NIH Public Access
Author Manuscript
J Water Health. Author manuscript; available in PMC 2013 December 01.

Published in final edited form as:
J Water Health. 2012 December ; 10(4): 565–578. doi:10.2166/wh.2012.102.

Unsealed Tubewells Lead to Increased Fecal Contamination of Drinking Water

Peter S. K. Knappett[1,2,*], **Larry D. McKay**[1], **Alice Layton**[3], **Daniel E. Williams**[3], **Md. J. Alam**[4], **Brian J. Mailloux**[5], **Andrew S. Ferguson**[6], **Patricia J. Culligan**[6], **Marc L. Serre**[7], **Michael Emch**[8,9], **Kazi M. Ahmed**[4], **Gary S. Sayler**[3], and **Alexander van Geen**[2]

[1]Department of Earth & Planetary Sciences, University of Tennessee, Knoxville, TN 37996-1410

[2]Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY 10964

[3]Center for Environmental Biotechnology, University of Tennessee, Knoxville, TN 37996-1605

[4]Department of Geology, University of Dhaka, Dhaka 1000, Bangladesh

[5]Department of Environmental Science, Barnard College, New York, NY 10027

[6]Civil Engineering & Engineering Mechanics, Columbia University, New York, NY 10027

[7]Department of Environmental Sciences & Engineering, University of North Carolina - Chapel Hill

[8]Department of Geography, University of North Carolina – Chapel Hill

[9]Carolina Population Center, University of North Carolina – Chapel Hill

Abstract

Bangladesh is underlain by shallow aquifers in which millions of drinking water wells are emplaced without annular seals. Fecal contamination has been widely detected in private tubewells. To evaluate the impact of well construction on microbial water quality 35 private tubewells (11 with intact cement platforms, 19 without) and 17 monitoring wells (11 with the annulus sealed with cement, 6 unsealed) were monitored for cultured *E. coli* over 18 months. Additionally, two “snap shot” sampling events were performed on a subset of wells during late-dry and early-wet seasons, wherein the fecal indicator bacteria (FIB) *E. coli*, Bacteroidales and the pathogenicity genes *elt*A (ETEC *E. coli*), *ipa*H (*Shigella*) and 40/41 hexon (adenovirus) were detected using qPCR. No difference in *E. coli* detection frequency was found between tubewells with and without platforms. Unsealed private wells, however, contained cultured *E. coli* more frequently and higher concentrations of FIB than sealed monitoring wells ($p<0.05$), suggestive of rapid downward flow along unsealed annuli. As a group the pathogens ETEC, *Shigella* and adenovirus were detected more frequently (10/22) during the wet season than the dry season (2/20). This suggests proper sealing of private tubewell annuli may lead to substantial improvements in microbial drinking water quality.

Keywords

adenovirus; Asia; Bacteroidales; *E. coli*; shigella; tubewells

Corresponding Author: P. S. K. Knappett, Lamont-Doherty Earth Observatory of Columbia University Route 9W, Palisades, NY 10964. Tel: 917-797-8371. Fax: 845-365-8154. pknappet@utk.edu.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Introduction

Fecal bacteria and viruses have been detected in groundwater wells emplaced in aquifers of diverse geologic material (Rudolph et al. 1996; Abbaszadegan et al. 2003; Embrey & Runkle 2006; Borchardt et al. 2011; Johnson et al. 2011; Kozuskanich et al. 2011). In developed countries like the United States wells are typically sealed with an expanding clay which fills the annulus between the well casing and the surrounding aquifer sediments from ground surface to approximately 1 m above the screened interval to prevent "short circuiting" by downward flow of contaminated surface water or shallow groundwater. In developing countries, such as Bangladesh, the annulus of shallow drinking water wells, referred to as tubewells, is typically filled with soil or sediments obtained during drilling; a measure unlikely to prevent rapid annular flow. Recent programs have encouraged the construction of concrete platforms and drainage channels around well heads to drain wash water before it infiltrates down the tubewell annulus, but there is insufficient information to indicate whether this is successful (Luby et al. 2008; Leber et al. 2011; van Geen et al. 2011). Although tubewells are considered an "improved" drinking water source by the World Health Organization (WHO 2000), these tubewells still do not have adequate protection from annular flow and are considered unsealed (Personal Communication, Peter Ravenscroft). The goal of this study is to evaluate the impact of tubewell construction (specifically annular seals and concrete platforms) on the levels of human exposure to fecal contamination and pathogens.

Over ten million of these tubewells provide drinking water for millions of inhabitants throughout rural Bangladesh. In this country 11% of all deaths are estimated to be caused by diarrheal disease (Streatfield et al. 2001) with recent studies suggesting that sustained levels of diarrheal disease are caused in part by drinking untreated groundwater (Escamilla et al. 2011; Wu et al. 2012; Escamilla et al. 2013). The widespread fecal indicator bacteria (FIB) and bacterial and viral pathogens revealed in tubewells (Luby et al. 2008; Leber et al. 2011; van Geen et al. 2011; Ferguson et al. 2012) are known to be predictive of diarrheal disease in diverse populations around the world (Gundry et al., 2004).

Bangladesh is underlain by shallow aquifers consisting of unconsolidated sand and silt laid down by streams and rivers flowing through the Ganges-Brahmaputra delta (Goodbred & Kuehl 1998; Weinman et al. 2008). In addition to annular flow around the tubewell, it is possible that fecal contamination enters aquifers and tubewells through infiltration from latrines or seepage from the many ponds and canals found in rural villages (Knappett et al. 2011a). Recent field experiments in Bangladesh indicate efficient spatial removal of FIB in typical medium-grained aquifers, with 7-$\log_{10}$ removal within 13 m from leaking latrine ponds (Knappett et al. 2012). This finding suggests that fecal contamination of unconfined aquifers from latrines, ponds and other sources should have relatively limited spatial extent. Contamination would be expected to be even more limited in areas where the aquifer is overlain by silt or clay layers. This is not consistent, however, with the widespread occurrence of FIB in tubewells located in both unconfined sandy aquifers and aquifers overlain by silt (Leber et al. 2011); suggesting the presence of rapid flow pathways, such as annular flow around the tubewell casing.

Recent programs in Bangladesh and other developing countries have encouraged the construction of 2–3 m^2 cement platforms with drainage channels around tubewells to remove standing water and reduce the likelihood of annular flow of contaminated water (Luby et al. 2008; WHO/UNICEF 2012). The utilization of concrete platforms is supported by studies in rural Africa (Godfrey et al. 2006), which found links between the presence of standing water around the well head and poor microbial water quality in the well.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Studies spanning multiple villages and seasons in Bangladesh, however, have shown that *E. coli* detection frequency in private tubewells is typically insensitive to both the presence and quality of a platform (Luby et al. 2008; Leber et al. 2011; van Geen et al. 2011) leading some to suggest that tubewells are not subject to annular flow (Luby et al. 2008). Only one study (Escamilla et al. 2013), considering a subset of approximately 90 wells used in the study by van Geen et al. (2011), found that well platform presence correlated to lower *E. coli* detection frequency in private wells. This effect was only significant during the early monsoon period (Apr–Jun) and no other time of year (Escamilla et al. 2013). Likely more critical than the presence of an intact platform in preventing annular flow is the presence of a seal between the borehole and the annulus of a well from surface to screened depth.

E. coli prevalence peaks in tubewells during the wet season (Leber et al. 2011), being closely associated with antecedent rainfall events (van Geen et al. 2011). Increases in allochthonous bacteria concentrations in aquifers following rainfall events are attributed to vertical flushing when bacteria are both introduced to the vadose zone and mobilized from grain surfaces due to increasing water content and shear velocity (DeNovio et al. 2004; Pronk etal. 2007). Pit latrines and ponds are the primary repository of human feces in Bangladesh and the water table is very shallow, lying 1 to 5 m below the surface, giving ample opportunity for contamination of the water table from these numerous point sources in densely populated rural villages. Further, flooding is widespread during the late wet season, potentially widening the spatial extent of fecal contamination sources at the surface during the time of year when the vadose zone is thinnest (van Geen et al. 2011; Knappett et al. 2012).

The objective of this study is to quantify the impact of private well construction on the frequency and concentrations of FIB detected in tubewells within a sandy aquifer underlying a village in rural Bangladesh. A further objective is to assess the utility of molecular FIB markers in predicting the year round risk of fecal contamination of a well emplaced within a shallow, reducing aquifer. The pathogenicity genes *elt* A (ETEC *E. coli*), *ipa*H (*Shigella*) and 40/41 hexon (adenovirus) were detected using qPCR to assess the ability of FIB's to indicate the presence/absence of pathogens.

Part of the monthly cultured *E. coli* measurements reported in this study for private tubewells has been published previously in the context of a larger study finding a broad negative correlation between *E. coli* detection frequency and arsenic (van Geen et al. 2011). The previously published monthly *E. coli* detection frequencies for 35 private wells at Site K is compared here to synoptic measurements made on 17 additional monitoring wells. Additionally, the previously unpublished results of two snap shot monitoring events for FIB DNA and pathogens on a subset of private and monitoring wells are presented here.

Methods

Site Description

The village of Char Para is referred to herein as Site K (Knappett et al. 2011a, b). Char Para overlies a sand bar deposit of the neighboring "Old Brahmaputra" river, which flows throughout Araihazar upazilla and used to transmit far more water and sediment than it does today (Weinman et al. 2008). Bangladesh has a dry season during which the region receives little rain from November through May. The dry season is followed by the monsoon, a period of 4 months during which Bangladesh receives the vast majority of its total rainfall for the year. this study rainfall was measured using a HOBO weather station (ONSET, Bourne, MA) in the region of Matlab, 50 Km south of Site K. The unconfined water table at Site K fluctuates throughout the year from 4 m below the surface at the end of the dry season to within 1 m of the surface during the wet season (Knappett et al. 2012). Although

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

there is an influence of the local river which bounds three sides of the village, lateral hydraulic gradients throughout Site K are small and affected by irrigation pumping in the surrounding rice fields. Approximately 1500 people live in Site K. Roughly 50 ponds and 180 latrines are scattered throughout the site. Half of these latrines spill effluent onto the open ground (Knappett et al. 2011a), consistent with the country-wide improved sanitation coverage for rural Bangladesh in 2010 of only 43% (WHO/UNICEF 2012).

Private wells in Bangladesh, referred to as “tubewells”, are inexpensive PVC pipes with 1 to 1.5 m screened intervals equipped with iron hand pumps and are typically screened at depths ranging from 8 to 30 m below the ground surface (van Geen et al. 2003). As of 2009 our exhaustive survey of Char Para (Site K) indicated that it contained 144 private tubewells. Therefore 1 tubewell supplies drinking water for 10 people, as there 1500 inhabitants in the village (Knappett et al. 2011a). Drillers will typically drill no further than the depth necessary to ensure year round supply. In the absence of poorly conductive surface deposits, tubewells will be quite shallow as they are at Site K (Leber et al. 2011). The minimum and maximum depths of these private tubewells reported by the owners varied from 6.1 to 91.5 m, respectively with a median depth of 9.1 m. The positions of all wells in this study were determined using high accuracy (sub-meter) GPS using a Trimble GeoXH receiver and Terrasync 2.4 software. GPS data were post-processed using Pathfinder Office 3.0 (Trimble Navigation Ltd., Sunnyvale, CA).

Drillers in Bangladesh do not typically use any material to seal the outside of the PVC pipe (Personal Communication, Peter Ravenscroft) from rapid annular flow or “short-circuiting” of surface water or near-surface water to screened interval depth. In developed countries, typically bentonite, an expanding clay, will be used to seal wells. To control for the possibility of annular flow, eleven monitoring wells with cement seals (MS) were installed in January 2008 throughout Site K. Ten of these had 1.5 m screens at the same depth as a nearby private well and one was not installed nearby one of the 35 monitored private wells (Fig. 1) but was screened at a typical depth. In addition six monitoring wells were installed within two multi-level nests, previous to 2008 without cement seals (M). These unsealed monitoring wells served as intermediates between private (P) and sealed monitoring (MS) wells to test for an effect of regular pumping only (as opposed to pumping absence and seal presence combined) on the frequency of *E. coli* detections in the well. The depths of the 35 class P wells varied between 5.8 and 30.5 m (5^{th} and 95^{th} percentile were 6.1 and 15.2 m, respectively) and the median depth was 7.6 m. Depths of the 11 MS wells varied between 7.2 and 15.4 m and the median depth was 7.7 m. All wells were monitored for *E. coli* monthly for the period from April 2008 through November 2009.

Well Sampling

Continuous pumping from both private (P) and sealed (MS) tubewells has been observed to dramatically decrease measured concentrations of *E. coli* over a 24 hour period (Knappett et al. 2011b), therefore prior to sampling all wells were purged for a consistent 3 wellbore volumes. Duplicate 100 ml water samples were taken from every well. Private tubewells were sampled using the existing iron hand pump whereas monitoring wells were pumped using submersible electric pumps (Typhoon, Groundwater Essentials, LLC) at flow rates ranging from 2 to 8 L/min. In between monitoring wells, all tubing and electric pumps were flushed with a dilute bleach and TWEEN solution derived from the well just sampled, followed by rinsing once with well water and once with Na-Thiosulfate as detailed in (Knappett et al. 2011b).

Two “snap shot” sampling events were performed, once during the dry season (March 16–18) and once during the wet season (July 3–7) in 2009 to analyze for a broad spectrum of

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

fecal indicator bacteria (FIB) and pathogens. Cultured *E. coli* was measured concurrently, only during the wet season sampling event.

Microbial Analyses

E. coli was quantified using the MPN based Colilert™ test kit (IDEXX Laboratories, Inc.). Duplicate 100 mL groundwater samples were collected in sterile containers and measurements were carried out in a lab within 8 hours of sample collection. Most Probable Numbers (MPN) of *E. coli* were determined by combining the numbers of discrete positive wells in both trays (Hurley & Roscoe 1983; Knappett et al. 2011b).

For enumeration of fecal bacteria genomes, 4–8 liters of groundwater was filtered onto 0.22 μm nitrocellulose filters. The filters were removed from the plastic housing, placed in sterile petri dishes, frozen and transported on dry ice to the University of Tennessee. DNA was extracted and purified from the filters using the FastDNA® SPIN for Soil Kit (MP Biomedicals, LLC, Solon, Ohio) following the manufacturer's protocols. DNA was measured using a nanodrop and the extracts were diluted to 5–10 ng/μl of total DNA to avoid inhibition which was further verified by measuring the amount of a known plasmid spike added to each sample for each PCR run.

Quantitative PCR was performed to detect *E. coli* and Bacteroidales using the identical assays and laboratory methods as described in our previous study (Knappett et al. 2011b). The gene targets for the *E. coli* (herein referred to as *mE. coli*) and Bacteroidales assays were the 23S rRNA gene and the 16S rRNA gene, respectively (Bernhard & Field 2000; Scott et al. 2002; Layton et al. 2006; Noble et al. 2006; Kildare et al. 2007) and the primer and probe sequences are provided in Table 2. Pathogen genes (*elt*A, *ipa*H and adenovirus 40/41) were assayed in duplicate or triplicate using the primers, probes and master mix types listed in Table 2 and following standard quantitative PCR protocols described in previous studies (Knappett et al. 2011b). Standards for the quantitative PCR reactions were made from a relevant gene fragment cloned into PCR4-TOPO cloning vector (Layton et al. 2006). The MDL was determined from the standard curve to be 20 gene copies per qPCR reaction. Data were calculated only for samples in which at least two PCR reactions had >1 gene copy and were quantified as copies/μl nucleic acid extract. Gene copies were adjusted to copies/100 mL for tubewell water based on the fraction of the filter extracted, multiplied by the volume of DNA extract and divided by the filtered sample volume. Due to differences in the volume of water filtered for each sample the MDL varied somewhat with each sample (Knappett et al. 2011b) with a mean detection limit of 4 copies/100 mL for the groundwater samples (Ferguson et al. 2012).

Experimental Design and Statistical Analyses

The three well types (P, M and MS) were compared for *E. coli* prevalence using binned wet/dry season box plots and a monthly time series comparison between P and MS wells. A six month dry season was defined here from November 15 through May 15 with the wet season being the other half of the year. A total of 18 months were available (April, 2008 through October, 2009) for which all three classes of wells were sampled each month (class P wells were also sampled from January 2008 through April 2008). For statistical testing on binned wet/dry season data a minimum of five sampling events were required in each season for each well, causing the numbers of wells in each category to be reduced to 33, 6 and 11 for Private (P), Monitoring (M) and Monitoring wells with seals (MS), respectively. ANOVA was performed three times with well class as the "treatment" and *E. coli* frequency during year round, wet and dry seasons as response variables, to determine differences between the classes of wells. Further, the non-parametric Kruskal-Wallis test was performed on the ranks to confirm statistical differences between paired classes of wells using the statistical

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

software NCSS (version 07.1.14, NCSS, LLC, Kaysville, Utah). *E. coli* prevalence in a given well (across time) or during a given month (across space) was accompanied by the approximation ± 2 $[p (1-p)/ n]^{1/2}$ used to estimate 95% CIs for the proportion of wells p with detectable *E. coli* where n is the total number of sampling events or wells respectively (Gelman & Hill 2007).

Results and Discussion

Monthly E. coli Detection Frequency in Sealed and Unsealed Wells

E. coli prevalence in tubewells was observed to be substantially higher in the monsoon than the dry season (Fig. 2b,c). An ANOVA on the *E. coli* prevalence data confirmed that well class (P, M, MS) significantly impacted the frequency of *E. coli* detected in a well, both year-round (Fig. 2a) and during the wet season ($p<0.05$) (Fig. 2b), but not during the dry season (Fig. 2c). Non-parametric Kruskal-Wallis tests between pairs of well classes confirmed that private tubewells (P) were more frequently contaminated than monitoring wells with seals (MS) year-round and during the wet season ($p<0.05$) (Fig. 2a,b). *E. coli* prevalence in M wells was intermediate and not significantly different from either P or MS wells. The lesser *E. coli* prevalence in M wells than P wells suggests that daily pumping from private tubewells plays a role in fecal contamination. Other possible causes of more frequent private tubewell contamination over monitoring wells (M and MS) include biofilm growth within the iron hand pump (Ferguson et al., 2011) and the introduction of *E. coli* into the well following pump priming of private wells (van Geen et al. 2011).

Although annular sealing appeared to substantially reduce *E. coli* detection frequency, the presence of an intact cement platform had no impact on the microbial drinking water quality from a private tubewell in either the wet or dry seasons (Fig. 2e,f). The WHO/UNICEF (2012) well construction guidelines emphasize the importance of an adequate drainage channel to allow the spilled water to leave the well head area however, strictly speaking this is not required for the classification of a tubewell as an “improved” drinking water source (WHO/UNICEF 2012). As of 2010 80% of the rural population in Bangladesh was recorded drinking from “improved” water sources (WHO/UNICEF 2012). In our exhaustive survey of the 144 private tubewells at Site K in 2009, however, only 42% (61/144) of private tubewells had intact platforms, and many of these did not have drainage channels. In a related study combining 32 wells from Site K with 93 wells in Matlab upazilla, 50 km south of Site K, only 51% (64/125) of all tubewells had intact platforms (van Geen et al., 2011). In the present study, we did not differentiate between intact platforms with and without good drainage channels. The sample size in the present study for which monthly *E. coli* measurements were available is small, with 11 tubewells having good platforms and 19 that did not have platforms or had broken platforms. Other studies, however, have reported the insensitivity of platform quality and presence on FIB detection frequency in private tubewells in Bangladesh (Luby et al. 2008; Leber et al. 2011; van Geen et al. 2011). This finding has led some to conclude that annular flow is not important in Bangladesh where wells are drilled and not dug (Luby et al. 2008). Based on the information presented in this study, annular flow seems to degrade microbial water quality, but the presence of an intact platform has little protective effect. This may be due to the general absence of good drainage channels, or the flat terrain which leads to ponding around well heads, rendering intact platforms and long drainage channels irrelevant following large rainfall events.

It was hypothesized that *E. coli* detection frequency would decrease with depth. Well depth was not found to correlate to *E. coli* detection frequency for any class (P, M, MS) of wells in this study for any month or binned season. This is consistent with other studies measuring FIB contamination risk factors in private tubewells in Bangladesh (Luby et al. 2008; Leber et al. 2011; van Geen et al. 2011). One study showed consistent decreases in FIB

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

concentration with depth in paired sealed monitoring wells (MS) installed only 3 m apart vertically in highly contaminated aquifers in the vicinity of latrine ponds (Knappett et al. 2012). It is likely that sediment heterogeneity and well-specific processes such as biofilm growth (Ferguson et al. 2011) and rapid flow down unsealed annuli confound a simple relationship between depth and *E. coli* detection frequency in studies where comparatively shallow tubewells (<36 m) are compared across village(s) (Leber et al. 2011; van Geen et al. 2011).

Throughout the 18 month monitoring period MS wells were typically less frequently contaminated on a month to month basis (Fig. 3b). During the early monsoon season *E. coli* was just as prevalent in MS wells as P wells, however *E. coli* prevalence tended to decrease later in the monsoon in MS wells but continued to increase in P wells (Fig. 3b). Two exceptions to this pattern were when *E. coli* prevalence in MS wells exceeded P wells, following major rainfall events in the 2008 wet season.

These findings can be explained the following way. Sealed monitoring wells sample aquifer water that becomes contaminated via vertical infiltration through the vadose zone in a “first flush” event when the water table is still >4 m below the surface (Fig. 3a). Since 90% of all wells in this study have total depths that vary between 6 and 15 m this “first flush” water reaches the saturated water table only 0.5 to 9.5 m above the screened interval (1.5 m). As the water table rises the impetus for downward movement of infiltrating rainwater through sediment decreases with the thinning of the vadose zone. Similarly, *E. coli* prevalence increases in private wells at the start of monsoonal rains, but in contrast to the sealed monitoring wells, as the water table rises unsealed private wells become more frequently contaminated (Fig. 3b). This can be explained by vertical flow along the annulus of the private wells enhanced by regular pumping.

Dry and Wet Seasons Snapshots of Molecular FIB’s and Pathogens

During the wet season snap shot sampling event concentrations of molecular *E. coli* and Bacteroidales in MS wells were significantly lower than in P wells as assessed by the Kruskal-Wallis test ($p<0.05$). Although FIB DNA concentrations were high during the dry season, no significant difference in concentration was observed between MS and P wells (Fig. 4a,c). This contrasts with the cultured *E. coli* data which shows all three classes of tubewells have lower frequency of *E. coli* detected during the dry season than the wet (Fig. 2b,c). The similar concentrations of molecular FIB’s in MS and P wells during the dry season suggests that short circuiting is not active during the dry season, and rather these concentrations of FIB DNA represent background levels in the aquifer sustained throughout much of the year.

This finding suggests that FIB DNA persistence in these shallow anaerobic aquifers is longer than cultured *E. coli*. This agrees with findings in Knappett et al. (2012) where *mE. coli* and Bacteroidales were detected well above detection limit in fine sediments adjacent to latrine ponds in the absence of cultured *E. coli*. Even in sediments containing abundant cultured *E. coli, E. coli* and Bacteroidales DNA was observed to be transported further laterally and especially vertically (Knappett et al. 2012). Another study found widespread high concentrations of FIB DNA in private wells across several villages and seasons (Ferguson et al. 2012). It’s likely that the reduced metabolism and size of starving fecal bacteria in oligotrophic aquifers allows them to pass much further than readily culturable *E. coli*. This was found in a study by Jansen et al. (2010) in sand columns with *Pseudomonas fluorescens* where the starved, coccoid shaped bacteria were less efficiently removed than metabolically active rod shaped bacteria. This phenomenon has important implications for understanding the perceived threat of FIB and pathogen DNA in groundwater. Traditionally cultured *E. coli* has been relied upon to indicate the relative health risk from drinking water

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

(WHO 2008). Fecal bacteria DNA, contained in dead or starving cells, however, may be transported further and persist longer in an aquifer than the more metabolically active culturable cells. Therefore, the relative health risk represented by different concentrations of FIB and pathogen DNA occurring in groundwater (Ferguson et al. 2012) is currently unknown and needs to be assessed as it has been for recreational water exposure (Wade et al. 2008).

One advantage of enumerating FIB DNA over cultured *E. coli* is that it is present in high concentrations year round at multiple field sites (Fig. 4) (Ferguson et al. 2012). When concentrations of *mE. coli* and Bacteroidales from the dry and wet season snap shot sampling events are plotted against each other for 14 overlapping wells, Bacteroidales emerges as more consistent year round, whereas *mE. coli* concentrations are uncorrelated in the same wells in different seasons (Fig. 5a). Furthermore, wet season Bacteroidales were the only FIB (and the only season) found to be somewhat predictive (R^2 =0.33, $p<0.05$) of annual *E. coli* detection frequency (Fig. 5b). Bacteroidales may be a more seasonally unbiased estimate of year round susceptibility of a groundwater well to fecal contamination than either *mE. coli* or the sporadically detected cultured *E. coli*. Sampling at one or two points in time, rather than frequent monitoring over years, may save substantial labor.

Although correlated with each other during the wet season snap shot, no combination of FIB's was found to be predictive of pathogen presence/absence (Fig. 6a,b), with 10/22 samples found to be positive for at least one pathogen. There were several false negatives with respect to the indicators, with pathogens being found in water samples containing low concentrations of *E. coli, mE. coli* and Bacteroidales. Annual *E. coli* detection frequency did not predict the wet season presence of pathogens (Fig. 5b), either. In contrast, although only 2/20 dry season snap shot wells were positive for pathogens, both of these wells were highly contaminated with *mE. coli* and Bacteroidales (cultured *E. coli* was not measured simultaneously during the dry season snap shot) (Fig. 6c). It is noteworthy that pathogens weredetected in approximately equal numbers of both unsealed (P) and sealed (MS) wells, reinforcing that although well construction effects lead to increased contamination of the tubewells themselves, fecal bacteria and pathogen DNA are clearly infiltrating the broader aquifer beyond the near-well environment (Knappett et al., 2012).

Conclusion

Multiple differences, including annular sealing and usage frequency, between private wells and monitoring wells led to more frequent detections of *E. coli* in unsealed tubewells. Clearly some simple well construction improvements can be made that will lead to decreases in FIB detections and cases of diarrheal disease obtained by drinking untreated groundwater (Gundry et al. 2004). In a related study, hand pumps themselves were found to harbor *E. coli* long after being exposed to high levels of *E. coli* (Ferguson et al. 2011). This effect could have accounted for some of the more frequent contamination seen here in private wells. Amongst unsealed private wells, however, platform presence/absence had no impact on microbial water quality. This finding could possibly be due to the lack of adequate drainage channels accompanying intact platforms, however, this lack of sensitivity to platform presence and quality has been reported previously in Bangladesh (Luby et al. 2008; Leber et al. 2011; van Geen et al. 2011). Together these findings suggest that annular sealing or another private well construction factor may be much more important in determining microbial drinking water quality than platforms and drainage channels in Bangladesh.

The molecular FIB Bacteroidales sampled in 22 wells during the wet season was found to be predictive of year-round fecal contamination in both unsealed and sealed wells, as assessed by annual *E. coli* detection frequency and dry season Bacteroidales. Pathogens were more

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

frequently detected in the wet season, but their presence was uncorrelated to any FIB or well type. The high prevalence of *E. coli*, and high concentrations of FIB DNA, in sealed monitoring wells, especially during the early monsoon season, indicate that fecal contamination is indeed infiltrating and spreading over broad volumes of the aquifer. Infiltration pathways likely include both leaky tubewell annuli and infiltration from ponds (Knappett et al. 2012). Other factors potentially contributing to *E. coli* prevalence in tubewells in Bangladesh include the size distribution (Leber et al. 2011; Knappett et al. 2012) and mineralogy (Ryan et al. 1999; Flynn et al. 2004) of the overlying sediment, pore-water ionic strength and chemical composition (Fontes et al. 1991; Loveland et al. 1996), and the local spatial density of contamination sources (Knappett et al. 2011a; van Geen et al. 2011). Multi-season FIB monitoring could be conducted on both unsealed and sealed private wells with similar usage frequency to isolate for an effect of well sealing only apart from regular pumping and well head effects (Ferguson et al. 2011). Further, private wells could be tested for leaks in the PVC pipe to determine how prevalent this is and whether leaking well pipes can explain the fecal contamination patterns in the unsealed private wells.

Acknowledgments

This study was supported by grant 5 R01 TW008066 from the NIH/FIC Ecology of Infectious Disease program. Additional funding was provided by the Center for Environmental Biotechnology, the Institute for a Secure and Sustainable Environment at the University of Tennessee and the Marie Curie Training Network GOODWATER program. An anonymous reviewer greatly contributed to the final product. Thank you to V. Escamilla for carrying out a detailed survey of Char Para in June 2009; to K. Radloff for providing long term groundwater levels; and to Md. R. Huq for his assistance in the field.

References

Abbaszadegan M, LeChevallier M, Gerba CP. Occurrence of viruses in U.S. groundwaters. J. Am. Water Works Assoc. 2003; 95:107–120.

Bernhard AE, Field KG. Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. Appl. Environ. Microbiol. 2000; 66:1587–1594. 2000. [PubMed: 10742246]

Borchardt MA, Bradbury KR, Alexander EC Jr, Kolberg RJ, Alexander SC, Archer JR, Braatz LA, Forest BM, Green JA, Spencer SK. Norovirus Outbreak Caused by a New Septic System in a Dolomite Aquifer. Ground Water. 2011; 49:85–97. [PubMed: 20199588]

DeNovio NM, Saiers JE, Ryan JN. Colloid movement in unsaturated porous media: Recent advances and future directions. Vadose Zone J. 2004; 3:338–351.

Embrey, SS.; Runkle, DL. Microbial Quality of the Nation's Ground-Water Resources 1993–2004. National Water-Quality Assessment Program Principal Aquifers. Reston, Virginia: US Geol. Survey; 2006. US Geol. Survey Scientific Investigations Report 2006-5290

Escamilla V, Wagner B, Yunus M, Streatfield PK, van Geen A, Emch M. Effect of deep tubewell use on childhood diarrhoea in Bangladesh. Bull. WHO. 2011; 89:521–527. [PubMed: 21734766]

Escamilla V, Knappett P, Yunus M, Streatfield PK, Emch M. Influence of Latrine Proximity and Type on Tubewell Water Quality and Diarrheal Disease in Bangladesh. Ann. Assoc. Am. Geogr. 2013 In Press.

Ferguson AS, Layton AC, Mailloux BJ, Culligan PJ, Williams DE, Smartt AE, Sayler GS, Feighery J, McKay L, Knappett PSK, Alexandrova E, Arbit T, Emch M, Escamilla V, Ahmed KM, Alam Md J, Streatfield PK, Yunus Md, van Geen A. Comparison of Fecal Indicators with Pathogenic Rotavirus in Groundwater. Sci. Tot. Environ. 2012; 431:314–322.

Ferguson AS, Mailloux BJ, Ahmed KM, van Geen A, McKay LD, Culligan PJ. Hand-pumps as reservoirs for microbial contamination of well water. J. Water Health. 2011; 9:708–717. [PubMed: 22048430]

Flynn R, Hunkeler D, Guerin C, Burn C, Rossi P, Aragno M. Geochemical influences on H40/1 Bacteriophage inactivation in glaciofluvial sands. Environ. Geol. 2004; 45:504–517.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Fontes DE, Mills AL, Hornberger GM, Herman JS. Physical and Chemical Factors Influencing Transport of Microorganisms through Porous Media. Appl. Environ. Microbiol. 1991; 57:2473–2481. [PubMed: 1662933]

Gelman, A.; Hill, J. Data analysis using regression and multilevel/hierarchical models. Cambridge University Press; 2007.

Godfrey S, Timo F, Smith M. Microbiological risk assessment and management of shallow groundwater sources in Lichinga, Mozambique. Water Environ. J. 2006; 20:194–202.

Goodbred SL, Kuehl SA. Floodplain processes in the Bengal Basin and the storage of Ganges-Brahmaputra river sediment: an accretion study using 137Cs and 210Pb geochronology. Sediment. Geol. 1998; 121:239–258.

Gundry S, Wright J, Conroy R. A systematic review of the health outcomes related to household water quality in developing countries. J. Water Health. 2:1–13. [PubMed: 15384725]

Hurley MA, Roscoe ME. Automated Statistical-Analysis of Microbial Enumeration by Dilution Series. J. Appl. Bacteriol. 1983; 55:159–164.

Jansen S, Vereecken H, Klumpp E. On the role of metabolic activity on the transport and deposition of *Pseudomonas fluorescens* in saturated porous media. Water Res. 2010; 44:1288–1296. [PubMed: 20153499]

Johnson TB, McKay LD, Layton AC, Jones SW, Johnson GC, Cashdollar JL, Dahling DR, Villegas LF, Fout GS, Williams DE, Sayler G. Viruses and Bacteria in Karst and Fractured Rock Aquifers in East Tennessee, USA. Ground Water. 2011; 49:98–110. [PubMed: 20331750]

Kildare BJ, Leutenegger CM, McSwain BS, Bambic DG, Rajal VB, Wuertz S. 16S rRNA-based assays for quantitative detection of universal, human-, cow- and dog-specific fecal Bacteroidales: A Bayesian approach. Water Res. 2007; 41:3701–3715. [PubMed: 17644149]

Knappett PSK, Escamilla V, Layton A, McKay LD, Emch M, Williams DE, Huq Md R, Alam Md J, Farhana L, Mailloux BJ, Ferguson A, Sayler GS, Ahmed KM, van Geen A. The impact of sanitation on fecal bacteria and pathogens in village ponds in Bangladesh. Sci. Tot. Environ. 2011a; 409:3174–3182.

Knappett PSK, Layton A, McKay LD, Williams D, Mailloux BJ, Huq Md R, Alam Md J, Ahmed KM, Akita Y, Serre ML, Sayler GS, van Geen A. Efficacy of hollow-fiber ultrafiltration for microbial sampling in groundwater. Ground Water. 2011b; 49:1–124. [PubMed: 21039447]

Knappett PSK, McKay LD, Layton A, Williams DE, Alam Md J, Huq Md R, Mey J, Feighery JE, Culligan PJ, Mailloux BJ, Zhuang J, Escamilla V, Emch M, Perfect E, Sayler GS, Ahmed KM, van Geen A. Implications of Fecal Bacteria Input from Latrine-Polluted Ponds for Wells in Sandy Aquifers. Environ. Sci. & Technol. 2012; 46:1361–1371. [PubMed: 22191430]

Kozuskanich J, Novakowski KS, Anderson BC. Fecal Indicator Bacteria Variability in Samples Pumped from Monitoring Wells. Ground Water. 2011; 49:43–52. [PubMed: 20497242]

Layton A, McKay LD, Williams D, Garrett V, Gentry R, Sayler G. Development of *Bacteroides* 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. Appl. Environ. Microb. 2006; 72:4214–4224.

Leber J, Rahman MM, Ahmed KM, Mailloux BJ, van Geen A. Contrasting Influence of Geology on *E. coli* and Arsenic in Aquifers of Bangladesh. Ground Water. 2011; 49:111–123. [PubMed: 20236332]

Loveland JP, Ryan JN, Amy GL, Harvey RW. The reversibility of virus attachment to Mineral Surfaces. Colloid Surface A. 1996; 107:205–221.

Luby SP, Gupta SK, Sheikh MA, Johnston RB, Ram PK, Islam MS. Tubewell water quality and predictors of contamination in three flood-prone areas in Bangladesh. J. Appl. Microbiol. 2008; 105:1002–1008. [PubMed: 18422953]

Noble RT, Griffith JF, Blackwood AD, Fuhrman JA, Gregory JB, Hernandez X, Liang X, Bera AA, Schiff K. Multitiered approach using quantitative PCR to track sources of fecal pollution affecting Santa Monica bay, California. Appl. Environ. Microbiol. 2006; 72:1604–1612. [PubMed: 16461716]

Persson S, Olsen KEP, Scheutz F, Krogfelt KA, Gerner-Smidt P. A method for fast and simple detection of major diarrhoeagenic *Escherichia coli* in the routine diagnostic laboratory. Clin. Microbiol. Infec. 2007; 13:516–524. [PubMed: 17331124]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Pronk M, Goldscheider N, Zopfi J. Particle-Size Distribution As Indicator for Fecal Bacteria Contamination of Drinking Water from Karst Springs. Environ. Sci. Technol. 2007; 41:8400–8405. [PubMed: 18200870]

Rajal VB, McSwain BS, Thompson DE, Leutenegger CM, Kildare BJ, Wuertz S. Validation of hollow fiber ultrafiltration and real-time PCR using bacteriophage PP7 as surrogate for the quantification of viruses from water samples. Water Res. 2007; 41:1411–1422. [PubMed: 17313967]

Rudolph DL, Barry DAJ, Goss MJ. Contamination in Ontario farmstead domestic wells and its association with agriculture: 2. Results from multilevel monitoring well installations. J. Contam. Hydrol. 1998; 32:295–311.

Ryan JN, Elimelech M, Ard RA, Harvey RW, Johnson PR. Bacteriophage PRD1 and Silica Colloid Transport and Recovery in and Iron Oxide-Coated Sand Aquifer. Environ. Sci. Technol. 1999; 33:63–73.

Scott TM, Rose JB, Jenkins TM, Farrah SR, Lukasik J. Microbial source tracking: current methodology and future directions. Appl. Environ. Microbiol. 2002; 68:5796–5803. [PubMed: 12450798]

Streatfield, K.; Persson, LA.; Chowdhury, HR.; Saha, KK. Disease Patterns in Bangladesh: Present and Future Needs. Dhaka, Bangladesh: International Centre for Diarrhoeal Disease Research, Bangladesh; 2001.

Wade TJ, Calderon RL, Brenner KP, Sams E, Beach M, Haugland R, Wymer L, Dufour AP. High Sensitivity of Children to Swimming-Associated Gastrointestinal Illness. Epidemiology. 2008; 19:375–383. [PubMed: 18379427]

Weinman B, Goodbred SL Jr, Zheng Y, Aziz Z, Steckler M, van Geen A, Singhvi AK, Nagar YC. Contributions of floodplain stratigraphy and evolution to the spatial patterns of groundwater arsenic in Araihazar, Bangladesh. Bull. Geo. Soc. Am. 2008; 120:1567–1580.

World Health Organization. Global Water Supply and Sanitation Assessment 2000 Report. Geneva, Switzerland: World Health Organization; 2000. http://www.who.int/water_sanitation_health/monitoring/globalassess/en/

World Health Organization. Guidelines for Drinking-water Quality 3rd Ed. Incorporating the First and Second Addenda Volume 1, Recommendations. Geneva, Switzerland: World Health Organization; 2008. http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/index.html

World Health Organization & United Nations Children's Fund Joint Monitoring Programme. Geneva, Switzerland: World Health Organization & United Nations Children's Fund; 2012. http://www.wssinfo.org/data-estimates/table/

Wu J, van Geen A, Ahmed KM, Akita Y, Alam Md J, Culligan PJ, Escamilla V, Feighery J, Ferguson AS, Knappett P, Mailloux BJ, McKay LD, Serre ML, Streatfield PK, Yunus M, Emch M. Increase in diarrheal disease associated with arsenic mitigation in Bangladesh. PLoS One. 2011; 6:e29593. [PubMed: 22216326]

van Geen A, Ahmed KM, Akita Y, Alam MJ, Culligan PJ, Emch M, Escamilla V, Feighery J, Ferguson AS, Knappett P, Layton AC, Mailloux BJ, McKay LD, Mey JL, Serre ML, Streatfield PK, Wu J, Yunus Md. Fecal Contamination of Shallow Tubewells in Bangladesh Inversely Related to Arsenic. Environ. Sci. & Technol. 2011; 45:1199–1205.

van Geen A, Zheng Y, Versteeg R, Stute M, Horneman A, Dhar R, Steckler M, Gelman A, Small C, Ahsan H, Graziano JH, Hussain I, Ahmed KM. Spatial variability of arsenic in 6000 tubewells in a 25 km^2 area of Bangladesh. Water Resour. Res. 2003; 39:1140.

von Seidlein L, Kim DR, Ali M, Lee H, Wang X, Thiem VD, Canh DG, Chaicumpa W, Agtini MD, Hossain A, Bhutta ZA, Mason C, Sethabutr O, Talukder K, Nair GB, Deen JL, Kotloff K, Clemens J. A Multicentre Study of Shigella Diarrhoea in Six Asian Countries: Disease Burden, Clinical Manifestations, and Microbiology. PLoS Med. 2006; 3:e353. [PubMed: 16968124]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

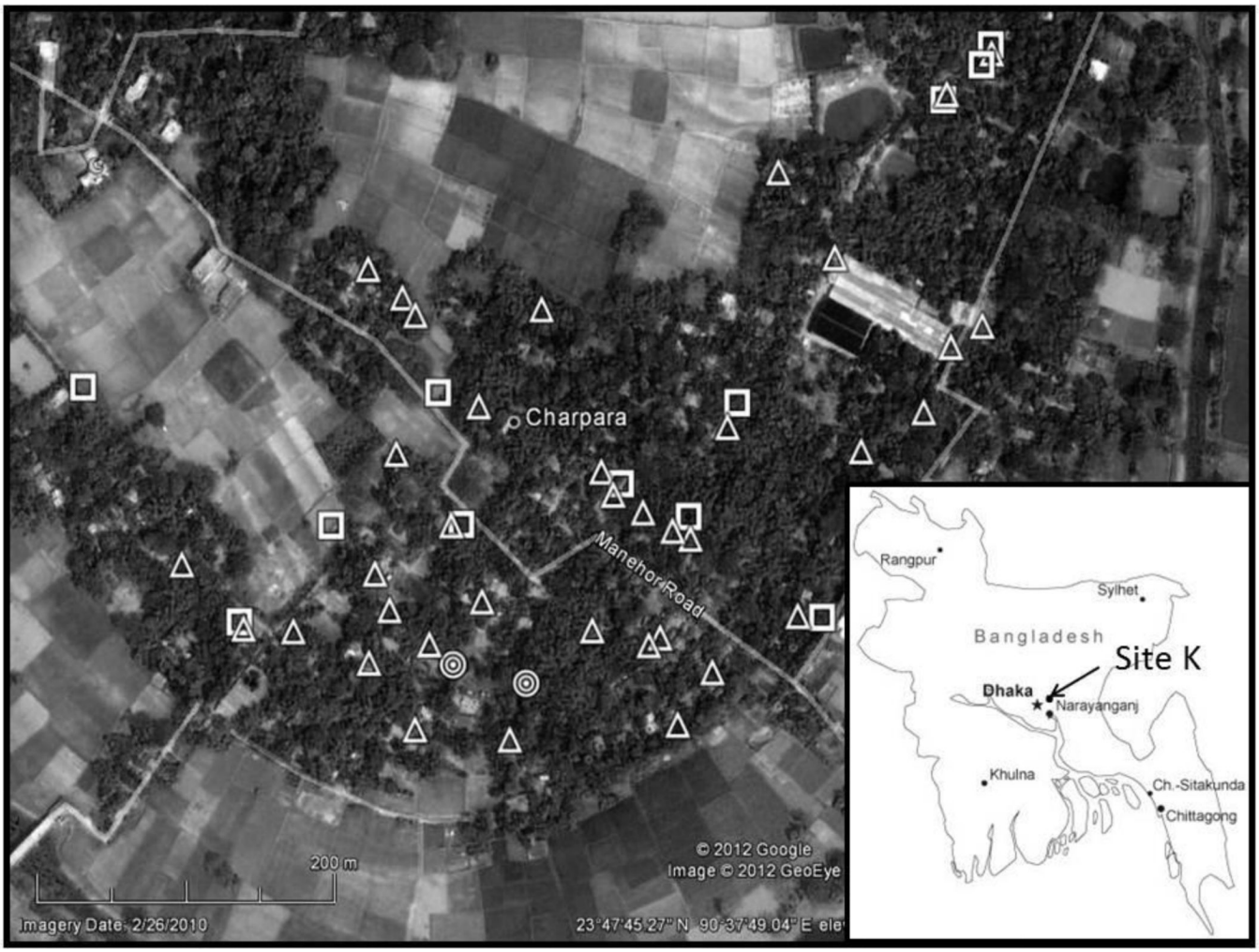

Figure 1.

Locations of 35 unsealed private tubewells (triangles), 6 unsealed (circles) and 11 sealed monitoring wells (squares) within Char Para (Site K). The 6 unsealed monitoring wells are contained within two multilevel piezometer nests. Image produced in Google Earth©. The inset country map is from www.mapresources.com. The scale bar in the bottom left corner represents 200 m.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

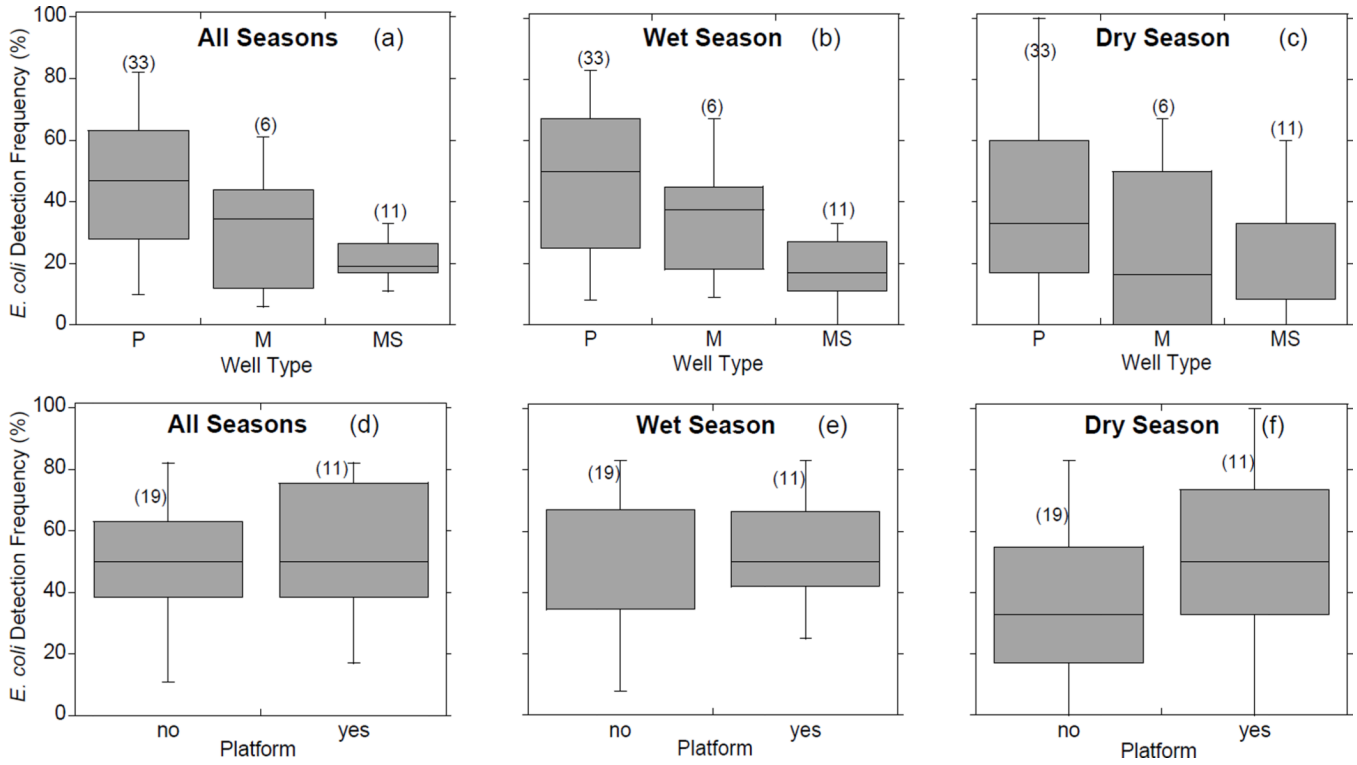

Figure 2.

Detection Frequencies of *E. coli* in monthly monitored private (P), unsealed monitoring (M), and sealed monitoring (MS) wells from April 2008 through November 2009. For the Well Type plots (a–c) the number of wells with at least five months of monthly data in each season were 33, 6 and 11 for P, M and MS respectively. There were a total of 12 possible wet season sampling events and 6 dry season months. In the Platform Presence plots (d–f), only private wells are presented here since no monitoring wells had cement platforms. A reduced number of private wells (n=30) was available due to missing information.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

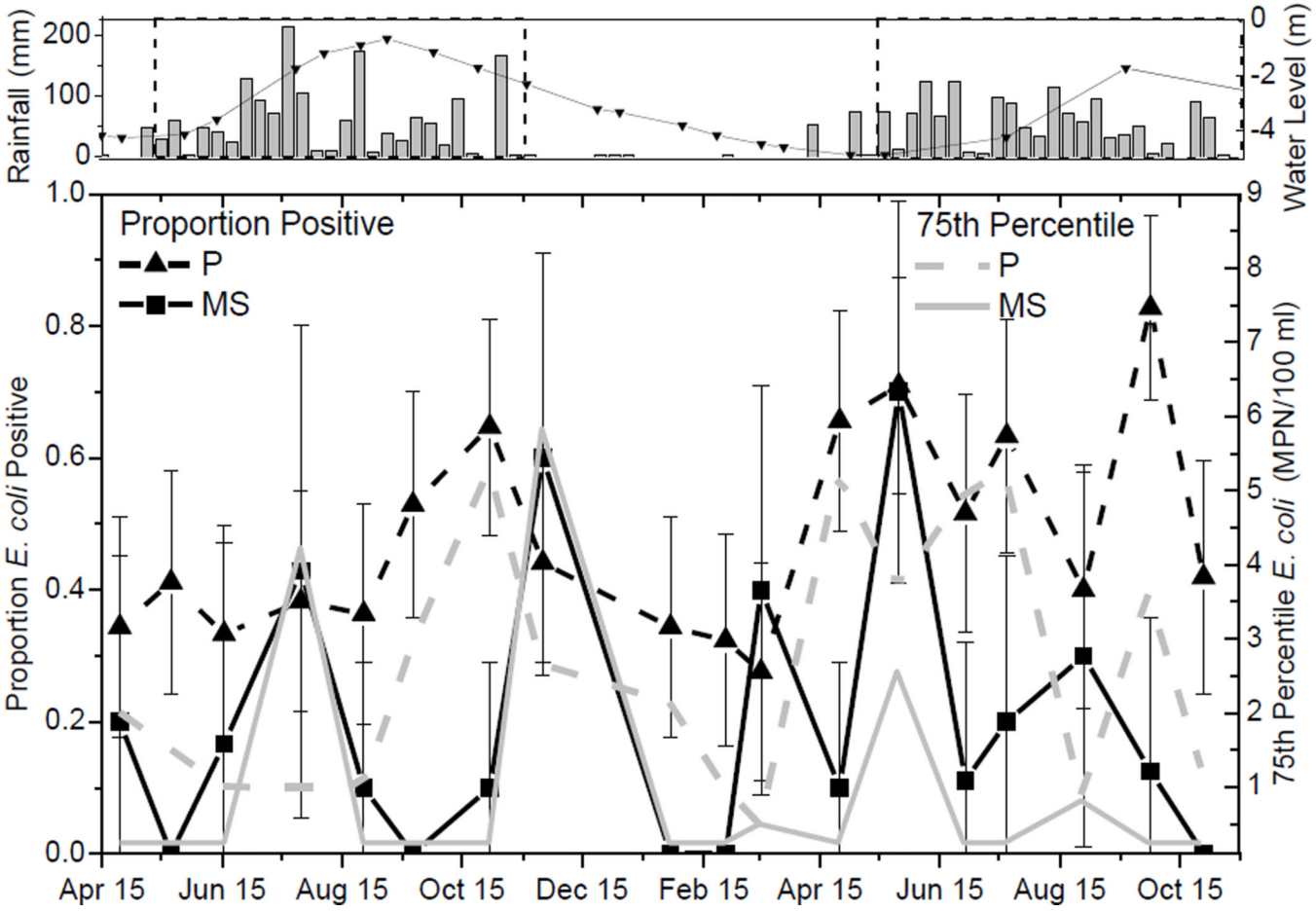

Figure 3.

Comparing cultured *E. coli* prevalence in wells with rainfall and water table levels. (a) Weekly precipitation (vertical grey bars) for Matlab located 50 Km south of Site K (left-axis). Manual groundwater levels are displayed at Site K (black line with grad symbols) from 04/01/08 through 11/1/09 (right-axis). Months assigned to the wet season are indicated by boxes outlined by dashed lines. (b) Monthly proportion of private (P) (n=35) and sealed monitoring (MS) (n=11) wells testing positive for cultured *E. coli* (left-axis). 75th percentile cultured *E. coli* concentrations (MPN/100 mL) for both P (dashed grey line) and MS (solid grey line) wells (right-axis).

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

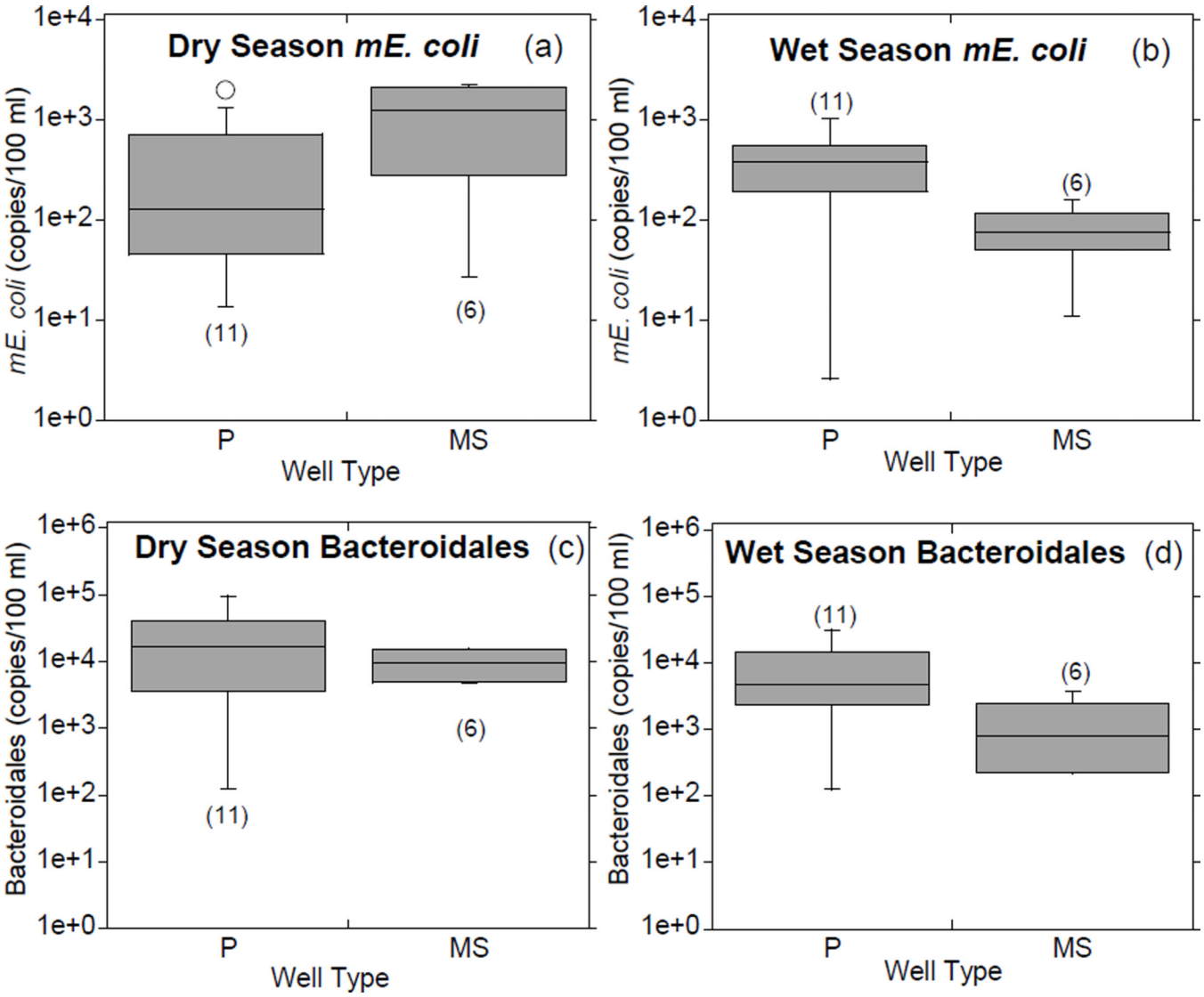

Figure 4.

Concentrations of *mE. coli* and Bacteroidales DNA in private (P) and sealed monitoring wells (MS) during dry season and wet season snap shot sampling events. Sample size for each group is indicated in parentheses. Unsealed monitoring wells (M) were not included in this analysis due to low sample numbers (n<5).

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

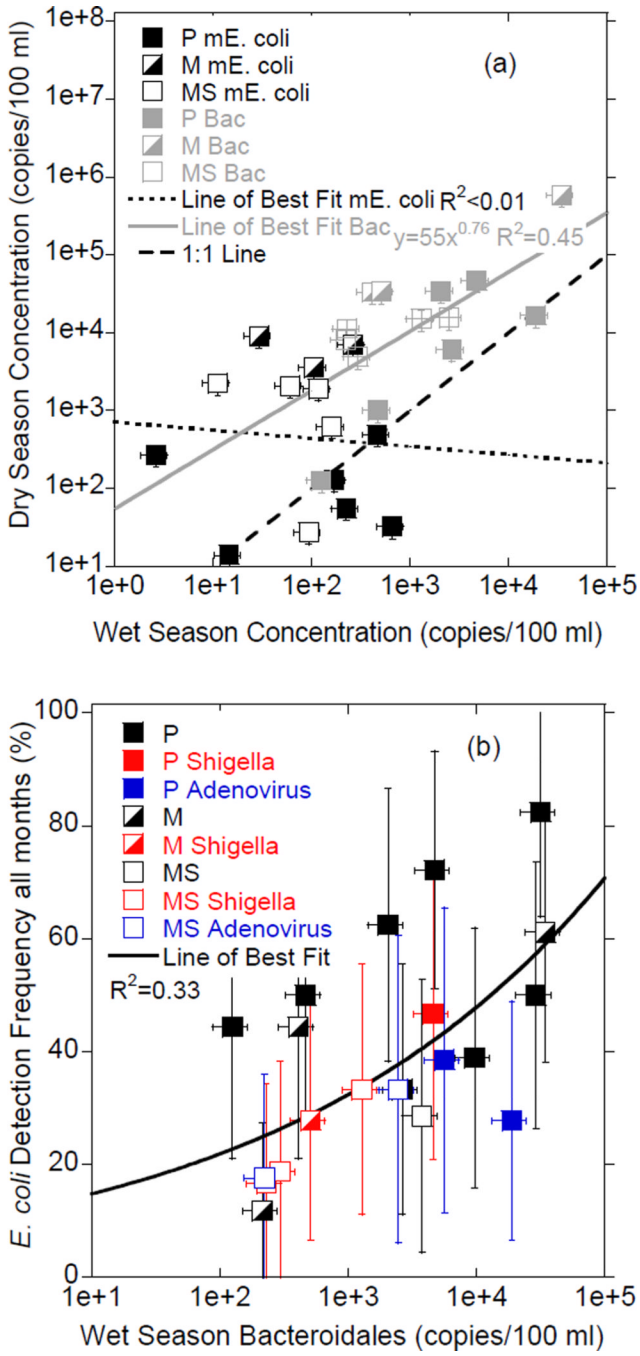

Figure 5.

Inter-seasonal comparisons of FIB and pathogens detected in unsealed private wells (P), unsealed monitoring wells (M) and sealed monitoring wells (MS). (a) Measured FIB marker gene concentrations in wells from during the wet and dry season snap shot sampling events (n=14). Only the equation for the line of best fit for Bacteroidales is displayed since the fit was very poor for *mE. coli*. (b) Wet season concentrations of Bacteroidales 16S genes and pathogen presence/absence plotted against cultured *E. coli* detection frequency for all months (n=22). Samples where no pathogens were detected are indicated by black symbols. Samples positive for *Shigella* and Adenovirus are indicated by red and blue, respectively.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

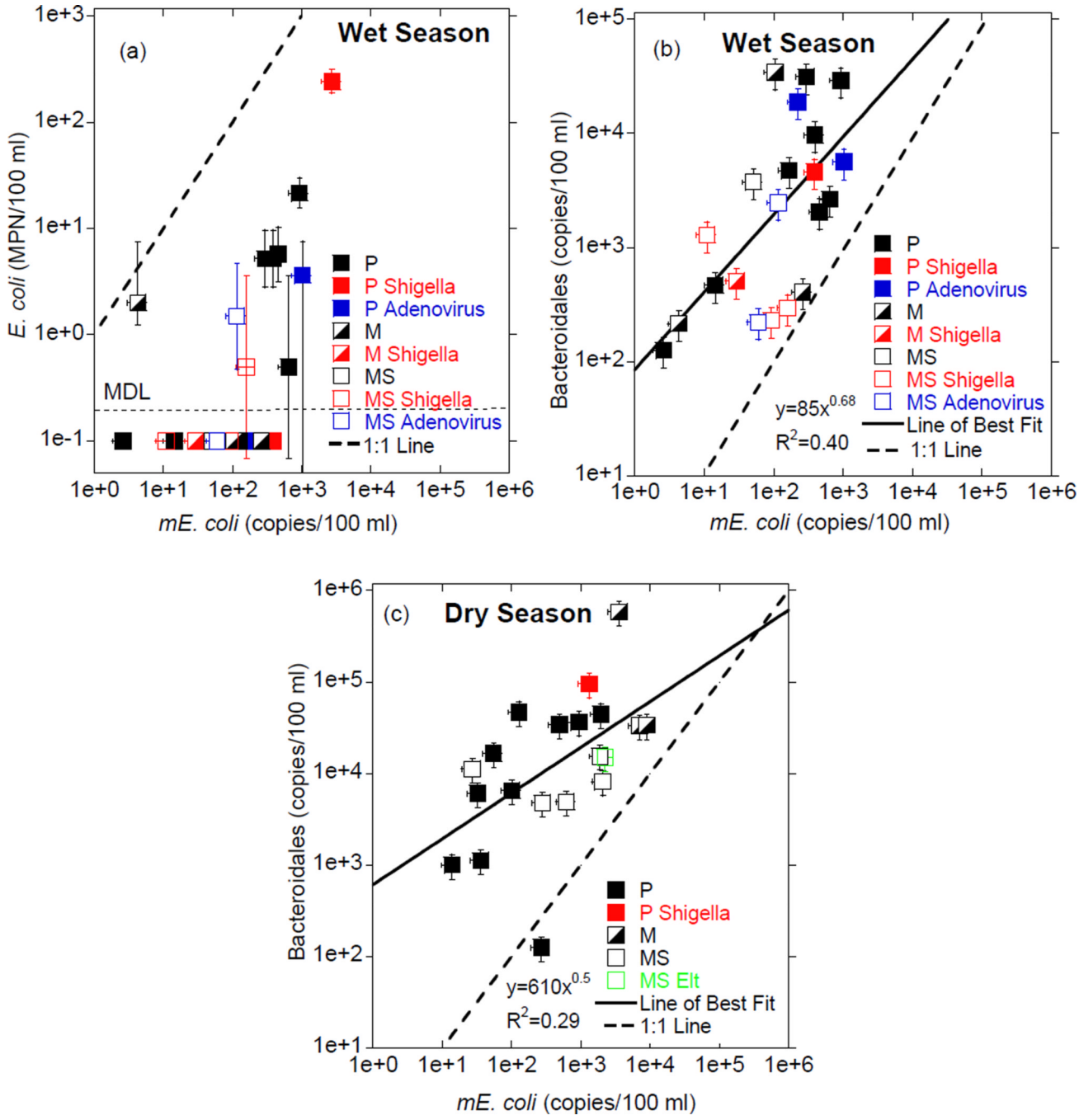

Figure 6.

Comparison of FIB concentrations and pathogen presence/absence during wet (n=22) and dry season (n=20) snap shot sampling events. (a) Comparison of synoptic measurements of cultured *E. coli* (MPN/100 mL) and *mE. coli* (copies/100 mL) from the wet season snap shot sampling event. The cultured *E. coli* method detection limit (MDL) was 0.5 MPN/100 mL. (b) Comparison of Bacteroidales and *mE. coli* concentrations during wet season snap shot. (c) Comparison of Bacteroidales and *mE. coli* during dry season snap shot. Cultured *E. coli* was not measured synoptically during the dry season snap shot sampling event. Samples where no pathogens were detected are indicated by black symbols. Samples positive for

Shigella, EltA (ETEC *E. coli*) and Adenovirus are indicated by red, green and blue, respectively.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1

Classification of tubewells at Site K.

Well Type (Notation)	Seal (y/n)	Pumping Frequency	Count
Private (P)	n	daily	35
Monitoring (M)	n	monthly	6
Monitoring (MS)	y	monthly	11
		Total	*52*

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 2

Quantitative PCR primer and probes used to target specific genes and organisms in tubewell water samples.

Target organism (Relevance)	**Assay Gene Target (Assay type and annealing temperature)**	**Oligonucleotide Sequences[a]**	**Reference**
EC23S (*E. coli*)	23S rRNA (Fluorogenic Probe, 55°C)	EC23Sf, 5’ GAG CCT GAA TCA GTG TGT GTG 3’ EC23Sr, 5’ ATT TTT GTG TAC GGG GCT GT 3’ EC23Srv1Taq, 5’ CGC CTT TCC AGA CGC TTC CAC	Knappett et al. 2011b
AllBac (all *Bacteroides*)	16S rRNA (Fluorogenic Probe, 60°C)	AllBac296f, 5’-GAGAGGAAGGTCCCCCAC-3’ AllBac412r, 5’-CGCTACTTGGCTGGTTCAG-3’ AllBac375Taq, 5’CCATTGACCAATATTCCTCACTGCTGCCT(BHQ-1)-3’	Layton et al. 2006
Shigella and EIEC *E. coli* (Dysentery type *E. coli* and *Shigella*)	*ipa*H (Fluorogenic Probe, 60°C)	*Ipa*H U1f- 5’ CCTTTTCCGCGTTCCTTG A-3’ *Ipa*H L1r- 5’- CGGAATCCGGAGGTATTG C-3’ *Ipa*H Taq- 5’-CGCCTTTCCGATACCGTCTCTGCA-3’	von Seidlein et al. 2006
ETEC *E. coli* (Enterotoxigenic *E. coli* strains)	*elt*A (Heat labile toxin LT) (Fluorogenic Probe, 60°C)	*Elt*311f- 5’ TCTGAATATAGCTCCGGCAGA-3’ *Elt*414r-5’ CAACCTTGTGGTGCATGATGA-3’ *Elt*383Taqr -5’ TTCTCTCCAAGCTTGGTGATCCGGT-3’	Modified from Persson et al. 2007
Adeno (adenovirus 40/41 hexon gene)	*Hexon* (Fluorogenic Probe, 60°C)	AV40/41-117f, 5’- CAGCCTGGGGAACAAGTTCAG 3’ AV40/41-258r, 5’ -CAGCGTAAAGCGCACTTTGTAA 3’ AV40/41-157Taq, 5’ ACCCACGATGTAACCACAGACAGGTC 3’	Rajal et al. 2007

[a] all probes synthesized with FAM (fluorescein) and black hole quencher 1 (BHQ1) from Biosearch Technologies.