Bacteriological assessment of drinking water supply options in coastal areas of Bangladesh

Md. Atikul Islam, Hiroyuki Sakakibara, Md. Rezaul Karim, Masahiko Sekine and Zahid Hayat Mahmud

ABSTRACT

This study was conducted to assess the bacteriological quality of alternative drinking water supply options in southwest coastal areas of Bangladesh. A total of 90 water samples were collected during both dry and wet seasons from household based rainwater harvesting systems (RWHSs), community based rain water harvesting systems (CRWHSs), pond-sand filters (PSFs) and ponds. The samples were evaluated for faecal coliform, *Escherichia coli* and Heterotrophic Plate Count, as well as *Vibrio cholerae*, *Salmonella* spp., *Shigella* spp. and *Pseudomonas* spp. Physico-chemical parameters (pH, electrical conductivity, and color) were also examined. In addition, sanitary inspections were conducted to identify faecal contamination sources. All options showed varying degrees of indicator bacterial contamination. The median *E. coli* concentrations measured for RWHSs, CRWHSs, PSFs, and ponds were 16, 7, 11, and 488 cfu/100 ml during the wet season, respectively. *Vibrio cholerae* O1/O139, *Salmonella* and *Shigella* spp. were not found in any samples. However, *Vibrio cholerae* Non-O1/Non-O139 and *Pseudomonas* spp. were isolated from 74.4% and 91.1% of the water samples collected during the wet season. A maximum pH of 10.4 was found in CRWHSs. Estimation of the disease burden for all options in disability adjusted life years (DALYs) showed an increased disease burden during the wet season. According to sanitary inspections, poor maintenance and unprotected ponds were responsible for rainwater and PSF water contamination, respectively. The findings of the present study suggest that alternative drinking water supply options available in southwest coastal Bangladesh pose a substantial risk to public health.

Key words | coastal Bangladesh, drinking water, indicator bacteria, pathogens

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INTRODUCTION

Coastal areas of Bangladesh comprise an area of 47,211 km^2, which is about 32% of the country's geographical area. In addition, 35 million people, or 28% of the country's total population, live in coastal areas (BBS 2001). In terms of administrative consideration, 19 out of 64 districts are considered coastal districts. These districts have been identified as problematic areas in which complex hydro-geological conditions and adverse water quality make it difficult to supply drinking water when compared to other parts of the country. There are certain areas in the coastal districts where shallow and deep tubewells are not useful due to high salinity in groundwater. In many

places in these areas, rainwater is preserved in natural reservoir ponds and collection of rainwater is the only source of drinking water (Ahmed & Rahman 2000; Kamruzzaman & Ahmed 2006).

The proportion of rain-fed pond water and other alternative options for drinking purposes used in the three southwestern coastal districts (Bagerhat, Khulna and Satkhira) varies from 23.1 to 34.1%, while the national average is 3% (BBS/UNICEF 1999). About 5 million people in the southwestern coastal region suffer from drinking water problems (Uttaran 2004). The alternative drinking water supply options

currently available in southwest coastal areas of Bangladesh are household based rainwater harvesting systems (RWHSs), community based rainwater harvesting systems (CRWHSs), pond-sand filters (PSFs), and rain-fed pond water. Thus, the current water supply options are mainly dependent on rainwater. For RWHSs and CRWHSs, corrugated iron (CI) sheet roofs are commonly used as catchment areas. In the case of RWHSs, the water collection tanks are mainly plastic, ferrocement and burnt clay pots (Motkha), while CRWHS tanks are made of reinforced cement concrete (RCC) or ferrocement. PSFs are small scale slow sand filtration units used to treat rain fed pond water.

In coastal areas of Bangladesh, the government is currently promoting community based water supply options such as PSFs and CRWHSs since these are considered to be the safe options for drinking water. However, few areas have such facilities. Additionally, people generally use rain fed pond water during the dry season because most households do not have a tank large enough to provide year round rain water storage. As a result, individuals drink pond water, which is often microbially unsafe (Frisnie et al. 2002). Previous studies have also shown that pond water in Bangladesh is heavily contaminated with faecal coliforms and other pathogenic bacteria (Islam et al. 1994, 1995, 2000; Albert et al. 2000; Alam et al. 2006). Thus, the use of pond water for drinking purposes may threaten public health. Howard et al. (2006) investigated the microbial quality of PSF water in arsenic affected areas of Bangladesh and found that more than 90% of the samples exceeded the WHO standard for thermotolerant coliforms (TTC) during both dry and wet seasons. The faecal coliform (FC) count in PSFs was found to range from <1 to over 150 cfu/100 ml (Kamruzzaman & Ahmed 2006). Several studies from other countries also showed that harvested rain water is often contaminated with various pathogenic bacteria and protozoa (Crabtree et al. 1996; Uba & Aghogho 2000; Simmons et al. 2001; Despins et al. 2009; Horak et al. 2010). Previous studies of alternative water supply options in Bangladesh have primarily focused on TC (Total Coliform) and FC (Ahmed et al. 2005; Howard et al. 2006; Kamruzzaman & Ahmed 2006). However, there has recently been concern regarding the suitability of traditional indicators for assessing the microbial safety of harvested rainwater, which may be contaminated with a variety of opportunistic and pathogenic microorganisms (Uba & Aghogho 2000; Lye 2002). To date, no studies have been conducted to evaluate the prevalence of pathogenic bacteria in alternative water supply options employed in the southwest coastal areas of Bangladesh. Therefore, it is crucial to identify the pathogenic organisms present in these water supply options to ensure a safe and sustainable water supply in coastal areas of Bangladesh.

There were three specific goals of this study: (1) to evaluate the prevalence of bacterial contamination and physico-chemical water quality; (2) to estimate the health risk associated with currently available water supply options; and (3) to estimate the relationship between sanitary risk factors and faecal contamination.

MATERIALS AND METHODS

Study area and sampling

Water samples were collected from the Dacope and Mongla Upazilas (sub-district) of the Khulna and Bagerhat districts located in the southwest coastal areas of Bangladesh (Figure 1). Samples were collected from RWHSs, CRWHSs, PSFs, PSF ponds and ponds. We collected water from both PSFs and PSF ponds to examine the removal efficiency of bacteria by PSFs. For RWHSs, plastic and ferrocement tanks were considered in the present study. A total of 90 water samples were collected (Table 1) during both the dry (March 13 to March 20, 2009) and wet seasons (August 10 to August 16, 2009) to account for seasonal changes. However, cyclone Aila hit the study area on May 25, 2009, which resulted in many ponds being flooded with saline water and damage to some of the PSFs. As a result, it was necessary to introduce 32 different, but representative samples during the wet season.

Water from different water supply options was collected following the standard procedures (APHA (American Public Health Association) 1998). For microbiological analysis, 500-ml water samples were aseptically collected in sterile Nalgene plastic bottles. All samples were placed in an insulated box filled with ice packs (Johnny Plastic Ice; Pelton Sheperd, Stockton, CA, USA) and transported to the Environmental Microbiology Laboratory of the International Center for
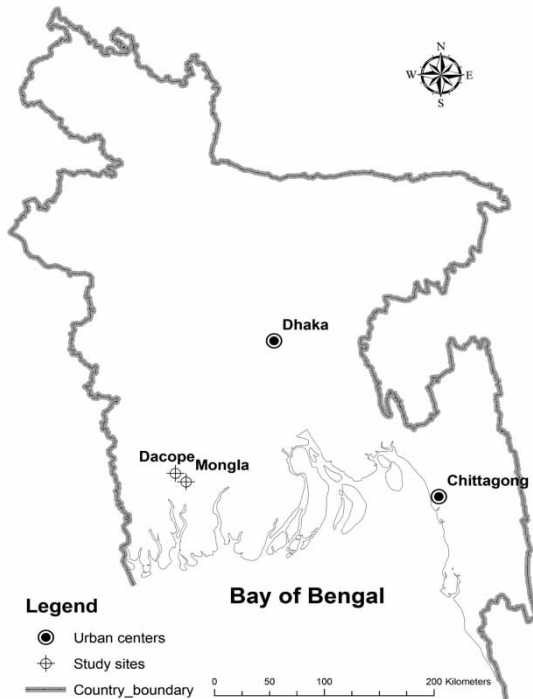
Figure 1 | Map of Bangladesh showing the study area.

Table 1 | Number of samples taken from each drinking water option

Sampling sources	Dry season	Wet season
RWHSs	20	23
CRWHSs	14	14
PSFs	17	14
Ponds	39	39
Total	90	90

Diarrhoeal Disease Research, Bangladesh (ICDDR, B) for bacteriological analysis immediately after collection.

Detection of indicator bacteria

For enumeration of FC and *Escherichia coli*, 100 ml water samples were filtered through a 0.22 μm pore-size membrane filter (Millipore Corp., Bedford, MA, USA), and the filters were then placed on membrane faecal coliform (mFC) and m-TEC agar plates, respectively, following procedures that have been described elsewhere (APHA 1998; Islam *et al.* 2001). The mFC plates were incubated at 44 °C

for 18 to 24h to enumerate the faecal coliforms and the mTEC agar plates were incubated at $35 \pm 0.5\,^{\circ}\text{C}$ for 2 h followed by further incubation at $44.5 \pm 0.2\,^{\circ}\text{C}$ for 22–24 h to enumerate the *E. coli*. Characteristic blue colonies were counted as FC and red or magenta colonies were counted as *E. coli*. All samples were expressed as colony forming units (cfu) per 100 ml. The samples were tested for heterotrophic plate count (HPC) using a previously described method (Islam *et al.* 2001).

Isolation of pathogenic bacteria

For qualitative analysis of *Vibrio cholerae*, 50 ml water samples were enriched with 25 ml triple strength alkaline peptone water (APW) and incubated for 6 h at 37 °C. Next, two loops of the enriched sample were plated onto thiosulfate citrate bile salt sucrose (TCBS) agar (BD, USA) and CHROMagar Vibrio (CV) agar (CHROMagar, Paris, France) plates. Following overnight incubation at 37 °C, yellow colonies with a diameter of 2–3 mm on TCBS agar plates and pale blue colonies on CV agar plates were presumptively selected as *V. cholerae* (Hara-Kudo *et al.* 2001). The selected colonies were then confirmed based on their colonial characteristics after transferring the same colony to fresh TCBS and CV agar plates using sterile toothpicks. Following overnight incubation at 37 °C, characteristic colonies of *V. cholerae* were selected and further characterized using a previously described procedure (Islam *et al.* 1995). Briefly, strains were only identified as *V. cholerae* if they fulfilled the following criteria: Gram negative, oxidase positive, produced acid from sucrose but not inositol and decarboxylated lysine and ornithine but not arginine. Strains were serotyped according to the procedure described by Kelly *et al.* (1992).

For the qualitative analysis of *Shigella*, *Salmonella* and *Pseudomonas* spp., 50 ml water samples were enriched in 25 ml triple strength Selanite broth and then incubated overnight at 37 °C. For isolation of *Shigella* and *Salmonella* spp., two loopfuls of overnight enrichment broth were subcultured on *Salmonella Shigella* agar and then incubated overnight at 37 °C. After overnight incubation, characteristic colonies of *Shigella* and *Salmonella* were confirmed by a battery of biochemical tests (Baron & Finegold 1990). For isolation of *Pseudomonas* spp., two loopfuls of enrichment

broth were taken and inoculated onto Cetrimide agar, which were then incubated overnight at 37 °C. After overnight incubation, green-yellow to blue-green colonies were identified as *Pseudomonas* spp. (United States Pharmacopeial Convention 2007).

Physico-chemical analysis

All water samples were tested for pH, Electrical Conductivity (EC), and color. Physico-chemical analyses were performed according to the APHA (1998). Analysis was conducted at the Environmental Laboratory of Khulna University of Engineering & Technology (KUET), Khulna, Bangladesh.

Health risk assessment

A Quantitative Health Risk Assessment (QHRA) model was used to quantify the likely disease burdens presented by alternative water supply options. QHRA is a simple deterministic spreadsheet model developed by the Arsenic Policy Support Unit (APSU) of the Government of Bangladesh. The model estimates microbial disability-adjusted life years (DALYs) for three reference pathogens, rotavirus, cryptosporidium and *E. coli* for viral, protozoal and bacterial disease, respectively, to determine the total disease burden. The details of the model assumptions regarding pathogen and indicator organisms and the dose response relationship are beyond the scope of this paper, but can be found elsewhere (Ahmed *et al.* 2005; Howard *et al.* 2006; Howard *et al.* 2007). The data describing *E. coli* were used to estimate the likely disease burden using DALYs as recommended by the WHO (2004), which is the globally applied method used to compare different disorders and diseases with different health outcomes.

Sanitary inspections

Sanitary inspection forms were prepared based on the *WHO Guidelines for Drinking Water Quality* (WHO 1997) and adopted for RWHSs, CRWHSs, and PSFs. The sanitary inspection forms used included ten risk factors on potential sources of pollution. At each site, a sanitary inspection was conducted when the sample was collected. Sanitary risk factors were in the form of binomial

categorical data. To examine the relationship between the risk factors and faecal contamination, *E. coli* data were transformed into categorical variables. The sample size for each option was not large; hence for analyses we used the combined data for both dry and wet seasons.

Statistical analysis

Statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) version 16.0. Data analysis was carried out using nonparametric tests. Specifically, the Wilcoxon signed rank t-test was performed to evaluate the seasonal variation. The Kruskal-Wallis test was used to compare between options for drinking water, while the Mann Whitney U test was used to analyze the differences in bacterial concentrations between PSF ponds and PSFs. The Chi-square test was used to assess the association between sanitary risk factors and faecal contamination.

RESULTS

Indicator bacterial contamination

Table 2 shows the concentration of FC, *E. coli* and HPC in samples. The mean, median, minimum and maximum concentrations for each option in dry and wet seasons are shown. Figure 2 is the box and whisker plot of distributions of the concentration of *E. coli*.

The findings presented in Table 2 and Figure 2 are as follows:

- Both mean and median *E. coli* concentrations for ponds were very high when compared with other options. The mean and median FC concentrations for ponds were also highest during both seasons.
- The pond water had the widest interquartile range of *E. coli* concentrations during the wet season, with 25th and 75th quartiles of 260 and 1000 cfu/100 ml, respectively (Figure 2). Conversely, other drinking water options showed comparatively narrow interquartile ranges.
- In CRWHSs and PSFs, there were significant differences in the mean *E. coli* and FC concentrations between the dry and wet season ($p < 0.05$). Samples from CRWHSs

Table 2 | Seasonal change in concentration of indicator bacteria

Indicator bacteria	Sampling sources	Dry season				Wet season			
		Mean	Med	Min	Max	Mean	Med	Min	Max
FC (cfu/100 ml)	RWHSs	465	22	<1	5,000	1,509	84	<1	18,000
	CRWHSs	170[a]	11	<1	1,000	856[b]	128	<1	5000
	PSFs	299[a]	13	<1	4,000	414[b]	80	4	1900
	Ponds	2,012	1,000	12	10,000	4,438	2,000	175	50,000
E. coli (cfu/100 ml)	RWHSs	74	5	<1	900	288	16	<1	6,000
	CRWHSs	24[a]	<1	<1	204	233.64[b]	7	<1	1,000
	PSFs	6.88[a]	2	<1	44	105[b]	11	1	1,000
	Ponds	498	190	<1	3,000	767	488	5	4,000
HPC (cfu/ml)	RWHSs	1,971	725	20	13,000	5,486	2,500	40	71,500
	CRWHSs	3,493	1,270	60	12,600	1,564	1,065	10	5,600
	PSFs	5,275	3,900	80	19,500	4,039	4,000	400	9,000
	Ponds	3,107	1,950	220	13,500	2,972	3,000	310	6,000

RWHSs, Household Rain Water Harvesting Systems; CRWHSs, Community Rain Water Harvesting Systems; PSFs, Pond Sand Filters.
Med, Median; Min, Minimum; Max, Maximum; FC, Faecal coliform; HPC, Heterotrophic Plate Count.
[a,b]Significantly different between dry and wet seasons at $p < 0.05$ by Wilcoxon signed rank t-test.
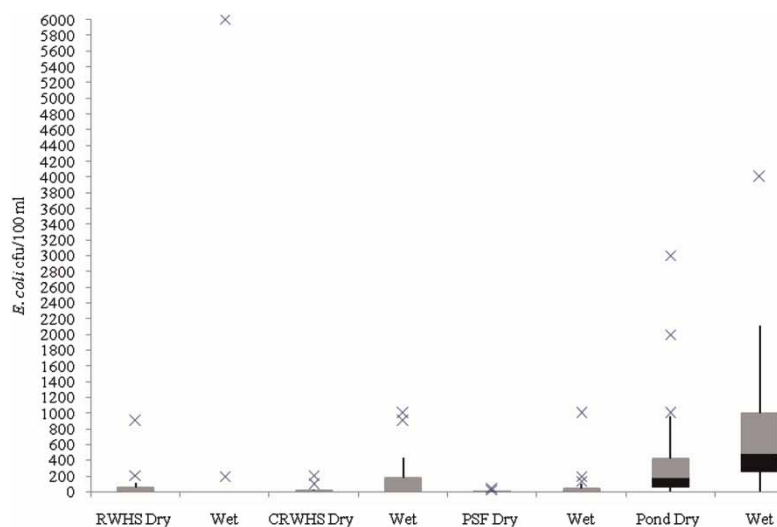

Figure 2 | Box and whisker plot showing the range of concentrations of *E. coli* in different drinking water options during the dry and wet season. For each box, the line within the box represents the median, while the top and bottom of the box represent the 75th and 25th percentiles, respectively, and the top and bottom whisker extend to the 90th and 10th percentiles, respectively. Observations beyond the 10th and 90th percentiles are shown as outliers 'x'.

and PSFs tend to be more polluted during the wet season. Conversely, differences between dry and wet seasons were not significant in RWHSs and ponds.

- The minimum concentrations of *E. coli* and FC for RWHSs and CRWHSs during both seasons were less than 1 cfu/100 ml, which indicates that harvested rainwater can ensure good microbial quality.

- Higher HPC concentrations were found in all options during both seasons; however, the greatest median concentration of HPC was observed in PSF water.

- The concentrations of FC, *E. coli* and HPC differed significantly among the different options in the wet season as indicated by the Kruskal-Wallis test ($p < 0.05$). While, in the dry season, FC and *E. coli* differed significantly.

According to the World Health Organization's guidelines, drinking water with a concentration of FC or *E. coli* larger than 0 cfu/ml is classified as 'unacceptable' (WHO 1997). Table 3 shows the percentage of drinking water samples with unacceptable levels of FC and *E. coli*. The percentage of unacceptable samples for the CRWHSs during the dry season was lowest for both FC and *E. coli*. However, in the case of FC, more than half of the CRWHS samples were unacceptable. Almost all samples from ponds were unacceptable for both seasons.

Table 4 shows mean values for FC, *E. coli* and HPC concentrations for PSF pond water and PSF water. The PSFs reduced the FC and *E. coli* concentrations significantly. No removal of HPC by PSFs was observed. The mean HPC in PSF water was higher than in PSF pond water. We also examined the removal efficiency of FC and *E. coli* by PSFs; however, some PSFs showed higher FC and *E. coli* levels

than the PSF ponds and were therefore considered as 0% removal for estimating the removal efficiency. The removal efficiency of indicator bacteria by PSFs is shown in Table 4. Specifically, the *E. coli* removal by the PSFs was about 83% during the dry season, but only 76% during the wet season. For FC, the removal efficiency was about 71% and 74% during the dry and wet seasons, respectively. The removal of FC and *E. coli* by PSFs was not sufficient to meet drinking water standards in either season.

Pathogenic bacterial contamination

Vibrio cholerae Non-O1/non-O139 were isolated from about 95% (37/39) of the pond samples during both seasons (Table 5). For RWHSs, CRWHSs, and PSFs, the proportion of samples containing *V. cholerae* Non-O1/non-O139 increased from 20% to 35%, 29% to 57% and 47% to 100%, respectively, during the wet season. However, no toxigenic *V. Cholerae* O1/O139 or *Salmonella* and *Shigella* spp. were isolated from any of the samples. In general, isolation of *Pseudomonas* spp. increased from 10% (9/90) to 91% (82/90) during the wet season.

Physico-chemical characteristics

A summary of the physico-chemical data for the water samples is shown in Table 6. The highest pH of 10.4 was recorded in the CRWHSs. During the dry season, 78.6% (11/14) of the CRWHS samples had a pH that fell outside the guideline values for drinking water of 6.5–8.5. The pH values were relatively higher during dry season. For RWHSs, the highest pH for the plastic and ferrocement tank was 8.45 and 9.92, respectively. The pH of the ponds varied significantly between dry and wet seasons ($p < 0.05$).

Table 3 | Percentage of unacceptable drinking water samples according to level of indicator bacteria

| Options | Season | Unacceptable (%)* | |
		FC	E. coli
RWHSs	Dry	90.0	75.0
	Wet	100	82.6
CRWHSs	Dry	64.2	42.8
	Wet	78.5	71.4
PSFs	Dry	94.1	64.7
	Wet	100	100
Ponds	Dry	100	97
	Wet	100	100
Total	Dry	91.1	77.77
	Wet	92.2	91.1

*According to the World Health Organization guidelines, the unacceptable level of faecal coliform or *E. coli* is > 0 per 100 ml.

Table 4 | Removal efficiencies of indicator bacteria by PSFs

| Organisms | PSF ponds | | PSFs | | Average % removal by PSFs | |
	Dry (mean)	Wet (mean)	Dry (mean)	Wet (mean)	Dry	Wet
FC (cfu/100 ml)	1,134[a]	2,545[c]	298[b]	432[d]	71.44%	74.11%
E. coli (cfu/100 ml)	294[a]	534[c]	6.88[b]	106[d]	83.27%	76.32%
HPC (cfu/ml)	3,540	2,575	5,275	3,997	–	–

[a,b]Significantly different between PSF ponds and PSFs in the dry season at $p < 0.05$ by Mann-Whitney U test.
[c,d]Significantly different between PSF ponds and PSFs in the wet season at $p < 0.05$ by Mann-Whitney U test.

Table 5 | Isolation of *Vibrio cholerae, Shigella, Salmonella* and *Pseudomonas* spp.

Drinking water Options	Vibrio Cholerae O1/O139		Vibrio Cholerae non-O1/ non-O139		Salmonella and Shigella spp.		Pseudomonas spp.	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
RWHSs	0	0	4 (20)	8 (35)	0	0	2 (9)	21 (91)
CRWHSs	0	0	4 (29)	8 (57)	0	0	2 (14)	10 (71)
PSFs	0	0	8 (47)	14 (100)	0	0	1 (7)	14 (100)
Ponds	0	0	37 (95)	37 (95)	0	0	4 (10)	37 (95)

Note: Figure in the parenthesis indicates the percent of samples isolated.

Table 6 | Seasonal change in physico-chemical parameters

Parameters	Sampling sources	Dry season			Wet season		
		Mean	Min	Max	Mean	Min	Max
pH	RWHSs	7.85	5.65	9.92	7.79	6.90	9.09
	CRWHSs	8.93	7.63	10.40	8.14	6.81	9.17
	PSFs	7.60	6.84	8.47	7.68	7.28	8.40
	Ponds	7.78[a]	6.90	8.83	7.50[b]	6.55	8.11
EC*	RWHSs	142[a]	24	470	70[b]	11	316
	CRWHSs	168.9[a]	94	340	80.37[b]	40	124
	PSFs	1,266	338	3,343	1,196	193	3,330
	Ponds	1,227[a]	344	3,930	3,700[b]	203	40,400
Color**	RWHSs	28.65	0	325	7.30	0	55
	CRWHSs	8.29	0	28	7.79	0	29
	PSFs	50.59[a]	0	182	22.64[b]	0	77
	Ponds	169	0	1,000	97.67	0	268

*μS/cm, micro siemens per centimeter.
**TCU, True Color Units.
[a,b]Significantly different between dry and wet seasons at $p < 0.05$ by Wilcoxon signed rank t-test.

The average EC value for ponds and PSFs were found to be more than 1000 μS/cm during both seasons. For RWHSs, CRWHSs, and ponds, EC varied significantly between seasons ($p < 0.05$). During the dry season, the water color was found to be very high for ponds and PSFs. Better water color was observed during the wet season for all of the water options. The PSFs showed significant variation in color between seasons ($p < 0.05$).

Health risk assessment

The results of lower (5th percentile), median and upper (95th percentile) disease burden estimates for each drinking water option by season are shown in Figures 3–5, respectively. According to the lower estimates (Figure 3), RWHSs,

CRWHSs, and PSFs showed little disease burden. However, ponds showed higher disease burden, even among lower estimates. The median disease burden estimates (Figure 4) showed that the disease burden increases for all options during the wet season. The burden of disease for bacteria was greater than the viral and protozoal burden when higher concentrations of indicator organisms were present (Figure 5). The viral and bacterial pathogen concentrations dominated the disease burden estimates when the contribution by protozoal pathogens to the total microbial DALY was negligible. CRWHSs and PSFs showed increased disease burden during the wet season, while RWHSs and ponds had nearly the same disease burden in both surveys.

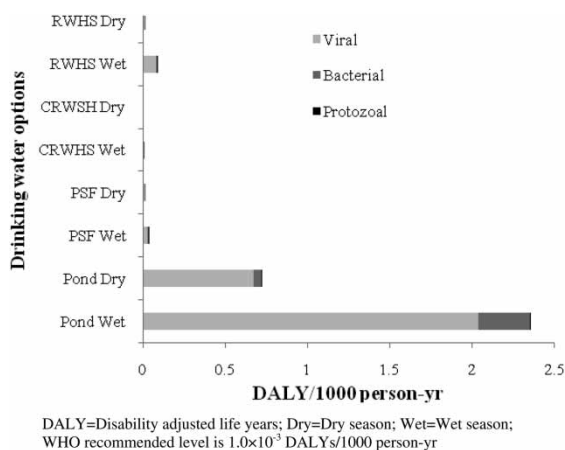The third edition of the guidelines for drinking water quality (WHO 2004) recommends a reference level of risk per

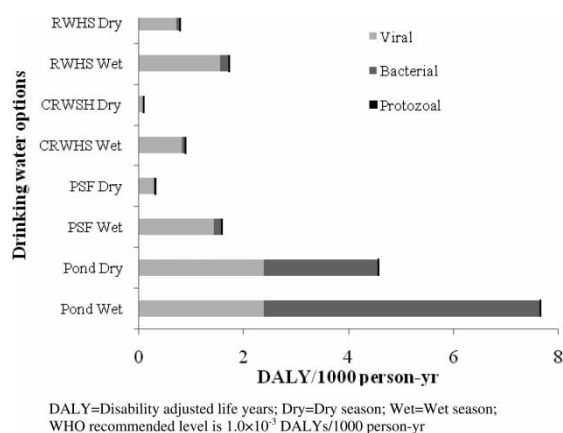**Figure 3** | Lower (5th percentile) disease burden by drinking water options and seasons.

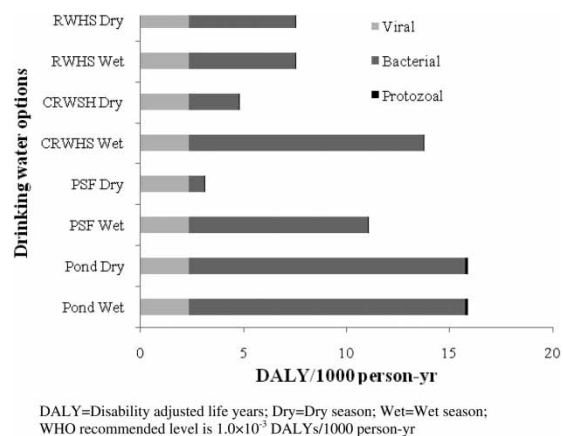**Figure 4** | Median disease burden by drinking water options and seasons.

Figure 5 | Upper (95th percentile) disease burden by drinking water options and seasons.

contaminant of 10^{-6} DALYs/person-yr. The upper bound of the disease burden estimates for ponds was about four orders of magnitude greater than the WHO reference level in both dry and wet seasons. During the dry season, the upper bounds of the disease burden estimates for CRWHSs and PSFs were approximately one order of magnitude lower than for the wet season, but were still about three and two orders of magnitude greater than the reference level of risk, respectively. During the dry season, the upper bound risk estimates for RWHSs were similar to those of the wet season.

Effects of sanitary risk factors

The results of health risk assessment (Figures 3–5) showed a high microbial risk for all options. The variation in DALYs for the same options was very large. Therefore, data from sanitary inspections were used to evaluate the sources of contamination. The relationships between individual risk factors and the presence of E. coli in RWHSs, CRWHSs, and PSFs are shown in Tables 7 and 8.

If the odds ratio (OR) is greater than 1, the corresponding factor has an effect on the E. coli concentration. In this study, two thresholds were introduced, E. coli <1 cfu/100 ml and E. coli ≤10 cfu/100 ml. These thresholds were selected because <1 cfu/100 ml is compliant with the WHO guidelines for Drinking Water Quality, and the use of ≤10 cfu/100 ml has been suggested by the WHO as an appropriate relaxation for small water supplies (WHO 1997). Table 7 shows the results for RWHSs and CRWHSs. Two risk factors were found to be significantly associated with the E. coli concentration >0 cfu/100 ml for RWHSs and CRWHSs: water collection from the tank manually, and no first flushing. When the threshold was 10 cfu/100 ml, water collection from the tank manually, and no first flushing were found to be significantly related to the E. coli concentration at the 1% level for both RWHSs and CRWHSs, whereas storage tank not clean, and gutter dirty or blocked showed a relationship with E. coli concentration at the 5% level for CRWHSs.

Table 8 shows the results for PSFs. When the threshold was 10 cfu/100 ml, a significant relationship with polluted stream flows into the pond and latrine within 10 m from the pond was found at the 5% level. In contrast, when the threshold was <1 cfu/100 ml, no risk factor was found to be significantly related to the E. coli concentration.

Table 7 | Contingency table analysis of sanitary risk factors and *E. coli* presence in RWHS and CRWHS to determine sources of faecal contamination

| Variables (responsible factor) | Criteria for qualitative risk factor | RWHSs (n = 43) | | | | CRWHSs (n = 28) | | | |
| | | *E. coli* > 0 cfu/100 ml | | *E. coli* > 10 cfu /100 ml | | *E. coli* > 0 cfu /100 ml | | *E. coli* > 10 cfu /100 ml | |
		OR	p	OR	p	OR	p	OR	p
1. Storage tank not clean inside (maintenance)	Dirty tank surface/ visible insects or pollutants to naked eye	1.78	0.440	3.05	0.081	3.08	0.152	6.42	0.034*
2. Pollution sources around the tank (maintenance)	Faecal droppings from birds or animals/ Unhygienic surrounding environment	0.429	0.304	1.58	0.541	0.333	0.378	0.889	0.927
3. Floor under tap dirty (maintenance)	Faecal droppings from birds or animals/visible pollutants to naked eye	0.775	0.356	0.425	0.092	0.714	0.755	2.0	0.520
4. Tap leaking/ damaged (maintenance)	Tap leaking/not working	0.786	0.603	0.452	0.278	0.556	0.378	0.333	0.172
5. Water collection from tank manually/ directly (water uses)	No tap/outlet pipe for hygienic abstraction of water	11.375	0.002**	11.70	0.001**	35.0	0.000**	2.66	0.001**
6. Gutter dirty or blocked (maintenance)	Leaves/faecal droppings from birds or animals/ visible pollutants to naked eye	0.710	0.036	3.16	0.099	5.0	0.069	5.25	0.046*
7. Visible sign of contamination on roof (maintenance)	Leaves/faecal droppings from birds or animals/ contaminated litter on roof	5.0	0.051	1.33	0.639	1.20	0.820	2.60	0.240
8. Water tank cracked/ damaged (maintenance)	Visible cracked in the tank	0.786	0.603	0.452	0.278	0.556	0.378	0.333	0.172
9. Rain water collection in open container (maintenance)		0.769	0.280	3.88	0.230	0.840	0.823	2.0	0.387
10. By-pass line or flush line missing (maintenance)	No first-flush diverter/no detachable down-pipe for manual first-flushing	11.42	0.011*	5.33	0.010**	33.0	0.000**	4.33	0.000**

n, Number of samples; OR, Odds Ratio; p = Chi-square value.
*Significant at the 0.05 level.
**Significant at the 0.01 level.

DISCUSSION

Water quality of ponds and PSFs

The worst bacterial quality was found in ponds, which are the principal drinking water option during the dry season. Environmental circumstances around the drinking water options are also very important considerations for keeping the options safe. During field surveys, almost all of the ponds were found to be affected by surface runoff, and some were used for washing and bathing purposes. It is likely that the high level of contamination is due to the flow of poorly disposed faecal matter into the ponds. The association between *E. coli* and polluted stream flows into

Table 8 | Contingency table analysis of sanitary risk factors and *E. coli* presence in PSF to determine sources of faecal contamination

| Variables (responsible factor) | Criteria for qualitative risk factor | PSFs (n = 31) | | | |
| | | *E. coli* > 0 cfu/100 ml | | *E. coli* > 10 cfu/100 ml | |
		OR	p	OR	p
1. Missing fence (maintenance)	No fence to protect animals	1.06	0.948	0.727	0.667
2. Hand pump loose at attachment (maintenance)		0.833	0.656	0.433	0.263
3. Fish culture in the pond (unprotected pond)		0.667	0.686	0.733	0.690
4. PSF roof cover open (maintenance)		0.196	0.113	0.250	0.217
5. Pond not protected by fence (unprotected pond)	No fence to protect animals	1.81	0.555	0.554	0.457
6. Lack of minimum head on filter bed (maintenance)	Negative head on the filter bed	1.77	0.627	0.238	0.101
7. Cracked/dirty drainage channel (maintenance)	Drainage channel is cracked/water logged/ visible insects or pollutants to naked eyes	0.952	0.968	1.27	0.791
8. Pollution source within 10 m from the pond (unprotected pond)	Faecal droppings from birds or animals/ Unhygienic surroundings	0.794	0.818	1.8	0.436
9. Polluted stream flows into the pond (unprotected pond)	Pond embankment is not sufficient to ensure protection from surface run off entering into the pond	0.783	0.150	5.62	0.049*
10. Latrine within 10 m from the pond (unprotected pond)		2.5	0.429	6.22	0.022*

n, Number of samples; OR, Odds Ratio; p = chi-square value.

* Significant at the 0.05 level.

the pond and latrine within 10 m from the pond suggests that unprotected ponds were the major sources of faecal contamination for PSFs. Rural ponds in Bangladesh that are used for bathing, washing utensils and drinking water options have high concentrations of FC (Islam *et al.* 2000). However, Islam *et al.* (1994) found that if a pond is protected from human use, has a high bank and no drain, it can provide water with a FC count <1 cfu/100 ml year round. Therefore, to improve the quality of pond water the ponds should be protected from surface runoff and human use.

A decline in microbiological quality during the wet season was evident for ponds and PSFs. For ponds, one of the major reasons for the decreased water quality was surface runoff during the wet season. Deterioration of pond water quality may influence the efficiency of PSFs. Howard *et al.* (2006) also found decreased PSF water quality during the wet season. However, few PSFs samples showed no or lower removal of FC and *E. coli* in both seasons. This may have contributed to the reduced overall removal efficiency

of PSFs, especially for *E. coli* in the wet season. Moreover, proper maintenance of PSFs is always a matter of concern.

The mean HPC concentrations in PSFs were higher than in the PSF ponds. More than 50% of the PSFs showed increases in HPC after filtration. The possible sources of this contamination may have been supply lines, sand beds or collection taps. In the present study, most of the PSF taps were found to be defective. Pepper *et al.* (2004) demonstrated that distribution lines in households and household taps can increase the HPC level dramatically for both surface and groundwater supply. However, further research is required to identify the sources of contamination.

V. cholerae non-O1/non-O139 were isolated from approximately 95% of the ponds, which revealed the extent of contamination of ponds by potentially pathogenic bacteria. According to field observations, few people treat pond water before consumption; therefore, drinking pond water may cause gastroenteritis and bacteremia (WHO 2004). In the present study, *V. cholerae* non-O1/non-O139

was isolated from almost 94.1% (16/17) of the PSF ponds during the dry season. However, following filtration by PSFs, 50% (8/16) of those samples no longer contained *V. cholerae* non-O1/non-O139. These findings suggest that PSFs can remove *V. cholerae* non-O1/non-O139, but the removal efficiency may depend on the contamination level of the ponds and the efficiency of the PSFs. This is because *V. cholerae* non-O1/non-O139 was isolated from all PSFs during the wet season. However, one PSF pond showed absence of *V. cholerae* non-O1/non-O139 during the wet season. The most probable reason for isolation of *V. cholerae* non-O1/non-O139 from the mentioned PSF water was contamination from defective supply lines between PSF pond and PSF. The possibility of bacteriological contamination through the leakages in distribution lines was suggested in a study by Chowdhury *et al.* (2001). No toxigenic *V. cholera* was observed in the present study. Nevertheless, toxigenic *V. cholerae* have been isolated from ponds in coastal areas of Bangladesh (Huq *et al.* 2005; Alam *et al.* 2006; Stine *et al.* 2008) and Momba *et al.* (2006) found toxigenic *V. cholerae* in surface water that is actively used for drinking purposes in rural areas of South Africa. *Pseudomonas* spp. were isolated from few PSFs and ponds during the dry season; while, in the wet season almost all samples showed presence of *Pseudomonas* spp. It seems that the major source for such contamination was surface runoff into the ponds during the wet season.

Water quality of RWHSs and CRWHSs

The CRWHSs appeared to be a comparatively better option according to the water quality data (Table 3). However, these facilities showed higher microbial contamination during the wet season. In the present study, few samples were found to be acceptable for drinking purposes based on the FC and *E. coli* counts. Several studies (Lye 1987; Crabtree 1996; Uba & Aghogho 2000; Simmons *et al.* 2001; Handia 2005; Despins *et al.* 2009; Horak *et al.* 2010) conducted in various parts of the world clearly showed that harvested rainwater often does not meet the microbiological drinking water quality standards. This is because although rainwater is safe in terms of pollution by pathogens, its quality may deteriorate during the process of harvesting. The median *E. coli* concentrations were low

for RWHSs and CRWHSs during both seasons, but there were a small number of facilities with very high counts, which reflects poor maintenance of the facilities. For CRWHSs, the dirty storage tank risk factor was found to be related to faecal contamination (Table 7); however, the relationship between faecal contamination and this risk factor was not found to be significant for RWHSs. The reason for this difference may have been the size of the tank. Because RWHSs have smaller tanks, they are generally washed more frequently than CRWHSs. During the sanitary inspections, the local residents reported that they do not always wash CRWHSs tank annually. Lack of first flushing during rainwater collection was also a common problem in the study area. However, contamination on the roof was not found responsible factor for RWHSs and CRWHSs. Conversely, contamination in the absence of first flushing was mainly from the roof and gutter of the collection system. Additionally, we found that water collection from the tank manually can contaminate harvested rainwater. Therefore, the results of this study clearly demonstrate the importance of proper collection and extraction of rainwater for both RWHSs and CRWHSs. Sanitary inspections were only conducted during the collection of water samples; therefore, repeated inspections may be useful to gain a better understanding of maintenance problems.

The maximum permissible limit of HPC in drinking water is 500 cfu/ml (US EPA 2003). As a group, organisms identified in heterotrophic plate counts do not present a risk to water consumers, although a HPC >500/ml indicates that more hygienic practices are required to maintain the drinking water quality. In the present study, the highest HPC concentration was recorded from the RWHSs. Several studies have shown that roof-collected rain water may contain higher HPC values than were observed in the present study (Lye 1987; Crabtree *et al.* 1996; Simmons *et al.* 2001).

Both RWHSs and CRWHSs showed some degree of contamination by *V. cholerae* non-O1/non-O139 that may have been due to poor operation and maintenance. Uba & Aghogho (2000) also found a high prevalence of *Vibrio* spp. in rainwater although collected from different types of roof catchments. *Salmonella* and *Shigella* spp. were not isolated from any of the samples in the present study; however, the authors in the mentioned study found a high prevalence of *Salmonella* and *Shigella* spp. in rainwater (Uba & Aghogho

2000). *Salmonella* spp. were also isolated from roof collected rainwater in New Zealand (Simmons *et al.* 2001). In the present study, *Pseudomonas* spp. were the most dominant opportunistic pathogens isolated from rain water samples during the wet season. For RWHSs and CRWHSs, roof and gutter of the collection systems and water collection from the tank manually may have contributed to higher contamination. The presence of *Pseudomonas* spp. in drinking water may cause infections to immunocompromised populations.

Relationship between drinking water quality and diseases

The most common category of water-borne diseases is diarrhoea (Copeland *et al.* 2009). The records of the Dacope and Mongla Upazila Health complex showed higher diarrhoea complaints during the wet season (June to October) (Figure 6), which supports the results of the present study. It should be noted that only diarrhoea records were used to evaluate water-borne diseases because records of other common water-borne diseases such as cholera and typhoid fever were not available. Nevertheless, the information presented here can provide an overall picture of waterborne diseases in the study area.

In the Philippines, the occurrence of diarrhoeal disease was found to increase when the *E. coli* count per 100 ml of water was greater than 1000. Such levels of contamination in drinking water sources have been identified as the major sources for the transmission of diarrhoeal pathogens
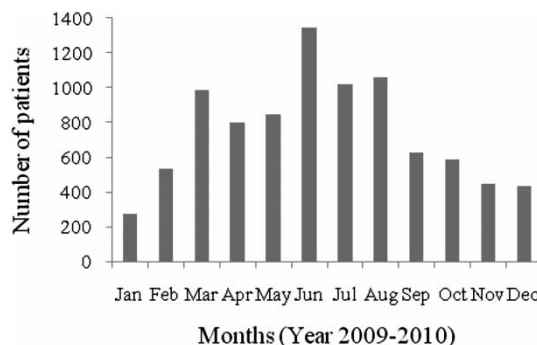

Figure 6 | Patients visiting Dacope and Mongla Upazila Health Complex with diarrhoea complaints in 2009–2010 (Source: Upazila Health Complex Dacope and Mongla).

(Moe *et al.* 1991). In the present study, 10.25% and 12.82% of the ponds showed *E. coli* counts >1000 cfu/100 ml in the dry and wet seasons, respectively. The high counts of *E. coli* in drinking water are obviously a risk for public health. PSF can reduce *E. coli* and FC significantly; therefore, use of PSF water instead of pond water may reduce diarrhoeal diseases. Indeed, although the FC and *E. coli* levels did not satisfy the drinking water standard, the use of protected ponds and proper maintenance can improve the removal efficiency.

Health risk

All of the water supply options evaluated in this study showed significantly higher disease burden when compare to recommended disease burden of 1×10^{-3} DALYs/1000 person-yr (WHO 2004). It should be noted that the 1×10^{-3} DALYs/1000 person-yr reference level indicates the risk per contaminant, but the water supplies containing many contaminants at their guideline values would possibly present a disease burden in excess of 1×10^{-3} DALYs/1000 person-yr. The lower disease estimates indicate that pond water has a risk of disease and that other options can ensure the reference level of disease burden if the systems are well maintained. Additionally, the median estimates showed the general tendency of risk associated with the drinking water options. The median disease burden associated with arsenic in the water supply of Bangladesh, which has a standard of 50 µg/l, is 185 µDALYs /person-yr (Howard *et al.* 2007). In this study, the median disease burden (microbial risk) from alternative water supply options compared with the median disease burden (arsenic risk at 50 µg/l) from arsenic contaminated water revealed that the burden was about 8, 4, 8, and 41 times higher in the wet season for RWHSs, CRWHSs, PSFs, and ponds, respectively. The upper bound of the disease burden estimated by Howard *et al.* (2006) for TTC was about 16 and 6 DALYs/1000 person-yr for PSFs and RWHSs, respectively, which was different from the present findings of about 14 and 8 DALYs/1000 person-yr, respectively. Although, CRWHSs and PSFs showed comparatively lower disease burdens during the dry season, the upper bound of the disease burden was very high during the wet season. The higher disease burden for RWHSs, CRWHSs, and PSFs may have been due to poor maintenance of the facilities. In general,

RWHSs, CRWHSs, and PSFs showed the potential to be better alternative drinking water options.

CONCLUSION

This study examined the prevalence of indicator and pathogenic bacteria in alternative drinking water supply options present in southwest coastal areas of Bangladesh. The high numbers of indicator bacteria and prevalence of *V. cholerae* and *Pseudomonas* spp. in the water supply options represent a potential source of human illness. To the best of our knowledge, this is the first study to broadly examine pathogenic organisms from PSFs and harvested rainwater in Bangladesh. The estimated disease burdens associated with the currently practiced water supply options are much higher than the WHO recommended risk level of 1×10^{-3} DALYs/1000 person-yr. The disease burden was primarily dominated by bacterial and viral pathogens. In most cases, people drink pond and rain water without any treatments, even though the efficiency of PSFs did not satisfy the drinking water standard. Thus, microbial health risks associated with alternative drinking water options require proper attention to enabling a safe and sustainable water supply to coastal areas of Bangladesh. According to sanitary inspections, water collection from the tank manually, gutter dirty or blocked, no first flushing, and an unclean storage tank were responsible for contamination of collected rain water. For degradation of PSF water, polluted stream flows into the pond and latrine within 10 m from the pond were the responsible factors. Therefore, proper maintenance and unprotected ponds were found to be vital factors for maintaining water quality in rain water collection systems and PSFs, respectively. However, the relations between sanitary risk factors and faecal contamination for water supply options need to be investigated in future on seasonal basis using a larger sample size. In general, the operation and maintenance of all types of facilities evaluated in this study require improvement. All of the options showed a high microbial risk; thus, measures such as in-house filtration or disinfection must be implemented to make the water safe for drinking purposes. The Department of Public Health & Engineering and other working NGOs should provide adequate information and training to the coastal communities to let them understand the operation and maintenance of facilities and importance of hygienic handling of water. Moreover, a protocol of design and construction, regular sanitary inspection and maintenance for harvested rainwater and PSFs should be adopted to protect the quality of water.

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