بـــــــم الله الرحمن الرحيم

Republic Of Sudan

Ministry of Higher Education and Scientific Research

University of Shendi

Faculty of Graduate Studies and scientific research

ISOLATION AND IDENTIFICATION OF BACTERIAL CONTAMINATION WATER SUPPLY IN SHENDI, NILE STATE, SUDAN

A thesis Submitted for partial fulfillment for the requirement of M.Sc. in Medical Laboratory Sciences(Microbiology)

By:

Hala krim Eldin Abd Alrazig Alhosin

Bsc. (Shendi University)
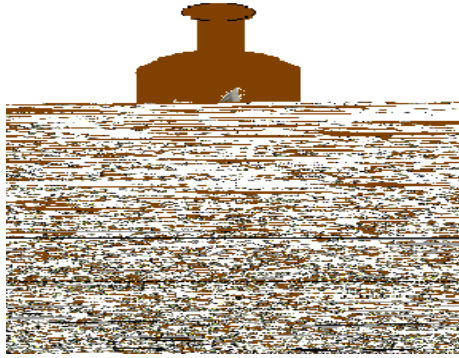
Supervisor

Dr.*Ahmed Mohamed Ahmed*

2018

الآية

قال تعالى:-

﴿ وَوَصَّيْنَا الْإِنسَانَ بِوَالِدَيْهِ إِحْسَاناً حَمَلَتْهُ أُمُّهُ كُرْهاً وَوَضَعَتْهُ كُرْهاً وَحَمْلُهُ وَفِصَالُهُ ثَلَاثُونَ شَهْراً حَتَّى إِذَا بَلَغَ أَشُدَّهُ وَبَلَغَ أَرْبَعِينَ سَنَةً قَالَ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَى وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحاً تَرْضَاهُ وَأَصْلِحْ لِي فِي ذُرِّيَّتِي إِنِّي تُبْتُ إِلَيْكَ وَإِنِّي مِنَ الْمُسْلِمِينَ ﴾

صدق الله العظيم

سورة الأحقاف الآية (15)

Dedication

I would like to dedicate dissertation to who gave me love , comfort throughout my life.

My mother

To who is always there for me and never let me need.

My father

To my greatest and dear

My brothers and sisters

To my teachers, aunts, uncles, whom are always there when I need them.

To my beloved friends whom shared me happiness and sadness.

الاهداء

الى من جرع الكاس فارغا ليسقني قطره حب، الى من كلت انامله ليقدم لنا لحظه سعاده ، الي من حصد الاشواك عن دربي ليمهد لي طريق العلم الي القلب الكبير

والدي الغالي

يامن ارضعتني الحنان والى رمز الحب وبلسم الشفاء ، يامن افتقدك من سنوات يامن يرتعش قلبي لذكرك يامن اودعتني لله اهديك هذا البحث

امي الغاليه

الى من ارى التفاؤل بعينيه والسعاده في ضحكه الى شعله الذكاء الي الوجه المفعم بالبراءه ولمحبتك لازهرت ايامي وتفتحت براعم للغد .

زوجي الغالي

الى من اثروني على انفسهم الى من علموني علم الحياه الي من اظهروالي ماهو اجمل من الحياه

اخواني واخواتي

الي من التجي اليها عندما تقسو الدنيا الي من علمتني بعض دروس الحياه

خالتي الغاليه

الى الاخوات اللواتي لم تلدهن امي الي من تحلو بالاخاء وتميزو بالوفاء والعطاء الي ينابيع الصدق الصافي الي من سعدت وبرفقتهم في دروب الحياه الحلوه والحزينه سعدت الى من كانوا معي على طريق النجاح الي من عرفت كيف اجدهم وعلموني ان لا أضيعهم .

صديقاتي

Acknowledgement

First of all I thank Allah for giving me the strength and populationto do this work. I would like to thank my supervisor

Dr: Ahmed Mohammed Ahmed

Who helped and supported mepopulationly to complete this work .

I would like to thank the individual help in the collection of specimens.

I would like to thank my colleagues for there great help valuable comments.

Finally I would like to thank all people who helped me to perform this work.

List of abbreviation

Abbreviation	Referent
APHA	American Public Health Association
AWWA	American Water Works Association
CDC	Center for Diseases Control and prevention
DAEC	Diffusely Adherent Escherichia Coli
DNA	Deoxy ribo Nucleic Acid
EAEC	Entero Aggregative Escherichia Coli
EHEC	Entero Hemorrhagic Escherichia Coli
EIEC	Entero Invasive Escherichia Coli
EPEC	Entero Pathogenic Escherichia Coli
ETEC	Entero Toxogenic Escherichia Coli
IPCC	Intergovernmental Panel on Climate Change
IWSC	International Water and Sanitation Center
MF	Membrane Filtration
MF	Micro Filtration
NF	Nano Filtration
ORT	Oral Dehydration Therapy
PCR	Poly myrase Chain Reaction
RO	Reverse Osmosis
SSMO	Sudanese standards Metrology Organization
TCU	True Color Unit
UF	Ultra Filtration
UNHCR	United Nations High Commission for Refugees
USEPA	United State Environmental Production Agency
UV	Ultra Violet
WEF	Water Environmental Federation
WHO	World Health Organization

ملخص البحث

التلوث الميكروبي للماء يعتبر مشكلة مستمرة والسبب هو التلوث بالمواد البرازية للحيوانات والإنسان والتي تدخل المنظومة المائية.

إن وجود البكتريا والميكروبات المرضية في المياه المعتبرة صالحة للشرب أمر خطير جداً لأنها يمكن أن تسبب الإسهالات المائيه واالكوليرا وحمى التايفويد، وغيرها من الإصابات الخطرة.

جمعت عينات المياه للفحص المختبري من سته وعشرين مربع موزع على مدينه شندي والتي صنفت إلى مياه الحنفيات، جري على كل العينات دراسة مجهرية وبعدها تم عزلها في الوسط الزراعي الماكونكي والتاكد منها بواسطه الاختبارات الكيميائيه.

أثبتت الدراسة أن عينات مياه الابار في مدينة شندي كانت ملوثة بنسبه 23.1 % (3عينات) وكان الموشر للتلوث بالمواد البرازيه للحيوان والانسان عزل الاشيرشيا القولنيه.

وتم عزل أنواع أخرى من البكتريا المعويه سالبه الجرام مثل السالمونيلا والسودوموناس وهذه ليست مؤشرعلمي لتلوث المياه بالمواد البرازيه.

خلاصة الدراسه أنه بعض مياه الشرب في مدينه شندي ملوثه بالمواد البرازيه للانسان والحيوان وبالتالي يجب مراجعه هذه الابار.

Abstract

Microbial contamination of water persists to be a major problem; the usual source is human and animal fecal matters that have contaminated the water systems. The presence of bacteria and pathogenic organisms is of great concern when considering the safety of drinking water, as pathogenic organisms can cause watery diarhea , cholera,typhoid fever and other illnesses . Water samples for laboratory examination were collected from 26 square from different area of Shendi town, which were classified as Tap water. Each sample it was collection in sterile contanier ، then isolated in MaCconky agar media andConfirmation by biochemical test. Tap water samples were found to be contaminated about 23.1% (3sample) and indicator of contaminationwith fecal matter of animal and human is isolation ofE.Coli .

It was isolation other type ofGram negative enteric bacteria such as *Salmonella*and pseudomona these were not scientific indicator of water contamination by animal and human fecal matter.

In conclusion of study that some drinking water in shendi town contaminated with animal and human fecal matter thus is must review these wells.

List of Tables

List of Content

Chapter One

Introduction

Rationale

Objective

1.1: Introduction:

Water is the most important substance on earth .It makes about 80% of our body weights, comprises about 92% of the blood and nearly 98% of gastrointestinal secretions. Water holds all nutrient factors in solution and acts as a transportation medium for these substances. One of the most important functions of water is to flush toxins from the body. (MurrayMT-1997) According to World Health Organization, (Who-2003) there were an estimate of 4 billions cases of diarrhea and 2.2 million deaths annually in these countries and(Diab, A. M., *etal* ,(2000) In Cameroon for example, they are the most prevalent water born disease among children below five years of age. In Yaoundé in particular, the prevalence of diarrhea is increasing studies conducted in the city amongst children under five years of age show that the prevalence rate has shifted from 13.01% in 2004 to 14.4% in 2005 .(DHS-Cameroon Demographic and Health Surveys,(2004) , Henock, N.Y. and Do vie D.B.,(2007).

Tap water is used to meet daily needs that require water, such as drinking, preparing food, washing, and for various personal hygiene purposes. When control and safety conditions are in place, tap water can be used to drink directly without subsequent treatment, but if necessary, household water purification devices such as water filters or even boil should be made.

Water pollution: Pure uncontaminated water does not occur in nature. It contains impurities of various kinds natural and manmade. (e.g. nitrogen, carbon dioxide, hydrogen sulfate, and etc, which may pollution is that caused by human activities (Park, 2005). Water may be contaminated by microorganisms (bacteria, viruses, helminthes, and parasites) usually of fecal origin. The most common and wide-spread danger associated with drinking water is contamination either directly or

indirectly by sewage, waste, or by human or animal excrement. If such contamination is recent and if among the contributors there are carrier of communicable enteric diseases, some of the living causal agents may present (WHO, 1984). The sources of drinking water pollution are:

*Sewage which contains decomposable organic matter and pathogenic agents.

*Agricultural pollutants which comprise fertilizers and pesticides.

*Physical pollutant such as thermal pollution and radioactive substances (Park, 2005) Using of contaminated water for drinking or in food preparation may then result in new cases of infections (WHO, 1997).

In most countries the principal risks to human health associated with the consumption of polluted water are microbiological in nature.

1.2: Rationale:

Water is essential to life and normal supply of clean safe drinking water is requiring for sustenance of life.
Concern regarding safe clean drinking water commenced at the turn of 20 century.

Briefly- the purpose of having drinking water quality line and regulation is to ensure that all human being with a country have access to safe drinking water.

It is estimated over 80% of disease caused by contaminated drinking water. WHO reported that every year more than 5 million human being die from illnesses linked to unsafe drinking water and 1-2 million deaths caused by diarrheal diseases.

This study aim for testing   for the presence of normal fecal organism in water is way of determining whether a water supply is fecally polluted.

1.3: Objectives:

1.3.1: general objective:

To isolation and identification bacterial contamination water supply in Shendi town.

1.3.2: specific objectives:

1-To isolate and identify bacteria that cause water contamination.

2- To find out the frequency of tap water contamination in Shendi town.

Chapter Two

Literature Review

2. Literature Review

Definitions

2.1: Water definition

Water is a binary compound that occurs at ambient temperature as clean, colorless, tasteless, liquid, freezes into ice at 0 deg. C and boils at 100 deg. C; it is of vital importance for human life (SSMO, 2002).

Safe drinking water definition

This is defined by the guide lines as water that does not represent any significant risk to health over a life time of consumption, including different sensitivities that may occur between life stages (WHO, 2006).

2.2: Water sources

There are basically three categories of naturally occurring water sources they are ground water, rain water, and surface water (UNICEF, 1999).

Ground water

Ground water occurs under most of the world land surface but there are great variation in the depths at which it is found ,its mineral quality ,the quantities present and the rate of infiltration and the nature of the ground above it (thus accessibility).

Therefore the pollution of water resources need a serious and immediate attention through periodical checkup of water quality (WHO, 2011).

Rain water

Rain water collection from roofs or larger catchment areas, can be utilized as source of drinking water particularly where there are no other safe water sources available (for example where ground water is polluted or to deep).

Rain Water may be collected (harvested) from surfaces by roof catchment and ground catchment after passing through a screen and or

filter the water is conducted through gutters to cisterns. These cisterns can be large enough to serve a community or an institution (UNICEF, 1999).

Surface water

Surface water in stream, rivers, lakes and ponds is readily available in many populated areas, but it is almost always polluted often grossly so it should be only used where there are no other safe sources of water available, where no other sources are readily available. Surface water can be contained, collected and used after some form of filtration (UNICEF, 1999).

2.3: Water requirement

The basic physiological requirements for drinking water have been estimated at about 2 liters per head per day, this is just for survival. But from the stand point of the public health and improvement of the quality of life, water should be provided in adequate volume. A daily supply of 150-200 liters per capita is considered as adequate. The consumption of water depends upon climatic conditions, standard of living and habits of the people (Park, 2005).

2.4: Uses of water

Water is absolutely essential to life. From 50-65 per cent of human body is composed of water and variations of as little as 1-2 per cent will cause thirst or pain. The loss of 5 per cent of body water can cause hallucination; loss 10-15 per cent can be fatal. Although human can live several months without food but they can survive only a day or two without water (Moeller, 2005). The uses of water in a community are.0 many and the requirement in quantity and quality are varied. Conventionally it has been convenient and economical to provide a

Single water supply sufficient in quantity to serve all uses and suitable in quality to meet drinking water requirement. The uses of water include:

*Domestic use (drinking, cooking, washing, bathing, flushing of toilet,

Gardening, etc).

*Public purposes (cleaning streets, recreation purpose, fire protection, and Public parks).

*Industrial purposes (for processing and cooling).

*Agricultural purposes such as irrigation of crops. (Park, 2005).

2.5: Drinking water quality

The quest for pure water dates back to antiquity. In modern times it has led to the formulation of specific standards to provide a basis for judging the quality of water. These standards are exposure limits for physical, chemical, viral and bacteriological agents that have been adopted by governments or appropriate authorities and therefore have legal force. The purpose of standards is to minimize all the known health hazards, since it is impossible to prevent all pollution (Park, 2005). The primary concern with health problems caused by water supply is infectious diarrheal diseases transmitted by the fecal – oral route, these are caused by disease-causing micro –organisms, or pathogens.

Physical quality: The ordinary consumer judges the water quality by its physical parameters.

The most important physical parameters are temperature, taste, odor, color, conductivity, salinity, solids contents, density, and turbidity (Abd el-magid, 1995).

Temperature

Cool water is generally more potable. Low water temperature tends to decrease the efficiency of treatment process, including disinfection, and may thus have a deterious effects on drinking water quality. However high water temperature enhances the growth of microorganisms, taste, odor, color and corrosion problem may be increased. No guide line value is recommended for water since its control is usually impracticable (Park, 2005). Design and construction of water systems should provide for

purying or convening of cool and also prevent freezing in cold climate (Salvato, 1982).

Taste:-Usually drinking water must be almost tasteless to consumer. Taste is a subjective property that is rather difficult to measure. Presence of taste may be due to some dissolved impurities that have found their way into water.) that is produced by decomposition of organic matter by microorganisms (Abd el magid, 1995).

Odor:-Odor should be absent or very faint for water to be acceptable, not greater than three threshold odor numbers (Salvato, 1982). Existence of odors in water may be due to number of reasons such as:

*Biodegradation of organic and inorganic compounds of nitrogen, phosphorus and sulfur.

*Decomposition of algae and other microorganisms

* Generation of substances such as ammonia, sulphides and hydrogen sulphides (Abd el-magid, 1995).

Color:-Pure water is colorless; color in water may result from the presence of natural metallic ions such as iron oxides (cause red color) and manganese oxides (cause brown or black color). Other sources are humus and peat material, plankton, weeds, and industrial wastes. Color is classified as:

*True color (true color units, TCUs). Due to substances in solution

*Apparent color (due to suspended matter).

Water from which turbidity has been removed by methods such as filtration or centrifugation where the color was due to vegetable or organic extracts that are colloidal (Abd el-magid, 1995). Drinking water should be free from color which due to the presence of colored organic matter (primary humus substances) and metals such as iron and manganese. The guide line value is up to 15 true color units (TCUs),

although level of color 15 TCU can be detected in a glass of water (Park, 2005).

Conductivity:-Conductivity may be defined as electrical conductance of a conductor of unit length and unit cross –section area, and commonly expressed in micro mhos / cm. Pure water is normally not a good conductor of electricity, the increase of dissolved salts in water increase its conductivity.

Salinity:-Salinity is the total dissolved solids in water after all carbonate have been converted to oxide, all bromide and iodide have been replaced by chloride and all organic matter has been oxidized (Abdel-magid, 1995).

Solids contents:-Solids content is defined as the matter that remains as residue upon Evaporation and drying at 103 to 105 deg. C.

Microbiological quality:- Other organisms naturally present in the environment and not regarded as pathogens in drinking water may also cause occasional opportunist disease such as organisms in drinking water may cause infection predominantly among people whose local or general natural defense mechanisms are impaired, this is most likely to be the case in very old and young children, those organisms such as pseudomonas, flavor bacteria, acineto bacteria, klebsiella, and serratia (WHO, 1984).

Free living organisms:-Free living organisms that may occur in drinking water supplies include fungi, algae, and etc. The most common problems with these are their interference in operation of water treatment process, color, turbidity, taste, and odor of finished water, thus drinking water must be free from these free-living organisms (Park, 2005).

Bacteria:-The word bacteria (singular bacterium) come from the Greek word meaning (rod) or (staff). Bacteria are single celled microscopic organisms that multiply by spitting in to binary fission. In order to

multiply they need carbon obtained from carbon dioxide (CO_2), if they are autotrophic or from organic compounds (dead vegetation and meat) if they are heterotrophy.

Their energy comes either from sunlight if they photosynthetic or from chemical reaction if they are chemosynthetic. Bacteria are present in air, water, earth, rotting vegetation, and the intestines of human and animals.

Under ideal conditions bacteria may be divided (generation time) every 20 minutes. Never the less they are taking up food quickly that they are likely to be limited by shortage food, oxygen, or water (Abdel- magid, 1995).

Coli form group bacteria:-The coli form of organisms includes all the aerobic and facultative an aerobic, gram-negative, non- spores- forming, rod-shaped bacteria that ferment lactose with acid and gas formation within 24-48 hours at 35-37 deg. C (s alvato، 1982). Coli form bacteria defined here are as facultative an aerobic, gram negative, non-spore-forming rods that ferment lactose with gas formation within 48 hours at 35 deg. C or as applied to the membrane filter method a dark red colony with metallic sheen within 24 hours on an endo-type medium contain lactose. However an acrogenic (non gas producing) lactose fermenting strains of E. coli and coli forms that do not produce metallic sheen on endomedium may be encountered. These organisms as well as typical coli forms can consider indicator organisms (APHA, AWWA & WEF, 1998). The indicator bacteria that most surveillance bodies use in routine assessment of risk of fecal contamination is Escherichia coli (E. coli) or as an alternative thermo tolerant coli form. E. coli provides the closest match to criteria for an ideal indicator, however it is not perfect and it is possible to find pathogens in drinking water supplies when E. coli is absent.

Basic characteristics of the ideal indicator are:

*Present wherever pathogens are present.

*Present in the same of higher numbers than pathogens.

The second edition of the WHO guide lines for drinking water quality published in 1993 strongly recommended the use of E. coli as the preferred fecal indicator because its provides the closest match to the criteria for an ideal indicator (WHO, 2002).

Escherichia coli pathogenic strains:-Escherichia coli is present in large numbers in normal intestinal flora of humans and animals, where it's generally causes no harm. However in other parts of the body E. coli can cause serious diseases such as urinary tract infections, bacteraemia and meningitis. A limited number of entero pathogenic strains can cause acute diarrhea. Several classes of entero pathogenic E. coli have been identified on the basis of different virulence factors, including entero hemorrhagic E. coli (EHEC), entero toxinogenic E. coli (ETEC), entero pathogenic E. coli (EPEC), entero invasive E. coli (EIEC), entero aggregative E. coli (EAEC),and diffusely adherent E. coli (DAEC).EHEC organisms can cause infections, ETEC produces heat labile or heat stable E. coli entero toxin, or both toxin simultaneously and is an important cause of diarrhea in developing countries specially in young children, infection with EPEC has associated severe, chronic, non bloody diarrhea, vomiting and fever in infants,

This occurs commonly in developing countries and rare in developed counties.

EIEC causes watery and occasionally bloody diarrhea. Entero pathogenic E.coli are I enteric organisms and humans are the major reservoir, particularly of EPEC, ETEC, and EIEC strains. Lives stock such as cattle, sheep, goats, pigs, and chickens are major source of EHEC strains (WHO, 2004).

Thermo tolerant bacteria:-Thermo tolerant coli form bacteria are coli form organisms that are able to ferment lactose at 44-45 deg. C., the group include the genus E. coli and some species of klebsiella, Entero bacter and citro bacter. Because thermo tolerant coli form organisms are readily detected they have an important secondary role as indicators of the efficiency of water treatment process in removing fecal bacteria (WHO, 1997).

 Water associated diseases:-The most common and widespread health risk associated with drinking water is microbial contamination which has the potential to cause large outbreaks of waterborne diseases like dysentery, cholera, typhoid, skin infections etc . The chemical contaminations do not cause immediate acute health problems unless they are present in massive quantities through some accident and use of chemical fertilizers and pesticides in crop near the drinking water sources. It therefore becomes essential to regularly control the quality of groundwater and to device ways and means to protect it.

 Water has a profound effect on human health both as a means to reduce Disease and as a media through which disease-causing agents may be transmitted. The impact of water on health derives principally from the consumption of water containing pathogenic organisms or toxic chemicals and the use of inadequate volumes of water that lead to poor personal and domestic hygiene, The risk of acquiring a waterborne infection increases with the level of contamination by pathogenic micro-organisms. However, the relationship is not simple and depends on factors such as infectious dose and host susceptibility. Drinking-water is only one way for the transmission of such pathogens; some agents may be transmitted from person to person, or through the contamination of food. In many cases, poor personal hygiene may lead to the transmission of pathogenic organisms through contamination of water stored within the

home or by preparation of food. Poor hygiene practices often result from the use of inadequate volumes of water and therefore water quantity is also important in controlling infectious diarrheal diseases. In general terms, it is better to provide larger volumes of reasonable quality water than to provide very limited quantities of excellent quality (UNICEF, 1995; WHO, 1997).

Safe water is a precondition for health and development and a basic human right, yet it is still denied to hundreds of millions of people throughout the developing world.

Water related diseases caused by insufficient safe water supplies coupled with poor sanitation and hygiene cause 3.4 million deaths a year, mostly among children.

Despite continuing efforts by governments, civil society and the international community, over a billion people still do not have access to improved water sources (UNISEF, 2008).

Water-associated diseases are classified into five main groups (according to Bradley, 1974):

Water-washed (water-hygiene) diseases:-Occur due to the lack of adequate water supply for washing, bathing and cleaning.

Pathogens are transmitted from person to person or by contact with contaminated surfaces. Eye and skin infections as well as diarrheal illnesses occur under these circumstances. Waterborne pathogens include bacteria, viruses, protozoa and helminthes. A short list of the most important pathogens and their significance in water supplies (WHO,2011). Control of water-washed diseases depends more on the quantity of water than the quality. Most of the diarrheal diseases should be considered to be water-washed as well as water-borne, helminthes; acute respiratory infections (ARI); skin and eye diseases; and diseases caused by fleas, lice, mites or ticks. For all of these, washing and

improved personal hygiene play an important role in preventing disease transmission(UNICEF, 2008).

Water-scarce diseases:- Occur due to the lack of water available for washing, bathing and cleaning. Hence, pathogens are transmitted from person to person or from contaminated surfaces to a person and are spread by the fecal–oral route.

In particular, eye (trachoma) and skin infections (scabies), as well as diarrheal diseases occur under those conditions.

Water-based diseases:-Are caused by organisms, in particular by different species of worms that spend parts of their life-cycle in different habitats.

Vector-borne diseases: Are caused by bites from insects that breed in water. Insect vectors such as mosquitoes transmit diseases such as malaria, Chikungunya and other diseases (WHO, 2011).

These diseases are not directly related to drinking-water quality. The most common vector insects are mosquitoes and flies (UNICEF, 2008).

Waterborne diseases:-Are caused by the ingestion of faecally contaminated water. Cholera and typhoid fever are classical examples of waterborne diseases, where only a few highly infectious pathogens are needed to cause severe diarrhea. Shigellosis, hepatitis A, amoebic dysentery and other gastrointestinal diseases can also be waterborne (WHO, 2011).

Most water-borne pathogens infect the gastrointestinal tract and cause diarrheal disease. In most cases, the specific pathogen responsible for infection is not identified, and case identification and treatment is fairly generic. Two very serious forms of diarrheal disease, cholera and shigellosis, should be considered separately because of their severity and tendency to create epidemics (UNI CEF, 2008).

Shigellosis:-Shigellosis or bacillary dysentery is an acute bacterial disease characterized by bloody diarrhea. Shigellae spp. are small Gram-negative bacteria that belong to the Enterobacteriaceae family. The genus Shigellae comprises four species: *S.* dysenteries', S. flexneri, *S. boydii* and S. sonnei. Bacillary dysentery is the most communicable of the bacterial enteritis. Symptoms are fever nausea, vomiting, cramps and tenesmus. Mild and asymptomatic cases occur. The illness is usually self-limited and lasts 4–7 days. The incubation time is 1–7 days for all Shigellae spp. infectious diseases (WHO, 2011).

Shigellosis, commonly known as acute bacillary dysentery, is manifested by the passage of loose stools mixed with blood and mucous and accompanied by fever, abdominal cramps and tenesmus (a symptom characterized by incomplete sense of evacuation with rectal pain)(Sur et al, 2004).

Shigellosis is a bacterial infection of the colon that causes diarrhea and can lead to death. Dysentery (frequent mucoid or bloody stools) when caused by Shigellae is called Shigellae dysentery. Of the estimated 164.7 million Shigellae diarrheal episodes occurring globally every year, most occur in developing countries (99%) and mainly in children (69%) (WHO 2006).

Transimition: - People infected with this bacterium may experience mild to severe diarrhea (which can be watery, bloody or mucousy). There may also be vomiting, a fever, nausea and cramps. It can last for 4 to 7 days. After you come in contact with this bacterium, you will usually feel symptoms in 1 to 3 days, but may range from 12 hours to one week.(Heymans DL, 2004).

Shigellae dysenteries', *S. flexneri, S. sonnei*, and *S. boydii* are the four species of small, Gram-negative, non-motile bacilli that cause shigellosis, and all but *S.sonnei* have more than one genetically distinct subtype

(serotype) (von Seidlein 2006). The species distribution varies globally; for example, *S. flexneri* was reported to be most prevalent in India (58%,Dutta 2002) and Rwanda (68%,Bogaerts1983), while *S. sonnei* was the most frequently detected species in Thailand (85%, von Seidlein 2006), Israel (48.8%, Mates 2000).

Shigellae are transmitted by the faeco-oral route, via direct person to- person contact, and via food, water, and inanimate objects. Only a small number of ingested bacteria are required to produce illness. The disease is communicable as long as an infected person excretes the organism in the stool, which can extend up to four weeks from the onset of illness. Secondary attack rates, the number of exposed persons developing the disease within one to four days following exposure to the primary case(Park 2005).

Complications:-Shigellosis may be associated with a large number of mild to severe life threatening complications, particularly due to *S.dysenteriae type* 1. Children may have high fever, rectal prolapsed and convulsions. Arthritis and arthralgia are complained by some patients. Intestinal perforation, hemorrhage, toxic mega colon and protein loosing enteropathy may complicate a shigellosis case. Leukemoid reaction (WBC count > 50,000/ cmm) and hemolytic uremic syndrome (a triad of microangiopathic hemolytic anemia, thrombocytopenia and renal failure) are seen in *S.dysenteriae* type 1 infection and may be fatal (Sur, 2004).

Diagnosis:-

Diagnosis of shigellosis is made clinically by the typical features of Bacillary dysentery with blood and mucus in stool although some cases may present with mild to moderate watery diarrhea initially. Dehydration is usually not a conspicuous feature. Microscopic examination of fecal smear stained with iodine shows presence of plenty of fecal leucocytes (>

10/high power field). Confirmation is made by stool culture, serological and biochemical. Tests (Sur et al, 2004).

Shigellae species die rapidly in unfavorable environments and stool culture should ideally be supplemented by attempts to identify Shigellae DNA using polymerase chain reaction (PCR) (von Seidlein 2006).

Typhoid:-The disease has received various names, such as gastric fever, abdominal typhus, infantile remittent fever, slow fever, nervous fever or pathogenic fever. The name "typhoid" means "resembling typhus" and comes from the neuropsychiatric symptoms common to typhoid and typhus (oxford, 2011).

Typhoid fever or enteric fever is a major health burden in developing countries. It is caused by Salmonella typhi and Salmonella paratyphoid. The faeco-oral route is the commonest mode of transmission and poor sanitation and reduced access to clean drinking water increases its prevalence and incidence (Ratnayake et al. , 2011).

Typhoid (typhoid fever) is a serious disease. It is caused by bacteria called Salmonella Typhi.

The causative agent of typhoid fever is *Salmonella typhi*, which is an enter pathogenic organism among other *Salmonella* spp.

They belong to the family Enterobacteriaceae and are Gram negative facultative anaerobic bacteria.

Today Salmonella spp. are classified by DNA stereotyping into different serotypes. Common human Salmonella serotypes are *S.* typhi, S. paratyphoid, S. enteritidis and *S.* typhimurium which cause enteric fever or gastroenteritis (WHO, 2011).

Typhoid causes a high fever, fatigue, weakness, stomach pains, headache, loss of appetite, and sometimes a rash. If it is not treated, it can kill up to 30% of people who get it(CDC, 2012). *S.* typhi has been isolated from water and sewage.

The persistence in water supplies is moderate; the survival time of *Salmonella* spp. in drinking-water ranges from a few days to over100 days. Resistance to chlorine is low.

Faecal contamination of groundwater and surface water, and insufficient disinfection practices are the main cause of waterborne outbreaks (WHO, 2004).

Diagnosis:-Diagnosis is made by any blood, bone marrow or stool cultures and with the Widdel test (demonstration of salmonella antibodies against antigens O-somatic and H flagellar).

In epidemics and less wealthy countries, after excluding malaria, dysentery or pneumonia, a therapeutic trial time with chloramphenicol is generally undertaken while awaiting the results of The Widdal test is time consuming and often, when a diagnosis is reached, it is too late to start an antibiotic regimen The term "enteric fever" is a collective term that refers to typhoid and paratyphoid (Parry CM, Beaching NJ, 2009).

Treatment:-The rediscovery of oral rehydration therapy in the 1960s provided a simple way to prevent many of the deaths of diarrheal diseases in general where resistance is uncommon; the treatment of choice is a fluoroquinolone such as ciprofloxacin (Parry CM, Beaching NJ, 2009; Effa EE, et al., 2011).

Prevention:-There are two vaccines licensed for use for the prevention of typhoid, the live, oral Ty21a vaccine (sold as Vivo if Berna) and the inject able Typhoid polysaccharide vaccine (sold as Typhim Vi by Sanofi Pasteur and Typherix by GlaxoSmithKline).

Cholera:-Cholera is an acute diarrheal illness that is caused by the bacterium *VibrioCholera*. It can be very mild, but in about one in 20 cases, it is severe. Severe cases are characterized by profuse watery diarrhea, vomiting, and leg cramps. In these cases, fluid loss is rapid and can quickly lead to dehydration and shock. In severe cases, without

treatment, cholera can be one of the most rapidly fatal infectious diseases: 50 percent of patients with severe cases die without treatment, and death can occur within hours(WHO,2009). Cholera was the first disease for which modern public health surveillance and reporting was carried out in an organized way.

It is one of the three diseases currently reportable under the International Health Regulations (IHR) of1969.

According to those regulations, national health, administrations should report the first cases of cholera on their territory to WHO within24 hours of their being informed (WHO, 2000). Cholera is an acute bacterial infection of the intestine caused by ingestion of food or water containing *Vibrio cholera*, serogroups O1 or O139.

Symptoms include acute watery diarrhea and vomiting which can result in severe dehydration or water loss. When left untreated, death can occur rapidly – sometimes within hours(WHO, 2000). Cholera is a diarrheal disease caused by infection of the intestine with the bacterium Vibrio cholera, either type 01 or 0139.

Both children and adults can be infected. About 20% of those who are infected develop acute, watery diarrhea – 10–20% of these individuals develop severe watery diarrhea with vomiting. If these patients are not promptly and adequately treated, the loss of such large amounts of fluid and salts can lead to severe dehydration and death within hours(WHO,2005).

The primary symptoms of cholera are profuse, painless diarrhea and vomiting of clear fluid.

These symptoms usually start suddenly, one to five days after ingestion of the bacteria. Diarrhea is frequently described as "rice water" in nature and may have a fishy odor.

An untreated person with cholera may produce 10 to 20 liters of diarrhea a day(Sack DA, Sack RB, Nair GB,2004).with fatal results.

Transmission:-Cholera is typically transmitted by contaminated water. In the developed world, seafood is the usual cause, while in the developing world it is more often] water.

Cholera has been found in only two other animal populations: shellfish and plankton (Sack DA, Sack RB, Nair GB, 2004).

Cholera is transmitted through contaminated food or drinking-water, as well as by person-to person contact through the fecal-oral route. Sanitary conditions in the environment play an important role since the *V. cholera* bacterium survives and multiplies outside the human body and can spread rapidly where living conditions are crowded and water sources unprotected and where there is no safe disposal of feces (WHO, 2000).

Anyone who ingests contaminated water can get cholera, regardless of their age or health status. Its incubation period is short—two hours to five days—and it can spread from place to place as people travel (WHO, 2008).

Diagnosis:-A rapid dip-stick test is available to determine the presence of V. cholera , In those samples that test positive, further testing should be done to determine antibiotic resistance (Sack DA, Sack RB,2006). In epidemic situations, a clinical diagnosis maybe made by taking a patient history and doing a brief examination. Treatment is usually started without or before confirmation by laboratory analysis, Stool and swab samples collected in the acute stage of the disease, before antibiotics have been administered, are the most useful specimens for laboratory diagnosis If an epidemic of cholera is suspected, the most common causative agent is V. cholera O1. If V. cholera serogroup O1 is not isolated, the laboratory should test for V. cholera O139.

However, if neither of these organisms is isolated, it is necessary to send stool specimens to a reference laboratory. Infection with V. cholera O139 should be reported and handled in the same manner as that caused by V. cholera O1 (CDC, 2010).

Treatment:-

Cholera patient being treated by:

Fluids: In most cases, cholera can be successfully treated with oral rehydration therapy (ORT), which is highly effective, safe, and simple to administer, Rice-based solutions are preferred to glucose-based ones due to greater efficiency [5. In severe cases with significant dehydration, intravenous rehydration may be necessary. Ringer's lactate is the preferred solution, often with added potassium ((Sack DA, Sack RB, Nair GB, 2004; WHO, 2005).

Electrolytes:- As there frequently is initially acidosis, the potassium level may be normal, even though large losses have occurred ,As the dehydration is corrected, potassium levels may decrease rapidly, and thus need to be replaced(Sack DA, &etal., 2004).

Antibiotics:- Antibiotic treatments for one to three days shorten the course of the disease and reduce the severity of the symptoms, Use of antibiotics also reduces fluid requirements , People will recover without them, however, if sufficient hydration is maintained(Sack DA, Sack RB , 2006).

Doxycycline is typically used first line, although some strains of V. cholerae have shown resistance , Testing for resistance during an outbreak can help determine appropriate future choices(Sack DA, Sack RB, Nair GB, 2004).

Vaccination :-

A number of safe and effective oral vaccines for cholera are available , Dukoral, an orally administered, inactivated whole cell

vaccine, has an overall efficacy of about 52% during the first year after being given and 62% in the second year, with minimal side effects(Sinclair D et al, 2011). It is available in over 60 countries. However, it is not currently recommended by the Centers for Disease Control and Prevention (CDC) for most people traveling from the United States to endemic countries (CDC, 2010).

However, as of 2010, it has limited availability, Work is under way to investigate the role of mass vaccination (WHO,2010). The World Health Organization (WHO) recommends immunization of high risk groups, such as children and people with HIV, in countries where this disease is endemic, If people are immunized broadly, herd immunity results, with a decrease in the amount of contaminant ion in the environment (Sack DA, Sack RB, 2006).

2.6: sampling:-

General Guidelines for Sampling:-

• Rinse the sample container three times with the sample before it is filled.

• Leave a small air space in the bottle to allow mixing of sample at the time of analysis.

• Label the sample container properly, preferably by attaching an appropriately inscribed tag or label. The sample code and the sampling date should be clearly marked on the sample container or the tag.

• Complete the sample identification form for each sample.

2.7:drinking water treatment

Surface water may contain pathogenic organisms, suspended or organic substances. Appropriate treatment may be necessary to render the water supply bacteriological safe, physical and chemical acceptable. Modern technology provides a choice of treatment methods to produce water of a desired quality from any given source (WHO, 2002). The

purpose of water treatment is to produce water that is safe and wholesome. The method of treatment to be employed depends upon the nature of raw water and the desired standards of water quality. Ground water (wells and springs) may need no treatment other disinfection, surface water (rivers, stream and lakes) which tends to be turbid and polluted required extensive treatment (Park, 2005). Water can be treated at various stages between the source and the end users, a limited number of technologies can be applied at source but most are used after water has been abstracted (IWSC, 2006). The concept of multiple barriers for water treatment is the cornerstone of safe drinking water production, traditionally the barriers have included:

- Protection of source water (screening and straining).

- Storage.

- Filtration.

- Disinfection.

- Protection of the distribution system (UNHCR, 1992; WHO, 2004).

Protection of source water:-

Protection of water sources can minimize the need for complex, costly or time and energy consuming treatments (IWSC, 2006). Protection of source water can help to minimize microbial risk associated with the water entering a drinking water treatment plant. Possible control measures to protect source water include land acquisition, water shed-inspection programmed. Water used for drinking should be originating from the highest quality source possible (WHO, 2004).

Storage:-Storage provides a reserve of water from which further pollution is excluded. As a result of storage a vary considerable amount of purification takes place, this natural purification (Park, 2005)

Filtration:-Filtration is the second stage in the water purification, and a quite an important stage because 98-99 % of the bacteria are removed by filtration. Two types of filters are in use, the slow sand filters (biological filters) and the rapid sand filters (mechanical filters)(Park, 2005).

Coagulation:-The water is first treated with a chemical coagulant such as alum, the dose of which varies from 5-40ml or more per liters depending upon the turbidity, color, temperature, and the PH value of water (Park, 2005).-

Sedimentation:-The coagulated water is led into sedimentation tanks where is detained for period varying from 2-4 hours when the flocculent precipitate together with impurities and bacteria settle down in the tanks. For proper operation the taks should be cleaned regularly from time to time to remove precipitate which settles at the bottom for avoid a breeding ground of molluses and sponges (Park, 2005). Sedimentation is a solid- liquid separation process in which particles settle under the force of gravity (WHO, 2004).

Filtration:-The clarified water is subjected to rapid sand filtration; filtration removes microbial pathogens mainly by size exultation that is microbes larger than the membrane pores are removed. Chemical coagulation is not usually needed before membrane treatment for removal of microbes (WHO, 2004).

The membrane filtration process most commonly used to remove microbes from drinking water are microfiltration (MF), ultra filtration (UF), nano filtration (NF),and reverse osmosis(RO) (AWWA, 1996; Taylor & Wiesner, 1999).

Disinfection:- Disinfection serves to destroy pathogenic organisms which may cause various types of water- borne diseases and it can considered as the final stage in the water treatment process (UNHCR, 1992). Disinfection method may be either physical or chemical:

*Physical methods including boiling, ultra violet (UV), irradiation etc.

*Chemical methods including use of oxidants (halogens, and halogen compounds such as chlorine, bromine, iodine, ozone, potassium perma nganate and hydrogen peroxide etc. (SSMO, 2003).

Chlorination: Chlorination the most important technological developments in the water treatment, during the twentieth century introduction in 1908, it's provided a cheap reproducible method of ensuring the bacteriological quality of drinking water (Moeller, 2005). Chlorination can be achieved using liquefied chlorine gas, sodium hypo chlorite, solution or calcium hypochlorite granules and on-site chlorine generators (WHO, 2004).

Water Distribution Network:- Drinking water monitoring based upon tests for coliform bacteria as indicators of fecal contamination originated approximately 100 years ago (Cox, 1997). At that time, most waterborne disease outbreaks were caused by pathogenic organisms and could be clearly traced to fecal contamination of drinking water.

conditions that may provide information on distribution system deficiencies and integrity problems is an important tool for protecting the public health (EPA, 2006).

Water can be transported from the source to the treatment plant, if any, and the distribution system, and eventually reach consumers through one of the following methods:

Through direct pumping to the distribution system:-In this system, water is pumped directly from the source to the distribution system to the consumers.(world bank,2012).

There are two type of Water Distribution Network Branch Network and Loop Network (Niklesh R .Murekar, et al.,2011). Treated water conveyed through a piped network is exposed to numerous surfaces. It is important that no materials placed in contact with the

drinking water in the network promote microbial growth or leach any contaminants into the water that can support microbial growth (WHO.2004) .

Branched System:-Also Branched systems referred to as a Dead-end System, the size of the main line in this distribution system decreases as its distance from the source increases, in consideration that the further pipes have to carry less water. The design of a branched system is generally straightforward, where the direction of water flow in all pipes and the flow rate can be readily determined , illustrates a branched or dead-end system. One of the advantages of a branched system is generally lower costs (World bank,2012)

Distribution System Problems

The distribution system problems are grouped into the following sequential focus areas:

Pathways that Breach Distribution System Integrity

Distribution System Contamination:-

• Fecal Contamination

• Toxic or carcinogenic contamination

Public Health Risk:-the pathways that breach distribution system integrity can generally be thought of as external (i.e., cross connection, intrusion, main breaks, etc.) or internal (i.e., biofilms, corrosion and leaching).(EPA,2006).

Flushing and pigging are routine maintenance practices often conducted within the distribution system to address consumer complaints and to reduce the retention time of water to improve water quality. (Brandt et al., 2004).

Operation and Maintenance Deficiencies:- Flushing and pigging are routine maintenance practices often conducted within the distribution system to address consumer complaints and to reduce the retention time

of water to improve water quality. Utilities have typically manually flushed water from the system using fire hydrants or flushing hydrants to control microbial growth, These practices can affect the distribution system water quality in a negative manner if not conducted properly. Improper flushing can result in moving a contaminant further into the distribution system (Brandt et al., 2004).

Finished Covered Storage Tank Deficiencies:-Storage tank deficiencies, such as vents without screens, inadequate hatches, access hatches that are not locked, and physical openings in storage tank roofs, can result in the entry of contaminants. Coatings on the storage tank interior can also result in contamination if the coating fails or is not properly cured. Potential public health issues associated with finished water storage facilities are described in a distribution system white paper on covered storage (AWWA & EES, 2002).

Biofilms:-Biofilms are defined as a complex mixture of microbes, organic, and inorganic material accumulated amidst a microbial produced organic polymer matrix attached to the inner surface of the distribution system (USEPA, 2002). Contaminants, including total coli forms and some pathogens, may attach to or become enmeshed in biofilms on pipe walls in distribution systems. Many pathogens have been found to survive, if not grow, in these pipe biofilms where they are protected from disinfectants. Over time, coliform bacteria may detach or slough from the biofilm, causing persistent total coli form detections. Pathogens may also be included in the detached material and may result in waterborne disease. The biofilm can result in total coli form positive detections and other contamination events if disturbed, Organisms that have been found in biofilms include bacteria, viruses, protozoa, invertebrates, algae, and fungi (USEPA, 2002).

Protection of distribution system:- Protection of the distribution system is the last and one of the most Important of the multiple barriers necessary for provision of safe drinking water. Any microbial contamination of this point has a high probability of Resulting in public health risk even if previous control steps have been applied effectively. Because of the extensive nature of the distribution system, with many kilometer of pipe (Geld rich, 1996; Geldericg & Lechevallier, 1999; Ainsworth, 2004).

Hazard control strategies should be focus on three essential elements as following:

*Maintaining the quality of the treated water by adequate maintance of distribution system.

*Minimizing bacteria growth.

*Preventing recontamination of water during distribution (WHO, 2004). Contamination of water supplies should be avoided at all times. In most small water supply systems, however, economic reasons prevent 24-hour daily water service. This creates a risk of polluted water infiltrating into the pipelines through leaks in pipe Joints and service taps. To counter the health risk, 0.3 mg/L residual chlorine should be maintained throughout the distribution system.

2.8.1 Effects of More Intense Rainfall Events:

1. Increased turbidity and sedimentation;

2. Loss of reservoir storage;

3. Water filtration or filtration/avoidance treatment challenges;

4. Increased risk of direct flood damage to water utility facilities. (world bank,2012)

Increased precipitation will increase the risk of flooding in many areas of the world.

Floods can increase human exposure to pathogens, as contaminants are spread by floodwaters. Developing countries are particularly susceptible to this, as water carries wastes, and drainage and sewage systems can become backed up. Water treatment facilities can become damaged, which can result in the distribution of untreated or improperly treated water. Sewer and water pipes can break, which can cause drinking water to become contaminated with sewage. Floods can also transport fecal matter from the ground or sewers that have overflowed, and contaminate wells, boreholes and surface waters.

There are main categories of diseases that result from floods. The most important includes waterborne diseases, the most common being a variety of diarrheal illnesses (WHO,2007).

Sea level rise will increase salinisation of groundwater, seriously impacting the health of the population; this will promote algal blooms and increase the bacterial and fungal content. This will, in turn, impact adversely upon ecosystems, human health, and the reliability and operating costs of water systems, rapidly growing urbanization combined with increasing demand for freshwater and non-existent or inadequate sanitation infrastructure poses a threat to public health and increases water-borne diseases. Sanitation systems may be damaged by flooding and infrastructural deterioration caused by extreme weather conditions, interrupting services and further compromising the quality of drinking water (IPCC, 2008).

2.9: Bacteriological analysis:

2.10: Physical analysis:

Turbidity: reagents and equipment (plainest color/ turbidity set, plainest automatic wavelength selection photometer).

2.11: Chemical analysis:

PH: reagents &equipment (palintest phenol red clear tablets, palintest automatic wavelength selection photometer and round test 10ml glass).

shallow wells are still used beside the other source in some of the selected village in Shendi .all selected shallow wells were liable to pollution by neighboring source of contamination such as latrines .

all of these wells were not conforming to the criteria and quality standards of safe and whole some water are define by who or the Sudan (SSMO, 2002)**.**

Chapter Three

Material and Method

Material and Methods

3-1 Study design:

Across sectional descriptive study conducted in shendi town in the period from March 2018 to sebtmber2018.

3-2 Study area:

Shendi Town is well known historically, and it is the third largest Town in River Nile State. It is in River Nile State, where the Headquarter of Shendi locality is located.

Shendi is located about 176 km north of Khartoum , and 130 km south of El damer (capital of River Nile State).It is bound by River Nile in the west and Kasala State in the East, also bound by south Shendi administrative unit in the South and north Shendi administrative unit in the North.

3-3: Sample size:

26sample of tap water from different area of shendi town was collected in sterilized container (50ml).

3-4: Data Collection:

The data were collected by observation and facilities of storage at house hold and laboratory sample were collection from identification site of drinking water supply and were analyzed to determine the bacteriological quality of drinking water.

3-:5: Data analysis:

This was descriptive study.

3:6: Bacteriological analysis:

3-**6-1: sample collection:**

After obtaining sterilized bottles, samples were collected from identified sites in Shendi Town.

3-6-2: material, apparatus 'and equipment:

Sterilized bottles, Flask, gloves, Loops, Petri dishes, Oven, autoclave, incubator, Refrigerator, Kov'cs, cotton, Peptone water, distilled water, Flame and marking pencil.

Sterilization of media:

Media were sterilized by autoclaving at 121 ºC for 15 min (haring 1998) .

3-6-3:Bacteriological Media Used:

Motility media: -

MacConkey Agar

MacConkey Agar is recommended for selective isolation of Escherichia coli from pharmaceutical products and is in accordanceonized methodology of BP. It is also recommended for selective isolation and differentiation of lactose fermenting and lactose non fermenting enteric bacteria.

Directions

Suspend 49.53 grams of dehydrated medium in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes i.e. validated cycle. AVOID OVERHEATING.

Cool to 45-50°C. Mix well before pouring into sterile Petri plates. The surface of the medium should be dry when inoculated.

Principle And Interpretation

MacConkey Agar is the earliest selective and differential medium for cultivation of coli form organisms (MacConkey, 1900 , 1905). Subsequently MacConkey Agar and Broth have been recommended for use in microbiological examination of foodstuffs (Downes F P and Ito K(Eds.), 2001) and for direct plating / inoculation of water samples for coli form counts (A W.,(Eds.), 2005) has recommended this medium for

the subculture and identification of Escherichia coli . It is also cited as Agar Medium H., The United States Pharmacopoeia 2011. European Pharmacopoeia 2011. Japanese Pharmacopoeia, 2008).

Pancreatic digest of gelatin and peptones (meat and casein) provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms. Lactose monohydrate is the fermentable source of carbohydrate. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria.

Sodium chloride maintains the osmotic balance in the medium.

Interpretation of culture growth:

The plates were examined for any significant bacterial growth. The isolated bacteria were then identified by colonial morphology, Gram stain and biochemical tests and   count .

Microscopic examination:

1- **preparation of smear:**

Gram stain:

Principle:

Differences in Gram reaction between bacteria is thought to be due to differences in the permeability of the cell wall of Gram positive and Gram negative organisms during the staining process. Following staining with a triphenyl methane basic dye such as crystal violet and treatment with iodine, the dye–iodine complex is easily removed from the more permeable cell wall of Gram negative bacteria but not from the less permeable cell wall of Gram positive bacteria. Retention of crystal violet by Gram positive organisms may also be due in part to the more acidic protoplasm of these organisms binding to the basic dye (helped by the iodine) (Cheesbrough, 2006).

Biochemical tests:

Oxidase test (Cytochrome oxidase test):

The oxidase test is used to assist in the identification of *Pseudomonas*, Neisseria*, Vibrio*, Brucella*, and* Pasteurella species, all of which produce the enzyme cytochrome   oxidase.

Urea's test: Testing for urease enzyme activity is important in differentiating enter bacteria.

Principle:

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in color of the indicator to pink-red.

Indole test:

Principle:

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dim ethyl aminobenzaldehyde. This reacts with the indole to produce a red colored compound. Kovac's reagent is recommended in preference to Ehrlich's reagent for the detection of indole from enterobacteria.

Citrate utilization test:

This test is one of several techniques used occasionally to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon.

Kliger's Iron Agar (KIA):

This is a differential medium. It tests for organisms' abilities to ferment glucose and lactose to acid and acid plus gas end products. It also allows for identification of sulfur reducers. This media is commonly used

to separate lactose fermenting members of the family Enterobacteriaceae (e.g. Escherichia coli*)* from members that do not ferment lactose.

Principle: The first differential ingredient, glucose, is in very short supply. Organisms capable of fermenting this sugar will use it up within the first few hours of incubation. Glucose fermentation will create acidic byproducts that will turn the phenol red indicator in the media yellow. Thus, after the first few hours of incubation, the tube will be entirely yellow. At this point, when the glucose has been all used up, the organism must choose another food source. If the organism can ferment lactose, this is the sugar it will choose. Lactose fermentation will continue to produce acidic byproducts and the media will remain yellow (picture on the far left below). If gas is produced as a result of glucose or lactose fermentation, then fissures will appear in the agar or the agar will be lifted off the bottom of the tube. If an organism cannot use lactose as a food source it will be forced to use the amino acids / proteins in the media. The deamination of the amino acids creates NH_3, a weak base, which causes the medium to become alkaline. The alkaline pH causes the phenol red indicator to begin to turn red. Since the incubation time is short (18-24 h), only the slant has a chance to turn red and not the entire tube. Thus an organism that can ferment glucose but not lactose will produce a red slant and a yellow butt in a KIA tube (second from the left below). These organisms are the more serious pathogens of the GIT such as Shigellae dysenteries' (McFadden, 1980).

Chapter Four

Results

-Atotal of 26 water sample were cullured on Macconkyagarو 16 sample(61%)Showed positive bacterial growth .Table(1)

-The most common isolated bacteria from tap water were E.coli ,pseudomonas and less common Sallmonella pra tyhi A. .Table(2).

-The positive growth showed3samples (18.8%) Whitch were Gram positive and 13sample(81.2%)whitchwere gram negative. Table(3).

-The distrubition of type of microorganism in tap water .Table (4)

Table(1)shows growth of bacteria in macconky agar:

Bacterial growth	Frequency	Percentage
Growth	16	61.5
No growth	10	38.5
Total	26	100

Table(2)shows the isolated bacteria in tap water

Number of sample	Gram staining	Bacterial isolated
1	Gram -bacilli	E.coli
2	Gram -bacilli	pseudomonas
3	Gram -bacilli	E.coli
4	Gram -bacilli	pseudomonas
5	No growth	
6	Gram -bacilli	pseudomonas
7	No growth	
8	Gram + cocci	
9	No growth	
10	Gram -bacilli	Salmonella pratyphiA
11	No growth	
12	Gram -bacilli	pseudomonas
13	Gram + cocci	
14	No growth	
15	No growth	
16	No growth	
17	Gram -bacilli	pseudomonas
18	Gram -bacilli	pseudomonas
19	Gram -bacilli	pseudomonas
20	Gram + cocci	
21	No growth	
22	Gram -bacilli	pseudomonas
23	No growth	
24	No growth	
25	Gram -bacilli	E.coli
26	Gram -bacilli	Salmonella pratyphiA

Table(3)shows distributions of microorganism according to Gram staining :

Gram staining	Frequency	Percentage
Gram positive	3	18.8
Gram negative	13	81.2
Total	16	100

Table(4) shows distribution of type of microorganism in tap water :

Bacterial isolated	Frequency	Percentage
E.coli	3	23.1
Pseudomonas	8	61.5
Salmonella pratyphiA	2	15.4
Total	13	100

Chapter Five

Discussion

Conclusion

Recommendations

Discussion

This is prospective study done for tap examination to find out ground water microbiological fecal contamination either from animal or human. The city tap water supply from citywells source. 26samples were taken randomly from the wells.

This samples were cultured in Macconkey agar for isolation of enterobacterceae . The growth was identiefed in 16 Samples (61.5%). the growth rate of E.coli which was indicator of fecal contaminatationwas found in 3samples well (23.1%). about the others Gram negative bacilli isolated were salmonella pratyphi A in 2 well samples (15.4%) and pseudomonas 8 wells sample (61.5%), so it may be environmental contamination. This result aggreed with study done in Basrach. which concluded that Tap water samples were found to be highly contaminated with many types of pathogenic bacteria such as Coli form (E.coli64.28% occurrence in tap water)*Salmonella, Shigella, and Pseudomonas, the study showed lower contamination than the above study. .(Hussein K. and Kassim 2012).*

The frequency of contamination ot drinking tap water in Shendi water suppling wells was 13well samples (23.1%).
significant E.coli count which indicate real contamination were encuontered in sample (1) and (25), and sample (3) .

In comparsion betweeen the study and study done by Eltagni et al which found the frequency of drinking water contamination was 27.1% in Shendi town approximately the same result as this study.(Eltigani O et al.2012) .

In other study conducted in urban Slums،India showed a rate of 43% drindig water contamination. Subbaraman etal. which was higher than the contamination rate in this study.

45

5.2:Conclusion:

   some of tap water from well were contaminated with bacteria such as E.coli, pseudomonas ,sallmonella.

5.3: Recommendation:

- So based on finding of this study and conclusion -thesis recommend related authorities by the following :-
- Health authority to find source of tap water contamination   and treated.
- Any tap water from well should be investigation and follow up.
- Health authority of locality should be establish closed surveillance system to follow up quality of drinking water according to who guidelines and selected suitable time.
- Shendi university coordination with of public health should raise and spread awareness about importance of safety and adequate drinking also must me created -encourage and support more study about drinking water quality and other water associated disease .

References& appendices

References

- Abdel–magid I. M. (1995). Hand book of waste water Reclamation & Reuse, Lewis publisher is an imprint of CRC press.

- Ainsworth R. ed (2004). Safe pipe water: managing microbial water quality in piped distribution system. World Health Organization, Geneva.

- APHA, AWWA and WEF (1998). Standards methods for the examination of water and waste water, 20th ed. , Washington.

- AWWA and EES. (2002a). New and Repaired Mains. Distribution System White Paper. Available online at: http://www.epa.gov/safewater/tcr/tcr.html. Accessed 12/12/2006.

- AWWA, Lyonnais des Eaux and water research commission of South Africa (1996). Water treatment membrane process. New York , Mc Graw Hill, Inc

- Brandt, M., J. Clement, J. Powell, R. Casey, D. Holt, N. Harris, and C. Ta.(2004) . Managing Distribution Retention Time to Improve Water Quality - Phase I. AWWA RF. Denver, CO.

- CDC (2010). Laboratory Methods for the Diagnosis of Epidemic Dysentery and Cholera (Atlanta, GA: CDC. .http://www.cdc.gov/ncidod/dbmd/diseaseinfo/cholera/top.pdf.

- CDC (2010). Community Health Worker Training Materials for Cholera Prevention and Control (Atlanta, GA).

- CDC (2012).Typhoid Vaccines, Vaccine Information Statement. www.cdc.gov/ncidod/dbmd/diseaseinfo/typhoidfever_g.htm

- Cheesbrough M. (2006). District laboratory practice in tropical countries. 2nd edition. Part 2.United States of America: Cambridge University Press.

- Cox, W. E. (1997). Evolution of the Safe Drinking Water Act: A Search for Effective Quality Assurance Strategies and Workable Concepts of Federalism. William and Mary Environmental Law and Policy Review 21.

- Diab, A. M., Abdel Aziz, M. H. and Abel Hamid, S. ,(2000). Drinking Water Qualities of Different Purification Systems in Ismailia Governorate. Egypt J. Microbiol. 35, No.4, pp. 533-534.

- DHS-Cameroon Demographic and Health Surveym,(2004). Mother and Child Mortality.Macro International,Caverton,MD. pp:135,138.

- Downes F P and Ito K(Eds.), 2001, Compendium of Methods For The Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C

- Eaton A. D., Clesceri L. S. and Greenberg A W.,(Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington, D.C.5. ,,

- Effa EE, Lassi ZS, Critchley JA, . (2011). Bhutta, Zulfiqar A. ed. "Fluoroquinolones for treating typhoid and paratyphoid fever (enteric fever)". *Cochrane Database Syst Rev* (10): CD004530

- Eltigani O M Omer, Sallam T (2012) Bacteriogical Investigation of Drinking Water' Department of Public Health, Faculty of Medicine and Health Sciences, University of Shendi, Shendi,

- EPA(2006). Distribution system monitors of drinking water quality , U S EPA office of ground water and drinking water.

- European Pharmacopoeia 2011, European Dept. for the quality of Medicine.

- Gelderich E.E. (1996). Microbial quality of water supply in distribution system. Boca Raton, Fl, Lewis publishers.

- Gelderich E.E., LEchevallier MW (1999). Microbial water quality in distribution system. In: letter man RD, ed. Water quality and treatment 5th ed. New York, Mc Graw. Hill.

- Harrigan, W.F.1998.laboratory methods in food microbiology. 3rd edition Academic Press Limited ,London.

- Henock, N.Y. and Dovie D.B.,(2007). Diarrheal disease in the history of public health .Arch Med.Rese.,38:159-163.

- Heymann, DL,. (2004). Control of Communicable Diseases Manual. 18 nth edition. Washington, DC: American Public Health Association .

- Hussein K . Abdul-Sadda and Kassim Fawzi Abdul-kareem Department of Pharmacology and Clinical laboratory Sciences College of pharmacy/ University of Basrah2012.

- IPCC (2008). Climate Change and Water. Technical Paper of the Intergovernmental Panel on Climate Change. Bates, B. C., Kundzewicz, Z. W., Wu, S., Palutikof, J. P. (eds), Geneva .

- IWSC (2006). Land scalping of technologies. An out put of the projects landscaping and review of approaches and technologies for water, sanitation and hygiene, cranfied University.

- Japanese Pharmacopoeia, 2008

- John P, Harley & Lansing M. Prescott (2002). Laboratory Exercises in Microbiology, 5th edition, USA .The McGraw-Hill Companies.

- MacConkey, 1900, The Lancet, ii:20.

- MacConkey, 1905, J. Hyg., 5:333.

- McFadden JF. (1980). Biochemical Tests for Identification of Medical Bacteria, 2nd Edition. Williams and Wilkins, Baltimore.

- Moeller W. D. (2005). Environmental health, 3rd edition, printed by TJ international (Ltd) pad stow, Cornwall, UK.

- Murray, M. T. ,(1997). Stomach Ailments and Digestive Disturbances. Rocklin, CA: Prima Publishing,pp:98-101.

- Niklesh R. and Murekar N. R. (2011). Design Of Distribution Network Of Water Supply For Kudwa And Katang i-Kala Villages, International Journal Of Advanced Engineering Sciences And Technologies Vol No. 7, Issue No. 2, 178196 Gasketed Pipe Joints Journal AWWA, Vol. 84(7):92-100.

- Oxford English Dictionary (2011). *Typhoid, adj. and n.* and *typhus, n.* Online version March 2011. Retrieved May 2011.

- Park K. (2005). Text book of preventive and social medicine 18th, ed, Ms/banarsidas bhanot publishers 1167, prem nagar, Jabalpur, 482001 (India).

- Parry CM, Beeching NJ (2009). "Treatment of enteric fever". *BMJ* 338:b1159–b1159.

- Ratnayake Eranda C., Chrishan Shivanthan and Bandula C Wijesiriwardena(2011). Cholestatic hepatitis in a patient with typhoid fever - a case report, Annals of Clinical Microbiology and Antimicrobials 2011.

- Sack DA, Sack RB, Nair GB, Siddique AK. (2004). "Cholera". *Lancet* 363 (9404): 223–33. doi:10.1016/S0140-6736(03)15328-7.

- Sack DA, Sack RB and Chaignat CL (August 2006). "Getting serious about cholera". *N. Engl. J. Med.* 355 (7): 649–51.

- Salvato, P.E. Joseph A. (1982). Environmental engineering and sanitation, John Wiley & Sons 3rd ed. United States of America, New York State.

- Sinclair D, Abba K, Zaman K, Qadri Fand Graves PM (2011). "Oral vaccines for preventing cholera". *Cochrane Database Syst Rev* (3): CD008603. □SSMO (2002). Drinking water guide lines, first edition, republic of Sudan, March 2002, Khartoum.

- SSMO (2006). Drinking water treatment guide lines, Khartoum.

- Sur D. (2004). Cholera disease ,challenges and management issues ,international vaccine institute , south Korea.

- Taylor J.S., Wiesner M. (1999). Membranes in letter man RD. ed., water quality and treatment. New York, Mc. Graw Hill, Inc.

- The United States Pharmacopoeia 2011, The United States Pharmacopoeial Convention. Rockville, MD.

- UNHCR (1992). Water manual for refugees situations programme and technical support section, Geneva.

- UNICEF (1999). A water hand book, water, Environment and sanitation technical guide lines series –No 2. New York.

- USEPA (2002). Health Risks From Microbial Growth and Biofilms in DrinkingWaterDistributionSystems.
  http://www.epa.gov/safewater/tcr/pdf/biofilms.pdf.

- UNICEF (2008). Hand book on water quality, plaza, new York, USA.

- WHO (1997). Guide lines for drinking water quality, volume 3, surveillance and control of community supplies, 2nd ed. ,Geneva.

- WHO (2002). Guide lines for drinking water standards in developing countries, pan American health organization, Regional office of the world health organization, Lima.

- WHO (2004). Water treatment and pathogen control, process efficiency in achieving safe drinking water, first published printed by Tj international (Ltd), Dad stow, cornwall, UK.

- WHO (2004). Guide lines for drinking water quality, 3rd ed. Volume 1, recommendations, Geneva.

- WHO (2004). Communicable disease control in emergencies, Afield manual guide line for facility managers, 2nd ed. .

- WHO (2006). Guide lines for drinking water quality, volume 1, a first addendum (recommendations), 3rd ed. , Geneva.

- WHO (1997). "*WHO/PAHO/UNESCO report. A consultation with experts on amoebiasis. Mexico City, Mexico 28–29 January 1997.".* Epidemiological Bulletin *18 (1): 13–14. PMID 9197085*

- WHO (2007). World Health Organization.. Water - related diseases: Diarrhoea.
  http://www.who.int/water_sanitation_health/diseases/diarrhoea/en/.

- WHO (2008). "Cholera: prevention and control". *Health topics.* http://www.who.int/topics/cholera/control/en/index.html. Retrieved 2008-12-08.

- WHO (2011). Hardness in drinking water, background document for development of WHO guidelines for drinking water quality, Geneva, Switzerland.

- WHO (2000). Report on global surveillance of epidemic water-borne infectious diseases, department of communicable diseases sueveillance and response.

- WHO (2007). Compacting water –borne diseases at the household level, WHO document production services, Geneva Switzerland.

- WHO (2008). Water quality interventions to prevent, diarrhea , cost and cost effectiveness, WHO document production services, Geneva Switzerland.

- WHO (1984). Guidelines for drinking Water Quality, World Health Organization Press.

- WHO. Healthy situation in Basra-UN .humanitarian briefing .2003.

- WHO (2004). Guidelines for drinking-water quality. Third edition.Volume 1 Recommendations. Geneva, World Health Organization.

- WHO.,(2005).Guidlines for the control of shigellosis, including epidemics due to *Shigella dysenteriae* type .Child adolescent health documents. Vol.34:98v

- WHO (2006). Guidelines for drinking-water quality. Volume 1 Recommendations. First addendum to the third edition. Geneva, World Health Organization.

- WHO (2007). Chemical safety of drinking-water – assessing priorities for risk management Geneva, World Health Organization.

- WHO (2008). Guidelines for drinking-water quality. Third edition, incorporating the first and second addenda. Volume 1 Recommendations. Geneva, World Health Organization.

- WHO (2009). Water safety plan manual: step-by-step risk management for drinking-water suppliers. Geneva, World Health Organization.

- WHO (2005). The treatment of diarrhea, a manual for physicians and other senior health workers,) and esp. chapter ―5. management of suspected cholera, pages 16-17
http://www.who.int/emc(department of communicable diseases and response)

- whocdscsredc200112/en/index1.html.Retrieved 26 January 2012.

- World bank (2012). Rural water supply, design volume I manila , Philippines.

Appendix

Collection of sample:-

As follows:

- Clean the tap to remove any attachments that may cause splashing by using clean cloth.

- Open the tap at maximum flow and let the water run for 1-2 minute

- Sterilize the tap for a minute with cigarette lighter.

- Open the tap before sampling, allow the water to flow for 1-2 minutes at medium flow rate.

- Open the sterilized bottle and took out bottle carefully unscrew the tap.

- Fill the bottle and immediately hold the bottle under the water jet and fill it and leave small air space to shaking before analysis.

- Cap the bottle carefully and keep it in the ice box before Transportation to laboratory.

1 - 1 ml of the sample of water by pipette of it was poured into the Macconky agar medium and left for a period of five minutes .

2- Get rid of excess.

3- We put the agricultural medium in incubator at a temperature of 37 ℃ for 24 hours under an

Microscopic examination:

Preparation of smear:

1- On clean dry slide one drop of normal saline was putted and by loop after sterilization small amount of well grown single bacterial colony was taken from the agar plate and mixed with normal saline.

2- bacteria and normal saline were well mixed and spread on slide in area about 1 cm.

3- Slide was left to air dry then fixed by heating by flame by passing the slide in flame 3 times.(cheese brough-2006).

Gram stain:

Procedure:

1- After making heat fixed smear, the slide was putted in staining rack.

2- The smear was covered with the basic stain crystal violet then left for 1 minute.

3- Washed by tape water then covered the smear with the mordant lugol´s iodine for 1 minute then washed by tape water.

4- The smear was covered with the decolorizer 95% acetone alcohol for 5 seconds then washed by tape water.

5- Finally the smear was covered with the counter stain Saffranin and left it for 2 minutes then washed by tape water.

6- The smear was dried by air and examined under microscope using 100X lance.

Results Gram positive bacteria . . . . Dark purple.

Gram negative bacteria . . . . Pale to dark red. (Cheesbrough, 2006).

Oxidase test:
Method using an oxidase reagent disc:
1. One disc was putted of oxidase disc on flat surface.
2. By using a piece of stick or glass rod (not an oxidized wire loop) a colony of the test organism was removed and rubbed on the disc.
3. A purple color was looked within 10 seconds.
Indole test:

Detecting indole using peptone water:

1. The test organism was inoculated in a tube containing 3 ml of sterile peptone water.

2. Then Incubated at 37ºC for 24 h.

3. Indole was tested by adding 0.5 ml of Kovac'sreagent. Shaked gently. A red color in the surface layer within 10 minutes were examined
Citrate method using Simmon's citrate agar:

Method:

1. Slopes of the medium were prepared in bijou bottles as recommended by the manufacturer.

2. Using a sterile straight wire, firstly the slope was streaked with the test organism and then stab the butt.

3. At 35ºC for 24 hours media was incubated. Then looked for a bright blue color in the medium.(cheese brough-2006).

kligler,s iron agar:

Procedure:

1. The KIA agar slants were labeled with the name of the bacterium to be inoculated. One of the tubes was used as a control.

2. Aseptic technique was used, the slant was streaked with the appropriate bacterium and then the butt was stabbed. The caps on the tubes were screwed but do not tighten!

3. Only for 18 to 24 hours at 35°C media was incubated for changes in the butt and on the slant. Tubes should be incubated and checked daily for up to seven days in order to observe blackening (John, 2002).

Bacteriological Media Used:

Motility media:

Gelatin	80g
Peptone	10g
Beef extract	3g
NaCl	5g
Agar	4gm
Distilled water	1liter

MacConkey Agar

Composition**

Ingredients Gms / Litre

Peptones (meat and casein) 3.000

Pancreatic digest of gelatin 17.000

Lactose monohydrate 10.000

Bile salts 1.500

Sodium chloride 5.000

Crystal violet 0.001

Neutral red 0.030

Agar 13.500

pH after sterilization(at 25°C) 7.1±0.2   (MacConkey, 1900 , 1905).