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Presence of *Escherichia coli* and Coliform Bacteria in Drinking water sources as indicator to other fecal contamination

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Abstract
The main objective of this research project is to find a best source for drinking water with low concentration of *E.coli* and to predict a better treatment method(s) to produce low contaminated water in order to achieve best conditions for reasonable public health in Omdurman (Sudan) through guiding people to use adequate and de contaminated drinking water. Coliforms are good indicator of potential of water contamination. Testing for coli form bacteria is faster and cheaper than testing for specific organisms and pathogens. Water is important for life of human and animals hence purity of water from pathogenic microorganism is very important public health goal.
Random samples from different water drinking sources were tested and cultured for the presence and/or concentration of *E. coli*. These samples were River Nile, surface water collected water from Almanara Company (treated by chlorine) before entering to the water dispensers system, Almanara normal house hold water after entering the dispensers system, Wells water and Mineral bottled water (treated by ozone) from three different industrial water treatment companies, these were Mizo, Cristal and Anhar. Sample from each water sources was cultured separately in the microbiology lab at Ahfad University for woman for coli form and *E. coli*. Surface water collected directly from the river Nile (neither decontaminated by chlorine, ozone) was found to be the most contaminated water with the *E. coli*, Almanara house hold water after entering the water dispensers system was found to be next in terms of contamination. Collected water from the tankers of Almanara before entering the dispenses system occupies the third position concerning the degree of contamination indicating that the dispensers system was already contaminated with the *E. coli*. Well water (treated with chlorine) and industrial Mineral bottled water (treated with ozone) were found to be coli form and *E. coli* decontaminated samples. Finally we concluded that drinking water contamination depends on the water source and the type of water decontamination treatment method. Results were evaluated and recommendations were drawn.

Keywords: public health, contamination, *E. coli*, bacteria, pathogens and ozonation

1. Introduction

1.1 Water

In living organisms, water acts as a temperature buffer and a solvent, is a metabolite, and creates a living environment. Water is an effective and necessary solvent in living organisms.

1.2 Source of water

1.2.1 Surface water

Surface water is water in a river, lake or fresh water wetland. Surface water is naturally replenished [1].

1.2.2 Under river flow

Throughout the course of a river, the total volume of water transported downstream will often be a combination of the visible free water flow together with a substantial contribution flowing through rocks and sediments that underlie the river and its floodplain called the hypothetic zone [1].

1.2.3 Groundwater

Groundwater is fresh water located in the subsurface pore space of soil and rocks.

1.3 Water contamination

When we think of contaminated water we tend to focus on many different things. These can be chemicals from industrial plant, heavy metals from pollution and sewage release. One of largest concern is to find pathogens in drinking water; some of these microbes are bacteria. Drinking water that is not properly treated or traveled through piping system may create an environment of contamination.

1.4 Coli forms

Coli forms are enterobacteriaceae organism distributing worldwide and found on soil, water and plant. They are also present as part of normal flora intestinal of humans and animals. Member of this family move by peritrichous flagella or they may be non motile without any flagella also they are aerobic and facultative anaerobic and grow readily on ordinary media.

1.4.1 Human diseases caused by entero bacteriaceae organism

Some members of the family such as salmonella species always causes human disease such as diarrhea. In addition another group of Enterobacteriaceae organisms, which are found as normal commensally in humans but become pathogenic when they acquire virulence factor genes through plasmid bacteriophages, or Pathogen city islands, *E. coli* associated with gastroenteritis in the human is one such

example [2, 3].

1.4.2 Classification of *E. coli*
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: Escherichia
Species: *E. coli*

1.4.3 Escherichia
It's one of enterobacteriaceae which are animal and human pathogen. This genus consists of five species: *E. coli*, *Escherichia ferguson*, *E.bermanii*, *E.vulneris* and *E. blatted*.

1.4.4 *E.coli*
It's an aerobe and it's most common important types that concourse disease to human in enteric disease [4, 5].

1.4.4.1 Culture of *E.coli*
Fermented sugar with production of acid and gas or acid only reduce nitrate to nitrite and are catalase positive but oxidize negative, the oxidize test is important test by which the member of the enterobacteriaceae can be distinguished from many other fermentative and non fermentative gram negative bacilli. Member of the family show every wide biochemical and antigenic heterogeneity among them self. *E.coli* are aerobe and facultative an aerobe.
E. coli gives bright pink flat colonies due to lactose fermentation condition. In liquid broth culture gives turbid growth with deposit, which disperses completely on shaking.

1.4.4.2 Pathology of *E.coli* infection
Many diseases caused by *E.coli* examples are
1. Urinary tract infection: caused by *E.coli* normally found in the feces.
2. Gastroenteritis related to contaminated food, water with fecal *E.coli*.
3. Infant diarrhea caused by Enteropathoginic caused by *E.coli* in tropical countries.
4. Septicemia caused by entering *E.coli* to blood stream and cause toxicity.
5. Neonatal meningitis.

1.4.4.3 Laboratory diagnosis
Laboratory diagnosis is generally based on isolation of *E.coli* by culture and separation of *E.coli* from other different clinical specimen. Samples were collected from urine, feces, blood and CSF

1.4.4.5 Prevention and control
General good hygiene for food, water in taking and handling use, good cooking all types of meets and boiling water in proper ways [6].

1.5 Presence of *E. coli* or coli formas indicatior to other fecal Contamination
Certain bacteria can be used as good indicator organisms of the potential contamination in particular situations and used to evaluate the general quality of water. The presence of bacteria commonly found in human feces or animal waste product and the greater risk for exposure to pathogenic organisms. The total number of coli form discharged by a normal person ranged from 100 to400 billion per day, some animals discharge much more, coliform bacteria (e.g. *E. coli*), in the surface water is considered as a common indicator of fecal contamination. For this reason, sanitation programs often test water for the presence of these organisms to ensure that drinking water systems are not contaminated with feces. This testing can be done using several methods which generally involve taking samples of water, or passing large amounts of water through a filter to sample bacteria, then testing to see if bacteria from that water grow on selective media such as MacConkey agar. Alternatively, the sample can be tested to see if it utilizes various nutrients in ways characteristic of coliform bacteria [7].

1.6 Water treatment techniques
1.6.1 Ozonation and chlorination
Ozonisation: is chemical water treatment technique based on the infusion of ozone into water. The gas composed of three oxygen atoms (O3) which is one of the most powerful oxidant. Chlorination is another type of advanced oxidation process, involving the production of very reactive oxygen species able to attack a wide range of organic compound and all microorganisms [8].

2. Materials and methodology
2.1 Material
2.1.1 Water
Different source of water were used, these sources are River Nile, Manara water collected directly from municipal water supply, water from wells and Industrial treated water from different water treating companies, these are Mizo, Anhar and Crystal.

2.1.2 Reagents
MaCconcky broth, EMP, distilled water, cotton and Aluminum foil.

2.2 General method
Different portions from each water sample were cultured for presence and concentration of *E. coli* separately, these portions were 1, 0.1, 0.01mL.
Culture process was done in two different media, MaCckoncky broth for coli form and EMP selectively for *E. coli*; these media were prepared prior to the process of culture. Evaluation of gas during the culture indicates to present of coli form bacteria whereas change in color of EMP indicates growth and presence of *E. coli*.

2.3 Media preparation
2.3.1 MacConky broth
63 gm of MacConky broth was weighed using sensitive balance and dissolved in 630 mL distilled water. 10 ml of MacConky media was transferred to 63 test tubes separately. Durham tubes were linked to the test tubes to detect gas evaluation. The test tubes were then closed by cotton and aluminum foil to prevent loss of volume during autoclaving process. The test tubes were then sterilized by autoclaving for 15 minutes at 121oC [22].

2.3.2 EMP (ethylene blue)
18.7 gm of EMP powder was weighed using sensitive balance and dissolved in 500 mL distilled water in a conical flask. The conical flask was closed by cotton to prevent loss of volume, the EMP was sterilized by autoclaving for 15 minutes at 121oC. The prepared media was cooled and

poured into 9 ml disposable Petri dishes using aseptic technique. The media was allowed to stand at room temperature for further use [22].

2.3.3 Inculcation of Sample in MacConky broth

Different portions (1ml, 0.1ml and 0.01ml) from water sample containing MacConky broth were inoculated separately for2 days at 37OC. Each portion was cultured three times to obtain better results [22].

2.3.4 Inculcation of Sample in EMP selective media

MacConky broth was sub cultured after 2days of incubation into EMP agar using striking method, then inculpated at 37oC for 48 hours [22].

4. Results

Table 1: Gas production and color change inMacCkoncky broth accompanies culture of non-running river Nile sample in different volume for *E.coli*.

Volume of sample	Sample Number	Gas production	Change in color
1mL	1	+	From red to yellow
	2	+	From red to yellow
	3	+	From red to yellow
0.1mL	1	+	From red to yellow
	2	+	From red to yellow
	3	+	From red to yellow
0.01 mL	1	+	From red to yellow
	2	+	From red to yellow
	3	+	From red to yellow

Table 2: Gas production and color change in MacCkoncky broth accompanies culture of Manara company samples in different volumes after entering capillary (pipe) for *E. coli*.

Volume of sample	Sample No.	Gas production	Change in color
1mL	1	Nil	From red to yellow
	2	Nil	From red to yellow
	3	Nil	No change
0.1mL	1	Nil	No change
	2	Nil	From red to yellow
	3	Nil	No change
0.01mL	1	Nil	No change
	2	Nil	No change
	3	Nil	From red to yellow

Table 3: Gas productions and color change in MacCkoncky broth accompanies culture of Manara company samples before entering capillary pipe for *E. coli*.

Volume of sample	Sample No.	Gas production	Change in color
1mL	1	Nil	From red to yellow
	2	Nil	From red to yellow
	3	Nil	From red to yellow
0.1mL	1	Nil	From red to yellow
	2	Nil	From red to yellow
	3	Nil	From red to yellow
0.01mL	1	Nil	From red to yellow
	2	Nil	From red to yellow
	3	Nil	From red to yellow

Table 4: Gas production and change in color in MacCkoncky broth accompanies culture of Well sample in different volumes for *E.coli*

Volume of sample	Sample No.	Gas production	Change in color
1mL	1	Nil	No change
	2	Nil	No change
	3	Nil	No change
0.1mL	1	Nil	No change
	2	Nil	No change
	3	Nil	No change
0.01mL	1	Nil	No change
	2	Nil	No change
	3	Nil	No change

Table 5: Gas production and color change in MacCkoncky broth accompanies culture of Bottled water (Anhar Company for *E.coli*).

Volume of sample	Sample No.	Gas production	Change in color
1mL	1	Nil	No change
	2	Nil	No change
	3	Nil	No change
0.1mL	1	Nil	No change
	2	Nil	No change
	3	Nil	No change
0.01mL	1	Nil	No change
	2	Nil	No change
	3	Nil	No change

Table 6: Gas production and color change in MacCkoncky broth accompanies culture of bottled water (Mizoo Company) for *E.coli*

Volume of	Sample No.	Gas production	Change in color
sample			
1mL	1	N il	No change
	2	Nil	No change
	3	Nil	No change
0.1mL	1	Nil	No change
	2	Nil	No change
	3	Nil	No change
0.01mL	1	Nil	No change
	2	Nill	No change
	3	Nil	No change

Table 7: Gas production and change in color in MacCkoncky broth accompanies culture of bottled water (Crystal Company) for *E. coli*.

Volume of sample	Sample No.	Gas production	Change in color
1mL	1	Nil	No change
	2	Nil	No change
	3	Nil	No change
0.1mL	1	Nil	No change
	2	Nil	No change
	3	Nil	No change
0.01mL	1	Nil	No change
	2	Nil	No change
	3	Nil	No change

Table 8: Growth of colony and color change in selective media (EMP) accompanies culture of Non-running River Nile sample in different volumes (1 ml, 0.1ml, 0.01ml) for *E. coli*.

Volume of sample	No.sample	Growth and color
1mL	1	Green metallic sheen +pink colony growth
	2	Green metallic sheen.
	3	Green metallic sheen.
0.1mL	1	Green metallic sheen + pink colony growth
	2	Pink colony growth.
	3	Pink colony growth.
0.01 mL	1	Slightly pink colony growth. Slightly
	2	Pink colony growth. pink colony Growtha
	3	lot of

Table 9: Growth of colony and color change in selective media (EMP) accompanies culture of Manara company before entering line house sample in different volumes for *E.coli***.**

Volume of the sample	Sample No.	Growth and color
1mL	1	No growth
	2	growth
	3	No growth
0.1mL	1	No growth
	2	No growth
	3	No growth
0.01mL	1	No growth
	2	No growth
	3	No growth

Table 10: Growth of colony and color change in selective media (EMP) accompanies culture of Manara Company after entering line house sample in different volumes for *E. coli*.

Volume of the sample	Sample No.	Growth and color
1mL	1	Pink color growth.
	2	Pink color growth.
	3	No growth.
0.1mL	1	Pink color growth
	2	No growth.
	3	4 spot Pink color growth colony.
0.01 mL	1	No growth.
	2	No growth.
	3	No growth.

Table 11: Growth of colony and color change in selective media (EMP) accompanies culture of Well sample in different volumes for *E.coli*.

Volume of sample	Sample No.	Growth and color
1mL	1	No growth
	2	No growth
	3	No growth
0.1mL	1	No growth
	2	No growth
	3	No growth
0.01 mL	1	No growth
	2	No growth
	3	No growth

Table 12: Growth of colony and color change in selective media (EMP) accompanies culture of (ANHAR company) sample in different volumes for *E.coli*.

Volume of the sample	Sample No.	Growth and color
1mL	1	No growth
	2	No growth
	3	No growth
0.1mL	1	No growth
	2	No growth
	3	No growth
0.01 mL	1	No growth
	2	No growth
	3	No growth

Table 13: Growth of colony and color change in selective media (EMP) accompanies culture of (Crystal company) sample in different volumes for *E.coli*.

Volume of sample	Sample No.	Growth and color
1mL	1	No growth
	2	No growth
	3	No growth
0.1mL	1	No growth
	2	No growth
	3	No growth
0.01mL	1	No growth
	2	No growth
	3	No growth

Table 14: Growth of colony and color change in selective media (EMP) accompanies culture of (Mizoo company) sample in different volumes (1 ml, 0.1ml, 0.01ml) for *E.coli*.

Volume of the sample	Sample No.	Growth and color
1mL	1	No growth
	2	No growth
	3	No growth
0.1mL	1	No growth
	2	No growth
	3	No growth
0.01 mL	1	No growth
	2	No growth
	3	No growth

Table 15: Most Probable Number Determination [23]

Sample	1.0 ml	0.1 ml	0.01 ml	Combination of positive tube	MPN /gm.	Lower confidence	Upper confidence
Anhar	0/3	0/3	0/3	0-0-0	<0.3	<0.05	<0.9
Crystal	0/3	0/3	0/3	0-0-0	<0.3	<0.05	<0.9
Mizo	0/3	0/3	0/3	0-0-0	<0.3	<0.05	<0.9
Well	0/3	0/3	0/3	0-0-0	<0.3	<0.05	<0.9
Manara company	2/3	1/3	1/3	2-1-1	2.0	0.7	8.9
Manara pipe	3/3	3/3	3/3	3-3-3-	>100	>15	>480
River Nil	3/3	3/3	3/3	3-3-3	>100	>15	>480

5. Discussion

Drinking water sample from several different sources usually used in Omdurman (Sudan) were cultured for *E.coli*. These sources were water from Almanara (water treating governmental company) before pumping to the houses, Almanara water after pumping, water from the Wells, water from non-running river Nile and there different industrial water treated companies; these are Mizo, Anhar and Crystal. *E.coli* is considered to be the major cause of water contamination and mixing with human feces. Culturing takes place in two media; the MacCkoncky Broth for general contamination and (EMB) Eosin Methylene Blue selectively for *E.coli*. Gas evaporation and change in color were used as evidences for growth and contamination in MacCkoncky broth whereas green metallic sheen or pink colony was the evidences for growth of *E.coli* in EMB.

Concerning industrial water samples, no change in color and no gas production in MaCckonCcky broth and no even green metallic sheen or colony in EMB were detected. This is expected since they are well purified and decontaminated using ozone. (Table 5, 6, 7, 12, 13, and 14).

The non-running river Nile show pronounced change of color, and clear gas evaluation in MacConky Broth beside clear green metallic sheen and colony growth in EMB, during culturing (table 1, 8). Also this is expected since water from non-Running River Nile was not decontaminated by any water treatment methods. Water obtained from the Well showed no contamination (table 4 and 11) indicted by color change or gas evaluation in MaCconky Broth or growth formation in EMB.

Comparing Almanara water samples after and before pumping and reaching houses through the water pump lines system, it was found that water before pumping is less contaminated than water after pumping (table 3 and 9) and entering dispenser lines (table 3 and 10) which indicates that water pump lines have a considerable degree of contamination. Results were statistically calibrated and elaborated.

6. Recommendations

Better recommendations were elaborated and used to evaluate the general quality of water and Strategies to improve household stored drinking water quality in post-disaster situations. (11) In many areas of the developing world, drinking water is collected from unsafe surface sources outside the home and is then held in household storage vessels. Drinking water may be contaminated at the source or during storage; strategies to reduce water borne disease transmission must safeguard against both events. We describe a two-component prevention strategies which allow an individual to disinfect drinking water immediately after collection (point-of-use disinfection) and then to store the water in narrow-mouthed, closed vessels designed to prevent recontamination (safe storage). New disinfectant generators and better storage vessel designs make these strategies practical and inexpensive. This approach empowers households and communities that lack portable water to protect themselves against a variety of waterborne pathogens and has the potential to decrease the incidence of waterborne diarrheal disease [24]. Other suggestion is to boil the water before consumption in case of emergency situation. Also resins may be used as a traditional method to improve water quality.

7. Conclusion

Severe water infection occurs as results of poverty and poor educational knowledge of water treatment methods.

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