Full Length Research Paper

Microbiological quality of water, associated management practices and risks at source, transport and storage points in a rural community of Lungwena, Malawi

Taulo, S.[1, 3*], Wetlesen, A.[1], Abrahamsen, R.[1], Mkakosya, R.[2] and Kululanga, G.[3]

[1]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Science, P.O. Box 5003, N-1432 As, Norway.
[2]Department of Microbiology, College of Medicine, P/B 360, Blantyre, Malawi.
[3]Faculty of Engineering, University of Malawi, P/B 303, Blantyre, Malawi.

This study investigated and compared the microbiological quality of source, transported and stored water in Lungwena households. It also examined water management practices at all the investigated points. One hundred and eighty (180) water samples were collected from 6 villages and tested for *Escherichia coli, Salmonella, E .coli* 0157:H7 and *Campylobacter jejuni* using standard methods. Water contamination practices were observed in two hundred and eighty seven households. *E. coli, Salmonella, E. coli* 0157:H7 and *C. jejuni* were isolated in 54, 24, 6.7 and 2.2% of the samples, respectively. *S*ampling points revealed a significant difference (p = 0.001) in *E. coli* concentration. *Salmonella* concentration between sampling points was not significant (p > 0.05). *E. coli* concentration was significantly (p = 0.042) higher than that of *Salmonella* spp. The microbiological quality of water was found to be poor as a result of both poor water management practices and environmental sanitation. There were no significant differences (p > 0.05) in water management practices among the villages.

Key words: Pathogens, stored water, transport water, water contamination.

INTRODUCTION

The potential of water to harbour microbial pathogens and causing subsequent illness is well documented for both developed and developing countries (Younes and Bartram, 2001; Wright et al., 2004) . Water-related diseases continue to be one of the major health problems globally (UNESCO, 2003). It is estimated that 80% of all illnesses are linked to use of water of poor microbiological quality (WHO, 2002). One of the strategies for tackling this problem is the provision of protected sources such as boreholes, standpipes, protected wells and springs (Ahmed et al., 1998). However, such facilities are located some distances requiring transportation to homes. During transportation, water gets contaminated with bacteria which grow and proliferate during storage in the homes (Hoque et al., 2006). This contamination may lessen the health benefits of water source improvements (Wright et al., 2004).

In Malawi, access to safe water has increased over the past 8 years through the installation of the above mentioned protected sources. In Lungwena, about 82% of the households have access to portable water, with 78% of the households having access to borehole water sources (Lungwena NUFU census, 2004: unpublished). Despite this availability and promotion of the use of such facilities, water-related diseases remain the major cause of mortality and morbidity (Malawi College of Medicine, 2000 unpublished; Ministry of Health, 2004). The area experiences outbreaks of Cholerae every rainy season (D. Pondani, Medical Assistant, Lungwena Health Centre; personal communication). The above episodes suggest that consumption of water from contaminated sources and poor environmental sanitation continue to be practiced in Lungwena.

The present study aimed at assessing microbiological

*Corresponding author. E-mail: staulo@poly.ac.mw
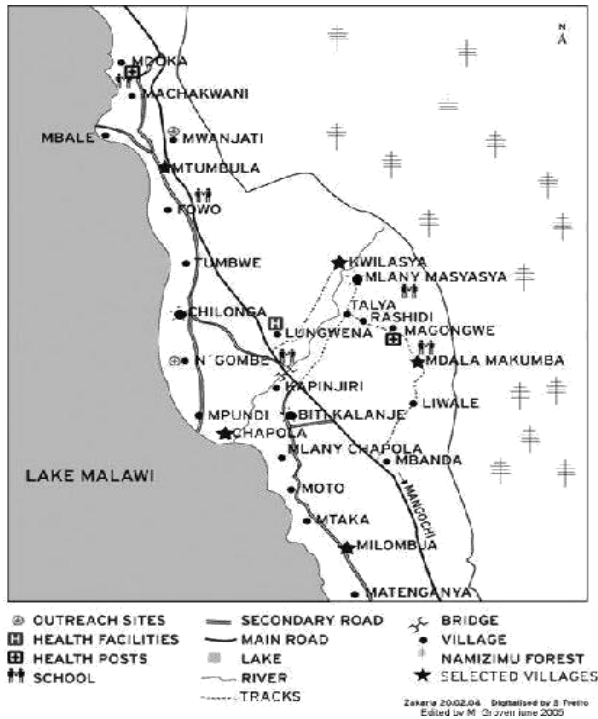
Figure 1. Map of Lungwena showing the sampled villages

the quality of the water at source, during transportation and storage in homes in Lungwena. It also attempted to investigate water management practices at the assessed points. It is expected that the findings of the study will assist Environmental Health Officers in developing appropriate health education campaign messages suitable for this Moslem dominated community.

MATERIALS AND METHODOLOGY

Study area

The study was conducted in Lungwena, a coastal area in the Southern part of Malawi. The area has 26 villages but only six villages (Figure 1) were randomly selected for the purpose of this study based on their geographical location.

Questionnaire

Data on water collection, transportation and storage practices were collected using structured questionnaires. The questionnaire was pre- tested in six households in the selected villages. Two hundred and eighty seven out of 350 randomly selected households were successfully interviewed.

Microbiological analysis

Sample collection and processing

Microbiological samples were collected from 60 households (10 per village), randomly selected from the 287 interviewed households. Samples were drawn from the source, transported and stored wa-

ter. Source samples consisted of 47 boreholes 4 protected wells, 4 unprotected wells and 5 lakes and/or rivers (2 replicates). Borehole samples were collected after running the water for about 1 min (to mimic normal practices). Samples from unprotected sources were collected using the same containers households used to draw the water. Samples from transported water were taken just before the water was brought into the house. Stored water samples were collected after 1- 3 h of storage using the usual cups house-holds use to draw the water from the storage container. Roberts et al. (2001) demonstrated that bacteria settle at the bottom of the container after 4 h of collection hence our sampling was conducted within 3 h. Collected samples were stored in a cooler box with

temperature maintained at between 4 -10 ^oC using ice packs. Samples were transported to the hospital laboratory where they were analysed within 3 h. To enumerate the samples, 10-fold dilutions were prepared using sterile Buffered Peptone Water (BPW) containing 0.1% peptone + 0.85% NaCl (CM0509: Oxoid, Basingstoke, London, UK).

Identification and enumeration of bacteria

E. coli and *E. coli* 0157:H7

E. coli was enumerated by spread plating an aliquot of 1 ml onto 3M[TM] Petrifilms[TM] for E. coli/Coliform Colony Count (EC – plates, 3 M Company, St. Paul, USA; Frampton and Restaino, 1993). The plated petrifilms were then incubated for 48 h at 37 ^oC (Nordic Committee on Food Analysis, 1993). Blue coloured colonies with gas entrapment on EC – plates were presumed to be *E. coli*. Presumptive colonies were confirmed by conducting an Indole test with Kovacs reagent (Merck, Midrand, South Africa) . Identification of *E. coli* 0157:H7 was conducted by streaking 100 l of the serial

diluted samples on to Cefixime Rhamnnose Sorbitol MacConkey agar plates (CR- SMAC, CM1005; Oxoid) and incubated at 37 ^oC for 24 h. Straw-coloured colonies were identified and recorded as *E. coli* 0157:H7 positive, (Vernozy-Rozand, 1997). Presumptive colonies were pooled and subsequently subjected to slide agglutination test using *E. coli* 0157:H7 antisera (DR0620; Oxoid).

Salmonella species

1 ml of the aliquot was added onto 10 ml of Selenite Cystine Broth (SCB base + 0.4% Sodium Biselenite, CM0395; Oxoid) and incubated at 37 ^oC for 18 h. The broth was then sub-cultured onto Salmonella-Shigella (SS) agar plates (CM0099; Oxoid) for 24 h at 37 ^oC. Big colonies with black centres were presumed to be *Salmonella* (Oxoid, London, UK). Presumptive colonies were exposed to biochemical test by inoculating a colony into Kliger Iron agar (CM0033; Oxoid) . Production of hydrogen sulphide, shown by blackening in part of butt was indicative of *Salmonella* (Oxoid, London, UK). These characteristic colonies were pooled and confirmed using *Salmonella* latex agglutination test kit (FT0203; Oxoid).

Campylobacter jejuni

An aliquot of 1 ml was inoculated into Preston Enrichment Broth (SR0117; Oxoid). The contents were incubated at 42 ^oC for 24 h (Scates et al., 2003). Two loopfuls of the broth were transferred onto Blood agar plates (Columbia blood + Lysed Horse Blood, Merck, Midrand, South Africa), wrapped in plastic pouches (AG0020C; Oxoid) and incubated at 42 ^oC for 4 days under micro-aerophilic conditions using anaerobic jar (HP0031; Oxoid) containing Campgen sachets (CN0020C; Oxoid). Colonies appear-

Table 1 Distribution of positive samples for the tested organisms in the six villages in Lungwena, Malawi.

Sampled villages	Number of positive samples per micro-organism (n)				
	Salmonella	*E.coli*	*0157:H7*	*C. jejuni*	Total
Milombwa	13 (7.2)	19 (10.6)	5 (2.8)	Nd[b]	37 (20.6)
Chilonga	5 (2.8)	16 (8.9)	2 (1.0)	Nd[b]	23 (12.8)
Chapola	3 (1.7)	16 (8.9)	Nd[b]	Nd[b]	19 (10.6)
Mdala Makumba	8 (4.4)	19 (10.6)	1 (0,6)	4 (2,2)	32 (17.8)
Kwilasya	9 (5.0	16 (8.9)	4 (2.2)	Nd[b]	29 (16.0)
Ntumbula	5 (2.8)	11 (6.0)	Nd[b]	Nd[b]	16 (8.9)
Total	43 (24)	97 (54)	12 (6.7)	4 (2.2)	156 (86.7)

NB: Figures in parenthesis are percentages based on total of 180 samples

[b] Nb: Not detected

ing round to irregular with smooth edges were presumed as *Campylobacter* (Lennette et al., 1985). A loopful of growth was placed in a drop of 3% hydrogen peroxide and appearance of bubbles was confirmed as positive for *Campylobacter*.

Statistical analysis

Data on the bacterial concentration for each sample were entered in Minitab version 14 worksheet (Minitab Inc, 2004), transformed into $\log_{10}$ Coliform Forming Unit per 100 ml (CFU/100 ml) of water sample. Significance of differences in pathogen concentrations among sampling points and villages were tested using one way analysis of variance (ANOVA) at 95% level of significance. Questionnaire variables were analysed using SPSS version 11.5.1 (SPSS Inc, 2002) and significant of differences tested using chi-square for categorical variables.

RESULTS

Incidence of water-borne pathogens in water samples

Results of the microbiological tests on the 180 samples are presented in Table 1. The tests detected *E. coli, Salmonella* spp.*, E. coli* 0157: H7 and *C. jejuni* in 54, 24, 6.7 and 2.2% of the samples, respectively. The highest (20.6%) incidence of pathogens was obtained in Milombwa and lowest (16%) in Ntumbula villages. Upon further inquiry, it was established that the community's borehole was broken for over 2 months and people were drawing water from uncovered protected well. *E. coli* and *Salmonella* were identified in all the six villages while *E. coli* 0157: H7 was identified in 4 villages. *C. jejuni* was isolated in Mdala Makumba only and the sample was from unprotected shallow well.

Bacterial counts

Mean counts of viable *E. coli* and *S. aureus* cells for each sampling point are presented in Figure 2. There was a significant difference (p = 0.001) in *E. coli* cells count between sampling points, with the highest count (3.71 ± 0.56) having been recorded in stored water samples.

Number of positive samples increased from source to storage. In contrast, *Salmonella* counts did not demonstrate any significant difference (p = 0.732) between sampling points possibly due to a few identified positive samples at the source (6 samples only) . As expected, viable *E. coli* and *S. aureus* cells counts were relatively higher in unprotected water sources (data not shown). *E. coli* counts were significantly (p = 0.042) higher than those of *Salmonella* spp. *C. jejuni* and *E. coli* 0157: H7 cells were detected in source samples (2 for each) and the same numbers were reflected in the trans-ported water, possibly indicating the significance of contamination of the pathogens at the source than during transportation.

Water management practices and risks

### Water collection and storage frequencies, and walking distances

Table 2 shows the relation between collection and storage frequencies, and distances from water sources to the households (round trip) . Frequency of collection was relatively higher among the category with less distance to the sources and shorter storage periods were observed in households living within a short distance (10 – 20 min). Viable *E. coli* and *Salmonella* spp. cells counts in 5 random samples collected from households that reported to have stored water for more than 2 days were surprisingly lower that those that were collected within 1 -3 h possibly due to settling of the organisms together with organic matter. Contamination frequency was relatively higher in households that collected water for more than 2 x a day (data not shown).

Source and transport sampling points practices

Observed and reported practices and risks at source and during transportation are presented in Figure 3. At the source, 97.8% of the women washed their hands in the

Table 2. Relation between collection and storage frequencies, and distances from water sources to homes (Round trip, min) in Lungwena Malawi.

Activity	Travel time to water source (round trip)				Total
	10 - 20	21 - 30	31 – 40	41 – 50	
Collection frequency					
Once a day	2 (0.7)	1 (0.3)	2 (0.7)	1 (03)	6 (2.1)
2 x a day	21 (7.3)	16 (5.6)	7 (2.4)	5 (1.7)	9 (17.1)
3 x a day	173 (60.3)	39 (13.6)	14 (4.9)	6 (2.1)	232 (80.8)
Total	196 (68.3)	56 (19.5)	23 (8.0)	12 (4.2)	287 (100)
Storage length (days)					
1 – 2	189 (65.9)	52 (18.1)	23 (8.0)	7 (2.4)	271 (94.4)
3 – 4	6 (2.1)	3 (1.0)	0 (0)	4 (1.4)	13 (5)
More than 4	1 (0.3)	1 (03)	0 (0)	1 (0.3)	3 (1.0)
Total	196 (68,3)	56 (195)	23 (8.0)	12 (4.2)	287 (100)
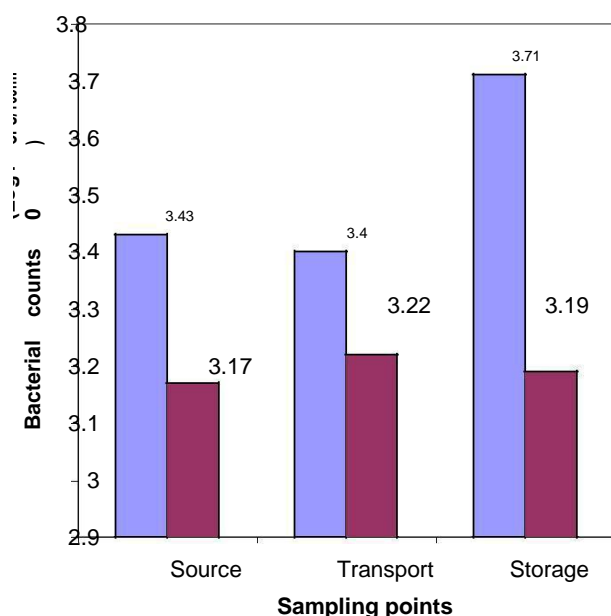
Figure 2: Mean concentrations of *E. coli* and *S. aureus* at source, transport and storage sampling points. Number of samples for *E. coli* at each sampling points were: source (n = 24), transportation (n = 33) and storage (n = 40) while those of *S. aureus* were; source (n = 6), Transportation (n=14), Storage (n =23).

conventional way (rinsing with water only) and subsequently washed their containers without using any form of cleansing agent. Most participants (68.9%) drew water from the wells using individual buckets (personal buckets) tied to a rope. At some borehole water sources (8.2%), children were observed drinking water directly from the borehole mouth with their mouth while at open water sources (7%), animals were observed drinking from the source. Growth of algae was observed in 5% of the brick-lined water sources. When containers were filled up, about 92% of the participants spilled out some water with

their fingers while 8.7% dipped leaves into the water to prevent the water splashing over their body. Fingers came in contact with water in the filled-up containers (25%) while women lifted the containers and while they transported the filled containers to their homes. Only 30% of the participants covered the container whilst carrying it.

Storage and handling of water in homes

Figure 4 shows the observed and reported water management practices in the households. Upon arrival in the homes, 84% of the participants transferred the water to another container. Water storage containers used in most households were; small- mouthed clay pot known as "mtsuko" (65%), aluminium metal containers (20%) and the rest were plastic buckets and drums. Mixing of the collected water with old stored water was observed in 16% of the households and no cleaning was done to the storage containers at the time of mixing. About 95.4% of the households covered their stored water, mostly with plates and winnowing baskets. Only 23% of the households used a 2-cup system when drawing water from the storage container. A comparison between use of 2-cup system and bacterial concentration demonstrated some correlation (r = 0.2). Treatment of water, mostly boiling and chlorination was practiced by 8.8% of the households. When the treated samples were tested, viable *E. coli* and *Salmonella* spp. *cells* were detected in all the samples (but in lower counts). Increase in bacterial counts in stored water was strongly associated (r = 0.312; p < 0.05) with low hygiene practices.

DISCUSSION

This study was carried out in order to assess the microbiological quality of domestic water, and also to investigate water management practices. The results have
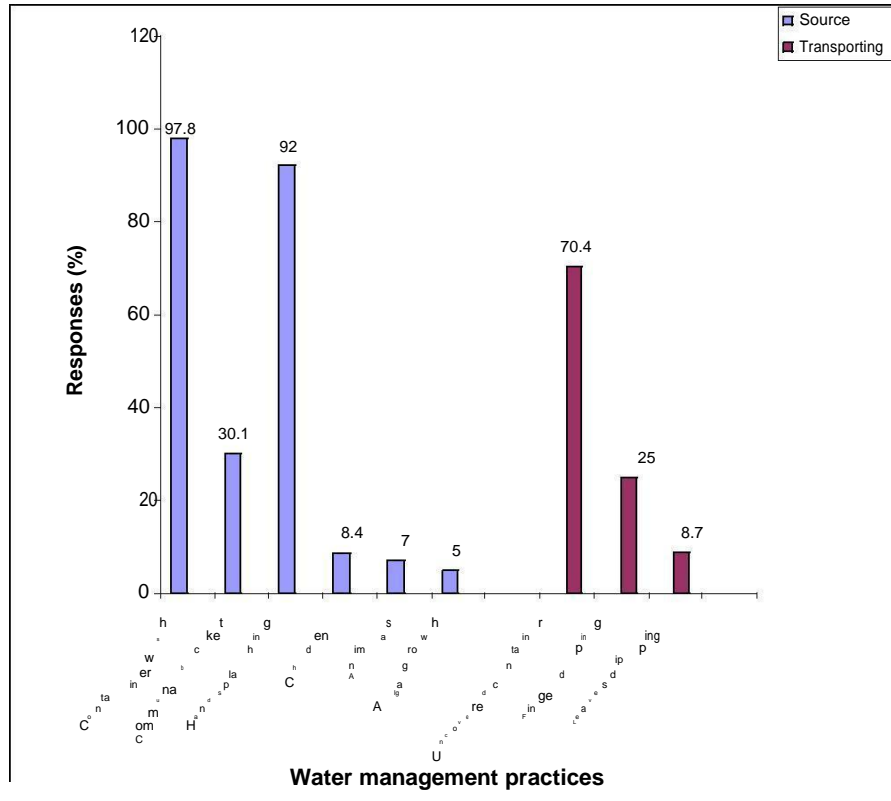
Figure 3. Reported and observed water management practices at source and during transportation in 287 households in Lungwena, Malawi.
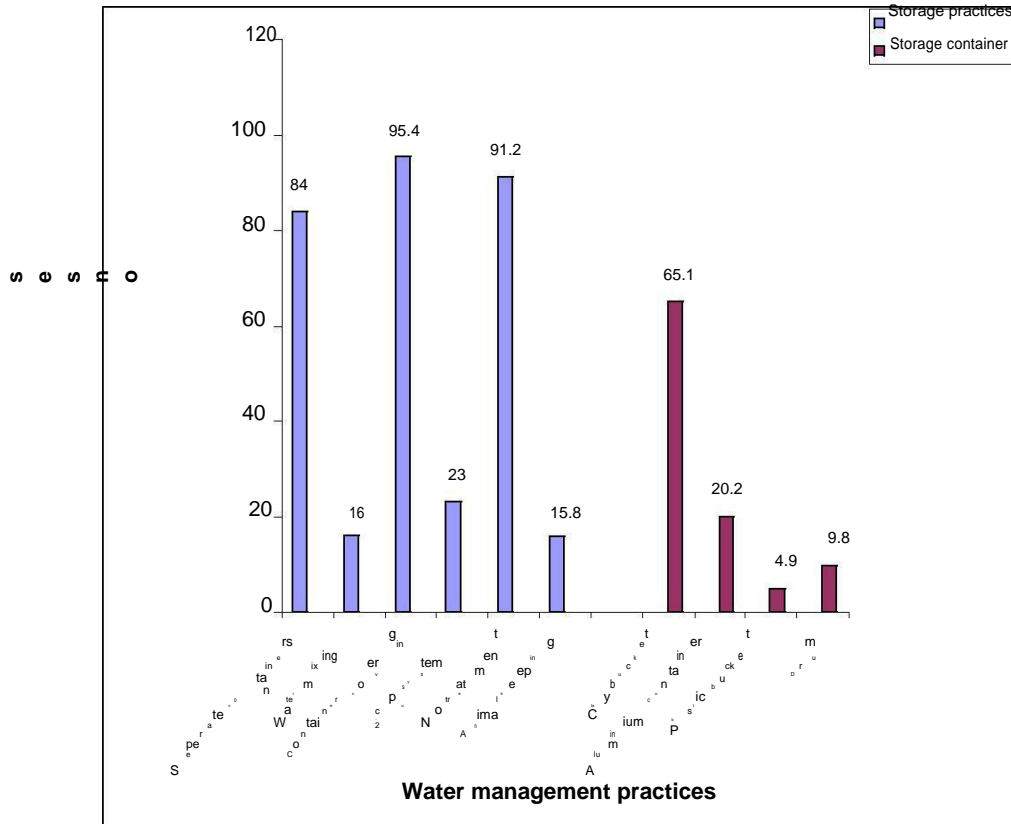
shown that there was faecal contamination of stored household water from both protected and unprotected water sources as illustrated by the presence of the test organisms. Incidences of *E. coli* and *E. coli* 0157: H7 found in this study are in agreement with those of Trevett et al. (2005) and Hogue et al. (2006) who found *E. coli* in 29% and *E. coli* 0157: H7 in 39% of stored water and borehole water samples, respectively. *E. coli* is an indicator of faecal contamination and faecal contamination is associated with poor environmental sanitation (Trevett et al., 2005). The high incidence of *E. coli* (13%) in boreholes is a concern as such sources are usually regarded as "safe". The observed pattern of low incidences of *E. coli* 0157: H7 and *C. jejuni* was consistent with earlier reports on water contamination (Botton et al., 1987). Botton et al. (1987) explains that *C. jejuni* is very difficult to isolate and is usually detected in small numbers. The presence of *E. coli* 0157: H7 in stored water demonstrates a potential health risk as the organism is pathogenic and causes complications in children.

Salmonella contamination is usually associated with contaminated food and animal feeds and its presence in water signals faecal contamination of both human and animal origin (Dondero, 1977). In our study, *Salmonella* was detected in 24% of the water samples and this finding is supported by that of Dondero et al. (1977) and

Phan et al. (2003). Contamination of *Salmonella* at the source was observed to be higher in samples that were collected from unprotected sources and possibly reflects exposure of the water to animals. It was alarming to observe people of Kwilasya abstracting water from a river bed sand (at a depth of less than 30 cm), sources that are associated with *Salmonella* contamination and other pathogenic micro organisms such as Vibrio cholera. In the case of sources that were Salmonella negative, contamination observed during transportation could have originated from washing of the dirty hands and containers (97.8%) observed at the source. Containers and hands are likely to have been pre-contaminated in the homes that kept animals in the same room where water was stored. Roberts et al. (2001) found that such rinsing practices are not effective in reducing bacteria.

Salmonella cells and the other tested organisms may have started growing soon after collection and reflected in the transported water.

The higher microbiological counts in the stored water samples compared to the source water samples possibly demonstrates a wide variation of poor hygiene practices in the homes. This is supported by the observed practices and their association with high bacterial counts. Water containers were covered with either winnowing basket or plates, materials which were also used for other

120

100

95.4

91.2

84

65.1

80

60

40

23

20.2

20

16

15.8

9.8

4.9

0

Storage practices
Storage container

Water management practices

Figure 4: Reported and observed water management practices in 287 households in Lungwena, Malawi.

household activities such as sifting maize flour and cutting of vegetables and meat. Attachment of micro-organisms on the surface walls of such materials and eventual contamination of the water is likely to have occurred (Roberts et al., 2001; Osmundsen, 2005). Collected water was transferred into storage containers, facilities that are not washed for several days, leaving sediments to settle at the bottom of the containers (Lindskog and Lindskog, 1988). These sediments which are mostly organic in nature, serve as nutrients for pathogens for their growth (Momba and Kaleni, 2001; Luby et al., 1999). Since most of the households did not treat their water (91.2%), it is possible that pathogens remained in the water at the bottom of the container together with the organic matter. Therefore, use of separate containers by most households (84%) coupled with use of "mtsuko" (Ogutu et al., 2001), mixing of fresh water with the water that was stored for more than 24 h could be possible causes of contamination observed in stored water.

Lungwena area has high pit latrine coverage (Lungwena NUFU census, 2004: unpublished) that usually collapse during the rainy season because of poor soils (sand). Leaching of pit latrine contents and flooding of human and animal wastes into the wells during rainy season could be other possible sources of contamination

in the wells and boreholes (Mathess et al., 1988). Individual water drawing containers, (especially those with ropes) practiced by most households were also prone to contamination in the homes. In view of the above findings and risks, we strongly recommend that immediate attention be focussed on ensuring a supply of biologically safe drinking water and improving its management from the source to the storage point. Education dealing with water management and imparting the community with simple and sound technologies aimed at reducing deterioration and algal growth in wells should be an integral component of water supply. Practices may be improved by covering containers, avoiding children (Maraj et al., 2006) and animals at water points in rooms where water is stored. Use of borehole water, home treatment of water and 2-cups system should still be encouraged.

Conclusion

The study has demonstrated that water used for both drinking and cooking in Lungwena is of poor quality (microbiologically) and the contamination is possibly due to poor management of water and existence of poor sanitation. The presence of *E. coli* in borehole water is of public significance as it is indicative of faecal contamina-

tion. Considering that fingers are prone to faecal contamination during toilet use (Shojaei et al., 2005), such practices can easily promote occurrence of diarrhoeal disease outbreaks through cross-contamination. In Lungwena community, implementation of interventions requires a careful consideration of local culture.

ACKNOWLEDGEMENTS

REFERENCES

Ahmed SA, Hogue BA, Mahmud A (1998). Water management practices in rural and urban homes: a case study from Bangladesh on ingestion of polluted water. J. Public. Health. 112: 317-321.

Botton FJ, Coates D, Hutchison, DN and Godfrey AF (1987) A study of thermophilic Campylobacters in a river system. J. Appl. Bacter. 62: 167- 176.

Dondero NC, Thomas CT, Khare M, Fukui GM (1977). Salmonella in surface waters of Central New York State. J. App. Envron. Microbiol. 4: 791- 801.

Feachem RG (1984). Interventions for the control of diarrhoeal diseases among young children: promotion of personal and domestic hygiene. Bull. WHO. 62: 467 - 476.

Frampton EW, Restaino L (1993). Methods for *Escherichia coli* identification in food, water and clinical samples based on β-glucuronidase detection. J. Appl. Bacter. 74: 223- 233.

Hogue BA, Hallman K, Levy J, Bouis H, Ali N, Khan F, Khanan S, Kabir M, Hossain S, Alam, MS (2006). Rural drinking water at supply and household levels: Quality and management. Int. J. Hyg. Environ. Health. 209: 451- 460.

Lennette EH, Ballows A, Hausler WJJr, Shadomy HJ (1985). Manual. Clin. Microbiol. American Society for Microbiol. Washington DC, USA.

Lindskog RU, Lindskog PA (1988). Bacterial contamination of water in rural areas: an intervention study from Malawi. J. Trop. Med. Hyg. 91: 1-7.

Luby S, Amber MD, Syed H, Atiullah N (1999). Limited effectiveness of home drinking water purification efforts in Karachi. J. Infect. Dis. 4: 3 - 7.

Maraj S, Rodda N, Jackson S, Buckley C, Macleod N (2006). Microbial deterioration of stored water for users supplied by stand-pipes and ground-tanks in a peri-urban community. J. South Afr. Water Res. 5: 693 -699.

Mathess G, Pekdeger A, Schoeter J (1988). Persistence and transport of bacteria and viruses in groundwater- a conceptual evaluation. J. Contamin. Hydrol. 2: 171-188.

Ministry of Health (2004). Mangochi District Health Profile 2003 - 2004. Ministry of Health. Lilongwe. Malawi.

Momba MNB, Kaleni P (2001). Regrowth and survival of indicator microorganisms on the surfaces of household containers used for storage of drinking water in rural communities of South Africa. South Afr. J. Wat.Resear.36: 3023- 3028.

Nordic Committee on Food Analysis, 1993. *Coliform* bacteria and *E. coli* TM in foods. Determination by the Plate Count Method with Petrifilm plates. No 147, NMKL, Nordisk Metodi Komite for Livsmedel, Esbo, Finland.

Ogutu P, Garrett V, Barasa P, Ombeki S, Mwaki A, Quick RE (2001). Seeking safe storage: A comparison of drinking water in clay and plastic vessel. American J. Publ. Health. 91: 1610 -1611.

Osmundsen B (2005). Drinking water safety; a study of collection and storage practices of drinking water in Lungwena. Masters Thesis. Norwegian University of Life Sciences. Ås. Norway.

Phan T, Khai L, BacLock C, Hayashidani H, Sameshima T, Watanabe T, et al. (2003). Isolation of Salmonella strains from aquatic environment and comparison with those of animal origin in Tan Phu Thanh village, Mekong Delta, Vietnam. Japan Agr. Res. Quarterly, 4: 237-241.

Roberts L, Chartier Y, Chartier O, Malenga G, Toole M, Rodka H (2001). Keeping clean water clean in a Malawi refugee camp: a randomized intervention trial. Bull. WHO. 79: 280 – 287.

Scates P, Moran L, Madden RH (2003). Effects of incubation temperature on isolation of *Campylobacter jejuni* genotypes from food enriched in Preston broth. J. Environ. Microbiol. 69: 4658 -4661.

Shojaei H, Shooshtaripoor J, Amiri M (2005). Efficiency of simple hand-washing in reduction of microbial hand contaminant in human food handlers. Intern. J. Food Res. 39: 525 - 529.

SPSS for windows Re. 11.5.1., 2002. SPSS Inc. Chicago.

Trevett AF, Carter RC, Tyrrel SF (2005). The importance of domestic water quality management in the context of faecal- oral disease transmission. J. Water Health. 3: 259- 270.

United Nations Educational, Scientific and Cultural Organisation (2003). Water for people for life: The United Nations World Water Development Report. UNESCO .

Vernozy – Rozand C (1997). Detection of *E. coli 0157:H7* and other verotoxin – producing *E. coli* (VTEC) in food. J. Appl. Microbiol. 82: 537 - 551.

World Health Organisation (2002). Water for Health enshrined as a human right. Available at: http://www.who.int/mediacentre/news/releases/prl/en.

World Health Organisation (WHO) (2006). Guidelines for drinking-water quality (3 rd Ed). World Health Organisation, Geneva, Switzerland. Pp. 121- 144

Wright J, Grundy S, Conroy R (2004). Household drinking water in developing countries: a systematic review of microbiological contamination between source and point-of-use. J. Trop. Med. Int. Health. 9: 106- 117.

Younes M, Bartram J (2001). Waterborne health risks and the WHO perspectives. Int.J. Hyg. Environ. Health. 204: 255- 263.