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Assessment of Drinking Water Microbial Contamination in Al-Butana Region of Sudan

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Abstract: The microbial quality of drinking water in the Al-Butana Region has not received much attention from environmentalists. In this study, a total of 100 drinking water samples were collected in sterile 50 mL medical laboratory plastic bottles. The samples were taken from different drinking water sources (1) direct well source (out-let tap water) (2) public reservoirs (3) distribution system (4) common public water stands (locally known as sabeel) (5) house pots (6) house refrigerators (7) donkey driven cart locally known as "karo" and (8) different drinking water utensils: (a) "ibreeg"; (b) drinking jug and (c) hand carrying cooler. Results revealed that bacterial contamination of well drinking water sources is variable depending on the hygienic status and behavior of users. None of the well water samples taken from direct well source showed any positive result of coliform test while 35% of those taken from public water stands (sabeels) showed a positive result of the test. The tests for faecal streptococci were unanimously negative in all of the investigated sites. Coverless reservoirs were found to be the main probable source of coliform bacteria via birds' feces while handless utensils used normally for drinking are the most source of coliform bacteria in public stands (sabeels).

Key words: Drinking water, bacterial contamination, coliform, hygienic status, Sudan

INTRODUCTION

Contamination of groundwater has been observed worldwide in aquifers underlie intensive populated communities. Bacteria are one of these contaminants that frequently found in groundwater (Yusuf, 2007). Bacterial contaminated drinking water and poor sanitation has been incriminated among factors responsible for over a million deaths per year (Akpor and Muchie, 2011; Kabir *et al*., 2011). Occurrence and growth of coliform bacteria in water distribution systems could be associated with many factors; rainfall, water temperature greater than 15°C, total organic carbon levels greater than 2.4 mg L^{-1} and assimilable organic carbon levels greater than 50 μg of acetate carbon equivalents per liter (Le Chevallier *et al*., 1991) and the availability of phosphorous compounds (Miettinen *et al*., 1997).

Microorganisms enter into drinking water body via humans and animals intestinal secretions in areas where sanitation conditions are poor or absent. When found in drinking water, microorganisms constitute a real indicator that it should not be used for human consumption if these contaminants found in excess of the maximum permissible level (1×10^2 CFU mL^{-1}) set by WHO (2008) and the other respective standards and guidelines. To minimize the risk of bacterial growth in drinking water, WHO (2004) reported that chlorination, ozonization, ultra violet radiation and advanced oxidation processes are widely accepted for the disinfection of drinking water.

Bacterial contamination can get into groundwater by many ways; Wild and domestic animals, birds and dairy farms wastes situated in a watershed area or within the hydrological catchments of groundwater however these, have been found to be a pathogenic contamination source of drinking water (Gleeson and Gray, 1997; Obiri and Jones, 2001), also the presence of campylobacter in waters within agricultural areas is a real evidence of environmental contamination by sewage effluent coming from agricultural areas (Obiri and Jones, 2001), biomass that resulted from degradable materials load into drinking water distribution pipes accumulates biofilms which accelerate growth of microorganisms and protect them against disinfection agents (Flemming, 1998; Lewis, 2001), long storage of good-quality drinking water is a main factor of faecal coliforms contamination through faecally contaminated hands or utensils (Jensen *et al*., 2002). In addition, coverless public reservoir contributes to pathogenic accession especially from birds feces. Contamination by microorganisms can occur through improperly installed or/and through undetected leaks in the water pipe system (Gleeson and Gray, 1997). It has been reported that contamination with fecal coliforms may be caused by infiltration of pollutants in the recharge area of the springs (Daghrah, 2009).

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The presence of microorganisms does not necessarily indicate that drinking water poses a health risk. The important consideration is the kind of microorganisms that are present (Al-Milleegi *et al*., 2007). Pathogenic effect on human life that related to water can be divided into two main groups; direct and indirect effects: 1) Direct effects of pathogens related to water consumption are health-based (WHO, 2004). Waterborne diseases can be divided into four group; (a) Bacterial infections i.e., meningitis (WHO, 2004), bacterial gastroenteritis disease (UMMC, 2008), cholera, bacillary dysentery (Shigellosis) (WHO, 2004) and typhoid and paratyphoid enteric fever (Gianella, 1996; Easman, 2005); (b) Viral infections i.e., viral gastroenteritis, hepatitis A virus (USFDA, 2007), dengue fever (WHO, 2004; Edelman, 2007), meningoencephalitis (WHO, 2004) and Hantavirus Pulmonary Syndrome (HPS); (c) protozoa infections i.e., Granulomatous Amoebic Encephalitis (GAE) (Martinez *et al*., 1997), amoebic dysentery (Amoebiasis) (AD) (WHO, 2004) and giardiasis (Huang and White, 2006) and (d) helminths infections i.e., fascioliasis (WHO, 2006a, b), Ascariasis (Wu and Jones, 2000), Trichuriasis. (Levy, 2004), Guinea worm disease (GWD) (Barry, 2007), Schistosomiasis, Filariasis (Philariasis), Onchocerciasis (Sightsavers International, 2008) and Strongyloidiasis (Jensen *et al*., 2002) and 2 indirect human health effects i.e., water-pipes corrosion (Pizarro *et al*., 2001; Arens *et al*., 1995) and contamination of both renal dialysis machines (Hoenich and Levin, 2003) and waterlines of dental units (Hoenich and Levin, 2003; Pankhurst, 2003). Drinking water pollution caused by faecal contamination is another serious problem due to the probable presence of diseases causing microorganisms (pathogens) (Le Chevallier *et al*., 1991). Moreover, radio iodine-125 could be absorbed by all part of algae found in the drinking water (Hosseini, 2010).

It has been found that the best means of water disinfection is boiling which destroys all the coliform in the drinking water while the use of solar could be considered a cheaper alternative although it does not eliminate completely the microbial load impeding suitability for drinking water. However, granular activated carbon filtration is recommended in order to decrease microbial content of the drinking water (Ibeto *et al*., 2010). Hence, periodical microbial analysis for drinking water is very important. Therefore, this study is conducted to investigate the hygienic status in the study area.

Study location: The study area was divided, administratively, into three regions as follows:

- The whole area of East of Gezira Locality, Gezira State
- The Western Administrative Units area of Umelghura Locality, Gezira State
- The Eastern Administrative Units area of Shark-el-Neel Locality, Khartoum State (Fig. 1)

MATERIALS AND METHODS

Collection of groundwater samples: Due to the lack of sterilized sample glass bottles in local markets, samples for bacteriological analysis were collected in sterile 50 mL medical laboratory plastic bottles. The samples used for bacteriological analysis were taken from different drinking water sources as follows: direct well source (out-let tap water), public supplying, distribution system, public water stands (sabeel), house pots, house refrigerators, "ibreeg", drinking jug, donkey driven cart locally known as "karo" and hand cooler. Samples used for bacteriological analysis were collected and prepared as follows: Samples taken from water taps were sampled after the tap had been sterilized by means of a lighter flame, then water was allowed to run for 2 min in order to flush any organisms that may exist, especially, if the pipe is left unused for a long time. The bottle was carefully opened under the water-flow current and entirely filled and then immediately closed while it was under the water-flow current in order to avoid any access of bacteria other than that already existing in the source of the intended sample. For the samples taken from water sources other than the tap water, for example, sabeel, the samples bottle was immersed, before opening, into the drinking utensil (cup) that was filled with the water from the pot, then opened inside the utensil and entirely filled and immediately closed while it was inside the utensil in order to prevent any contamination of bacteria from air or any other source of bacteria other than the intended source. The collected water samples were then immediately sent to the laboratory and stored in the refrigerator at 4°C pending analysis.

Statistical analyses of data: Basic statistics program (Microsoft Excel Spreadsheet) was used to calculate mean, range and standard deviation of the obtained data.

Microbiological analyses: Before sterilization, glassware, to be used in the analysis, was washed thoroughly with deionized water and left to dry and then sterilized in a hot oven at 160°C for at least 3 h (Harrigan and McCance, 1976). Instruments such as loops, needles, forceps, spoons and knives were sterilized by flaming directly after dipping in spirit. The media and chemicals used in this study were purchased from Elrebooa CO. Ltd., Assouk-el-Arabi, Khartoum, Sudan. These media and chemicals were used to detect and enumerate different types of microorganisms according to Harrigan (1998). The total viable count of bacteria was carried out by using the pour plate count method. For the determination of coliform bacteria in the water samples, the multiple tube technique

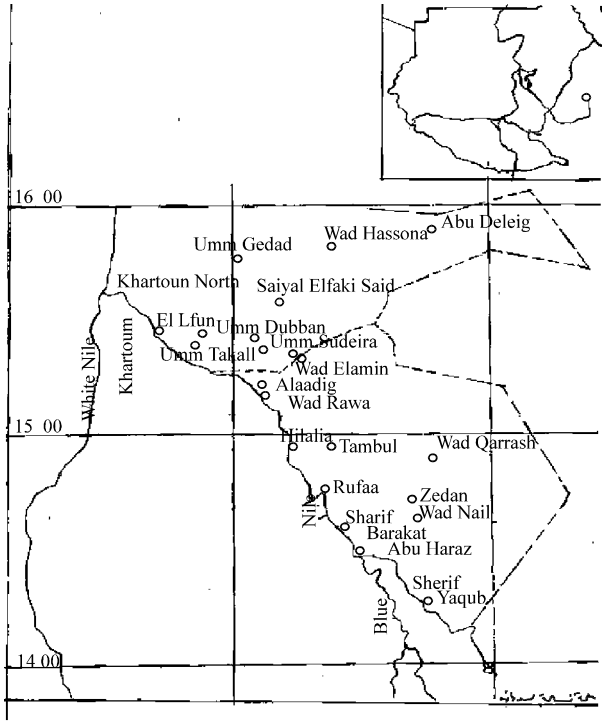

Fig. 1: Location map of the study area, Al-Butana region of Central Sudan, Source: Sudan National Survey Authority, prepared for the current study

was used according to Harrigan (1998). This method is known as the dilution method or Most Probable Number (MPN) technique. The number of positive tubes was calculated from the MPN tables and the results were recorded.

RESULTS AND DISCUSSION

For study convenience, the results obtained were classified and presented in Table 1.

Borehole sources: The range of total viable count of bacteria is between 2.1×10^2 and 6.4×10^2 CFU mL^{-1}. None of the 11 samples that were taken from the direct well-source (outlet-tap) has revealed positive results for the microbial indicators of pollution (total coliform, *E. coli* and *Streptococcus faecalis*). This result can be attributed to the nature of aquifer formation which lends protection against bacterial contamination as it acts as a natural filter that prevent leaking down of microorganisms. Abdel Magid *et al.* (1984) reported that the absence of faecal coliform in samples taken from deep boreholes is attributed to their complete protection from human and animal contact. Moreover, high Cl^- concentration (Brashfield, 1971) and high salinity (Abdel Magid *et al.*, 1984) were reported to correlate negatively with coliform counts. According to Pelczar *et al.* (1977) no plate-count standards have been suggested for water because water with a few pathogenic bacteria is obviously more dangerous than water containing many saprophytic bacteria. However, water of good quality is expected to give a low count, less than 100 mL^{-1}.

Public reservoirs supplying community groups: The range of total viable count of bacteria in common reservoir samples is between 5.2×10^2 and 1.1×10^3 CFU mL^{-1}. Only 2 out of 7 (29%) samples that were taken from different common reservoirs supplying each community have revealed a positive result for total coliform while *E. coli* was detected in only one sample (17%) and none of the samples was positive for *Streptococci faecalis*. The bacteriological contamination in common reservoirs in the study area may be attributed to avian wastes. Animal and avian wastes were previously reported by Gleeson and Gray (1997) as a causative agent of water sources contamination. Faecally-contaminated dust may also be implicated in coverless reservoirs. Reservoirs' cover may usually be removed by windstorms (Hamad and Dirar, 1982; Abdel Magid, 1997). Contamination of common reservoirs is, generally, self-

Table 1: Microbial analysis of the different drinking groundwater sources in the study area

Sample source	No. of sources investigated	Range of total viable count of bacteria CFU mL^{-1}	Total coliform count MPN/100			*E. coli* count MPN/100			*Streptococcus faecalis* MPN/100		
			-	+	%	-	+	%	-	+	%
Borehole sources (outlet-tap)	11	2.1×10^2-6.4×10^2	11.0	0.0	0.0	11.0	0.0	0.0	11.0	0.0	0.0
Public reservoirs	7	5.2×10^2-1.1×10^3	5.0	2.0	29.0	6.0	1.0	17.0	7.0	0.0	0.0
Distribution systems	36	8.0×10^2-1.2×10^3	32.0	4.0	11.0	36.0	0.0	0.0	36.0	0.0	0.0
Public water stand (sabeel)	23	1.84×10^5-9.1×10^5	15.0	8.0	35.0	17.0	6.0	26.0	23.0	0.0	0.0
Domestic house-pot (zeer)	12	9.6×10^3-1.2×10^4	10.0	2.0	17.0	10.0	2.0	17.0	12.0	0.0	0.0
House-refrigerator	4	3×10^2-4×10^3	4.0	0.0	0.0	4.0	0.0	0.0	4.0	0.0	0.0
Karo*	4	3.2×10^3-7.7×10^3	4.0	0.0	0.0	4.0	0.0	0.0	4.0	0.0	100
Different drinking water utensils	3	4.7×10^2-6.4×10^3	1.0	2.0	67.0	2.0	1.0	33.0	3.0	0.0	0.0
Mean		(1.05×10^5)	8.2	1.8	29.0	7.3	1.0	17.0	10.0	0.0	0.0
Range		4×10^2-2.26×10^5	0-32.0	0-8.0	0-100.0	0-36.0	0-10.0	0-100.0	1-36.0	0.0	0.0
SD		(2.5×10^5)	9.0	2.4	37.0	11.0	1.8	30.0	11.0	0.0	0.0

SD: Standard deviation, -: Negative test, +: Positive test, MPN: Most probable number of coliform organism, CFU: Colony forming units, *Karo is a local name for a cart pulled by donkeys or horses, usually used for fetching drinking water from public water sources to consumers

lasting due to flushing out via the pipeline system. Reservoir self-lasting contamination was demonstrated and confirmed by taking a water samples from Algara' village reservoir which showed positive results for each of the total coliform count (23 CFU mL^{-1}), the *E. coli* (4 CFU mL^{-1}) and the total viable count of bacteria 8.0×10^3 CFU mL^{-1}. Repeating the analysis of the same reservoir after a period of one month has revealed negative results for all of the above mentioned microbial parameters and the total viable count of bacteria was reduced to 4.74×10^3 CFU mL^{-1} (41.25%). It has been reported that the low levels of coliform in the surface and overhead tank water could be partially responsible for their low nitrite contents. Consequently, the higher coliform counts in rivers could explain, in part, their higher nitrite contents via nitrate reduction nitrite (Ukhun *et al*., 2005).

Distribution systems: The total viable count of bacteria in distribution systems ranged between 8.0×10^2 and 1.2×10^3 CFU mL^{-1}. Among the 36 samples taken from the distribution systems of several communities, only 4 (11%) samples have shown positive results for the total coliform count test, whereas both *E. coli* and *Streptococcus faecalis* have shown negative results. The low microbial contamination of the distribution systems in the studied communities may be attributed to the properly installed pipelines and lack of fractures. Fractures in distribution pipeline are considered as the main factor responsible for microbial contamination, especially, after treated water is being pumped along the distribution network. During its journey to consumers, water takes microbial contaminants through undetected leaks in the old fractured and worn distribution pipes. Microbial contamination in the distribution pipeline systems in the study area may be attributed to soil movement characterizing the heavy cracking clay soil of the study area. The soil movement may play a significant role in pipe-joint damages, especially when the pipes are installed at depths just at the subsurface layer of the soil. Moreover, improperly buried pipes are more vulnerable to microbial contamination caused by accidental fractures in pipelines. Previously, Gleeson and Gray (1997) reported bacterial contamination cases of drinking water as due to undetected fractures in the pipes.

Public water stands (sabeel): According to Table 1, the total viable count of bacteria in public water stands (sabeel) ranged from 1.84×10^5-9.1×10^5 CFU mL^{-1}. Generally, the relatively high-contaminated samples are those taken from the public water stands, locally known as sabeel (charity clay-pot stands that supply water to passing-by persons in streets and market places). Sabeel stands are situated in various locations throughout the study area. Positive total coliform count was detected in 8 samples out of 23 (35%). *E. coli* test was positive in 6 samples (26%) out of the 23 analyzed samples, whereas none of the examined water samples have shown any positive result for the *Streptococcus faecalis* test. Public water stands are the most vulnerable drinking water source to bacterial contamination in the communities of the study area. Generally, bacterial contamination in public water stands may be attributed to a variety of factors; among these factors, the microbial contaminated hands of some of the users, in particular children, is the most plausible. Handle-less cups used for drinking exacerbate bacterial transmission by the contaminated hands that are immersed to various depths into the bulk of the drinking water inside the pot. Coverless clay-pot facilitates the bacterial accession into drinking water; especially widely open clay-pots. Dust storms, unattended domestic animals and insects increase the potential for bacterial contamination as well (Hamad and Dirar, 1982). The absence of the daily cleaning practice, prior to filling the clay-pot, allows growth of bacteria, algae and other microorganisms,

especially, in overlooked locations. Furthermore, the hoses that are usually used for filling the pots are another potential source of bacterial contamination Abdel Magid (1997). The mechanism of pathogenic contamination in public water stands is independent of pollution at the groundwater source (*in situ*). Previous evidence (Abdel Magid *et al*., 1984) attributed the high total coliform and faecal coliform counts, in hand-dug wells, to dust storms and contamination of the buckets and the ropes used by humans for obtaining water from the well. Jensen *et al*. (2002) reported that even if drinking water of poor rural communities is obtained from 'safe' sources, it can become contaminated during storage in the house under non hygienic and non-sanitary conditions. Moreover, the role of livestock activity as a major factor of bacterial input cannot be excluded (Stephenson and Street, 1978).

Domestic house-pots (zeer): The total viable count of bacteria in the domestic house-pots ranged between 9.6×10^3 and 1.2×10^4 CFU mL^{-1}. Less bacterial contamination can be observed in samples that were taken from domestic house-pots when compared with those taken from public water stands pots. Only 2 samples (17%) out of the 12 samples investigated showed positive results with respect to both of the total coliform and *E. coli* tests. None of the samples tested have shown any growth of *Streptococci faecalis*. However, the relatively low bacterial contamination that was observed in domestic house-pot samples in comparison to the public water stands-pots can be attributed to the daily cleaning practice, limited usage and shelter from faecally contaminated sources such as dust storms. The in-house bacteriological water quality is independent of the initial bacteriological water quality of the source (Jensen *et al*., 2002). Bacterial contamination of household drinking water is a result of many factors (1) post-treatment contamination along the water distribution line (2) storage and intermittent provision by the drinking water company and (3) lack of or inadequate post-storage water treatment before consumption (Nguendo-Yongsi, 2011).

House-fridge (refrigerator): According to Table 1, the relatively, medium range of total viable count of bacteria (3×10^2-4×10^3 CFU mL^{-1}) for fridges, in comparison to house-pot samples, may be attributed to the limited growth of bacteria inside the refrigerator. The 4 groundwater samples which were taken from private house-refrigerators have revealed negative results for each of the total coliform, *E. coli* and *Streptococcus faecalis* tests.

Karo vendors: The range of total viable count of bacteria is between 3.2×10^3 and 7.7×10^3 CFU mL^{-1} in samples taken from karo vendors (Donkey or horse mounted water distribution carts). All of the four water samples investigated showed negative results for the presence of each of the coliform, *E. coli* and *Streptococcus faecalis*. In villages without water distribution systems, karo vendors are the main means of distributing water from a public water source to consumers. The karo vendors take the water directly from the well source or else and sell it to customers who evacuate the water barrels many times a day (filling and refilling repeatedly), thus flushing out any non-hygienic residues and consequently retard or prevent significant growth of bacteria (i.e., self-cleaning).

Miscellaneous drinking water utensils: According to Table 1, for the three accidentally obtained water samples that were taken from drinking jug, hand-cooler and ibreeg (utensil usually used in latrines) the total viable count of bacteria ranged from 4.7×10^2-6.4×10^2 CFU mL^{-1}. The samples of drinking jug and hand-cooler showed a positive result for total coliform test (67%). *E. coli* test was positive for the hand-cooler sample (33%), whereas negative results were obtained for the *Streptococcus faecalis* test for all of the samples of the various drinking water utensils tested. This bacterial contamination of the drinking jug and the hand-cooler may be attributed to the microbial contaminated hands of persons utilizing these facilities. Poor hygienic behaviors such as improper method of storage, handling and serving, deteriorate the quality of drinking water (Tambekar *et al*., 2006). However, in case of the ibreeg sample, the continuous and repeated use of this utensil assists in washing out any non-hygienic residues and consequently retards vigorous bacterial growth.

CONCLUSIONS AND RECOMMENDATIONS

In this study, it has been indicated that public water stands (sabeel) are the most vulnerable drinking water source to bacterial contamination in the study area when compared to other sinks and sources.

To minimize microbial contamination in storage tanks and common "sabeel" facilities, the study suggests that public health authorities should enforce adequate hygienic practices, beside preventing the use of handle-less utensils in common "sabeel", daily cleaning (before filling) of water-pot and common reservoirs and that domestic water storage tanks should be tightly covered and residues of precipitated mud should, at least, be monthly flushed and periodically disinfected.

To avoid microbial contamination via fractured distribution pipes; pipes of good noncorrosive material should be installed at the right depth in order to avoid probable disruption which may be caused by soil movement etc.

Public health authorities should undertake the initiative to encourage and spread the culture of sanitation among residents in the study area.

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