covered and left overnight. The following morning the Petri dishes were turned upside down and stored at 4°C (Clesceri and Greenberg, 1998).

3.2.6 Lauryl Sulfate Tryptose Broth

Lauryl Sulfate Tryptose Broth (HIMEDIA) was prepared according to the manufacturer's instructions labeled on the bottle. A 2L flask containing 1L deionized water and 35.6 g Lauryl Sulfate Tryptose Broth was heated and

stirred until the agar dissolved. The solution was allowed to boil for 1min, without being autoclaved. After that it was allowed to cool, and the broth was stored at 4°C (Eaton *et al.*, 1995).

3.2.7 Eosin Methylene Blue (EMB) Agar

(EMB) Agar (HIMEDIA) was prepared according to the manufacturer's instructions labeled on the bottle. A 2L flask containing 1L deionized water and 37.5 g EMB) Agar was heated and stirred until the agar dissolved. The solution was allowed to boil for 1min, and then was autoclaved at 121°C for 15 min. After that it was allowed to cool, and the agar was poured into sterile Petri dishes to have 20 ml that was covered and left overnight. The following day the Petri dishes were turned upside down and stored at 4°C (Atlas, 2010).

3.2.8 Citrate Agar

SIMMONS Citrate Agar was prepared according to the manufacturer's instructions labeled on the bottle. A 2L flask containing 1L deionized water and 22 g Citrate Agar was heated and stirred until the agar dissolved. The solution was allowed to boil for 1min, and then was poured into sterile tubes in a slanted position, that were covered and autoclaved at 121°C for 15 min. After that they were allowed to cool overnight. The following day the tubes stored at 4°C (Murray *et al.*, 1995). .

3.2.9 SIM Medium

SIM Medium (HIMEDIA) was prepared according to the manufacturer's instructions labeled on the bottle. A 2L flask containing 1L deionized water and 36.23 g SIM Medium was heated and stirred until the agar dissolved.

The solution was allowed to boil for 1min, and then was poured into sterile tubes in upright position, that were covered and autoclaved at 121°C for 15 min. After that they were allowed to cool overnight. The following day the tubes stored at 4°C (Murray *et al*., 1995).

3.2.10 Methyl Red Voges Proskauer (M.R.V.P) Medium

M.R.V.P Medium (OXOID) was prepared according to the manufacturer's instructions labeled on the bottle. A 2L flask containing 1L deionized water and 17 g M.R.V.P Medium was heated and stirred until the agar dissolved. The solution was allowed to boil for 1min, and then was poured into sterile tubes in upright position, that were covered and autoclaved at 121°C for 15 min. After that they were allowed to cool overnight. The following day the tubes stored at 4°C (Murray *et al*., 1995).

3.3 Detection of bacteria by culturing methods and biochemical tests

3.3.1 Detection of *TC*

By the Standard Method for the Examination of Water and Waste Water (1996), the water sample (1 L) was filtered through a 0.22 μm gridded nitrocellulose membrane filter by Membrane Filtration technique (MF) to retain the bacteria into filter paper by vacuum after sterilized 10 minute by exposure to UV (Clesceri and Greenberg, 1998).

Placed an absorbent pad in to the cover of M-Endo culture dish using sterile forceps, then (1.7 ml) of Lauryl Sulfate Tryptose Broth was added to the filter paper after filtration process was completed. Incubated the cover of M-Endo dish in the (SELECTA) incubator for 4 hours at 35°C, then the

cover was turned upside down to M-Endo dish and incubated in the (SELECTA) incubator for 24 hours at 37°C (Clesceri and Greenberg, 1998; Dezuane, 1997 and Spellman and Drinan, 2012) .

Upon completion of incubation period, pink to dark-red colonies with a metallic surface sheen are counted as *TC* colonies.

Count obtained was calculated by the following equation (Tomar, 1999 and Banu, 2007):

$$TC/_{100ml} = \frac{\text{No.of Colonies Counted}}{\text{Vol}} \times 100$$

TC : Total Coliform

Vol : Volume of water sample filtered in (ml)

3.3.2 Detection of *FC*

By the Standard Method for the Examination of Water and Waste Water (1996); the water sample (1 L) was filtered through a 0.22 μm gridded nitrocellulose membrane filter by (MF) technique to retain the bacteria in to filter paper by the vacuum after sterilized it 10 minute by exposure to UV (Clesceri and Greenberg, 1998).

Placed absorbent pad into cover of M-Fc culture dish using sterile forceps, then (1.7 ml) of Lauryl Sulfate Tryptose Broth was added to immerse the filter paper after filtration process completed. Incubated the cover of M-Fc dish in the (SELECTA) incubator for 4 hours at 35°C, then the cover was turned upside down to M-Fc dish and incubated in the (Lab Tech) Shaking incubator for 24 hours at 44.5°C (Clesceri and Greenberg, 1998; Dezuane, 1997; Spellman and Drinan, 2012).

Upon completion of incubation period, blue colonies are counted as *FC*.
Count obtained was calculated by the following equation (Tomar, 1999 and
Banu, 2007):

$$FC/_{100ml} = \frac{BC}{VOL} \times 100$$

    FC: Fecal Coliform

    BC: Blue Colonies

    VOL: Volume of water sample filtered in (ml)

3.3.3 Isolation of *E. coli* and *Klebsiella*

The *FC* colony were cultured on Macconkey and EMB plates and
incubated 24 hours at 37°C. Gram negative microorganism's colonies
isolated from Macconkey were used in subculture on to other Macconkey
plates to isolate pure species which used for biochemical tests (CUT, MR,
VP, TSI and IND tests) and DNA extraction (Clark *et al*., 2001).

3.3.4 Isolation of *salmonella*

The *FC* was enriched on Selenite Broth (SB) for 72 hours at 37°C. After
pre enrichment incubation period completed, loopful of enrichment SB was
streaked on to Salmonella-Shigella (SS) agar and incubated for 24 hours at
37°C, then the colonies with black head were used in subculture on to other
SS plates to isolate pure species which used for biochemical tests (CUT,
MR, VP, TSI and IND tests) and DNA extraction (Clark *et al*., 2001).

3.4 DNA Extraction

Pure colonies from fresh culture species were suspended in 1 ml TE buffer
[10 mM Tris-HCl, 1 mM EDTA, and (pH 8.0)] and the mixture was briefly
mixed on a vortex mixer. The suspension was centrifuge at 11,500 X g for

5 min then discards supernatant and added 400µl of sterile distilled H2O in to precipitant, and was boiled for 10-15 min. The cells then immediately were incubated on ice for 10 min. The debris was pelleted by centrifugation at 11,500 X g for 5 min. DNA which extracted from the supernatant was transferred to a sterile eppendrof tube and stored at -20°C until PCR testing (Dongyou, 2011)

The quantity and purity of DNA extract was determined spectrophotometrically at an absorbance of 260 nm and A260/A280. The optical density (O.D.) at 260 nm of diluted fractions of the isolated DNA samples was measured by (Spectro UV-Vis DUAL BEAM) spectrophotometer and the DNA concentration was calculated by considering 1 O.D. (260 nm) = 50 µg/ml DNA and taking into account the dilution factor using (Singleton, 2000 and Dongyou, 2011).

3.5 Detection of water borne pathogens by PCR

3.5.1 Detection of *E. coli Mdh* gene

E. coli were identified by PCR with specific primers for malate dehydrognase gene (*Mdh*) was described in (Hsu *et al*. 2006). The PCR reaction mix (25 µL) was performed using 12.5 µL of PCR premix with MgCl2 (Ready Mix™ Taq PCR Reaction Mix with MgCl2, Sigma), 11.1 µL of ddH2O, 0.4 µM of each primer, and 2 µL DNA template. DNA amplification was performed using thermal cycler (Mastercycler Personal, Eppendorf) according to the following thermal conditions: initial denaturation for 2 min at 94°C followed by 30 cycles at 94°C for 1 min for

denaturation, annealing at 59°C for 30 s and extension at 72°C for 1 min. Final extension was carried out at 72°C for 5 min. The amplified products were examined by (1.5%) agarose gel electrophoresis with (1 μL/100ml) Red gel staining of DNA bands in (1X TAE) buffer to determine the size of amplified fragment for each isolate. Electrophoresis was done for one hour in electrical field strength of 100 V, and then the gel was visualized by UV light.

DNA ladder of 100 bp was also included in all gels (100bp DNA ladder RTU, GeneDireX). Negative control was included in these experiments. Primer nucleotide sequences and expected size of amplicon are presented in Table 1.

Table 1. *E. coli* **target genes for PCR amplification, amplicon size, primer sequences and annealing temperature (AT).**

Organism	Gene target	Oligonucleotide sequence (5→3)*	Amplicon Size (bp)	AT	Reference
E. coli	*Mdh*	*Mdh* (**F**): ACT GAA AGG CAA ACA GCC AGG C *Mdh* (**R**): CGT TCT GTT CAA ATG CGC TCA GG	**392**	**59°C**	**Hsu** *et al*.**, 2006**

* The primer used in this study was synthesized by Sigma-Aldrich (USA)

3.5.2 Detection of *Salmonella IpaB* gene

Salmonella were identified by PCR with specific primers for Invasion Plasmid Antigene B gene (*IpaB*) was described in (Momtaz *et al*., 2013; Kong *et al*., 2002; Momba *et al*., 2006). The PCR reaction mix (25 μL) was

performed using 12.5 μL of PCR premix with MgCl2 (Ready Mix™ Taq PCR Reaction Mix with MgCl2, Sigma), 11.1 μL of ddH2O, 0.4 μM of each primer, and 2 μL DNA template. DNA amplification was performed using thermal cycler (Mastercycler Personal, Eppendorf) according to the following thermal conditions: initial denaturation for 2 min at 94°C followed by 35 cycles at 94°C for 1 min for denaturation, annealing at 62°C for 1 min and extension at 72°C for 2.5 min. Final extension was carried out at 72°C for 10 min. The amplified products were examined by (1.5%) agarose gel electrophoresis with (1 μL/100ml) Red gel staining of DNA bands in (1X TAE) buffer to determine the size of amplified fragment for each isolates. Electrophoresis was done for one hour in electrical field strength of 100 V, and then the gel was visualized by UV light.

DNA ladder of 100 bp was also included in all gels (100bp DNA ladder RTU, GeneDireX). Negative control was included in these experiments. Primer nucleotide sequences and expected size of amplicon are presented in Table 2.

Table 2. *Salmonella* **target genes for PCR amplification, amplicon size, and primer sequences and (AT).**

Organism	Gene target	Oligonucleotide sequence (5→3)*	Amplicon Size (bp)	AT	Reference
Salmonella	*IpaB*	*Ipa B* (F): GGA CTT TTT AAA AGC GGC GG *Ipa B* (R): GCC TCT CCC AGA GCC GTC TGG	314	62°C	Momtaz *et al.*, 2013

* The primer used in this study was synthesized by Sigma-Aldrich (USA)

3.5.3 Detection of *Klebsiella Gap A* gene

Klebsiella were identified by PCR with specific primers for Glyceraldehyde 3- phosphate dehydrogenase gene (*Gap A*) was described in (Siri *et al*., 2011). The PCR reaction mix (25 μL) was performed using 12.5 μL of PCR premix with MgCl2 (Ready Mix™ Taq PCR Reaction Mix with MgCl2, Sigma), 11.1μL of ddH2O, 0.4 μM of each primer, and 2 μL DNA template. DNA amplification was performed using thermal cycler (Mastercycler Personal, Eppendorf) according to the following thermal conditions: initial denaturation for 2 min at 94°C followed by 35 cycles at 94°C for 1 min for denaturation, annealing at 62°C for 1 min and extension at 72°C for 2.5 min. Final extension was carried out at 72°C for 10 min. The amplified products were examined by (1.5%) agarose gel

electrophoresis with (1 μL/100ml) Red gel staining of DNA bands in (1X TAE) buffer to determine the size of amplified fragment for each isolates. Electrophoresis was done for one hour in electrical field strength of 100 V, then the gel was visualized by UV light.

DNA ladder of 100 bp was also included in all gels (100bp DNA ladder RTU, GeneDireX). Negative control was included in these experiments. Primer nucleotide sequences and expected size of amplicon are presented in Table 3.

Table 3. *Klebsiella* target genes for PCR amplification, amplicon size, primer sequences and (AT).

Organism	Gene target	Oligonucleotide sequence (5→3)*	Amplicon Size (bp)	AT	Reference
Klebsiella	*GapA*	*Gap A* **(F):** TGA AAT ATG ACT CCA CTC ACG G *Gap A* **(R):** CTT CAG AAG CGG CTT TGA TGG CTT	**700**	**62°C**	**Siri *et al*., 2011**

* The primer used in this study was synthesized by Sigma-Aldrich (USA)

Chapter Four

Results

4.1 Bacterial enumeration and culture characterization

4.1.1 *TC* and *FC* enumeration

In this study, a total of 60 water samples collected from groundwater wells, network distribution systems, tankers, tap water (houses, restaurants and school) and rain fed cisterns, were examined for *TC* and *FC*. The results showed that 24 and 19 of water samples were positive for *TC* and *FC*, respectively. Results of *TC* and *FC* contamination generally from water resources in 14 locations in Tubas Governorate are represented in Figure 3.
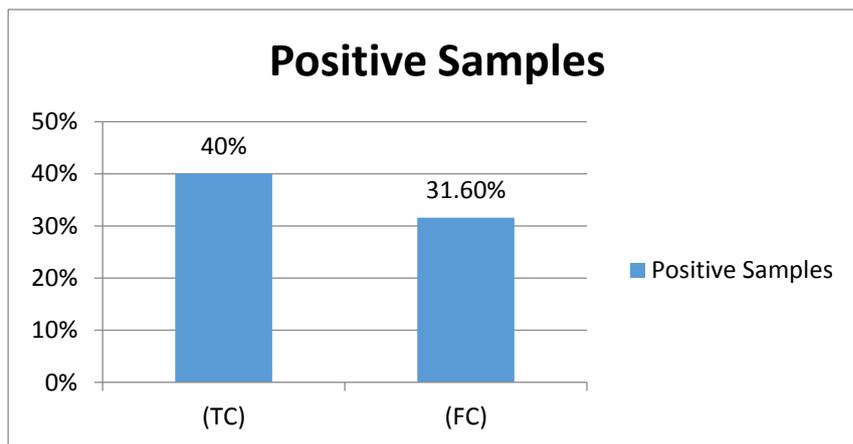
Figure 3. Percentage of *TC* and *FC* counts in water samples from different water resources.

Specific determination of (*TC*) contamination in each water resources in Tubas Governorate, the results showed that the high percentage of contaminations with (*TC*) was recorded from rain fed cisterns(15.00%), followed by tap water (houses, restaurants and schools) and tankers (13.33%; 8.33%, respectively). Meanwhile, the lowest percentage (1.67%) had been recorded from groundwater wells and network distribution systems (Fig. 4).

Regarding to (*FC*) contamination in each water resources in Tubas Governorate, the results showed that the percentage of contaminations were (13.33%; 11.67%; 6.67%), from rain fed cisterns; tap water (houses, restaurants and schools) and tankers, respectively. None of groundwater wells samples and network distribution systems samples had any positive *FC* (Fig. 4).
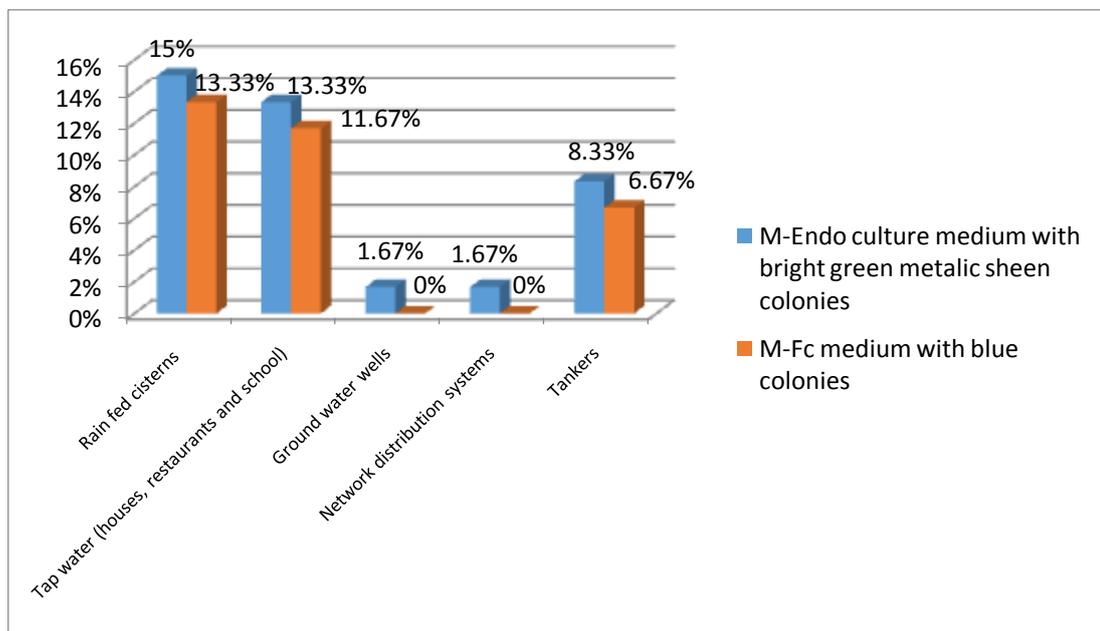
Figure 4. Results of (TC) and (FC) distribution in drinking water samples from rain fed cisterns, tap water (houses, restaurants and schools), groundwater wells, network distribution systems and tankers

Analysis of rain fed cisterns samples revealed that 1/15 (6.66%), 4/15 (26.67%) and 4/15 (26.67%) had (*TC*) count from 10-100, 1.01-9.99 and 0.01-1.0 (CFU/100 ml), respectively.

In the case of Thermotolerant Coliforms, 4/15 (26.67%) and 4/15 (26.67%) had (*FC*) count from 1.01-9.99 and 0.01-1.0 (CFU/100ml), while (*FC*) count from 10-100 (CFU/100 ml) didn't exist.

Analysis of tap water samples from homes, school and restaurants revealed that 6/15 (40%) and 2/15 (13.33%) had (*TC*) count from 1.09-9.99 and 0.01-1.0 (CFU/100 ml), and none (*TC*) count from 10-100 (CFU/100 ml).

In the case of Thermotolerant Coliforms, 5/15 (33.33%) and 2/15 (13.33%) had (*FC*) count from 1.01-9.99 and 0.01-1.0 (CFU/100 ml), respectively, and none (*FC*) count from 10-100 (CFU/100 ml).

Analysis of ground water wells samples revealed that 1/6 (16.67%) had (*TC*) count from 0.01-1.0 (CFU/100 ml), and none (*TC*) count from 1.01-9.99 and 10-100 (CFU/100ml). In addition to that, (*FC*) was not detected in any of these samples.

Analysis of network distribution system water samples revealed that 1/15 (6.67%) had (*TC*) count from 0.01-1.0 (CFU/100ml) and nothing detected of (*TC*) that count from 1.01-9.99 and 10-100 (CFU/100ml). In addition to that, none of these samples was to (*FC*) detected.

Analysis of tankers water samples revealed that 2/9 (22.22%) and 3/9 (33.33%) had (*TC*) count from 1.01-9.99 and 0.01-1.0 (CFU/100 ml), respectively, but none of (*TC*) count from 10-100 (CFU/100 ml).

In the case of Thermotolerant Coliforms, 1/9 (11.11%) and 3/9 (33.33%) had (*FC*) count from 1.01-9.99 and 0.01-1.0 (CFU/100 ml), respectively, but not of (*FC*) count from 10-100 (CFU/100 ml) (Table 4 and Table 5)

Table 4. Comparison of TC (3 CFU/100ml) range of water samples from tap water (houses, restaurants and school), ground water wells, rain fed cisterns, network distribution systems and tankers.

Recommended level of (TC) parameter CFU/100ml	Tap water (houses, restaurants and school)		Ground water wells		Rain fed cisterns		Network distribution systems		Tankers	
	No. of Samples	(%)	No. of Samples	(%)	No. of Samples	(%)	No. of Samples	(%)	No. of Samples	(%)
10-100	0	0	0	0	1	6.66	0	0	0	0
1.01-9.99	6	40	0	0	4	26.67	0	0	2	22.22
0.01-1.0	2	13.33	1	16.67	4	26.67	1	6.67	3	33.33
0	7	46.67	5	83.33	6	40	14	93.33	4	44.45
TOTAL	15	100	6	100	15	100	15	100	9	100

Table 5. Comparison of FC (0 CFU/100ml) range of water samples from tap water (houses, restaurants and school), ground water wells, rain fed cisterns, network distribution systems and tankers

Recommended level of (TC) parameter CFU/100ml	Tap water (houses, restaurants and school)		Ground water wells		Rain fed cisterns		Network distribution systems		Tankers	
	No. of Samples	(%)	No. of Samples	(%)	No. of Samples	(%)	No. of Samples	(%)	No. of Samples	(%)
10-100	0	0	0	0	0	0	0	0	0	0
1.01-9.99	5	33.33	0	0	4	26.67	0	0	1	11.11
0.01-1.0	2	13.33	0	0	4	26,67	0	0	3	33.33
0	8	53.34	6	100	7	46.6	15	100	5	55.56
TOTAL	15	100	6	100	15	100	15	100	9	100

4.1.2 Cultural characterization and colony morphology of *TC* and *FC*

Cultural characterization and colony morphology of (*TC*) were detected on M-Endo media which appeared with bright green metallic sheen color as in Figure 5. M- Endo media have Sodium Lauryl Sulfate and Sodium Deoxycholate which are selective agent used to inhibit of Gram-positive bacteria. In addition to that, the Basic Fuchsin supplement is a pH indicator, and responsible for green metallic sheen appearance due to lactose fermentation to acetyl aldehyde.

(*FC*) appeared with blue color colonies on M-Fc culture media as in Figure 6. M-FC media have Rosolic Acid supplement that inhibit non *FC* bacteria; Bile Salt that inhibit non-enteric pathogens, and Aniline Blue which is responsible of blue color colonies due to lactose fermentation to acid.
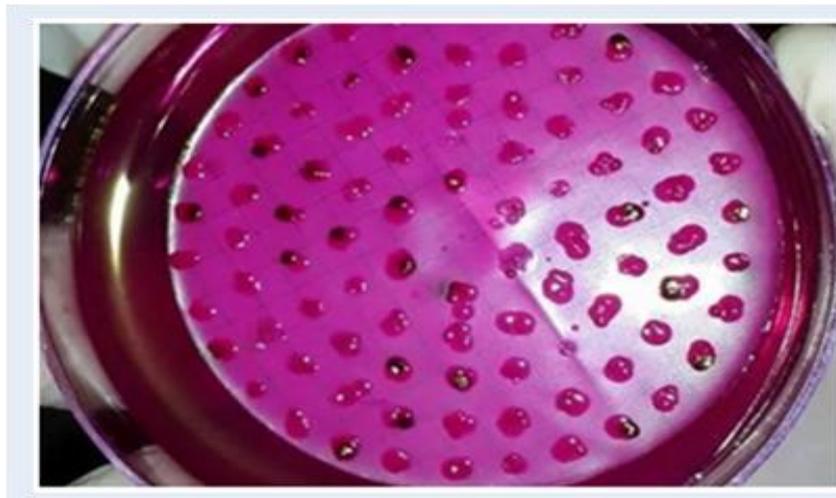

Figure 5. *TC* with bright green metallic sheen color colonies in M-Endo culture medium

Figure 6. *FC* with blue color colonies in M-Fc culture medium
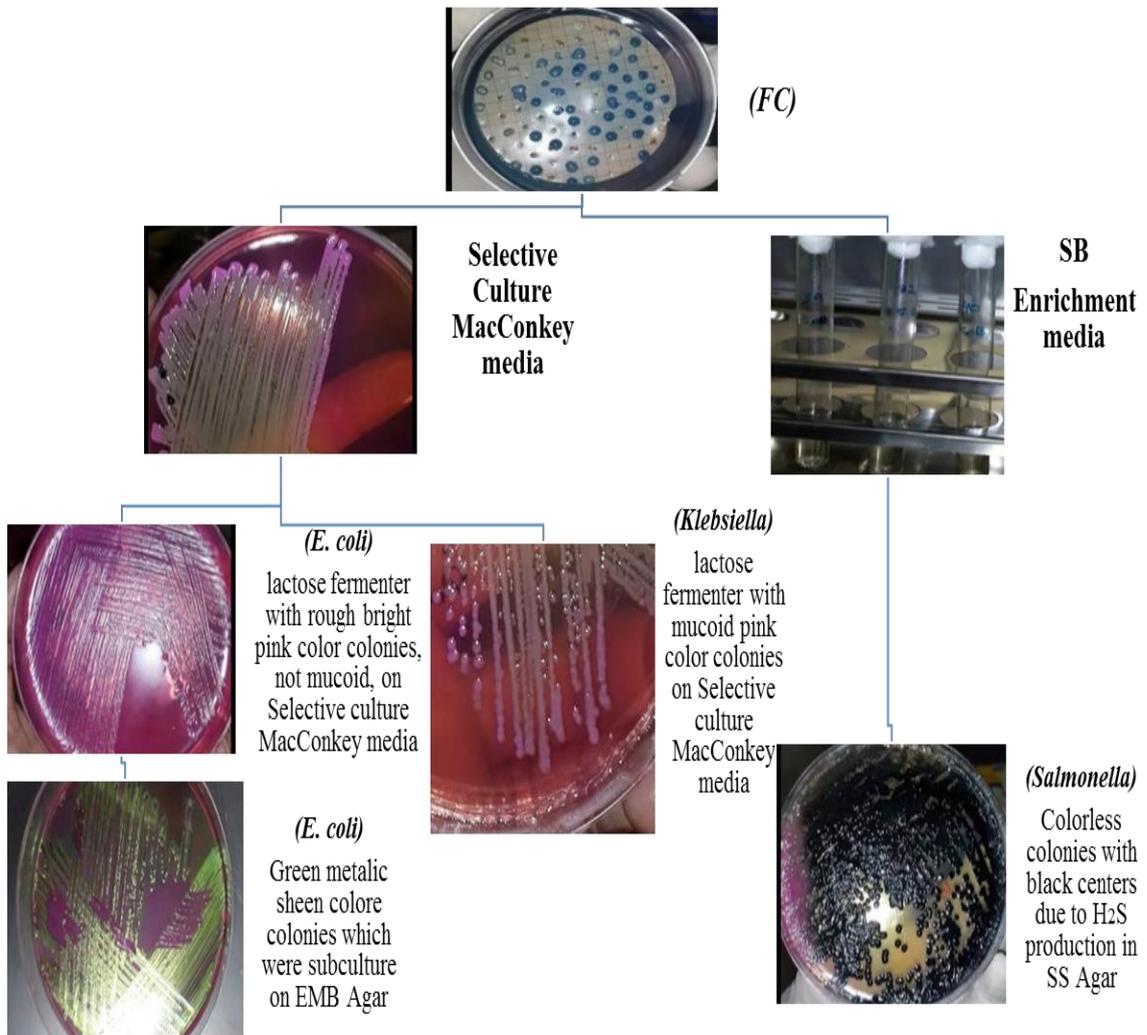
Figure 7. Colony morphology on different selective and differential media; M-Fc media, MacConkey agar, SS Agar and EMB

4.2 Biochemical tests results

A number of biochemical tests [(LF) Test, (IND) Test, (MR) Test, (VP) Test, (TSI) Test, Motility Test, (CUT) and (H2S) production] were performed for the identification of bacterial isolates from *E. coli, Klebsiella and Salmonella* colonies (*FC*).

E. coli and *Klebsiella spp*. had ability to ferment the lactose sugar and causes drop in the pH of the media was detected by neutral red indicator, which appeared as bright pink to red colonies on the agar. *Salmonella spp*. was grow on MacConkey agar but didn't ferment lactose, which appeared colorless on the medium with black centers.

In IND Test, *E. coli* was converted tryptophane to indole by tryptophanase enzyme causes red color in indole tube after addition Kovacs reagent, but *Klebsiella* and *Salmonella* spp. didn't changed the medium color as in Figure 8, 9 and 10.

In MR test, *E. coli* and *Salmonella* spp. were fermented the sugars by the mixed acid pathway, causes decrease in pH of MR-VP media after addition the methyl red indicator and had red color in medium. In contrast, *Klebsiella spp*. was fermented the sugars by the butylenes glycol pathway, causes increase in pH and yellow color in medium as in Figure 8, 9 and 10.
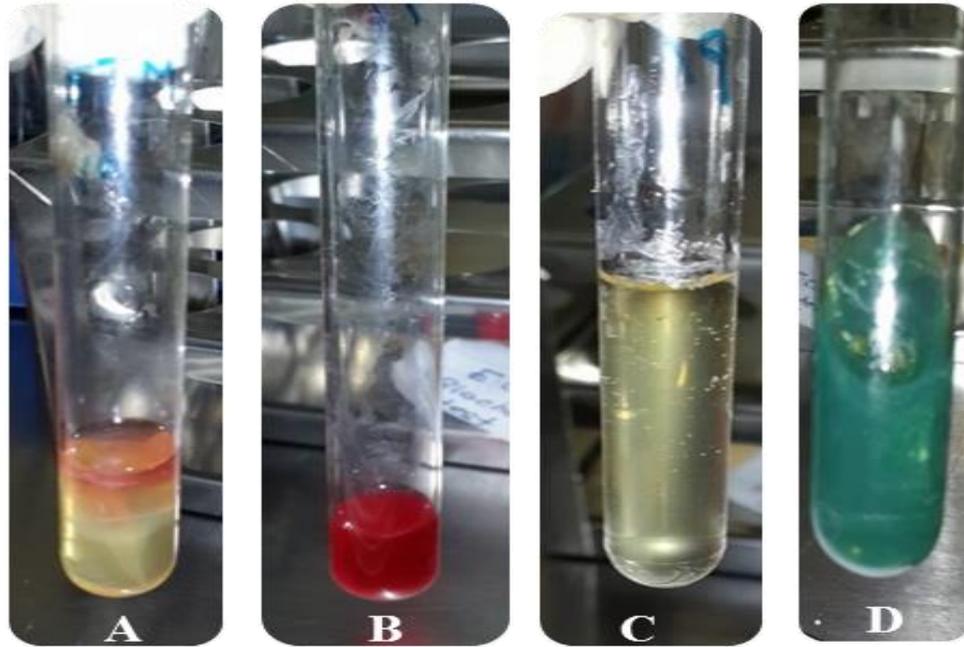
Figure 8. Biochemical test results of *E. coli*. **A** represent IND positive, negative motility result and negative H2S production ; **B** represent MR positive result; **C** represent VP negative result; **D** represent CUT negative result.

In VP test, *Klebsiella spp.* was converted the acetyl methyl carbinol to acetoin through the action of Barritt's reagent on MR-VP medium and changed the color to red, but *E. coli* and *Salmonella spp.* didn't changed the medium color as in Figure 8, 9 and 10.

In CUT test, *Klebsiella* and *Salmonella* spp. were utilized citrate as energy sources causes changed in citrate tube to blue, but *E. coli* didn't changed the medium color as in Figure 8, 9 and 10.
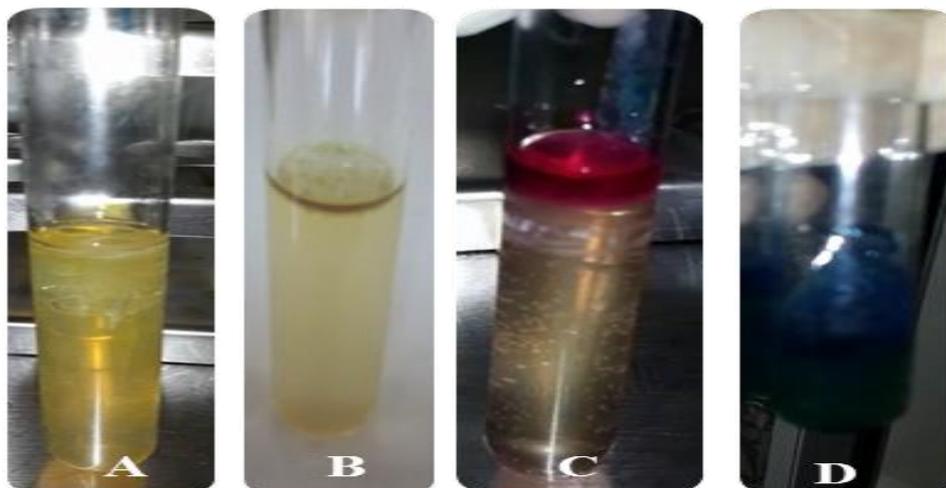
Figure 9. Biochemical test results of *Klebsiella*. **A** represent IND negative, negative motility and negative H2S production results; **B** represent MR negative result; **C** represent VP positive result; **D** represent CUT positive result.

Figure 10. Biochemical test results of *Salmonella*. **A** represent IND negative, positive motility and positive H2S production results ; **B** represent MR positive result; **C** represent VP negative result; **D** represent CUT positive result.

All the biochemical tests for *E. coli, Klebsiella* and *Salmonella* Spp. were compared with standard results of biochemical tests (Koneman, *et al*., 1996; Engelkirk, 2008; Parija, 2009; Mcdevitt, 2013; Acharya, 2013) as in Table 6.

Table 6. Biochemical test results of *E. coli, Klebsiella* and *Salmonella Spp*

Microorganism	LF	IND	MR	VP	CUT	Motility	H2S production
E.coli	+	+	+	_	_	_	_
Klebsiella	+	_	_	+	+	_	_
Salmonella	_	_	+	_	+	+	+

TSI results were confirmed the identification of microorganisms which isolated. An acid slant-acid butt (A/A; yellow/yellow) results were indicated of dextrose, lactose and/or sucrose fermentation by *E. coli* and *Klebsiella spp*. with gas and without H2S production.

An alkaline slant-acid butt (A/K; red/yellow) result was indicated of dextrose fermentation only by *Salmonella spp*. with gas and H2S production. TSI results are represented in Table 7.

Table 7. TSI results of *E.coli, Klebsiella* and *Salmonella*

Microorganism	Slant	Butt	Gas production	H2S production
E.coli	Acid	Acid	+	_
Klebsiella	Acid	Acid	+	_
Salmonella	Alkaline	Acid	+	+

4.3 Results of DNA extracted purity by using spectrophotometer

The quantity and purity of DNA extract was determined by spectrophotometer (Spectro UV-Vis DUAL BEAM) at an absorbance of 260 nm and A260nm/A280nm. The results of six samples were chosen randomly is presented in Table 8

Table 8. Results of absorbance at 260 nm and A260nm/A280nm by spectrophotometer

NO. of sample	A1 (260nm)	A2 (280nm)	A1/A2 (260nm/ 280nm)
1	1.209	0.682	1.772
2	1.156	0.822	1.406
3	1.300	0.775	1.677
4	1.398	1.001	1.396
5	1.100	0.881	1.248
6	1.338	1.001	1.336

The DNA concentration was calculated by considering 1 O.D. (260 nm) = 50 µg/ml DNA. The results showed the concentration of DNA ranged between (55µg-69.9µg) in the six samples which were chosen randomly. The ratio of 260nm/280nm of approximately between (1.2-1.8) indicates that the samples were free of protein contamination.

4.4 Detection of *E. coli* by PCR

DNA samples which tested positively for *E. coli* were able to amplify the expected PCR products of a size equal to 392 bp; using primers specific for malate acid dehydrogenase *(Mdh)* gene. Results about detection of *E. coli* using PCR in water samples are presented in Figure 11. It showed that 19/60 (31.67%) of drinking water samples were contaminated with *E. coli* as the main indicator for faecal contamination and possible associated human enteric pathogens. *E.coli* was presented in all *FC* positive water samples. In addition, the percentage of its existence was the highest among other types of bacteria.
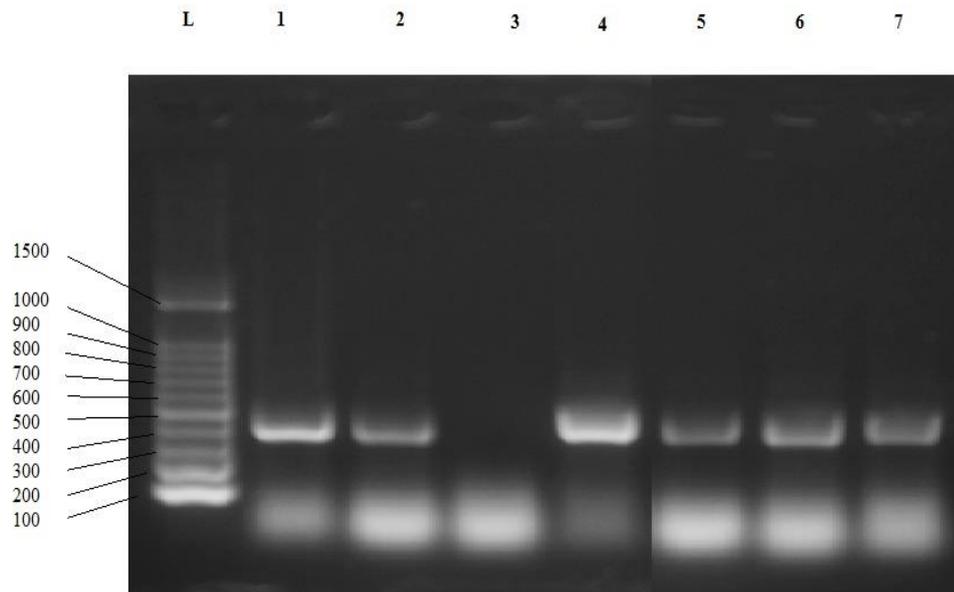
Figure 11. PCR results of *E.coli*: Lane **L** contained ladder (100bp DNA ladder RTU, GeneDireX), lane **1** represents *E. coli* positive control; lane **2** represents *E. coli* target gene *(mdh);* lane **3** represents *E. coli* negative control; lane **4-7** represents *E. coli* target gene *(mdh).*

4.5 Detection of *Klebsiella* by PCR

All of the DNA samples for *Klebsiella* species were tested positively using primers specific for Glyceraldehyde 3- phosphate dehydrogenase gene *(Gap A).* Positive samples generated the expected PCR products with size equals to 700 bp. The detection of *Klebsiella* using PCR in water samples are presented in Figure 12. Results of this research showed that 11/60 (18.33%) of drinking water samples were contaminated with *Klebsiella.*
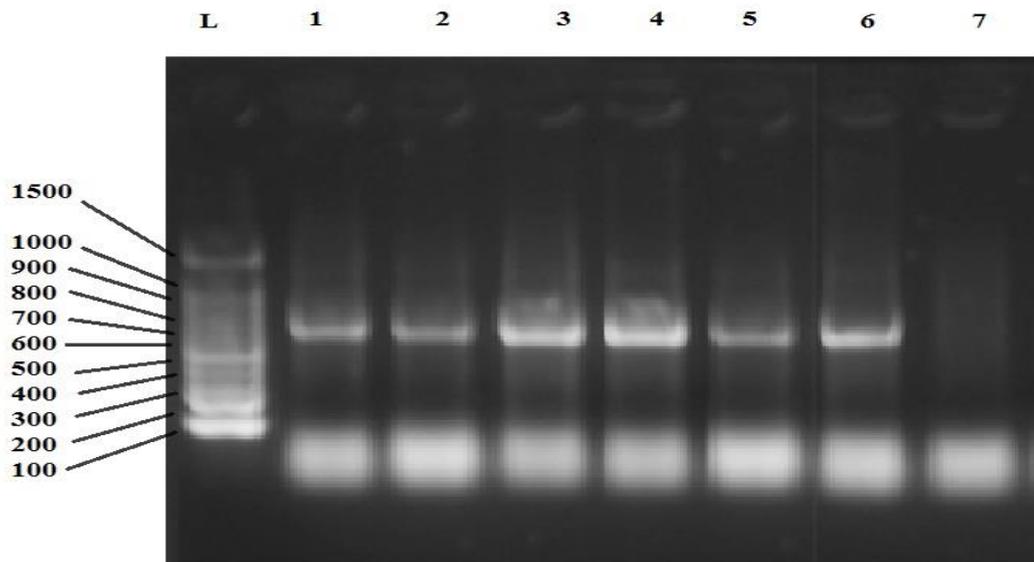
Figure 12. PCR results of *Klebsiella*: Lane **L** contained ladder (100bp DNA ladder RTU, GeneDireX), lane **1** represents *Klebsiella* positive control; lane **2-6** represents *Klebsiella* target gene (*GapA*); lane **7** represents negative control.

4.6 Detection of *Salmonella* by PCR

All of the DNA samples for *Salmonella* were tested using primers specific for Invasion Plasmid Antigene B gene (*IpaB*) of *Salmonella*. The size of 314 bp of (*IpaB*) gene of *Salmonella* was detected from positive water samples by PCR in as presented in Figure 13. Results of this research showed that only (10%) of water samples were contaminated with *Salmonella*.
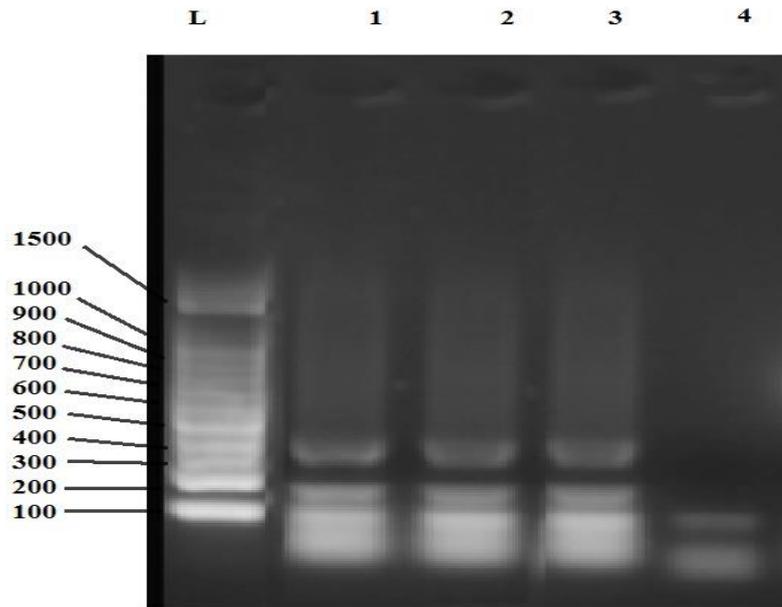
Figure 13. PCR results of *Salmonella*: Lane **L** contained ladder (100bp DNA ladder RTU, GeneDireX), lane **1** represents *Salmonella* positive control; lane **2 and 3** represents *Salmonella* target gene (*IpaB*); lane **4** represents negative control.

4.7 Comparison between waterborne bacterial pathogens Culture and PCR Assay

The prevalence of water borne pathogens (*E. coli; Klebsiella* and *Salmonella),* isolated from *FC* colonies of different water resources; were determined by using combination of culture, biochemical and molecular diagnostic techniques. The results showed that *E. coli* was detected in (26.67%) and in (31.67%) of the samples when analyzed by the culture and PCR, respectively. By the use of PCR we found that 3 of samples were *E. coli* positive by PCR but not by culture.

Klebsiella was detected in (16.67%) of the samples when analyzed by the culture and (18.33%) by PCR. By using of PCR we found one *Klebsiella* positive sample while it was not resolved by culturing method.

Salmonella spp was detected only in (10%) of the specimens for both culture and PCR as shown in Table 9.

In total the results found 4/60 (6.67%) of waterborne enteropathogen positive specimens by PCR where the culture revealed negative.

Table 9. Comparison between culture and PCR assay in detecting waterborne bacterial pathogens (*E. coli, Klebsiella and Salmonella*)

Bacteria	Assay compared		Difference	
	Culture positive	PCR positive	Counts	Percentage
E. coli	16/60 (26.67%)	19/60 (31.67%)	3/60	5%
Klebsiella	10/60 (16.67%)	11/60 (18.33%)	1/60	1.67%
Salmonella	6/60 (10%)	6/60 (10%)	0/60	0%

4.8 Prevalence of *E. coli, Klebsiella* and *Salmonella* distribution in different water resources

The results showed that the highest percentage of contamination of *E. coli, Klebsiella* and *Salmonella* which appeared in water samples were from rain fed cisterns. Almost 31.67% of water samples were found contaminated with *E. coli*. *E. coli* was presented in all *FC* contaminated water samples, and the percentage of its existence was the highest compared with other waterborne bacterial pathogens. *E. coli* was responsible of the highest percentage of contamination in the water samples from rain fed cisterns, tap water and tankers; with prevalence of 53.3%, 46.6% and 44.44%, respectively as in (Figure 14).
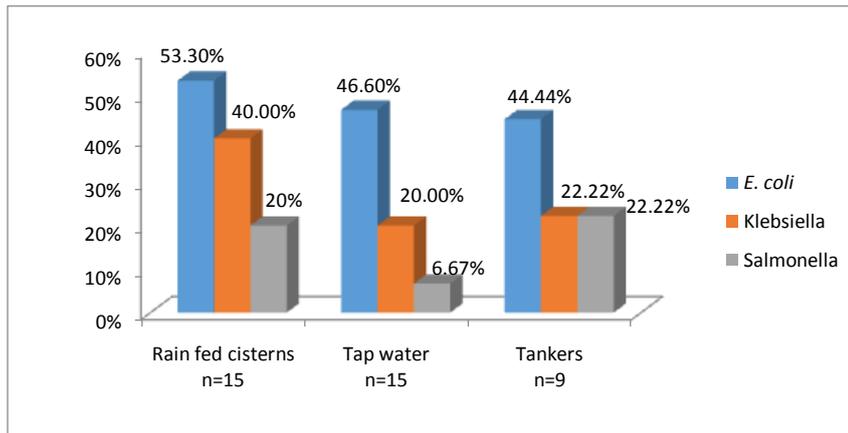
Figure 14. Prevalence of *E. coli, Klebsiella* and *Salmonella* distribution in water samples from rain fed cisterns, tap water and tankers

A total of 18.33% of water samples were contaminated with *Klebsiella. Klebsiella* were found in 40%, 20% and 22.22% isolated from rain fed cisterns, tap water and tankers samples, respectively.

Only 10% of water samples were found contaminated with *Salmonella. Salmonella* was detected in 20%, 6.67% and 22.22% of samples isolated from rain fed cisterns, tap water and tankers samples, respectively.

Chapter Five

Discussion,
Conclusion and Recommendation

5.1 Discussion

Waterborne bacterial pathogens are considered a major public health hazard, especially in developing countries. Control of water-borne pathogens spread is required accurate and cost-effective diagnostic tests.

Traditional enumeration and detection of waterborne bacterial pathogens have been based on the enrichment media, selective culture and standard biochemical test methods. These traditional methods have a number of disadvantages, such as, tedious, time-consuming and high chance of errors in sampling due to low concentrations of pathogenic bacteria in water samples. Recently, development of molecular technique can be used to complement culture method for the detection of the microorganisms in marine environment. PCR is an example of rapid and highly sensitive methods, and have the ability to produce extremely large numbers of copies of a specific nucleic acid segment for the specific detection of water borne pathogens (Toze, 1999; Fan *et al*. 2008). Several studies were published about the use of PCR method for detection of water borne pathogens (Faruque *et al*., 2002; Momba *et al*., 2006; Gomez-Duarte *et al*., 2009; Sharma *et al*., 2010; Siri *et al*., 2011).

TC and *FC* are the most common indicators of water quality, since they can be used as an indication of treatment effectiveness and to assess the cleanliness and integrity of distribution systems.

The obtained results in Tubas Governorate showed that 40% of water samples from different water resources were contaminated with *TC*, which were generally exceeding the Palestinian and WHO limits (3CFU/100ml)

(Fig. 2). However, the level of contamination in rain fed cisterns (15%) was higher than others (Fig. 3). Rain fed cisterns played as risky factor of contamination in drinking water. This is due to rain water; which stored in cisterns, had bad management practices in cleaning and storage conditions. In addition, the uses of pits in the discharge of wastewater with close distance to the rain fed cisterns in the homes (less than 16 meter). Tubas Governorate and neighboring communities related to governorate didn't have any infrastructure for centralized wastewater collection or treatment system. The uncontrolled discharge of untreated sewage has a negative impact on the underlying water resources.

Tap water (houses, restaurants and schools) ranking the second in % of contamination may due to factors related to domestic animals breeding and pet birds which feces home rooftops contaminating some of the open tanks. Much more guidance towards increase the education about hygiene and cleanness of water collection places at homes such as tanks and rain fed cisterns, were urgently needed.

Other results showed low percentage of *TC* contamination in ground water wells and network distribution systems were detected. This due to malfunction in the process of water treatment by disinfection or as intrusion through pipes and becomes trapped in the biofilm, where the *TC* remain in a viable active form. So the decision-makers must increase health surveillance on water resources and network distribution systems.

With comparison to local and international water quality standard, the *FC* contamination indicates the potential high *FC* contamination in Tubas

Governorate. The results showed that 31.67% of water samples from different water resources were contaminated with *FC* (Fig. 2), with 13.33% and 11.67% of water samples from rain fed cisterns, tap water (houses, restaurants and schools), respectively (Fig. 3). The results were generally exceeded the Palestinian and WHO limits (0 CFU/100ml). No ground water wells samples and network distribution systems samples had positively detected *FC* in Tubas Governorate, indicating of the lack of *FC* contamination in ground water wells and network distribution systems, while deterioration of water quality related to contamination of potable water collected from home tanks and rain fed wells. Due to the lack of water networks in some villages in Tubas Governorate such as, Bziq, Salhab, Kherbit Atof , Al-Aqaba and Al-Maleh, making the habitants mainly dependent on the tankers to supply their houses wells with drinking water. In addition to the absence of a supervisory role by village councils and municipalities in these areas in terms of control of chlorination process of tankers and wells in the household after filling, making them more susceptible to contamination appeared with *FC* tests. However, the level of contamination in rain fed cisterns was higher than others. The presence of *FC* in drinking water samples is indicative of fecal contamination and presence of enteric pathogens. Therefore it is necessary to establish cooperation between the Ministry of Health and PWA to increased health surveillance on tankers. It is although necessary to control the chlorination process in Tubas governorate by conducting periodical tests of potable water from home tanks and rain fed cisterns.

Wide spectrums of pathogens play an important role in waterborne disease. *E. coli* has been used to detect *FC* contamination in drinking water and as a risk indicator of waterborne disease. Results of this research showed that 31.67% of drinking water samples were contaminated with *E. coli* as the main indicator for faecal contamination and possible associated human enteric pathogens. *E. coli* was presented in all *FC* positive water samples. In addition, a percentage of its existence was the highest for other types of bacteria. *E. coli* was responsible of the highest percentage of contamination in the water samples from rain fed cisterns, tap water and tankers. *E. coli* was reached 53.3%, 46.6% and 44.44% of waterborne bacteria which isolated from rain fed cisterns, tap water and tankers samples, respectively (Fig. 14). *E. coli* strains have been implicated as an agent of diarrheal disease. Diarrheagenic strains of *E. coli* have been divided into five major categories on the basis of their virulence factors, clinical features and mechanisms by which causes disease association with certain serotypes: *EHEC EAEC, EIEC, EPEC* and *ETEC* (Szákal *et al*. 2003; Brown *et al*. 2010; Bugarel *et al*. 2011; Kinge *et al*. 2012).

Results of this research showed that 18.33% of drinking water samples were contaminated with *Klebsiella. Klebsiella* consists 40%, 20% and 22.22% of waterborne bacteria which isolated from rain fed cisterns, tap water and tankers samples, respectively (Fig. 14). *Klebsiella* spp. are belonging to the *Enterobacteriaceae* family, ubiquitous in its habitat associations, and this habitat divided in to two groups: clinical (human and hospital environment) and non-clinical (animals and water environment)

(Bagley, 1985). *Klebsiella* spp. are considered from pathogens causing nosocomial infections (Horan *et al*., 1988). *Klebsiella* spp. are excreted in the faeces of animal and human, and natural inhabitants in colonize form of different water resources especially in taps, 60–80% of *Klebsiella* spp. isolated from clinical, Environmental and faeces specimens are *K. pneumonia*, and are positive in the thermotolerant coliform test (WHO, 2004). *K. pneumoniae* can infect different parts of the body; such as, urinary tract, gastrointestinal tract and the respiratory tract system. The gastrointestinal tract is the most important reservoir for transmission of *K. pneumonia* infections which are preceded by gastrointestinal colonization (Struve and Krogfelt, 2004).

Results of this research showed that 10% of water samples were contaminated with *Salmonella. Salmonella* consists (3/15) 20%，(1/15) 6.67% and (2/9) 22.22% waterborne bacteria which isolated from rain fed cisterns, tap water and tankers samples, respectively*,* (Fig. 14)*. Salmonella* mostly detected from rain fed cisterns. *Salmonella* spp. are ubiquitous enteric bacteria belong to the Enterobacteriaceae family, and etiological agents of water and food-borne diseases (Crump *et al*., 2004; Lynch *et al.,* 2009; Kinge *et al*., 2012). *Salmonella* spp. are the most important group of waterborne bacterial pathogens affecting the public health of both human and animals. *Salmonella* spp. are the one causes of intestinal illness all over the world especially in developing countries, as well as the etiological agent of more severe systemic diseases such as typhoid and paratyphoid fevers (Levantesi *et al*.,2011). *Salmonella* spp. were found in different

water sources especially raid fed cistern, which had been contaminated with humans or animals feces, sewage overflows, storm water runoff, agricultural fertilizer and pesticides runoff (Gray, 2008).

The prevalence of waterborne pathogens from different water resources samples varies greatly due to several reasons. Studies referred that due to several factors such as differences of the region itself where the samples collected, in terms of the nature of geography, population, the awareness of the problem, ecology of the country in terms of agricultural, industrial and urban or rural and the water resources such as ground water, surface water, rain fed cistern, networks distribution systems and tankers. In addition to that, the number of samples, time of sample collection of the year, transportation, the percentage of disinfection, time of chloride addition, validity period and the effect of pipe material and age on the formation of disinfection by products in network distribution system. Other factor related to the sensitivity of detection methods (conventional method or molecular technique) and the virulence gene were detected.

Both conventional and molecular methods successfully identified bacteria of interest, however, the PCR was more accurate, sensitive and faster than conventional methods for detecting *E. coli, Klebsiella* and *Salmonella* from different water resources samples. PCR method had ability to detect the (392bp) *Mdh*, (700bp) *GapA* and (314bp) *IpaB* genes (Fig. 11, 12 and 13). These genes provided specific indicator for presence of *E. coli, Klebsiella* and *Salmonella,* respectively, in drinking water samples. The results showed that *E. coli* was detected in (26.67%) and in (31.67%) of the

samples when analyzed by the culture and PCR, respectively. By the use of PCR we found that 3 of samples were *E. coli* positive by PCR but not by culture. *Klebsiella* was detected in (16.67%) and in (18.33%) of the samples when analyzed by the culture and PCR, respectively. By use of PCR we found one sample was *Klebsiella* positive by PCR while it was not resolved by culture. *Salmonella spp* was detected in (10%) of the specimens both by culture, and PCR. In total the results found 6.67% of waterborne enteropathogen positive specimens by PCR that the culture revealed negative (Table 9).

PCR is considered as selective, sensitive, and specific assay that can detect a small number of cultural as well as non-cultural organisms. The PCR assay was extremely reliable, being able to detect 100% of culture confirmed bacterial infections in the study specimens. Furthermore, it also detected 4 (6.67%) culturally negative of a clinically important gastroenteritis bacteria, indicating the high level of efficiency of the assay system.

The PCR assay can be chosen as an alternative to the culture technique, and can further be used for identifying asymptomatic carriers who serve as potential reservoirs of *E. coli, Klebsiella* and *Salmonella* which silently transmit the disease within the community. Finally the applications of the PCR technique will improve the quality of detection of the pathogens. This will make the clinical diagnosis better and help the prevention of waterborne diseases.

5.2 Conclusion

1. The level of contamination of *TC* and *FC* in rain fed cisterns was the highest among the others.

2. The results showed that water samples from rain fed cisterns, tap water (houses, restaurants and schools), ground water wells, network distribution systems and tankers, were contaminated with (*TC*), some of them were exceeded the Palestinian and WHO limits (3CFU/100ml).

3. The results showed that water samples from rain fed cisterns, tap water (houses, restaurants and schools) and tankers, were contaminated with (*FC*), exceeded the Palestinian and WHO limits (0 CFU/100ml). Meanwhile, none of ground water wells samples and network distribution systems samples had any positive *FC*.

4. A percentage of *E. coli* existence was the highest for other types of bacteria. (31.67%) of drinking water samples were contaminated with *E. coli* as the main indicator for faecal contamination and possible associated human enteric pathogens.

5. Deterioration of water quality related to contamination of potable water in home tanks were coming after the rain fed cisterns, raising the demand for measurements and actions towards revising the conditions of these water reservoirs' by the corresponding authorities.

6. PCR method had ability to amplify the (392bp) *Mdh*, (700bp) *GapA* and (314bp) *IpaB* genes, provided specific detection for the presence of *E. coli, Klebsiella* and *Salmonella,* respectively, in drinking water samples.

7. PCR were more sensitive in detecting those water borne pathogens.

8. By the use of PCR negatively cultured samples for *E. coli and Klebsiella* were found positive. In total the results found 6.67% more of waterborne enteropathogen positive specimens by PCR that the culture revealed negative.

9. PCR assay can be chosen as an alternative to the culture technique, and it's a fast-growing area in microbiology and recent advances should be considered to simplify the procedure further.

5.3 Recommendations

Based on the study, the following are some of the recommendations to be considered for water improvement in Tubas Governorate:

- Increase the educational level for the habitants of Tubas governorates in the field of epidemiological studies on the water quality to bridges the gap between scientific investigations and implementation of preventative measurements to improve the public health.

- Increased health surveillance on water resources in Tubas governorate and ensure water quality to minimize the burden of water related diseases especially water that conserved tanks in houses, tankers and rains fed cisterns are urgently demanded, and should be continuously monitored by the Ministry of Health and PWA.

- Tankers should be tested periodically by the corresponding authority for their suitability and sanitation conditions of the water they are imported to houses.

- Monitoring the quantity of chlorine disinfection at Tubas distinct water resources according to identified limits of WHO or Palestinian

guidelines (0.8-1 mg/l). And intensification the surveillance of PWA and Ministry of Health on tankers especially when filling-up the home, school and restaurants drinking tank, a disinfection process for inlet and outlet should be applied.

- Other researches were advised to repeat this tests but in different seasons (wet season (April-June)) to assess the water quality in Tubas Governorate.

- Providing financial support to village councils and municipalities to install sanitation system for all towns and villages in the governorate which used cesspits' or pits for wastewater disposal by Ministry of Local Governorate and PWA.

- Implementation of a rapid and sensitive detection method of bacterial pathogens in drinking water in cases of water-borne disease outbreaks to protect human health and make assessment of water quality and water treatment process. The PCR assay can be chosen as an alternative to the culture technique, and guidance the decision makers in Service Provider and Ministry of Health to applicant the PCR technique to improve the quality of detection of the pathogens. This will make the clinical diagnosis better and help the prevention of waterborne diseases. PCR molecular technique is a fast-growing area in microbiology and recent advances should be considered to simplify the procedure further

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جامعة النجاح الوطنية

كلية الدراسات العليا

استخدام تقنية تفاعلات البلمرة المتسلسلة للكشف عن مسببات الامراض البكتيرية في مياه الشرب في محافظة طوباس- فلسطين

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الملخص

ترتبط عدم كفاية المياه الصالحة للشرب والصرف الصحي مع تدهور نوعية المياه والأمراض الوبائية التي قد تنقلها مياه الشرب، خاصة في البلدان النامية. لقد أجريت هذه الدراسة لتحديد الجودة الميكروبيولوجية لمياه الشرب وللبحث في انتشار القولونية الاجمالية، القولونية البرازية، الاشريكية القولونية، والكلبسيلا والسالمونيلا في (60) عينة من مياه الشرب التي جمعت من (6) ابار للمياه الجوفية، و (15) عينة من أنظمة التوزيع (الشبكات)، و(9) عينات من صهاريج نقل المياه، و(15) عينة من تنكات اسطح المنازل و (15) عينة من ابار جمع مياه الامطار في محافظة طوباس، وذلك باستخدام تقنية تفاعلات البلمرة المتسلسلة (PCR). أظهرت النتائج ان (40%) و (31.6%) من عينات المياه قد تجاوزت الحدود المسموح لها في فلسطين ومنظمة الصحة العالمية للقولونية الاجمالية والتي هي (3 مستعمرة لكل 100 مل) و القولونية البرازية والتي هي (0 مستعمرة لكل 100 مل) على التوالي. لقد أظهرت النتائج ان معدل انتشار الاشريكية القولونية، والكلبسيلا والسالمونيلا بلغت 31.67%، 18.33% و 10% على التوالي في مختلف مصادر جمع العينات. لقد وجدت الدراسة ان عينات المياه التي تم جمعها من ابار مياه الجمع للامطار هي الأكثر خطورة بالنسبة لغيرها من مصادر المياه، حيث شكلت ما نسبته (15%) و (13.33%) من التلوث بالقولونية الاجمالية والقولونية البرازية على التوالي، وتحديدا كانت نسبة التلوث (53.3%) بالاشريكية القولونية،(40%) الكلبسيلا و(20%)السالمونيلا. في حين كانت العينات المأخوذة من ينابيع الابار الجوفيه وأنظمة التوزيع (الشبكات) شبه خالية من

ج

القولونية البرازية لكنها ملوثة قليلا نسبيا بالقولونية الاجمالية (1.67%) ، مما يجعلها أقل خطورة من غيرها من مصادر المياه اعتمادا على الحدود المسموح بها في فلسطين ومنظمة الصحة العالمية.

لقد كشفت الدراسة عن سوء النوعية الميكروبيولوجية لمصادر مياه الشرب والتي قد تعود لعدة اسباب مثل التسريب من الحفر الامتصاصية لابار الجمع في المنازل، او تلوث ناقلات المياه (الصهاريج). وقد اوصت الدراسة بزيادة المراقبة الصحية على ابار الجمع وناقلات المياه في محافظة طوباس، وتأمين نظام صرف صحي و التحكم بكمية الكلور المستخدمة في التعقيم (0.8 - 1 ميلي غرام لكل لتر)، بالاضافة الى زيادة التوعية حول الأمراض المرتبطة بالمياه بالتعاون مع وزارة الصحة وسلطة المياه الفلسطينية. كما اوصت الدراسة بتطبيق تقنية تفاعلات البلمرة المتسلسلة للفحوص الروتينية في مختبرات المياه في فلسطين نظرا لدقتها في تحديد السلات المرضة بدقة وكفائة عالية.

الكلمات الدالة: الأمراض المنقولة بالمياه، القولونية الاجمالية، القولونية البرازية و تفاعلات البلمرة المتسلسلة.