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Microbiological assessment of drinking water with reference to diarrheagenic bacterial pathogens in Shashemane Rural District, Ethiopia

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Fecal contamination of drinking water is a major problem in rural communities of Ethiopia, where surface water sources like rivers, wells, and lakes are used for drinking. In spite of these problems, few data exist on the microbiological safety of water sources in these settings. Therefore, the aim of this study was to investigate the microbiological safety of drinking water from the sources and households in selected communities of Shashemane rural district, Ethiopia. A descriptive analytic study was used to examine the bacteriological quality of drinking water from sources and household containers. Data on water collection and storage practices were collected using structured questionnaires. Water samples were collected according to the WHO Guidelines for drinking water quality assessment from surface and ground water sources which are used directly for drinking purpose in the community. Water samples were examined for total coliforms and fecal coliforms using the most probable number methods. The detection of *Escherichia coli, Salmonella, Shigella*, and *Vibrio cholerae* were assessed by biochemical tests. Total coliforms were detected in higher proportion in all water source samples. Fecal coliform contamination was detected in all water sources, except in hand pipes. *E. coli, Salmonella* and *Shigella* species were detected in water samples from river and wells. Total coliforms, fecal coliforms, *E. coli* and *Salmonella* spp. were also detected in water samples from households. The bacteriological load of the sampled water from source and households was found to be higher than the maximum value set for drinking water. Therefore, enabling the community access to potable water through encouraging construction of toilets, creating proper domestic and animal waste disposal system and rendering health education and sanitation practices for the community is recommended.

Key words: Diarrhea, drinking water, Ethiopia, fecal coliforms, Shashemane, total coliforms.

INTRODUCTION

Access to safe drinking water is one of the basic human rights and is enormously crucial to health. For a nation to maintain optimal health and development, there has to be a continual supply of safe drinking water to its population.

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However, drinking water is also the most important source of gastroenteric diseases worldwide, mainly due to the fecal contamination of raw water, failure in the water treatment process or recontamination of drinking water at source and point of use (WHO, 2003; Pironcheva, 2004; Wright et al., 2004; Clasen et al., 2006, 2007; Miner et al., 2016).

About two thirds of drinking water consumed worldwide is derived from various surface water sources like: lakes, rivers and open wells. Hence, it can easily be contaminated microbiologically by sewage discharges or fecal loading by domestic or wild animals (WHO, 2003). As a result, water related diseases continue to be one of the major health problems globally (JMP, 2008). It is estimated that globally 80% of all illnesses are linked to use of unsafe and microbiologically poor water quality (WHO, 2002; Mpenyana-Monyatsi et al., 2012).

In developing countries, about 1.8 million deaths per year are attributed to unsafe water, sanitation and hygiene, mainly through infectious diarrhea (WHO, 2002). Diarrheal disease remains a major killer in children. It is estimated that 17% of all child deaths under the age of 5 years in developing countries result from diarrheal diseases (WHO, 2003). Hence, lack of safe drinking water supply, basic sanitation and hygienic practices is associated with high morbidity and mortality from feco-orally transmitted diseases.

About 1.1 billion people have no access to safe drinking water and diarrheal disease is highly endemic in these societies. This underlines the need for safe drinking water. However, the effectiveness of interventions aimed at improving the quality of drinking water alone may not solve the problem because people can become infected with organisms that cause diarrhea through multiple pathways. Even in developed countries with improved water supplies, diarrhea is often endemic (Wright, 2004; Colford et al., 2006; Roy et al., 2006; Clasen et al., 2007; Johnson et al., 2016). For example, it has been reported that environmental interventions have shown 15 to 17% median reduction in diarrhea from water quality Interventions (Clasen et al., 2007). This indicates that not only water improvements at the source or collection point (protected wells, boreholes, and tap stands) but also improvement at household level and other sources are equally important to minimize the risk of water born diarrhea (Zvidzai et al., 2007).

In Ethiopia, with a population of 75 million, more than half of the population has no access to safe water. It is estimated that about 35 million people do not have access to sanitation services and half of the population of the country are suffering from water related diseases (UNICEF, 2008).

Ethiopia is one of the developing countries with a population of 75 million where only 52 and 28% of its population has access to safe water and sanitation coverage, respectively (MoWR, 2007). For this reason,

60 to 80% of the population suffers from water-borne and water-related diseases (MoH, 2007). This burdens the country with enormous financial and social costs to take care of such a huge number of people suffering from these debilitating infections.

It was also estimated that over half a million children under the age of five die every year from diarrhea related diseases. In other words, for every five children born, one will die from diarrhea before they reach their 5th birthday (UNICEF, 2008; Clasen et al., 2007).

In developing countries such as Ethiopia, most of the rural communities are poverty-stricken, lack access to potable water supplies and rely mainly on river, stream, well and pond water sources for their daily water need. Water from these sources is used directly by the inhabitants and the water sources are fecally contaminated and devoid of treatment (WHO, 1993). Consequently, a significant proportion of residents in rural communities of Ethiopia are exposed to water-borne disease and their complications.

These pathogenic contaminants are derived from homeotherms including human beings and resulted in contamination of drinking water sources in areas with poor standards of hygiene and sanitation. The sanitation crisis heightens when it is accompanied by poor health protection system associated with poor life standard (Nath, 2003).

Microbiological water quality can also be deteriorated in the course of collection, transport, and home storage. Thus, access to a safe source alone does not ensure the quality of water that is consumed. Furthermore, a better water source does not lead to full health benefits in the absence of improved water storage and sanitation (Clasen et al., 2007). Hence, the objective of this study was to assess the bacteriological quality of drinking water from sources and households in the study area and to highlight the possible associated risk factors.

MATERIALS AND METHODS

Study area description

Shashemane district is found in West Arsi Zone of Oromia Regional State, Ethiopia. It is located at 250 km south of Addis Ababa on the way to Moyale-Keniya. The Woreda (district) is located within an altitude range of 1700 to 2727 m above sea level with annual rainfall record of 750 to 1200 mm/year. Drinking water and latrine coverage of the Woreda is 54 and 67%, respectively. The Woreda has 2 health centers and 36 health post which give service for the inhabitants of 37 rural kebeels (local administrative units).

Demographic characteristics of the study area

According to the 2012 national housing and population census, the population of the study area is estimated to 286,287 with 139,673 males and 147,114 females. There are 28,161 households with an average family size of 5.4 per household.

Table 1. Bacteriological analysis of drinking water samples from different sources.

Raw water samples	Total colifom (MPN/100 ml)	Fecal colifom (MPN/100 ml)	*E. coli*	*Salmonella* spp.	*Shigella* spp.	*Vibrio cholera*
Rivers	270 - 1600	70 - 1600	P	P	P	ND
Wells	67 - 1366	1 - 213	P	P	P	ND
Springs	2 - 70	4 - 27	ND	ND	ND	ND
Hand pipe	2 - 9	ND	ND	ND	ND	ND
Point of use	1 - 140	1 - 21	P	P	ND	ND

P: Present, ND: not detected.

Study design and sampling frame

A descriptive analytic study was used to examine the bacteriological quality of drinking water from sources and household containers. Data on water collection and storage practices were collected using structured questionnaires. Among 37 kebeles, 5 representative kebeles were selected purposively. The selection was based on the existence of diarrheal disease for the last five years in the area. In addition, geographical location was considered to attain representative kebeles. Availability of different types of sources of water for consumption was also considered. For water sample collection from each sample kebele, simple random sampling method was used.

Sample size determination

For water sample collection from the selected kebeles, a judgment sampling method was used. Accordingly, a total of 135 water samples (93 from source and 42 from households) were collected for bacteriological examination. For risk factor assessment, the sample size was determined by single population proportion formula. Accordingly, 291 households were selected randomly for interview.

Water sample collection

Water samples were collected according to the WHO Guidelines for drinking water quality assessment (WHO, 1993). The samples were collected from surface and ground water sources (protected and unprotected wells, hand pipes, rivers and springs) that are used directly for drinking purpose in the community. Water samples were also collected in the same area from household containers. Closed sterilized 500 ml glass containers were used to collect samples aseptically. Before taking sample from hand pipes, the hand pipes water was flushed for 5 min and then the mouth of the hand pipe was sterilized with a spirit of lamp flame and then cooled by running water. Sample from hand dung wells were collected by attaching a piece of string to the sampling bottle together with a clean heavy material that sink down the bottle into the well and unwinding the string slowly. Stored water samples were collected after 1 to 3 h of storage using the usual cups households use to draw the water from the storage container. All samples were transported to the laboratory on ice, kept at 4°C and analyzed within 2 h. The samples were drawn for 5 months (October 2012 to February 2013).

Microbiological analysis

Microbiological analyses of water samples were performed as described in Standard Methods for the Examination of drinking water (WHO, 1996; APHA, 2005). Total and fecal coliforms were determined by the most probable number (MPN) per 100 ml sample using multiple tube fermentation technique including presumptive, confirmed and completed phases (APHA, 2005). For samples which gave positive results for coliforms test, further identification was done using an IMVIC test as described by Niemi et al. (2003). The isolation of *Salmonella* and *Shigella* was done using pre-enrichment and enrichment techniques (APHA-AWWA, 1998). Double enrichment in alkaline peptone water was used for isolation of *Vibrio cholera* as described elsewhere.

Water sanitation survey

Using random sampling methods, representative samples of households were selected from each farmer's kebeles to assess the knowledge and hygienic practices of the community with respect to the bacteriological water quality. Standard and structured questionnaire were developed for the purpose of data collection. Questionnaires were made to the household head. Core points included in the questionnaires were: demographic details of the respondent, personal and domestic hygiene practices, water handling and usage, source and method of water collection, information regarding defecation (availability of toilet and their usage), and concepts of diarrheal diseases. The questionnaires were set according to Banda et al. (2006).

Statistical methods

Descriptive analysis, which included mean, proportions, percentages as well as descriptive graphs and tables were used. The bacteriological counts recorded were compared with the WHO guidelines for drinking water.

RESULTS

Prevalence of indicator organisms in water sample from sources

Total coliforms (TC) were detected in all samples taken from all surface waters (rivers and springs), wells and hand pipe water samples. The MPN/100 ml analysis of total coliforms ranges from 270 to 1600 and 67 to 1366 for rivers and wells, respectively (Table 1).

Fecal coliforms (FC) were detected in 27/27 (100%)

Table 2. Prevalence of indicator organisms and pathogens detected in water samples from source and households at contamination level at source.

Indicator organism	Water sample source			
	River water, n=27	Spring, n =27	Well, n=36	Hand pipe, n=3
Total coliforms (TC)	27 (100)	5 (83.0)	36 (100)	2 (66.7)
Fecal coliforms (FC)	27 (100)	1 (16.7)	33 (91.6)	0 (0.0)
E. coli	24 (88.8)	0 (0)	33 (91.6)	0 (0.0)
Salmonella spp.	8 (29.6)	0 (0.0)	8 (22.2)	0 (0.0)
Shigella spp.	3 (11.1)	0 (0.0)	3 (8.3)	0 (0.0)
V. cholera	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 3. Prevalence of indicator organisms and pathogens detected in water samples from source and households at contamination level at point of use.

Sample villages	Sample size	T C [n (%)]	FC [n (%)]	*E. coli* [n (%)]	*Salmonella spp.* [n (%)]	*Shigella spp.* [n (%)]	*V. cholera* [n (%)]
Edola	7	7 (100)	4 (57.14)	2 (28.5)	0 (0.0)	0 (0.0)	0 (0.00)
B. Guracha	7	5 (71.4)	1 (14.28)	1 (14.28)	0 (0.0)	0 (0.0)	0 (0.0)
Chabi	7	5 (71.4)	2 (28.57)	1 (14.28)	0 (0.0)	0 (0.0)	0 (0.0)
K. Rogicha	7	7 (100)	6 (85.7)	4 (57.14)	1 (14.28)	0 (0.0)	0 (0.0)
Sole	7	7 (100)	5 (71.4)	2 (28.5)	0 (0.0)	0 (0.0)	0 (0.0)
Awasho	7	7 (100)	7 (100)	4 (57.14)	1 (14.28)	0 (0.0)	0 (0.0)
Total	42	38 (90.5	18 (42.8)	14(33.3)	2 (4.76)	0 (0.0)	0 (0.0)

and 33/36 (91.6%) water samples from rivers and wells water, respectively (Table 2). Excessive fecal coliform bacteria were found along the course of the river stream. The level of fecal coliform bacteria ranges from 70 to 1600 MPN/100 ml and 1 to 213 MPN/100 ml in rivers and wells water samples, respectively (Table 1). Fecal coliforms were not detected in any samples taken from hand pipe water, while from spring water sources, only one out of 6 (16.6%) samples was found positive for FC test (Table 2). *Escherichia coli* was detected in 24/27 (88.8%) and 33/36 (91.6%) in water samples collected from rivers and wells, respectively (Table 2).

Prevalence of water borne bacterial pathogens in water samples from various water sources

Potential enteric bacteria such as *Salmonella* and *Shigella* species were isolated from various water sources in the area particularly from river water sources.

From 27 river water samples tested, *Salmonella* spp. were detected in 8/27 (29.6%), while *Shigella* spp. were detected in 3/27 (11.1%). *Salmonella* spp. were detected in 8/36 (22.2%) of water samples from the well water sources, while 3/36 (8.3%) of water samples from the wells were positive for *Shigella* spp. (Table 2).

Salmonella spp. were more frequently isolated from the rivers. *Salmonella* spp. and *Shigella* spp. were not found in samples from springs and hand pipe water samples. In all samples tested *Vibrio cholera* was not detected (Table 2).

Contamination of drinking water at point of use

Risk levels of bacterial indicator organisms as well as pathogens in water samples from the household water containers were investigated (Table 3). From a total of 42 water samples collected from the households, 38/42 (90.5%), 18/42 (42.8%), 14/42 (33.3%) and 2 (4.76%) samples were positive for: TC, FC, *E. coli* and *Salmonella* spp. respectively (Table 3). The average count of TC, FC and *E. coli* were beyond the recommended value by WHO which is 0 cfu/100 ml of sample. The results have shown that there is fecal contamination of stored household water in most surveyed households.

Water handling practices of the community in the study area

It was observed that 142/291 (48.8%) of the respondents

use traditional clay pots to collect water from the source, while 149/291 (51.2%) of them is used Jerican (plastic container) to collect water from the source. In the present study, 72% the respondents cover their containers with different materials after filling the container with water (Table 4). It was found that some of the households dipped leaves into the containers as a cover, while they transport the filled water containers to their home. Fingers come in contact with water in the filled-up containers to deep leaves during transportation. From 291 households interviewed, only 37% of the respondents cleaned their container before transferring water from collection to storage containers. Mixing of the collected water with old stored water was observed in most of the households and cleaning of the storage container is not practiced at the time of mixing. Those households which use hand dung well use rubber material to withdraw water from the well. Once they withdrew enough water for their daily consumption, they put the rubber material on the ground near the well and they re-use it whenever they need without any proper care (Figure1). It was also found that the same river water is used for bathing and drinking both for human and livestock (Figure 2).

Availability of hygienic facilities and the hygienic practice of the community in the study area

Among the 291 households interviewed, only 143 (49.1%) of them had latrine houses even though most latrine houses were not functional. From the interviewed households, 148/291 (50.9%) of them defecate in open fields, while 143/291 (49.1%) of them use a toilet. Only 130/291 (44.7%) of study subjects exercise hand washing after defecation (Table 4).

Regarding the knowledge and sanitary practices of the respondents, it was found that, among 291 households, only 103/291 (35.4%) of them knew that water can transmit diarrheal disease, while 188/291 (64.6%) of the households did not know whether water can transmit diarrheal disease or not. Out of these interviewed households, only 45/291 (15.5%) households believed that fecally contaminated water can cause diarrheal disease. Only 73/291 (25.1%) of the interviewed individuals understood that unsanitary hands can contaminate drinking water. About 100/291 (34.4%) of households responded that unclean containers can contaminate their drinking water and only 23/291 (7.9%) households knew that uncovered containers could be a source of water contamination (Table 4).

DISCUSSION

According to World Health Organization guideline (WHO, 2003), total coliform counts must not be detected in any 100 ml of drinking water samples. Therefore, results of total coliforms obtained in the present study showed that all examined samples from wells (100%) and most surface water (92%) exceeded the recommended values and not safe for drinking. Although, the WHO guideline for drinking water does not allow any detection of fecal coliforms and *E. coli*, in our study, it was found out that 92.6% of well water samples are contaminated with fecal coliforms, while 54.1% surface water samples are contaminated with *E. coli*. The contamination of these water sources is probably due to poor protections and exposure to contamination by human and domestic wastes. The behavioral and hygienic practices of the community might also be contributing to this high load of indicator organisms. During the survey it was observed that communities in the study area practice open field defecation, bathing and washing closes in rivers and the same water source is used for domestic animals (Figure 2). The river is located in sloppy area and most wells are found at lower elevation compared to the fields used for open defecation. Hence, fecal matter produced by the cattle and human inevitably reaching the water sources and increased the contamination.

Spring water was found less contaminated by coliforms and none of them were contaminated with *E. coli*. The low prevalence of coliforms and the absence of *E. coli* in spring water sample might be due to the geographical protection of springs from animal wastes. In addition, most springs in the area are located under the stones where it flows from a narrow outlet between the stones and as a result the probability of being contaminated by human or animal is low. The continuous flow of the spring water may also contribute for its low contaminations by washing away the microbes.

Salmonella and *Shigella* spp. were isolated from various water sources especially from rivers. The sources of contamination are probably due to human and animal feces and the introduction of microorganisms by birds and wild animals. The high prevalence of bacterial pathogens in river water sources might be due to behavioral practices related to use of the river water sources for bathing, washing cloth and disposing of wastes into the river. The high prevalence of *Salmonella* spp. isolation from surface water sources might be due to manure from free-grazing domestic animals and wild species that can spread onto adjacent fields. The bacteria from the manure enter surface waters with the correct combination of topography and flood. The non-detection of pathogen in some well water sample might be a reflection on the depth of the well among several other contributing risk factors. The detection of these enteric pathogens from drinking water in the study area will put the community at high risk of diarrheal diseases. Secondary data collected from the health centers in the study area (data not shown) indicate that there is high prevalence of diarrheal diseases. Consequently, this

Table 4. Sanitation knowledge and practice of the respondants.

Sanitation practice/sanitation knowledge /water collection and storage containers used	n	%
Presence of toilet at home (n=291)		
Yes	143	49.1
No	148	50.9
Hand washing after toilet with soap/water alone (n=291)		
Yes	130	46.7
No	161	53.3
Open air defecation practice (n-291)		
Yes	148	50.9
No	143	49.1
Knowledge that water transmit diarrheal diseases		
Yes	103	35.4
No	188	64.6
Knowledge that fecally contaminated water can cause diarrheal disease		
Yes	45	15.5
No	246	84.5
Knowledge that unsanitary hand can contaminate drinking water		
Yes	73	25.1
No	218	74.9
Knowledge that unclean container can contaminate drinking water		
Yes	100	34.4
No	191	65.6
Knowledge that uncovered container can contaminate drinking water		
Yes	23	7.9
No	268	92.1
Knowledge that water can be contaminated both at source and point of use		
Yes	76	26.1
No	215	73.9
Getting information about water handling practice		
Yes	86	29.6
No	205	70.4
Type of water collection container		
Clay pots	142	48.8
Jeri can (plastic material)	149	51.2
Covering water during transportation		
Yes	210	72.2
No	81	27.8
Containers rinsing before collection		

Table 4. Contd.

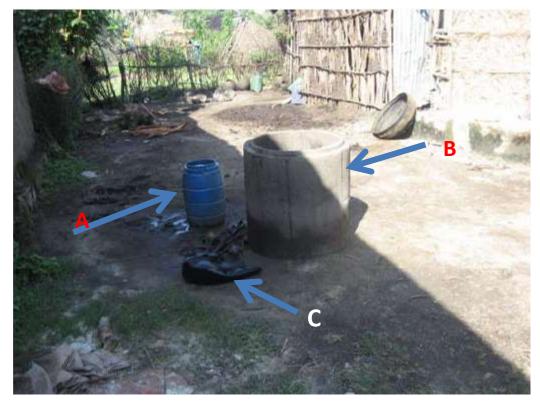|	n	%
Yes	107	36.8
No	183	63.2
Responsible body for water collection		
Mothers	183	62.9
Father	3	0.01
Son	54	18.6
Daughter	51	17.5

n: Total number.

Figure 1. Well water source: (A) container used for water holding and storage, (B) well, and (C) rubber material used to withdraw water from the well.

might be due to the significant contribution of contaminated water consumption by the community. Hence, this result underlines the need for proper action.

The microbiological analysis of water at point of use in the study area indicates that 90.5% of samples were contaminated with total coliforms, while 42.8 and 33.3% of them contaminated with fecal coliforms and *E. coli*, respectively. About 4.8% of the samples were also contaminated with *Salmonella* spp. Mixing of the collected water with old stored water was observed in most of the households and cleaning of the storage containers is not practiced at the time of mixing. The use of clay pots for water storage might also expose the water for contamination since it would not properly cover. The use of uncovered water containers is also likely to increase water contamination between source and point-of-use as hands are dipped into vessels to scoop cupful of water. Contamination through hands, unwashed containers and use of uncovered vessels would result in water quality deterioration from source to point-of-use. When water is transported from source to home, different types of material including leaves are used to cover the

Figure 2. The same water source is used for humans and animals (bathing and drinking).

container which contributes to further contamination. Containers and hands are likely to have been pre-contaminated in the homes in which animals are in the same room where water is stored.

The community in the study area uses the same material for water withdrawal from the storage and for drinking which may lead to a high probability of contamination. In spite of these mentioned risks, it was observed that contaminated water is consumed with remarkable frequency in these rural communities without treatment of water to any degree.

The poor water quality observed in storage containers may be the cumulative effect of collection and storage practices. Fecal pathogens from hands and household utensils appeared to contribute to point-of-use contamination, which highlights the need for improved personal and domestic hygiene practices. As long as water storage remains a fact of life in communities like rural communities around Shashemane, immediate action should be taken to address these risks. In most rural areas, households collect and store water with low

microbiological quality from rivers and wells and these waters were found highly contaminated. Furthermore, the contamination continues at all households included to this study at the point of use.

It was found that majority of the community exercised open field defecation in the study area. Similarly, it was reported that among study subjects interviewed in Southern Ethiopia, which is adjacent to the Shashemane district, almost all of them exercise open filed defecation (Teferi, 2007).

In rural areas of Ethiopia, most people practice open defecation, a tradition that has remained widespread due to inadequate hygiene awareness, lack of technical knowledge on the part of villagers, inadequate policy, investment and implementation. They use the surrounding fields, bushes and even their household compounds for defecation. They practice open defecation without understanding the potential health risks of fecal-related diseases transmission. This shows that community health education is still not exhaustive in the area.

The outbreak of acute watery diarrhea in the study area

in 2007/2008 motivated the villagers to build few toilets, but their use was not sustained for various reasons: the construction of the latrine houses was performed by mass campaign aiming at controlling the incidence of the outbreak which resulted in poor quality construction toilets. Poor construction methods mean that people are afraid of collapse; badly maintained latrines have created a perception of dirtiness leading to fears of becoming ill due to escaping toxic and smelly gases.

The sanitation and hygienic practices are essential in attempt to protect from diarrheal disease. The rural community in the study area does not have enough basic knowledge and awareness about the relationships between water quality and diarrheal disease. It is believed that better community sanitation reduces the concentration of thermo-tolerant coliforms by two orders of magnitude which lead to a 40% reduction in diarrhea. Further, providing private excreta disposal would be expected to reduce diarrhea by 42%, while eliminating excreta around the house would lead to a 30% reduction in diarrhea (Vanderslice and Briscoe, 1995). Hence, water handling and hygienic practices should be an integral part of health education even if treated water is provided as the water contamination history does not wind up at the source only.

In this study most drinking water sources tested were found to be contaminated. The sources of contamination in the area have been identified as coming from human and/or animal wastes. The waste is entering the water course and these highly contaminated water sources are being heavily extracted by the villagers. Other source of contamination was possibly due to poor management of water and poor sanitation practices which promote cross-contamination at household level. This situation continues to expose inhabitants to high risk health problems, particularly, to the serious outbreaks of diarrheal disease which is commonly reported in the study area since 2007.

In view of the aforementioned findings and risks, we strongly recommend that attention be focused on ensuring a supply of safe drinking water and improving its management from the source to the storage point. Education dealing with water management and empowering the community with simple and sound technologies aimed at reducing deterioration of their drinking water should be an integral component of water supply.

Hygienic practices at household level may be improved by covering containers, avoiding children and animals at water points in rooms where water is stored. Home treatment of water and 2-cups system to withdraw water for consumption should be encouraged. The health extension workers should educate the community members about the correct use and storage of water, the need for safe sanitation facilities, personal and environmental hygiene and diarrheal disease transmission,

aiming at reliable behavioral change.

Common management of the rural water can be adopted by extending hygiene education and avoiding illiteracy from the rural areas in general. Thus, proper use of solid waste disposal and sustainable use of latrine would be the option that has to be encouraged by community though teaching. Sanitation and hygiene education promotion should be done regularly, repeatedly and continuously to adopt hygienic behavior or practice on latrine utilization among the community. Monitoring activities by the extension health workers and others is very crucial to sustain and to bring impacts on the health of the community.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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