Sudan University of Science and Technology

College of Graduate Studies

Occurrence of *Escherichia coli* and Fecal Coliforms in Drinking Water from Selected Localities in Khartoum State , Sudan

وجود الإشريكية القولونية و البكتريا القولونية في مياه الشرب من محليات مختارة في ولاية الخرطوم السودان

A dissertation submitted in partial fulfillment for the requirements of

M.Sc.degree in medical laboratory sciences (Microbiology)

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2020

قال تعالى
بسم الله الرحمن الرحيم

(أَوَلَمْ يَرَ الَّذِينَ كَفَرُوا أَنَّ السَّمَاوَاتِ وَالْأَرْضَ كَانَتَا رَتْقًا فَفَتَقْنَاهُمَا وَجَعَلْنَا مِنَ الْمَاءِ كُلَّ شَيْءٍ حَيٍّ أَفَلَا يُؤْمِنُونَ)

صدق الله العظيم
سورة الأنبياء ، الآية 30

Dedication

I dedicate this work to my father, bless upon him, my mother who has been my constant source of support and love, my sisters, brothers, and friends who helped me in my life and gave me the force to continue.

Acknowledgments

All Thanks and gratefulness to my lord, ALLAH for helping me to complete this work. I would like to express my deep gratitude with special respect to those great people who have greatly supported this study , I am also very grateful to my great supervisor Dr. **Samar Mohammed Saeed** for her critical and close monitoring and supervision, constructive comments, checking and correcting this thesis

May thanks are also extended to my family for their great help and support during my work, my thanks extended to my friends, for their strong assistance while conducting my study.

Abstract

Little information is available on water borne diseases in Sudan which have poor water quality that is recognized as a public health threat, this probably is due to absence of an infrastructure for detection and recording such infection and its source.

This was cross sectional study aimed to determine the occurrence of *E.coli* and feacal coliforms bacteria in drinking water from different sources in Khartoum state , Sudan in the period from April 2019 to October 2020.

A total of 103 water samples were collected from three different localities in Khartoum State (Khartoum, Khartoum North , Omdurman(karary locality) to find out pathogenic bacteria, 42%(43) of samples were collected from Omdurman , 29% (30) from Khartoum North and 29% (30)from Khartoum.

Water samples were collected from different sources as follow, coolers 50 (48.5%) ,taps 40(38.8%) and tanks 13(12.6%).

Eighty nine (85.6%) water samples was showed growth with production of gas and 14(14.4%) did not. After identification *E.coli* 47(52.8%) followed by coliform distributed as follow : *Klebseilla pneumonaie* 27(30.4%), *Proteus vulgaris* 9 (10.1%) and *Serratia marcescens* 6 (6.7%).

The highest occurrence of isolates was in taps water and Omdurman city(karary locality)

This study conclude that improper disinfection of water supplies in Khartoum state hence, attach to WHO quality standard , measure for better public health so as to control disease outbreak by coliforms.

المستخلص

تتوافر معلومات ضئيلة عن الأمراض التي تنقلها المياه في السودان بسبب رداءة نوعية المياه التي يعتبر تهديدا للصحة العامة. ولعل هذا يرجع إلى غياب البنية التحتية من أجل اكتشاف وتسجيل هذه العدوى ومصدرها.

هذه الدراسة هى دراسة مقطعية تهدف الى تعيين حدوث البكتريا القولونية في مياه الشرب من مصادر مختلفة في ولاية الخرطوم – السودان – التى اجريت فى ابريل 2019م الى اكتوبر 2020م . في هذه الدراسة مجموعه 103عينة جمعت عينات من 3 محليات مختلفة في الخرطوم شعبة (الخرطوم بحري، أم درمان). في الخرطوم 30 عينة (29%)، 30 عينة (29%) من بحري و 43عينة (42%) من أم درمان على التوالي.

عينات الماء جمعت من مصادر مختلفة مبرد ماء (5, 48 %) مياه المواسير (8 , 38 %) و خزانات المياه (6 , 12 %) .(6 ,85 %)من عينات المياه اظهرت نمو البكتريا و(4 , 14 %) من عينات المياه اظهرت عدم نمو البكتريا . أربعة الأنواع بكتيرية وجدت : إشريكية قولونية ، تليها القولونيات: كليبسيللا العقدية المتقلبة الاعتيادية، السراتية. إشريكية قولونية أساسا عملة مسكوكة المعزولة التي تم تحديدها في 47 العينات (52.8%)، تليها كليبسيللا العقدية 27 عينة (30.4%)، المتقلبة الاعتيادية 9 العينات (10.1%)، السراتية 6عينات (6.7%).خلصت هده الدراسه إلى أن عملية تطهير المياه أن تنفذ إلى الحد من الأمراض التي تنقلها المياه، والمياهالتي تمدالى الاقسام المختلفة يجب أن تتبع معايير منظمة الصحة العالمية من أجل تحسين الصحة العامة ومكافحة الفاشية من القولونيات.

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List of abbreviation

MPN	Most probable number
SPC	Standard plate count
MF	Membrane filter
P-A Test	Presence absence test
KIA	Kligler iron agar
MAC Agar	Maconkey agar

Chapter I
Introduction

Chapter I
Introduction

1.1 Introduction

Earth consist of approximately 70% surface area covered with water and remaining is land which have only 2% water which is drinkable (Dong *et al.,* 2015).

Approximately three out of five persons in developing countries do not have access to safe drinking water, and only about one fourth hasn't any kind of sanitary facilities. Significant progress was made in water supply and sanitation since the International Drinking Water and Sanitation Decade (1981-1990), however the proportion of people with access to adequate water and sanitation has not increased due to population growth, insufficient continued investment, inefficient systems, and lack of training and spares to maintain systems in working order (Cheesbrough, 2011).

Good health depends on a clean, drinkable water supply. So water must be free of pathogens, dissolved toxins and disagreeable turbidity, color and taste (Adam, 2017). Through ordinary exposure to air, soil and effluent surface water usually acquire harmless, saprobic microorganisms, but also can pick up pathogenic contaminants ―waterborne pathogens like protozoa (Gardia and Cryptosporidium), bacteria (*Campylobacter*, *Salmonella*, *Shigella* and *Vibrio*) and Hepatitis A and Norwalk viruses (Adam, 2017). The determination that the water is consequently unsafe to drink is by focusing on detecting fecal contamination, high fecal levels can mean that water contains pathogens and other water sources can be analyzed for the presence of various indicator bacteria (Adam, 2017).

A major type of bacteria in polluted water is coliform bacteria, a group of Gram-negative non spore forming bacilli which inhabit human and animal intestines, they usually ferment lactose to acid and gas, *Escherichia coli*, *Klebsiella*spp. and *Enterobacter*spp are the most important species (WHO, 2014).

The greatest microbial risks are associated with ingestion of water that is contaminated with human or animal feces. Wastewater discharges in fresh waters and costal seawaters are the major source of fecal microorganisms, including pathogens(Adam ,2017). Acute microbial diarrheal diseases are a major public health problem in developing countries. People affected by diarrheal diseases are those with the lowest financial resources and poorest hygienic facilities. Children under five, primarily in Asian and African countries, are the most affected by microbial diseases transmitted through water (WHO,2014). Microbial waterborne diseases also

affect developed countries. In the USA, it has been estimated that each year 560,000 people suffer from severe waterborne diseases, and 7.1 million suffer from a mild to moderate infections, resulting in estimated 12,000 deaths a year (WHO,2014).

1.2 Rationale:

Most drinking water comes from rivers and well and used in its natural form, in most of towns, it must be clean and free of contaminants before distribution to consumers.(Adam ,2017). An understanding of the microbial ecology of distribution that will ensure safe and high quality drinking water. Water borne diseases are caused by pathogenic microorganisms that are transmitted in drinking contaminated water. Water borne diseases can be spread via ground water, which is contaminated with trench latrines causing of many types of diseases including cholera and other serious illnesses such as typhoid and dysentery (Moreira and Bondelind, 2016). Diarrheal and gastrointestinal tract infections was the fifth leading causes of hospitals admission in Khartoum state in 2015 (Abdeldafie, 2018). In recent year, Khartoum state witnessed a large movement of people from peripheral state to centre which make it a big community characterized by crowding poor sanitation and inadequate water supply. Hence the present study designed to carry out a set of microbiological analysis for drinking water of Khartoum state as well as the identification of pathogenic microorganisms in these water sources.

1.3 Objectives

1.3.1 General objectives

To determine the occurrence of *Escherichia coli* and fecal coliforms bacteria in drinking water from different source in Khartoum state Sudan.

1.3.2 Specific objectives

1. To isolate and identify coliform bacteria from different sources of water by using presence absence technique.

2. To determine the frequency of coliform bacteria in selected water sources and areas.

Chapter II
Literature Review

Chapter II

Literature review

2.1. Definition of Water pollution:

Water is typically referred to as polluted when it is impaired by anthropogenic contaminants. Water pollution result from contamination of water bodies, usually as a result of human activities. Water bodies include for example lakes, rivers, oceans, aquifers and groundwater. Water pollution results when contaminants are introduced into the natural environment. For example, releasing inadequately treated wastewater into natural water bodies can lead to degradation of aquatic ecosystems. In turn, this can lead to public health problems for people living downstream. They may use the same polluted river water for drinking or bathing or irrigation. Water pollution is the leading worldwide cause of death and disease due to water-borne diseases (Savedge, 2006 ; West, 2006).

2.2 Types of Water Pollution

Water pollution can be classified as surface water pollution (sub classified as nutrient water pollution or marine water pollution), or ground water pollution (Moss, 2008).

2.2.1. Surface water pollution

Surface water pollution includes pollution of rivers, lakes and oceans. A subset of surface water pollution are nutrient water pollution or marine water pollution (Moss, 2008).

2.2.1.1 Nutrient water pollution

Nutrient water pollution, a form of water pollution, refers to contamination by excessive inputs of nutrients. It is a primary cause of eutrophication of surface waters, in which excess nutrients, usually nitrogen or phosphorus, stimulate algal growth. Sources of nutrient pollution include surface runoff from farm fields and pastures, discharges from septic tanks and feedlots, and emissions from combustion (Wuepper *et al.,* 2020).

2.2.1.2 Marine water pollution

Marine water pollution occurs when harmful effects result from the entry into the ocean of chemicals, particles, industrial, agricultural and residential waste, noise, or the spread of invasive organisms. Eighty percent of marine pollution comes from land. Air pollution is also a contributing factor by carrying off iron, carbonic acid, nitrogen, silicon, sulfur, pesticides or dust particles into the ocean. Land and air pollution have proven to be harmful to marine life and its habitats (Duce *et al.,* 2009).

2.2.2. Ground water pollution

Groundwater pollution (also called groundwater contamination) occurs when pollutants are released to the ground and make their way down into groundwater. This type of water pollution can also occur naturally due to the presence of a minor and unwanted constituent, contaminant or impurity in the groundwater, in which case it is more likely referred to as contamination rather than pollution. Pollution can occur from on-site sanitation systems, landfills, effluent from wastewater treatment plants, leaking sewers, petrol filling stations or from over application of fertilizers in agriculture. Pollution (or contamination) can also occur from naturally occurring contaminants, such as arsenic or fluoride. Using polluted groundwater causes hazards to public health through poisoning or the spread of diseas (Denver, 1998).

2.3 Sources of Water Pollution

Sources of surface water pollution are generally grouped into two categories based on their origin:

2.3.1. Point sources

Point source water pollution comes from discrete conveyances and alters the chemical, biological, and physical characteristics of water. It refers to contaminants that enter a waterway from a single, identifiable source, such as a pipe or ditch. Examples of sources in this category include discharges from a sewage treatment plant, a factory, or a city storm drain (Harrison, 2001).

2.3.2. Non-Point sources

Nonpoint source pollution is pollution resulting from many diffuse sources, in direct contrast to point source pollution which results from a single source. Nonpoint source pollution generally results from land runoff, precipitation, atmospheric deposition, drainage, seepage, or hydrological modification (rainfall and snowmelt) where tracing pollution back to a single source is difficult. It may derive from many different sources with no specific solutions or changes to rectify the problem, making it difficult to regulate. Nonpoint source water pollution is difficult to control because it comes from the everyday activities of many different people, such as lawn fertilization, applying pesticides, road construction or

building construction (Young *et al.*, 1996).

2.4 Bacterial Pathogens in Drinking Water

Taking this in account, data are needed when the intent is to develop a comprehensive list of what are considered the most important agents (or potential agents) of waterborne disease. Heterotrophic Plate Counts and Drinking-water Safety (Tenaillon *et al.,* 2010). A large variety of bacterial are capable of initiating waterborne infections:

2.4.1. *Escherichia coli*

Facultative anaerobic gram-negative bacilli that belong to the family *Enterobacteriaceae*. Non spore forming, grow over wide range of temperature (15- 45°C), almost motile and some strains are express fimbriae, some strains produce polysaccharide capsule. Escherichia coli (E. coli) bacteria normally live in the intestines of healthy people and animals. Most types of E. coli are harmless or cause relatively brief diarrhea. But a few strains, such as E. coli O157:H7, can cause severe stomach cramps, bloody diarrhea and vomiting other strain causing diarrhea include Enteroaggregative *E.coli* (EAEC), Enterotoxigenic *E.coli* (ETEC), and Enteroinvasive *E.coli* (EIEC). The diarrheagenic *E.coli* pathotypes differ regarding their prefer initial host colonization sites, virulence mechanisms, and the inducng clinical symptoms and consequences, and are classified as Enteropathogenic *E.coli* (EPEC), Enterohemorrhagic *E.coli* (Shiga toxin producing), (EHEC/STEC), Enteroaggregative *E.coli* (EAEC), Enterotoxigenic *E.coli* (ETEC), and Enteroinvasive *E.coli* (EIEC) (Tenaillon *et al.,* 2010).

2.4.2. *Klebsiella pneumoniae*

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It appears as a mucoid lactose fermenter on MacConkey agar. Although found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human and animal lungs if aspirated, specifically to the alveoli resulting in bloody, brownish or yellow colored jelly like sputum. In the clinical setting, it is the most significant member of the genus Klebsiella of the Enterobacteriaceae. K. oxytoca and K. rhinoscleromatis have also been demonstrated in human clinical specimens. In recent years, Klebsiella species have become important pathogens in nosocomial infections (Ryan and Ray, 2004).

2.4.3. *Proteus species*

Proteus is a genus of Gram-negative Proteobacteria. *Proteus* bacilli are widely distributed in nature as saprophytes, being found in decomposing animal matter, sewage, manure soil, the

mammalian intestine, and human and animal feces. They are opportunistic pathogens, commonly responsible for urinary and septic infections, often nosocomial. Three species—P. vulgaris, P. mirabilis, and P. penneri—are opportunistic human pathogens. Proteus includes pathogens responsible for many human urinary tract infections.P. mirabilis causes wound and urinary tract infections. Most strains of P. mirabilis are sensitive to ampicillin and cephalosporins. P. vulgaris is not sensitive to these antibiotics. However, this organism is isolated less often in the laboratory and usually only targets immunosuppressed individuals. P. vulgaris occurs naturally in the intestines of humans and a wide variety of animals, and in manure, soil, and polluted waters. P. mirabilis, once attached to the urinary tract, infects the kidney more commonly than E. coli. P. mirabilis is often found as a free-living organism in soil and water. About 10–15% of kidney stones are struvite stones, caused by alkalinization of the urine by the action of the urease enzyme (which splits urea into ammonia and carbon dioxide) of Proteus (and other) bacterial species (Guentzel, 1996).

2.4.4. Pseudomonas SPP

Pseudomonas is a genus of Gram-negative bacteria belonging to the family Pseudomonadaceae and containing 191 validly described species. The members of the genus demonstrate a great deal of metabolic diversity and consequently are able to colonize a wide range of niches. Their ease of culture in vitro and availability of an increasing number of Pseudomonas strain genome sequences has made the genus an excellent focus for scientific research; the best studied species include P. aeruginosa in its role as an opportunistic human pathogen, the plant pathogen P. syringae, the soil bacterium P. putida, and the plant growth-promoting P. fluorescens, P. lini, P. migulae, and P. graminis (Madigan and Martinko, 2005).

2.4.5. *H.pylori*

Helicobacter pylori, previously known as Campylobacter pylori, is a gram-negative, microaerophilic, spiral (helical) bacterium usually found in the stomach. Its helical shape (from which the genus name, helicobacter, derives) is thought to have evolved in order to penetrate the mucoid lining of the stomach and thereby establish infection Studies of prevalence or seroprevalence suggested that drinking-water might play some role in infection with H. pylori. More and more data show that H. pylori DNA can be detected by polymerase chain reaction from faecal samples of infected individuals or patients with peptic ulcer, which strongly suggests faecal–oral transmission. However, many characteristics make H. pylori a special bacterium in the world of human pathogens, and a long way remains for the epidemiology of transmission and the environmental occurrence of this pathogen to be better defined (Yamaoka, 2008).

2.4.6. Campylobacter spp

Campylobacter (meaning "curved bacteria") is a genus of Gram-negative bacteria. Campylobacter typically appear comma- or s-shaped, and are motile. Some Campylobacter species can infect humans, sometimes causing campylobacteriosis, a diarrhoeal disease in humans.The most known source for Campylobacter is poultry, but due to their diverse natural reservoir, Campylobacter spp. can also be transmitted via water. Other known sources of Campylobacter infections include food products, such as un pasteurized milk and contaminated fresh produce. Sometimes the source of infection can be direct contact with infected animals, which often carry Campylobacter asymptomatically. C. jejuni is recognized as one of the main causes of bacterial food/water borne disease in many developed countries (Vandamme *et al.*, 2006).

2.4.7. Aeromonas

Aeromonas is a genus of gram-negative, facultative anaerobic, rod-shaped bacteria that morphologically resemble members of the family Enterobacteriaceae. Most of the 14 described species have been associated with human diseases. The most important pathogens are A. hydrophila, A. caviae, and A. veronii biovar sobria. The organisms are ubiquitous in fresh and brackish water. Aeromonas Gastrointestinal disease in children is usually an acute, severe illness, whereas that in adults tends to be chronic diarrhea. Severe Aeromonas gastroenteritis resembles shigellosis, with blood and leukocytes in the stool. Acute diarrheal disease is self-limited, and only supportive care is indicated in affected patients (Martinez-Murcia *et al.,* 1992).

2.5. Water transmitted diseases

Waterborne diseases are conditions caused by pathogenic micro-organisms that are transmitted in water. These diseases can be spread while bathing, washing, drinking water, or by eating food exposed to contaminated water. While diarrhea and vomiting are the most commonly reported symptoms of waterborne illness, other symptoms can include skin, ear, respiratory, or eye problems. Microorganisms causing diseases that characteristically are waterborne prominently include protozoa and bacteria, many of which are intestinal parasites, or invade the tissues or circulatory system through walls of the digestive tract. Various other waterborne diseases are caused by viruses (Janovy *et al.,* 1996).

2.5.1. Cholera

Cholera is an infection of the small intestine by some strains of the bacterium Vibrio cholerae. Symptoms may range from none, to mild, to severe. Transmission is usually through the fecal-

oral route of contaminated food or water caused by poor sanitation. Most cholera cases in developed countries are a result of transmission by food, while in the developing world it is more often water . The classic symptom is large amounts of watery diarrhea that lasts a few days. Vomiting and muscle cramps may also occur. Diarrhea can be so severe that it leads within hours to severe dehydration and electrolyte imbalance. This may result in sunken eyes, cold skin, decreased skin elasticity, and wrinkling of the hands and feet. Dehydration can cause the skin to turn bluish. Symptoms start two hours to five days after exposure. Prevention methods against cholera include improved sanitation and access to clean water. Cholera vaccines that are given by mouth provide reasonable protection for about six months (Finkelstein, 1996; Harris *et al.*, 2012).

2.5.2. Typhoid

Typhoid fever, also known simply as typhoid, is a bacterial infection due to a specific type of Salmonella that causes symptoms. Symptoms may vary from mild to severe, and usually begin 6 to 30 days after exposure. Often there is a gradual onset of a high fever over several days. This is commonly accompanied by weakness, abdominal pain, constipation, headaches, and mild vomiting. Some people develop a skin rash with rose colored spots. In severe cases, people may experience confusion. Without treatment, symptoms may last weeks or months. Diarrhea is uncommon. Other people may carry the bacterium without being affected, but they are still able to spread the disease to others. Typhoid fever is a type of enteric fever, along with paratyphoid fever (Magill, 2013).

2.5.3. Gastroenteritis

Gastroenteritis, also known as infectious diarrhea and gastro, is inflammation of the gastrointestinal tract—the stomach and intestine. Symptoms may include diarrhea, vomiting and abdominal pain. Fever, lack of energy and dehydration may also occur. This typically lasts less than two weeks. It is not related to influenza, though it has erroneously been called the "stomach flu". E. coli usually remains harmlessly confined to the intestinal lumen; however, in the debilitated or immunosuppressed host, or when gastrointestinal barriers are violated, even normal “nonpathogenic” strains of E. coli can cause infection. Moreover, even the most robust members of our species may be susceptible to infection by one of several highly adapted E. coli clones which together have evolved the ability to cause a broad spectrum of human diseases. Infections due to pathogenic E. coli may be limited to the mucosal surfaces or can disseminate throughout the body. Three general clinical syndromes result from infection with inherently pathogenic E. coli strains: (i) urinary tract infection, (ii) sepsis/meningitis, and (iii)

enteric/diarrheal disease. The diarrheagenic E. coli strains, which include several emerging pathogens of worldwide public health importance, and will specifically focus on pathogens afflicting humans. These strains include enteropathogenic E. coli (EPEC), enterohemorrhagic E. coli (EHEC), and enteroaggregative E. coli (EAEC). enteroinvasive E. coli (EIEC) and Enterotoxigenic E.coli (ETEC) this categories of diarrheagenic E. coli are differentiated on the basis of pathogenic Feature (Shors, 2013).

2.5.4. Dysentery

Dysentery is a type of gastroenteritis that results in diarrhea with blood. Other symptoms may include fever, abdominal pain, and a feeling of incomplete defecation. Complications may include dehydration. The cause of dysentery is usually the bacteria Shigella, in which case it is known as shigellosis, or the amoeba Entamoeba histolytica. Other causes may include certain chemicals, other bacteria, other protozoa, or parasitic worms. It may spread between people. Risk factors include contamination of food and water with feces due to poor sanitation (Marie and Petri, 2013).

2.5.5. Peptic ulcer

Helicobacter pylori is one of the major causative factors of peptic ulcer disease. It secretes urease to create an alkaline environment, which is suitable for its survival. It expresses blood group antigen adhesin (BabA) and outer inflammatory protein adhesin (OipA), which enables it to attach to the gastric epithelium. The bacterium also expresses virulence factors such as CagA and PicB, which cause stomach mucosal inflammation. The VacA gene encodes for vacuolating cytotoxin, but its mechanism of causing peptic ulcers is unclear. Such stomach mucosal inflammation can be associated with hyperchlorhydria (increased stomach acid secretion) or hypochlorhydria (reduced stomach acid secretion). Inflammatory cytokines inhibit the parietal cell acid secretion. H. pylori also secretes certain products that inhibit hydrogen potassium ATPase; activate calcitonin gene-related peptide sensory neurons, which increases somatostatin secretion to inhibit acid production by parietal cells; and inhibit gastrin secretion. This reduction in acid production causes gastric ulcers. On the other hand, increased acid production at the pyloric antrum is associated with duodenal ulcers in 10% to 15% of H. pylori infection cases. In this case, somatostatin production is reduced and gastrin production is increased, leading to increased histamine secretion from the enterochromaffin cells, thus increasing acid production. An acidic environment at the antrum causes metaplasia of the duodenal cells, causing duodenal ulcers (Lanas and chan, 2017).

2.6. Indicator microorganism

Certain bacteria can be used as indicator organisms in particular situations. The presence of bacteria commonly found in human feces, termed coliform bacteria (e.g. E. coli), in surface water is a common indicator of faecal contamination. For this reason, sanitation programs often test water for the presence of these organisms to ensure that drinking water systems are not contaminated with feces. This testing can be done using several methods which generally involve taking samples of water, or passing large amounts of water through a filter to sample bacteria, then testing to see if bacteria from that water grow on selective media such as MacConkey agar. Alternatively, the sample can be tested to see if it utilizes various nutrients in ways characteristic of coliform bacteria (Ashbolt *et al.,* 2016).

2.6.1. Coliform

Coliform bacteria are defined as Rod shaped Gram-negative non-spore forming and motile or non-motile bacteria which can ferment lactose with the production of acid and gas when incubated at 35–37°C. Due to the limited ability of certain coliform bacteria to ferment lactose, the definition has changed to bacteria containing the enzyme β-galactosidase. They are a commonly used indicator of sanitary quality of foods and water. Coliforms can be found in the aquatic environment, in soil and on vegetation; they are universally present in large numbers in the feces of warm-blooded animals. While coliforms themselves are not normally causes of serious illness, they are easy to culture, and their presence is used to indicate that other pathogenic organisms of fecal origin may be present. Such pathogens include disease-causing bacteria, viruses, or protozoa and many multicellular parasites. Coliform procedures are performed in aerobic or anaerobic conditions (Reddy *et al.,* 2010).

2.6.2. *E.coli*

Detection of E. coli in treated drinking water directly indicates contamination from human or animal waste, and indirectly, the potential presence of enteric viruses, bacteria and parasites. And to be sure, some strains of E. coli are pathogenic and can cause diarrhea, urinary tract infections, intestinal hemorrhage and kidney failure, and can even result in death. Almost all enteric pathogens are released in large numbers in the excrement of infected humans and animals, and many pathogens and diseases are “shared” between species (zoonotic). So wherever there is water, animals and people, one can find E. coli, but whether the water contains dangerous germs and at what levels is not always known (Ashbolt *et al.,* 2016).

2.6.3. *Enterococci*

Enterococci are also used as indicators of fecal contamination of recreational waters throughout the world. In the US, the fecal pollution standard for recreational bathing waters was originally set using concentrations of total coliforms, based on the results of a US Public Health Service study of swimmer health on Lake Michigan in Chicago, IL in 1948. In recognition of the fact that related Gram-negative bacteria are naturally present in water, that standard was subsequently revised to a "fecal" coliform standard, which assumes that only a fraction of total coliforms were of fecal origin. In the late 1970s and early 1980s, swimmer health studies were carried out to aid in the identification of new fecal indicator organisms that may be more reliable than fecal coliforms. Researchers determined that concentrations of enterococci concentrations measured in recreational marine waters polluted by treated wastewater were strongly correlated to the number of swimmers becoming sick with gastrointestinal illness (Stevenson, 1953).

2.6.4. *Clostridium*

This genus includes several significant human pathogens, including the causative agents of botulism and tetanus. The genus formerly included an important cause of diarrhea, Clostridioides difficile, which was separated after 16S rRNA analysis. They are obligate anaerobes capable of producing endospores. The normal, reproducing cells of Clostridium, called the vegetative form, are rod-shaped, or spindle. Clostridium endospores have a distinct bowling pin or bottle shape, distinguishing them from other bacterial endospores, which are usually ovoid in shape. Clostridium species inhabit soils and the intestinal tract of animals, including humans. Clostridium is a normal inhabitant of the healthy lower reproductive tract of females. It can be used as good water pollution indicator (Maczulak, 2011).

2.7. Bacteriological examinations of water

2.7.1. Standard plate count technique

The standard plate count method consists of diluting a sample with sterile saline or phosphate buffer diluent until the bacteria are dilute enough to count accurately. That is, the final plates in the series should have between 30 and 300 colonies. Fewer than 30 colonies are not acceptable for statistical reasons (too few may not be representative of the sample), and more than 300 colonies on a plate are likely to produce colonies too close to each other to be distinguished as distinct colony-forming units (CFUs). The assumption is that each viable bacterial cell is separate from all others and will develop into a single discrete colony (CFU). Thus, the number of colonies should give the number of bacteria that can grow under the incubation conditions employed. A wide series of dilutions (e.g., 10-4 to 10-10) is normally

plated because the exact number of bacteria is usually unknown. Greater accuracy is achieved by plating duplicates or triplicates of each dilution, although we will not be doing that in this exercise (Mishra *et al.*, 2018).

2.7.2. Multiple tube fermentation technique(Most probable number)

Although this test is simple to perform, it is time-consuming, requiring 48 hours for the presumptive results. There are a number of isolation media each with its bias and the bacteria enriched are not a strict taxonomic group. Hence, the total coliforms can best be described as a range of bacteria in the family Enterobacteriaceae varying with the changing composition of the media. Following presumptive isolation of coliforms, further testing is required for confirmation of the coliform type. During the late 1940s there was a divergence between the UK and US approaches to identifying the thermotolerant or socalled 'faecal' coliforms. Thus, over a period of some 50 years, water bacteriologists developed the concept of E. coli as the indicator of faecal pollution, but continued to attach significance to the total lactose fermenters, colon group or generally referred to as the 'total coliforms' group. Despite the obvious failings of the total coliform group to indicate health risk from bacterial pathogens, they provide valuable information on process efficiency which is clearly important in relation to health protection (Camper *et al.,* 1991).

2.7.3. Membrane filtration technique

Until the 1950s practical water bacteriology relied almost exclusively, for indicator purposes, on the enumeration of coliforms and E. coli based on the production of gas from lactose in liquid media and estimation of most probable numbers using the statistical approach initially suggested. In Russia and Germany, however, workers attempted to culture bacteria on membrane filters, and by 1943 Mueller in Germany was using membrane filters in conjunction with Endo-broth for the analysis of potable waters for coliform. By the 1950s membrane filtration was a practical alternative to the MPN approach, although the inability to demonstrate gas production with membranes was considered a major drawback. The arbitrary definitions adopted for E. coli and the related coliforms were all based upon cultural characteristics, including the ability to produce gas from lactose fermentation. Hence, the thermotolerant coliforms include strains of the genera Klebsiella and Escherichia, as well as certain Enterobacter and Citrobacter strains able to grow under the conditions defined for thermotolerant coliforms. This phenotypic approach has also resulted in E. coli or a related coliform being ignored simply because they failed to ferment lactose, failed to produce gas from lactose or were indole-negative at 44.5°C. The approach had

been repeatedly questioned, and was only resolved in the UK in the 1990s. It has long been recognised that artificial culture media lead to only a very small fraction (0.01–1%) of the viable bacteria present being detected. Since MacConkey's development of selective media for E. coli and coliforms at the beginning of the twentieth century, various workers have shown these selective agents inhibit environmentally or oxidatively stressed coliforms (Dufour., 1977; McCrady, 1915; Waite, 1997).

2.7.4. Defined substrate technology

Media without harsh selective agents but specific enzyme substrates allow significant improvements in recoveries and identification of target bacteria. In the case of coliforms and E. coli. What has evolved into the Colilert® technique has been shown to correlate very well with the traditional membrane filter and MPN methods when used to test both fresh and marine water. Furthermore, these enzyme-based methods appear to pick up traditionally nonculturable coliforms. These developments have led to further changes in definitions of total coliforms and E. coli. In the UK, for example, total coliforms are members of genera or species within the family Enterobacteriaceae, capable of growth at 37°C, which possess β-galactosidase. In an international calibration of methods, E. coli was enzymatically distinguished by the lack of urease and presence of β-glucuronidase. Furthermore, the International Standards Organisation has recently published miniaturised MPNbased methods for coliforms/E. coli and enterococci based on the defined substrate approach (Clark *et al*., 1991; Eckner, 1998).

2.7.5. Presence –absence test

The presence/absence (P/A) test is an inexpensive procedure for a rapid qualitative determination of bacterial indicators in drinking water (Ramteke *et al.* 1994). The P/A test kits have the advantage where resources and time factors are a major constraint. Also, if a significant number of water samples are expected to be free of fecal contamination, then it could be a waste of resources to conduct a quantitative analysis of each sample. Some test kits meant primarily for quantitative analysis are also available in the P/A format such as Colilert, Aquagenx, etc., but these are relatively costly. (Ramteke *et al.* 1994).

2.8. Quality of water

The most effective means of consistently ensuring the safety of a drinking-water supply is through the use of a comprehensive risk assessment and risk management approach that encompasses all steps in the water supply from catchment to consumer. In these Guidelines, such approaches are termed water safety plans (WSPs). The WSP approach has been developed to

organize and systematize a long history of management practices applied to drinking-water and to ensure the applicability of these practices to the management of drinking-water quality (Gómez *et al.*, 2016).

Safety and quality of water supply is an important priority to protect human health and wellbeing. Water of good drinking quality is of basic importance to human physiology as well as in dispensable to man‟s continued existenc. The supply of drinking water always requires storage, as consumption is varying. Storage involves a risk of contamination before use. Especially in non-piped systems and piped systems with intermittent supply, storage is a crucial factor regarding water quality and thus public health. This is because in such systems the risk of a water quality impairment is especially high. Since the major part of the world's population obtains drinking water via in-house or near-house storage, impairment during domestic storage is of high importance and therefore requires a closer look. (Slavik *et al.,* 2020).

2.9Previous study:

Study done by (Zeinab *et al.*, 2010) they enumerated the bacteriological contamination of the main sources of drinking water in Al Gedarif city. A total of 134 water samples (raw waters, treated waters, main reservoirs, main pipelines, and sabeelzeer waters) were tested for their total coliforms and *E. coli* counts, using the most probable number technique (MPN). The results indicated that the total coliform and *E. coli* counts were lower in the ground water sources (Al Azaza and Abu Al Naja boreholes) than that in the surface sources (Atbara River, Al Saraf and Dalassa dams). Moreover, both counts in most of the zeer water samples were higher than those of the other sources. It was also noticed that the zeers located in public areas (market) were more contaminated than the other sabeelzeers.

Another study done by (Ibrabim and Isam 2010)A total of 50 drinking water samples (one from each farm) were collected. All water samples were examined for bacterial viable count and cultured to isolate common bacteria present. Sixty three bacterial isolates were recovered from all samples. The isolated bacterial genera were *Bacillus, Corynebacterium, Enterobacteria,Staphylococcus, Streptococcus, Actinobacillus, Campylobacter, Moraxella, Aeromonas, Cardiobacteria, Pseudomonas and Branhamella*. All water samples examined showed moderately high viable count. The mean viable count from the 10 farms in each area was 12X107, 37X106, 22X106, 22X106.

20X106 (cell/ ml) for Shambat, HelatKogaly, Alsamrab, Alhalfaia and Helat Koko areas respectively. Most of the examined farms were with bad hygienespecially surround drinking water troughs. It could be concluded that drinking water quality in selected dairy farms was poor.

Previous study done by(Aliaa and Ali, 2005)they concluded that out of Fifty water samples were collected in Khartoum state from different sources, and investigated with the Multiple Tube Fermentation Technique for total and thermotolerant coliforms and with Membrane Filtration Technique for total coliforms only.

Results of the study showed that Multiple Tube Fermentation Technique was more sensitive than Membrane Filtration Technique. Thermotolerant coliforms were detected in (60%) of samples from tap water of well source while in only (30%) of samples from tap water of river source.

Thermotolerant coliforms were detected in 10% water samples from periodically cleaned water tanks and from 20% water samples from uncleaned tanks.

Chapter III
Materials and Methods

Chapter III

Materials and Methods

3.1 Study design

This was descriptive cross-sectional and laboratorys based study.

3.2 Study area

The study was done on Khartoum state (Khartoum North, Omdurman and Khartoum).

Source of water sample is taps water , coolers and tanks.

3.3 Study period

The study was carried out during the period from April 2019 to October 2020.

3.4 Sampling technique

Convenience method– non-probability sampling

3.4.1 Sample size

103 of water samples were collected from selected sources of water in sterile bottle and stored at 4C$^{\circ}$ for maximum of three days.

s3.5 Data collection

Data were collected by questionnaire that included date place ,type of water (Appendix111).

3.6 Sample processing

Water samples were obtained from place suspected of having fecal contamination. In successful isolation, some important factor was considered: The inside and outside the tap was cleaned and disinfected carefully, the tap was opened and water was flowed for 2-3minutes, the tap was turned off and spout was sterilized by alcohol and water sample was taken in sterile container "bottles".

3.7 Cultures of water sample

+Cultures of water sample was done by presence absences technique as follow:

Equal volume of collected water sample were added to 50ml of sterile selective culture broth containing lactose and indictor "Laurytryptose (lactose) broth" (Appendix-11) with the Durham tube and then incubated at 37C for 48 hours.

All positive bottles with gas production were subcultured on two bottle of birilent Green Bile broth(BGBB) media (Appendix11) with the Durham tube for 48 hours in two different temperature 37C and44C, then the bacteria were identified by subculturing Eosin Methylene Blue EMB and Indole test at 37C (VAZ-Moreira *et al*., 2011).

3.8 Identification of growth

One Loopful of the sample was placed in the top of the EMB agar plate and was Streaked down, the plate was then incubated over night in 37°C. Agreenish metallic 'sheen color was the indicator for appositive Reaction of the presence of *E.coli* in water specimen(Cheesbrough, 2011).

3.8.1 Gram stain method

A Primary stains "Crystal violetwas applied to the dry–heat–fixed smear of microorganism for1minute.Then the stain was washed with Water and cover with Lugol'siodinefor1minute.Stain was washed with distilled water. And decolorized by acetone, alcohol and washed with distilled water. Then the stain was covered with safarnin for 2 minutes. The slide was placed in a rack to dry. The specimen was examined at (X100)(Oil-immersion lens). (Cheesbrough,2011)

2.8.2 Biochemical tests

Four biochemical tests were used for the identification of bacterial isolates, Indole test – Urease test – Citrate test – KIA test.

Indole test method :

Several colonies of the microorganism were rubbed into the tube of tryptophan peptone water media with sterile loop, the tube was incubated overnight in 37°C in an incubator, after 18-24 hours Kovacs reagent was added, a bright pink – red color ring was developed in positive reaction (Niemi *et al.*, 2003).

Urease test method :

Colonies of the microorganism to be inoculated in the urea media by sterile loop, the tube was incubated overnight in 37°C , after incubation the media was tested for change in color if pink the test will be positive (Maroncle *et. al*, 2006).

Citrate test method :

Colonies of the microorganism were picked up by a straight wire and inoculated in sloped Simmon's citrate agar and incubated overnight at 37°C in an incubator, after incubation the media was tested for change in color if blue the test is positive (Cheng *et al.*, 2012).

KIA test method:

Colonies of the microorganism were picked up by a straight wire and inoculated in sloped (KIA) media, then incubated overnight at 37°C in an incubator after incubation if there was a change throughout the medium, butt and slant are yellow, gas bubbles in the butt and no blackening in the butt this may be *E.coli* (Cheesbrough, 2011).

3.9 Statistical analysis

Analysis of the data was performed by SPSS version 16. To check frequency. Data were presented in form tables and figures.

Chapter IV
Results

Chapter IV
Results

A total of 103 water sample were collected from different localities in Khartoum states distributed as follow Omdurman 43(42%) samples, Khartoum North 30(29%) samples ,and Khartoum 30(29%) samples as shown on figure 4.1

Figure 4.2 displayed the distribution of water samples according to type of water source.

According to present absent method (85.6 %) of samples showed signs of bacterial growth while (14.4 %) showed no evidence of growth as presented on figure 4.3 .

All isolate were identified and *E.coli* was the most frequent isolate 47(52.8%) followed by *K. pneumoniae* 27 (30.4%) , *P. vulgaris* 9(10.1) and *S. marcescens*6 (6.7%) as showed on table 4.1 .

The most predominant organisms were isolated from taps water 50(48.5%) followed by coolers 40 (38.8%) and tanks 13 (12.6) as presented in Fig 4.4

According to the selected area Omdurman was the most frequent distributed area of isolated bacteria as displayed on table 4.2.

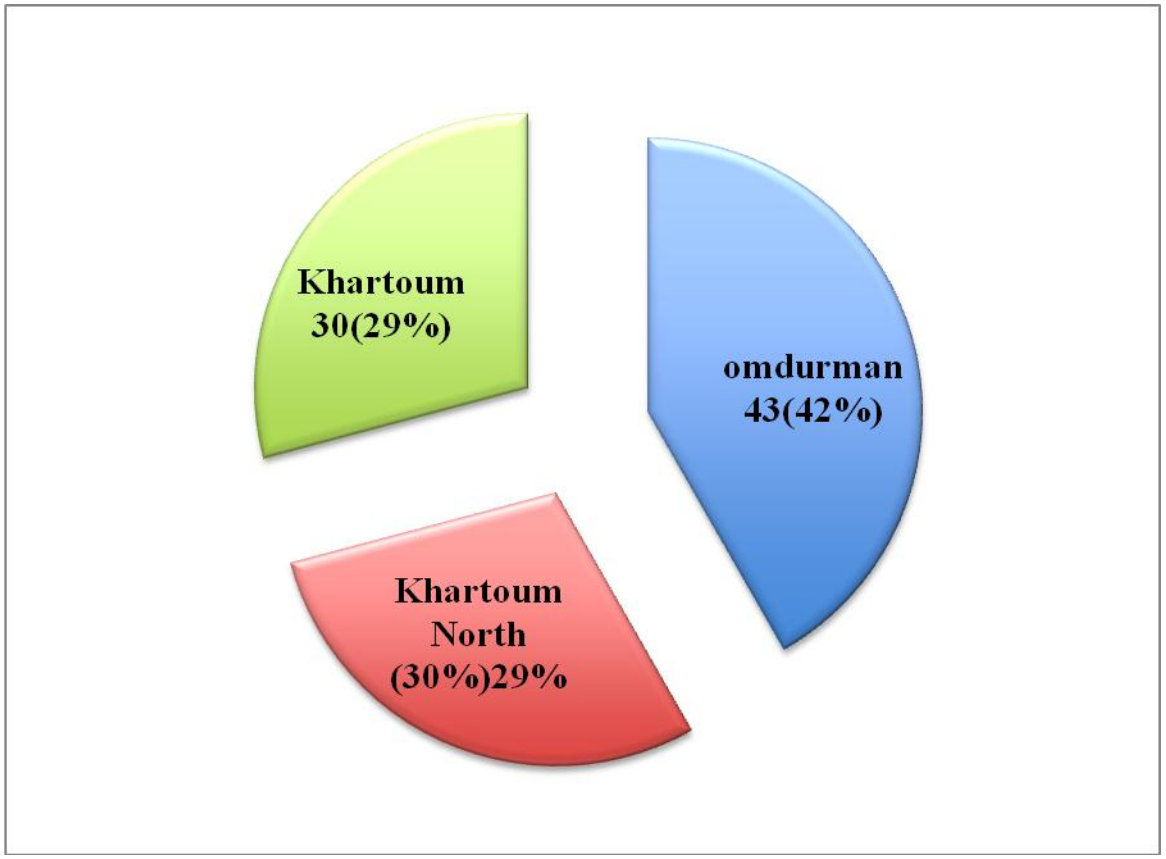

Fig (4.1): Frequency and Percentage of water samples according to source of water

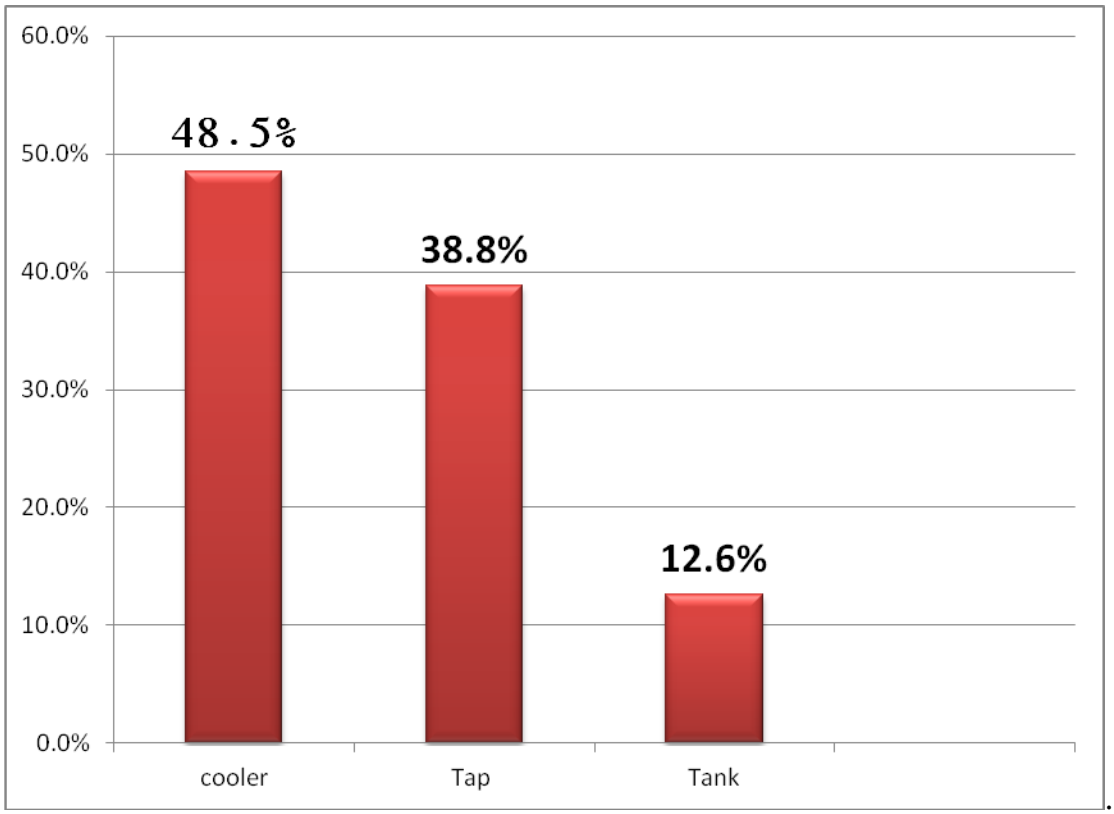

Fig (4.2): Percentage of water sample according to source of water

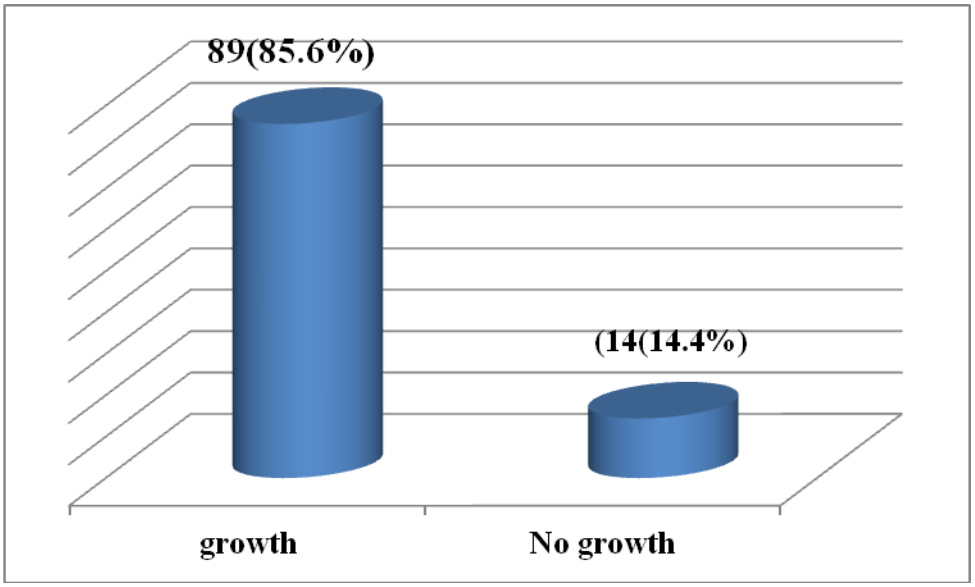

Fig 4.3: Frequency and Percentage of bacterial growth by presence absence technique

Table (4.1): Frequency and percentage of bacteria isolated from infected water sample

Bacterial isolates	Frequency	Percent
Escherichia coli	**47**	**52.8%**
Klebsiella pneumonia	**27**	**30.4%**
Proteus vulgaris	**9**	**10.1%**
Serratia marcescens	**6**	**6.7%**
Total	**8 9**	**100%**

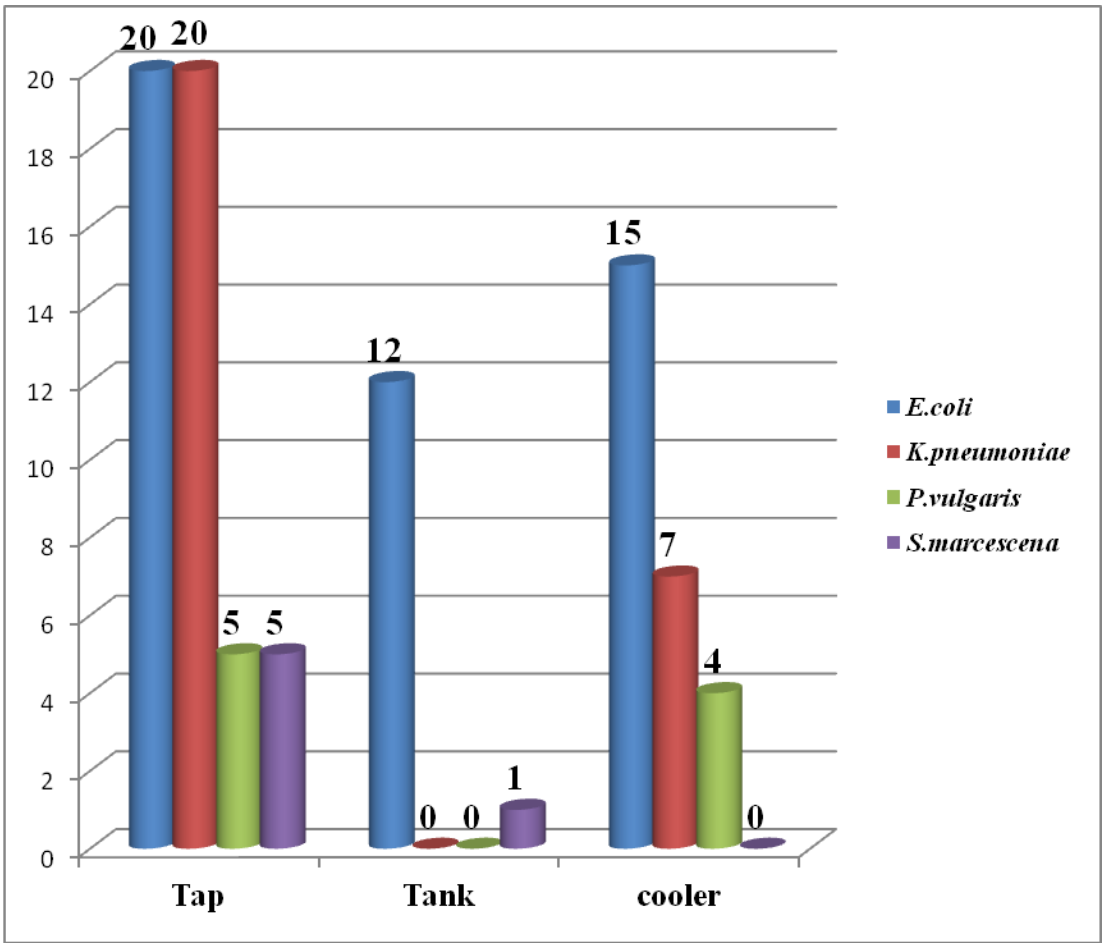

Fig 4.4 : Distribution of isolated bacteria according of water source

Bacterial isolates	Omdurman	Khartoum North	Khartoum	Total
Escherichia coli	29 (32.5%)	18(20.2%)	-	47(52.8%)
Klebsiella pneumoniae	7(7.8%)	-	20 (22.4%)	27(30.3)
Proteus vulgaris	-	9(10.1%)	-	9 (10.1%)
Serratia marcescens	-	-	6(6.7%)	6 (6.7%)
Total	36 (40.4%)	27(30.3%)	26(29.2)	89 (100%)

Table (4.2): Distribution of isolated bacteria according to collection area

Chapter V
Discussion, Conclusion and Recommendations

Chapter V
5. Discussion , Conclusions and Recommendations

5.1 Discussions

The routine monitoring and assessment of the microbiological quality of water is the key priority for both water suppliers and surveillance agencies. Microbiological quality is of principal concern because of the acute risk to health posed by viruses, bacteria and helminthes in drinking water.

Therefore, monitoring and assessment of drinking-water is primarily a health-based activity which influence the protection of public health through ensuring that the water supplied is of a good quality.

A total of 103 water sample were collected from different water sources (85.6%) showed signs of bacterial growth production of gas and (14.4%) did not .These finding is disagree with(Ibrahim and Isam,2010) who found (63%) showed signs of bacteria growth .

All isolates were identified and *E.coli* was the most frequent isolate 47(52.8%) followed by *klebsiella pneumonia* 27(30.4%), *Proteus vulgaris* 9 (10.1%) and *Serratia marcescens* 6(6.7%). This is in consistency with(Aliaa and Ali,2005; Zeinab,2010)who found *Escherichia coli* was the most predominant isolate in water samples and disagree with (Ibrahim and Isam ,2010; Abdulrahman *etal.,* 2016)who found *Bacillus* spesies was most common isolated water samples.

Another study in Nigeria done by (Bulakarima *et al*.,2016) who isolate *E.coli* and *K.pneumoniae* in drinking water.The finding of this study is also similar to the study in Kebbi state by (Kalpana *et al*., 2011) who were show drinking water contained *E .coli*, this due to *E,coli* was most bacterial pathogen in drinking water.

In the present study *E.coli* and coliforms bacteria was more frequent in Omdurman (karary Locality) (42%) followed by Khartoum North and Khartoum province (29%)(29%) respectively, this may be due to that Omdurman , is bigger than Khartoum North and consist of many wards.

Total coliform isolates were 48%(50) in Taps water followed by 38.8 %(40) in coolers water and (13)12.6% in tanks , these findings can be explained by the fact that we need a proper sterilization for water, and the cleaning procedures of water tanks in Khartoum state may be done by non-professional persons who contaminating tank instead than cleaning them.

The control of drinking water quality in distribution network remain a major challenge and protection of source, treatment and distribution management are all critical strategies in maintaining and improving water supply.

5.2 Conclusion

- There is high frequency of *Escherichia coli* and coliform bacteria in drinking water in Khartoum state

-Omdurman(karary locality) showed high frequency of coliform bacteria isolate in drinking water followed by Khartoum and Khartoum North

- Tap water was most the contaminated water from selected source where we all type of isolate species were found

5.3 Recommendation

-The drinking water should be periodically screened for the prescience of bacterial contaminate to prevent serious health risk

-Using large sample size, so as more area will be included

- Multiple PCR technique should be used beside traditional laboratory method as a routine technique in the diagnosis of coliform bacteria in drinking water

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Appendix I

1:Crystal violet (HilMedia Laboratories Pvt. Ltd. Mumbai, Inida) Ingredients

g/L

Crystal violet 20g/

Ammonium oxalate 9g

Ethanol or methanol, absolute 95 ml. Preparation:

Weigh the crystal violet on a piece of clean paper (pre weighed), transfer to abrown bottle, pre marked to hold I liter, add the absolute ethanol or methanol (technical grade is suitable) and mix until the dye is completely dissolved, weight the ammonium oxalate and dissolve in about 200 ml of distilled water ,add to the stain, make up to the I liter mark with distilled water, and mix well(Caution: Ammonium oxalate is a toxic chemical, therefore handle it with care), label the bottle, and store it at room temperature. The stain is stable for several months.

2: Lugol's iodine (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients g/L

Potassium iodide 20g Iodine 10g.

Preparation:

Weight the Potassium iodide, and transfer to a brown bottle pre marked to hold1 liter, add about a quarter of the volume of water, and mix until the potassiumiodide is completely dissolved, weight the iodine, and add to the potassiumiodide solution. Mix until the iodine is dissolved (Caution: iodine is injuriousto health if inhaled or allowed to come in contract with eyes., therefore handleit with care in a well ventilated room and make up to the I liter mark withdistilled water, and mix well. Label the bottle, and mark it Toxic. Store it in adark place at room temperature Renew the solution if it'scolour fades.

3:Acetone-alcohol decolorize (HiMedia Laboratories Pvt. Ltd. Mumbai, India).

Ingredients g/L

Acetone 500Ml

Ethanol or methanol, absolute 475 Ml

Preparation:

Mix the distilled water with the absolute ethanol (ethyl alcohol) or methanol (methy1 alcohol), transfer the solution to a screw-cap bottle of 1 liter capacity,technical grade is adequate, measure the acetone, and add immediately to thealcohol solution mix well (Caution: Acetone is a highly flammable chemical that vaporizes rapidly, therefore use it well away from an open flame) and label the bottle, and mark it Highly flammable. Store in a safe place at room temperature the reagent is stable indefinitely.

4: Safranin (HiMedial Laboratories Pvt. Ltd. Mumbai, India) Ingredients g/L

SafraninO 0.50

Ethyl alcohol, 95%100.11

5:EosinMethylene Blue –HiMediaLaboratoriesPvt.Ltd.Mumbai,India)

Ingredients g/L

Peptone10.000

Lactose10.000

Dipostassuim Hydrogen phosphate 3.000

Eosin yellow dye0.400

Methylene Blue dye0.065Agar

14.000

Directions:

Suspend 37.5 g in 1000 ml of cold distilled water. Heat to boiling, string constantly, distribute and autoclave at 121 °C for 15 min cool to about 60°C and before transferring to plates gently shake the flask to oxidize the medium and46to disperse the flocculent precipitate that forms darning sterilization final PH 7.1+/-0.2

6:Nutrient Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India).

Ingredients g/L

Peptic digest of animal tissue 5.00 Sodium

chloride5.00

Beef extract1.50

Yeast extract 1.50

Agar 15.00

Preparation

Suspend 28.0 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely.Sterilized by autoclaving at 15 Ibs pressure (121

°C) for 15 minutes. Pour into sterile Petri plates.

7: Kligler Iron Agar (HiMedia Laboratories Pvt. Ltd. Mumbai,Ingredients India). g/L

Peptic digest of animal tissue 15.00

Beef extract 3.00

Yeast extract 3.00

Protease peptone 5.00

Lactose10.00

Dextrose1.00

Ferrous sulphate0.20

Sodium chloride5.00

Sodium trisulphate0.30

Phenol red 0.024

Agar 15.00

Final pH 7.4 ±0.2(at 25 °C)

Preparation

Suspend 57.52 grams in 1000 ml distilled water. Heat to boil to dissolve the medium completely. Mix well and distribute into tubes. Sterilize by autoclaving at 15 lbs pressure (121 ° C) for 15 minutes. Cool the tubeson slopes with inch butts.

8: Peptone Water (HiMedia Laboratories Pvt. Ltd. Mumbai, India). Ingredients g/L

Peptic digest of animal tissue 10.00 Sodium

chloride 5.00

FinalpH7.2+/- 0.2(at 25°C).

Preparation

Suspend 15.0 grams in 100 ml distilled water. Mix well and dispense into tubes

with or without inverted Durham's tubes and sterilize by autoclaving at15 lbs pressure (121 °C) for 15 minutes.

9: Kovac’s Reagent (HiMedia Laboratories Pvt. Ltd. Mumbai, India) Ingredients g/L

P-dimethylaminobenzaldehyde 10 g

isoamyl alcohol 150mL

Concentrated hydrochloric acid 50mL

Preparation

Kovac's reagent is prepared by dissolving 10 gm of p-dimethyl aminobenzaldehyde in 150 ml of isoamyl alcohol and then slowly adding 50 ml of concentrated hydrochloric acid.

10: Urea Agar (Christensen) (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients g/L

Peptic digest of animal tissue 1 .00 Dextrose1.00

Sodium chloride 5.00

Dipotassiumphosphate 1.20

Monopotasiumphosphate0.80

Phenol red 0.012

Agar 15.00

Final pH 7.4±0.2(at25°C).

Preparation

Suspend 21 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121 ° C) for 15 minutes.

Cool to 50°C and aseptically add 50 ml of sterile 40% urea solution and mix well. Dispense into sterile tubes and allow setting on slanting position. Don't over heat or reheat the medium as urea decomposes very easily.

11: Simmons Citrate Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India).

Ingredients g/L Magnesium

sulphate0.20

Ammonium dihydrogen phosphate 1.00

Dipotassiumphosphate1.00

Sodium citrate2.00

Sodium chloride 5.00

Bromothymole blue 0.08

Agar 15.00

Final pH 7.4±0.2(at25°C).

Preparation

Suspend 24.28 grams in 100 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired in tubes or flasks, sterilize by autoclaving at 15 lbs pressure (121 °C) for 15 minutes.

14: Lauryltryptosebroth(HiMedia Laboratories Pvt Ltd.

Mumbai,India) Ingredients g/L

Tryptose 20.0 g Lactose

5.0g

Dipotassiumhydrdgen phosphate K2HPO4 2.75g Potassium dihydrogen phosphate KH2PO4 2.75g Sodium chloride Nacl5.og

Sodium Lauryl sulfate 0.1g Reagent

grad water 1L PH7.4+0.2(at 25°C)

Preparation

Suspend 35.6 grams of the medium in one liter of distilled water,mix well and dissolve by heating with frequent agitation .Dispense into tubes with Durham gas collecting tubes for gas detection .Sterilize in autoclave at 121c for 15 minutes .Cool as quickly as possible.The prepared medium should be stored at°8-2C.

15:BirilentGreenBileBroth (BGBB) (HiMedia Laboratories PvtLtd.Mumbai.

India2) Ingredients

gLTryptone 10.0g

Bacteriological ox bile 20.0g

Lactose10.0g

Brilliant green 13.3mg Distilled

water 1L

PH 7.2+ 0.2 (at 25°C)

Preparation

Dissolve4o.0gramsofdehydratedmediumin1literofdistilledwater

.Stirslowly until complete dissolution .Dispend in appropriately-sized

tubes containing a Durham tube .Sterilize in an autoclave at 121c for 15 minutes. After

cooling,the Durham tubes should not contain trapped air.

Appendix III

Questionnaire

Occurrence of *Escherichia Coli* and Fecal Coliforms in Drinking Water from Selected Localities in Khartoum State , Sudan

By:Nosiba Ahmed Hassan

Date…… :

No……:

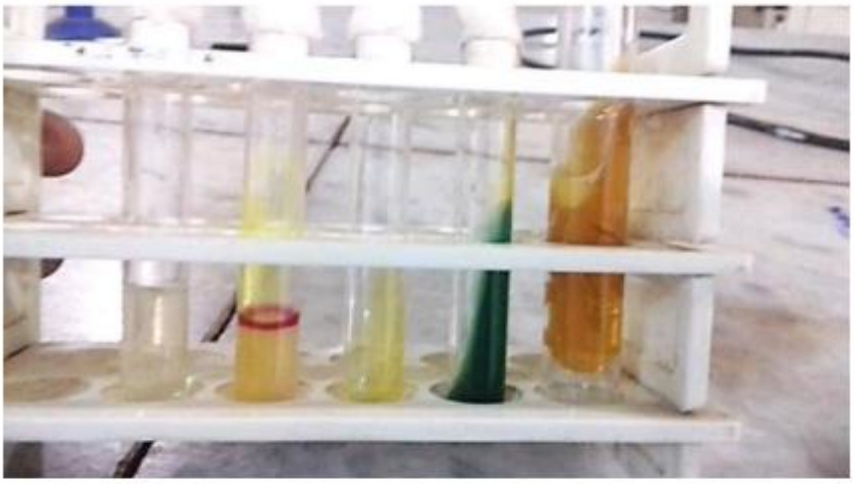
Type of water……:

Place…… :

Province: …….

Escherichia coli in Eosin Methyline Blue (EMB)

Klebsiella pneumoniae in Eosin Methyline Blue(EMB)



Colored plate (4): Show set of biochemical tests to identify *E.coli*

Colored plate (5): Show biochemical tests result to identify *K.pneumoniae*