Assessment of Bacterial Contamination in Drinking Water Sources in Khartoum/ Sudan

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Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information
DOI: 10.9734/JAMB/2022/v22i130430

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/83367

Original Research Article

ABSTRACT

Aim 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 pneumoniae, Enterobacter aerogene Enterobacter sakazaki, Enterobacter cloacae, Serratia marinoruba, Proteus mirabilis, Salmonella spp. Raoultella terrigena; planticola, Orchobacter anthrobi, Cronobacter spp., Aeromonas salmonica, Aeromonas hydrophilia, Pantoea agglomerans.

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**Vibrio parahaemolyticus.** Coliform bacteria, *Escherichia coli* and *Pseudomonas* spp were detected and most probable numbers (MPN) were counted using the previous kits according to manufacturer instructions. **Conclusion:** The water must be tested before using and quality control technique must be achievable to ensure continuously supply of pure drinking water.

**Keywords:** Drinking water; *E. coli*; pseudomonas spp; coliform; colilert; pseudalert; MPN.

### 1. INTRODUCTION

Accessible and safe water is essential to life. Significant benefits to health can be achieved by using good quality drinking water. Generally, the contaminated water with human or animal feces represents deep risk as it contains different microbes. Wastewater discharges in surface water are the great source of fecal microorganisms, including pathogens [1-4].

Bacteria associated with drinking water are those that originate from the gut of warm-blooded animals. Sources include wildlife, pets, and contamination due to improperly designed, failing, or overloaded waste treatment systems, including septic systems from private homes, and sanitation pipes. Human sources are important as they contain human pathogens. Flood water also contains high numbers of bacteria. Surface waters typically contain bacteria but groundwater should be free of animal sources.

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

The most important species of this group include *Escherichia coli*, *Klebsiella* spp and *Enterobacter* spp. The non-coliform bacteria were also found in contaminated water such as *Streptococcus*, *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) for detection of 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* to hydrolyze substrate in the Pseudalert reagent [8].

This approach is different from traditional media, which provide a nutrient-rich environment that supports all organisms. 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. such media often contain high levels of salts, detergents, or other selective agents.

### 2. MATERIALS AND METHODS

This study was designed to assess the bacterial contamination in drinking water sources in Khartoum/ Sudan using new technique (Colilert and Pseudalert test) for the first time in Sudan.

The Colilert test used to detects or quantifies both total coliforms and *E. coli*, with results in 24 hours. The mechanism of Colilert Test depend on using β-galactosidase to metabolize ONPG, the result obtain by changing the color to yellow. *E. coli* use β-glucuronidase to metabolize MUG and create fluorescence. The test differentiates between coliform and non-coliforms which not have these enzymes, so they are unable to grow and interfere. Pseudalert test also detects and quantifies *Pseudomonas aeruginosa*, actively growing strains have an enzyme that produces blue fluorescence under ultraviolet light when expose to substrate inside the reagent.

#### 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 containers then labeled and placed in an ice-boxes and immediately transported 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 of each 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°C. Well isolated colonies from filter paper as shown in (Fig. 1) were sub cultured on nutrient agar plates for purification then identified 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 each Pseudalert and Colilert. The reagent was added to sample then poured into Quanti-Tray that 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 recorded according to manufacturer instructors (IDEXX) and the Most Probable Number (MPN) was calculated as followed: Yellow wells indicate the presence of total coliforms, Yellow/fluorescent wells indicate the presence of E. coli, then positive wells were Count according 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 in Tables (1 and 2).

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.

Table 1. Bacterial contamination from surface water

| Area                  | No of samples | Coliform | Escherichia coli | Pseudomonas spp. |
|-----------------------|---------------|----------|-----------------|------------------|
| Khartoum              | 11            | 1 -ve    | 4 -ve           | 1 -ve            |
| Omdurman              | 19            | 1 0 +ve  | 7 +ve           | 10 +ve           |
| Kuhartoum north (Bahri)| 26            | 4 -ve    | 6 -ve           | 8 -ve            |
| Total                 | 56            | 15 +ve   | 13 +ve          | 11 +ve           |
|                       |               | 10 -ve   | 14 -ve          | 13 -ve           |
|                       |               | 16 +ve   | 12 +ve          | 13 +ve           |
|                       |               | 15 -ve   | 24 -ve          | 22 -ve           |
|                       |               | 41 +ve   | 32 +ve          | 34 +ve           |
Table 2. Bacterial contamination from ground water

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

Safety of drinking water is more important than the quantity as discussed 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 microbial contamination. Also, the 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].

The group of total coliform bacteria contains different kinds of bacteria that found in the environment, including soil, vegetation and untreated surface water. In this study, the percentage of coliform in surface (73.2%) (Table 1) and ground water (83.3%) (Table 2), were very high, these could be due to the different types of bacteria that found numerous 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 may cause a severe risk due to pathogens present (Table 3). Clinical symptoms may appear due to microbial infection, such as diarrhea, cramps, nausea, headaches. These may pose a special health risk for animal, infants, young children and immune compromised people. Septic tank which used for wastewater storage and treatment may contaminate the 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 using of animal manure in fertilizing the agriculture lands may pollute the ground water [10].

An important source of contamination of surface and ground waters is runoff water from agricultural, pasture lands and urban areas in a study reported by Doran and Linn (108), he mentioned that 5 to 10 times more fecal coliforms detected in eastern Nebraska (grazed area) comparing to un grazed area during the monitoring for three-year period [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, eliminates the subjective interpretation found in traditional methods. Also can be used to detect a single viable coliform or E. coli per sample. It is economical and gives reliable results.

None-coliform were also isolated during this study, this is a serious issue since they could harbor pathogenic and resistance genes difficult to treat. Pseudomonas spp and Aeromonas spp are not belong to fecal pollution, thus they are useful in assessing regrowth in distribution systems [19].

Vibrio parahaemolyticus was reported in this study from surface water, as mention by João, it is one of food-borne bacteria which cause gastroenteritis, especially in Japan and South East Asia. Main sources of infection are 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 Salmonella is normal inhabitant in the intestinal tract of humans and animals. It constantly found in environmental samples, as they are excreted by humans, pets, farm and wild animal. The main sources of this organism are sewage, agriculture pollution, and water. Salmonellae can survive for several weeks in water and in soil if the environmental conditions is suitable [20,21].

E. coli was isolated in this study (30 from surface water and 15 from ground water), it divided into six different groups, based on epidemiological evidence, phenotypic traits, clinical symptoms and virulence factors. These are, enterotoxigenic (ETEC, namely O148), enterohemorrhagic (EHEC, namely O157) and enteroinvasive serotypes (EIEC, namely O124) are of importance and can contaminated the water [22,23].

Recently, Aeromonas hydrophila has gained public health attention as an opportunistic pathogen. And a potential agent of
gastroenteritis, septicemia, meningitis and wound infections. It has an important role in intestinal disorders in children under five years old, the elderly and immune compromised people [24]. There were no bacteria reported in purified water bottles, this may be because of the regular disinfection of the water supply using different methods 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) from Eastern and Southern Sudan [25].

5. CONCLUSION
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. RECOMMENDATIONS
6.1 Assessment and monitoring of water system must be regularly done to find the source of contamination in order to address and correct the problem with repairs, treatment or improved operation and maintenance practices.

After addressing the contamination source, disinfection must be done using shock chlorination and testing the water for negative result before use.

Options of disinfection include 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 Pure drinking water without microbial contamination is one of the major challenges of the 21st century.

6.5 Drinking water should be checked regularly for microbiological risk

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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