Original Research Article

Assessment of Bacterial contamination in drinking water sources in Khartoum/ Sudan

ABSTRACT

Aims of the Study: To assess the bacterial contamination in drinking water sources in Khartoum/ Sudan

Place of Study: Central Veterinary Research Laboratory/ Bacteriology Department.

Study Design: One hundred water samples were collected from the three localities of Khartoum state (Khartoum= 33, Omdurman= 34, Khartoum north [Bahri] =25) and 8 from different companies of water supply.

Methodology: Fifty four Samples were collected from surface water and (38) from ground water [well]. These samples transported to bacteriology lab for microbiological analyses using filtration method and new technique Colilert and Pseudalert kits which used for the first time in Sudan.

Results: Filtration method revealed different bacterial species, they were: *Escherichia coli*, *Klebsiella pneumonia*, *Enterobacter aerogene Enterobacter sakazaki*, *Enterobacter cloacae*, *Serratia marinoruba*, *Proteus mirabilis*, *Salmonella* spp. *Raoultella terrigena\planticola*, *Orchobacter anthrobi*, *Cronobater* spp., *Aeromonas salmonicida*, *Aeromonas hydrophilia*, *Pantoea agglomerans*, *Vibrio parahaemolyticus*. Coliform bacteria, *Escherichia coli* and *Pseudomonas* app were detected and most probable numbers (MPN) were counted using the previous kits according to manufacture instructions.

Conclusions: The water must be tested before using and quality control technique must be achievable to ensure continuously supply of pure drinking water.

Key words: Drinking water, E. coli, Pseudomonas spp., Coliform, Colilert, Pseudalert, MPN

1. INTRODUCTION

Water is essential to life. An adequate, safe and accessible supply must be available to all. Improving access to safe drinking-water can result in significant benefits to health. Every effort should be made to achieve a drinking water quality as safe as possible. In general terms, 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 [1-4].

Bacteria of greatest concern in drinking water are those that originate from the gut of warm-blooded animals. Sources include wildlife, pets, and contamination problems arise from improperly designed, failing, or overloaded waste water treatment systems, including septic systems from private homes, and leaking sanitary sewer pipes. Human sources are a particular concern as they include bacteria of human origin and may include human pathogens. Floodwater commonly contains high levels of bacteria from numerous sources.

Surface waters typically contain bacteria. Groundwater should be free of those bacteria that arise from animal sources. Proper well design and construction should protect drinking water sources from contamination by surface waters, livestock on farms or in feedlots.

A total coliform bacterium is a group of different kinds of bacteria commonly found in the environment, including soil, vegetation and untreated surface water. Total coliform bacteria are generally not harmful. Fecal coliform bacteria are a subgroup of the total coliform group.

The most important species of the group include *Escherishia coli*, *Klebsiella spp* and *Enterobacter spp*. At the same time, non-coliform bacteria were also found in polluted water such as *Sterptococcus*, *Proteus* and *Pseudomonas* species [5-7].

In this study The Colilert test was used which is an advance test because it uses proprietary Defined Substrate Technology (DST) to simultaneously detect total coliforms and *E. coli* two nutrient-indicators, ONPG and MUG, are the major sources of carbon in Colilert and can be metabolized by the coliform enzyme β-galactosidase and the *E. coli* enzyme β-glucuronidase, respectively. The Pseudalert test was used to detect *Pseudomonas aeruginosa* in water sample. The test is based on a bacterial enzyme detection technology that signals the presence of *Pseudomonas aeruginosa* through the hydrolysis of substrate in the Pseudalert reagent. Pseudomonas cells rapidly grow and reproduce using the rich supply of amino acids, vitamins and other nutrients present in the Pseudalert reagent [8].

This approach is different from traditional media, which provide a nutrient-rich environment that supports the growth of both target organisms and non-targets. When non targets grow and mimic target organisms, false positives occur. Growth of non-targets can also suppress target organisms and give false negatives in traditional media. To suppress non targets, traditional media often include high levels of salts, detergents, or other selective agents that may inadvertently suppress target organisms and give further false negatives.

2. MATERIALS AND METHODS

This study was designed to evaluate drinking water hygiene, with special reference to the incidence of bacterial water-borne disease in some parts of Sudan using new technique (Colilert and Pseudalert test) for the first time in Sudan.

The Colilert test simultaneously detects or quantifies both total coliforms and *E coli*, with results in 24 hours. As coliforms grow in the Colilert Test, they use β-galactosidase to metabolize ONPG and change it from colorless to yellow. *E. coli* use β-glucuronidase to metabolize MUG and create fluorescence. Since most non-coliforms do not have these enzymes, they are unable to grow and interfere. The few non-coliforms that do have these enzymes are selectively suppressed

by the Colilert Test's specifically formulated matrix. Pseudalert test also detects and quantifies *Pseudomonas aeruginosa*, actively growing strains have an enzyme that cleave substrate in the reagent to produce blue fluorescence under ultraviolet light.

2.1 Samples collection:

One hundred water samples from three localities in Khartoum state (Khartoum, Khartoum north (Bahri), Omdurman were collected aseptically in sterile plastic container then labeled and placed in an ice-box and transported immediately to the Central Veterinary Research Laboratory, department of Bacteriology for microbiological analysis. Each sample consist of 300 ml distributed in 3 container (each one 100 ml). Thirty six samples were taken from well (ground water), 56 samples directly from pipe (surface water) and 8 purified drinking water samples from different companies. These samples were classified as followed:

The surface water samples were: 11 samples from Khartoum, 19 from Omdurman and 26 from Khartoum north (Bahri).

The ground water samples were: 14 samples from Khartoum, 15 from Omdurman and 7 samples from Khartoum north (Bahri).

The purified samples were: 8 samples from different companies

2.2 Methodology:

2.2.1 Filtration Method:

One hundred ml each of sample was filtered through filter papers (4.5 µm in diameter) using filtration system, then the membrane was put on sterile Petri dishes containing absorbable paper impregnated with 2.5 ml sterile Endo broth media then incubated for 24h at 37^{o}C. Well isolated colonies from filter paper as shown in (fig 1) were sub cultured on nutrient agar plates for purification, then identification using conventional methods and API kits.

2.2.2 Analysis of water sample using Pseudalert and Colilert (Kits):

One hundred ml of water was used for Pseudalert and another 100 ml for Colilert. Each was incubated at 37ºC for 24 hours. After incubation the results were recorded according to manufacturer instructors (IDEXX) and the Most Probable Number (MPN) was calculated.

For E.coli and coliform detection using Colilert test, reagent was added to samples and incubated at 37°C for 24 hours. the following results indicate the presence or absent of bacteria: Colorless = negative, Yellow = total coliforms and Yellow/fluorescent = E. coli (as shown in fig 2). In case of Presence/Absence of *Pseudomonas aeroginosa* detected by (Pseudalert), reagent was added to samples and incubated at 37°C for 24 hours. Under ultraviolet light blue, fluorescence indicates presence of *Pseudomonas aeuginosa*.

In case of Quantification, reagent was added to sample then poured into Quanti-Tray (counts from 1–200) or Quanti-Tray/2000 (counts from 1–2,419), Sealed and incubated at 37°C for 24

hours. After that results were read as followed: Yellow wells = total coliforms, Yellow/fluorescent wells = *E. coli*, then positive wells were Count and refer to MPN table.
Under ultraviolet light, Blue fluorescence indicates the presence of *Pseudomonas aeruginosa*.

3. RESULTS

3.1 Out of 56 surface water samples were revealed the following bacterial species: *Escherichia coli, Pseudomonas* spp, *Aeromonas salmonicida, Aeromonas hydrophila caviae/sobria, Enterobacter sakazaki, Enterobacter aerogene, Enterobacter cloacae, Proteus mirabilis* and other *proteus* spp, *Klebsiella pneumoniae, Pantoea agglomerans, pantoea spp. Raoultella terrigena, Staphylococcus lentus, Vibrio paraheamolyticus, Salmonella clori-arizona* and other *Salmonella spp.* Only 15 samples showed no bacterial growth.
3.2 Out of 36 ground water samples, 30 samples revealed the following bacterial species: *Escherichia coli, Pseudomonas* spp, *Enterobacter sakazaki, Orchobacter anthropi, Enterobacter cloaca, Cronobacter spp., Proteus* spp, *Klebsiella pneumoniae,* and other *Klebsiella spp., Aeromonas salmonicida, Raoultella terrigena/planticola, Serratia marinoruba, Orchobacter anthropi, Enterobacter sakazaki and Enterobacter cloaca.* Only 6 samples showed no bacterial growth.

3.3 Bacteria detected from surface and ground water using IDEXX were coliforms, *E. coli* and *Pseudomonas* spp. The most probable number (MPN) range from 3 to > 2419.6. The results obtained as followed:

Table 1: Bacterial contamination from surface water

Area	**No of samples**	**Coliform**	***Escherichia coli***	***Pseudomonas* spp.**
Khartoum	**11**	**1 –ve** **1 0 +ve**	4 **–ve** **7 +ve**	**1 –ve** **10 +ve**
Omdurman	**19**	**4 -ve** **15 +ve**	**6 -ve** **13 +ve**	**8 -ve** **11 +ve**
Khartoum north (Bahri)	**26**	**10 -ve** **16 +ve**	**14 -ve** **12 +ve**	**13 -ve** **13 +ve**
Total	**56**	**15 –ve** **41 +ve**	**24 –ve** **32 +ve**	**22 –ve** **34 +ve**

Table 2: Bacterial contamination from ground water

Area	No of samples	Coliform	*Escherichia coli*	*Pseudomonas* spp.
Khartoum	**14**	**5 -ve** **9 +ve**	**10 -ve** **4+ ve**	**10 - ve** **4 +ve**
Omdurman	**15**	**0 -ve** **15 +ve**	**7 - ve** **8 + ve**	**9 - ve** **6 +ve**
Khartoum north (Bahri)	**7**	**1 -ve** **6 +ve**	**6 -ve** **1 + ve**	**3 - ve** **4 + ve**
Total	**36**	**6 –ve** **30 +ve**	**23 –ve** **13 +ve**	**22 –ve** **14 +ve**

Table 3: Number of positive and negative sample

Source of sample	Total number	Positive	Negative
Surface water	56	41(73.2%)	15 (26.8%)
Ground water	36	30 (83.3%)	6 (16.7%)
Purified water	8	0 (0%)	8 (100%)
Total	100	71%	29%

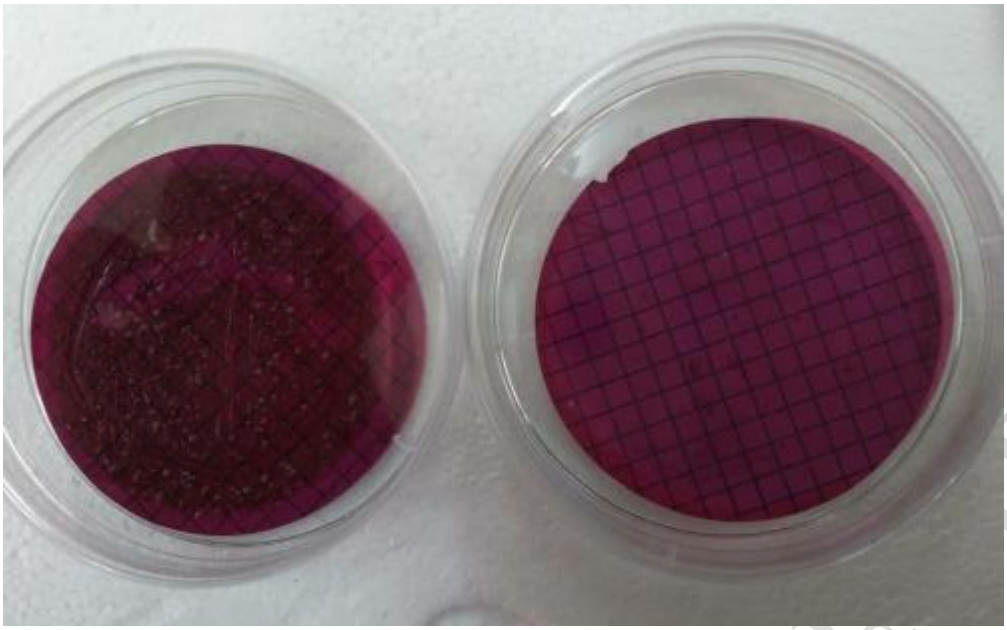

Fig 1: shows bacterial growth using filtration method

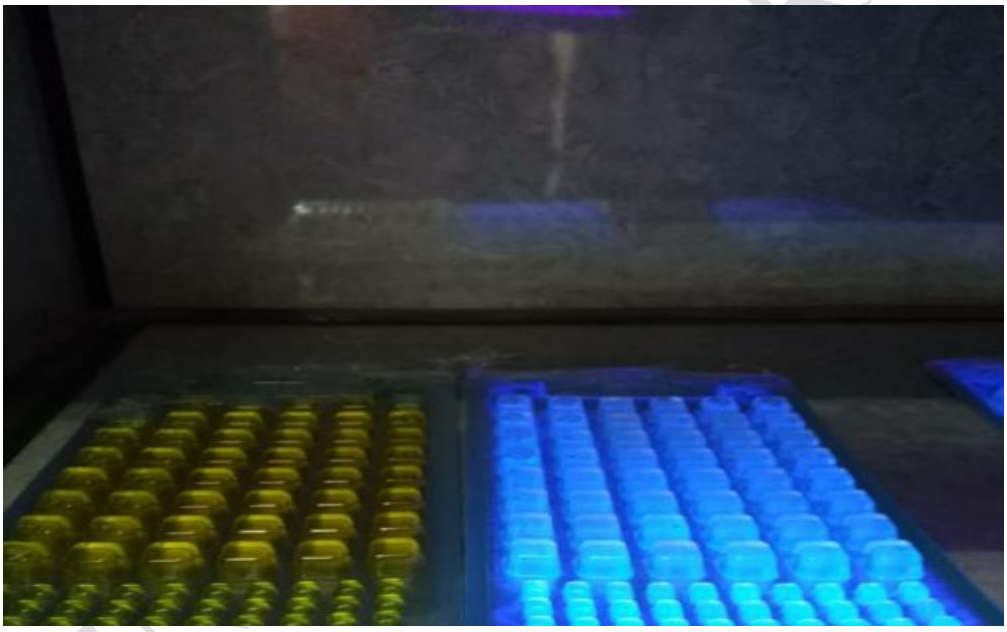

Fig 2: Negative and positive results of bacterial growth after exposing colilert and Pseudalert test to UV

3.4 Purified water samples were obtained from 8 companies named as follow: (Safia, Anhar, Crystal, Forat, Yes, Miso, Zulal and Care), all these samples were negative for bacterial growth when tested by filtration method and Colilert, Pseudalert test.

4. DISCUSSION

The need to increase attention on the safety of drinking water, rather than just the quantity has been acknowledged globally through the commitment to the Sustainable Development Goals (SDGs). SDG 6.1 focuses on achieving universal and equitable access to safe and affordable drinking water for all, and SDG 6.2 focuses on improving water quality by reducing pollution. The need for increased attention on drinking water safety (DWS) in Sudan has also been clearly illustrated by the AWD outbreak of 2016/17, which highlighted gaps and challenges being faced in ensuring DWS [9].

Total coliform bacteria are a group of different kinds of bacteria commonly found in the environment, including soil, vegetation and untreated surface water. Fecal coliform bacteria are a subgroup of the total coliform group. In this study, the percentage of coliform in surface (73.2%) (as shown in table 1) and ground water (83.3%)(as shown in table 2), were very high, these could be to the different types of bacteria that existed in great quantities in the intestines and feces of humans and animals. The presence of fecal coliform bacteria in drinking water is a strong indication of recent sewage or animal waste contamination, which should be interpreted as an indication that there is a greater risk that pathogens are present (table 3). Microbes in these wastes may cause short-term effects, such as diarrhea, cramps, nausea, headaches or other symptoms, as well as potentially pose long-term health effects. They may pose a special health risk for animal, infants, young children, some of the elderly and people with severely compromised immune systems. Septic tank widely used for wastewater storage and treatment may contaminate groundwater supplies.

Many farmers use cellars, tanks or landfills to store manure, water leaching from these storage sites may also contaminate groundwater, especially during periods of rainfall. The application of animal manure to agricultural lands as fertilizer is common practice throughout the world. The bacteria present in the manure may leach into the groundwater by João [10].

An important source of contamination of surface and ground waters is runoff water from agricultural and pasture lands, and urban areas. In a study reported by Doran and Linn [108], runoff from a cow-calf pasture in eastern Nebraska was monitored during a three-year period. Rainfall runoff from the grazed area contained 5 to 10 times more fecal coliforms than runoff from the fenced; un grazed area [11].

This study had verified the use of IDEXX Colilert (18-hour & 24-hour) as an acceptable alternative to other test methods for the recovery of *E. coli* from drinking water, source water, and wastewater [12-18], the test has many good features, such as ,ease of use, simplifies training, Unit-dosed packaging eliminates media preparation, no repeat testing due to clogged filters or heterotrophic interference. Also it is rapid in detecting coliforms and *E. coli* simultaneously in 24 hours or less, no confirmations needed, no glassware cleaning or colony counting. It is so

accurate, it can identify *E. coli* specifically, eliminate unnecessary public notification due to non-target organisms, suppresses up to 2 million heterotrophs per 100 ml. Eliminates the subjective interpretation found in traditional methods. It can also detect a single viable coliform or E. coli per sample. It is economical and flexible.

Other type of bacteria that not belong to coliform were also isolated during this study, this is a serious issue since they could harbor pathogenic and resistance genes difficult to treat. Neither *Pseudomonas* spp nor *Aeromonas* spp are indices of faecal pollution, but may be useful in assessing regrowth in distribution systems [19].

Vibrio parahaemolyticus was reported in this study from surface water, as mention by João, it is a well-documented causal agent of acute food-borne gastroenteritis, particularly in Japan and South East Asia. Cases are associated with the consumption of raw or undercooked shellfish such as oysters, shrimp, crabs, and lobster [10].

Also *Salmonella* spp were isolated from surface water, this is in agreement with Arvanitidou and Le who mentioned that, the principal habitat of *Salmonella* is the intestinal tract of humans and animals. Salmonellae are constantly found in environmental samples, because they are excreted by humans, pets, farm animals, and wild life. Municipal sewage, agriculture pollution, and storm water runoff are the main sources of these pathogens in natural waters. Salmonellae do not seem to multiply significantly in the natural environment, but they can survive several weeks in water and in soil if conditions of temperature, humidity, and pH are favorable [20,21].

E. coli was isolated in this study (30 from surface water and 15 from ground water), its strains isolated from intestinal diseases have been grouped into at least six different main groups, based on epidemiological evidence, phenotypic traits, clinical features of the disease and specific virulence factors. From these, enterotoxigenic (ETEC, namely O148), enterohemorrhagic (EHEC, namely O157) and enteroinvasive serotypes (EIEC, namely O124) are of outstanding importance and can be transmitted through contaminated water [22,23].

In recent years, *Aeromonas hydrophila* has gained public health recognition as an opportunistic pathogen. It has been implicated as a potential agent of gastroenteritis, septicemia, meningitis, and wound infections. It can play a significant role in intestinal disorders in children under five years old, the elderly, and immunosuppressed people [24].

There were no bacteria reported in purified water bottles, this may be because of the continuous disinfection of the water supply using many options like chlorination, ultraviolet radiation, distillation, and ozone treatment.

Enterobacter, Klebsiella, Proteus, Serratia, Salmonella, Pseudomonas were isolated in this study, this is in agreement with Ayman, (2006) rom Eastern and Southern Sudan [25].

5. CONCLUSIONS

The water hygiene situation is poor in Khartoum state due to contamination with many bacterial contaminants which constitute hazard to be spread to both man and animal.

6. RECOMENDATIONS

6.1 Water system must conduct an assessment to find out how the contamination got into the water. If the assessment identifies the cause of the contamination, the water system can usually correct the problem with repairs, treatment, or improved operation and maintenance practices.

If total coliform and/or E. coli bacteria are detected in a private water supply, an immediate effort should be made to identify and eliminate the source of contamination. After addressing the contamination source, disinfect the entire water system, using shock chlorination. After shock chlorination, submit another water sample for testing. The water should test negative before use.

If the source of bacterial contamination or well construction errors cannot be identified and eliminated, continuous disinfection of the water supply may be necessary. Options include continuous chlorination, ultraviolet radiation, distillation, and ozone treatment.

6.2 Establishing a well-planned water pollution researches in all these localities to check the microbial quality constantly.

6.3 Further studies needed to evaluate the water microbial situation.

6.4 Safe drinking water for all is one of the major challenges of the 21st century.

6.5 Microbiological control of drinking water should be the norm everywhere.

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