# Determining the Impact of Sanitation via Detection of Pathogenic *E. coli* in Environmental Samples from Rural Bangladesh

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Diarrhea is the second leading cause of death among under-five children. Specifically, 800,000 children under-five die every year from diarrhea in developing countries such as Bangladesh.[1] Infectious diarrhea is caused by human enteric pathogens via the fecal-oral route as fecal material can be transmitted via consumption of contaminated food or water, exposure to polluted soil, and exposure to fecal-contaminated hands.[2] Five pathotypes of *E. coli* cause gastroenteritis, including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EAEC). Diarrhea is preventable via interventions such as improved sanitation, access to safe drinking water, and access to vaccinations.

The WASH Benefits study is a multi-institutional public health study led by researchers from the University of California, Berkeley, Stanford University, and the International Centre for Diarrhoeal Disease Research, Bangladesh, among others. The WASH Benefits study's primary objective is to measure the effects of health interventions, including improved water quality, improved sanitation, access to hand-washing, and access to nutrition, on child health and diarrheal incidence among children. A cluster-randomized control trial started in Mymensingh, Bangladesh in 2012 as part of the WASH Benefits study. Seven intervention arms including water quality, sanitation, nutrition, hand-washing, a combination of water quality, sanitation and hand-washing, and a combination of water, sanitation, hand-washing, and nutrition, were implemented. The majority of the Mymensingh population has access only to unimproved sanitation, which consists of either a pit latrine with no concrete slab or open defecation.[3] The trial enrolled pregnant women in order to study children under two because during the first two years of life, children born in low-income countries are at high risk for enteric infections. Half of participating households received sanitation treatment including a new or upgraded dual pit latrine with a water seal and super structure, a child potty, and a sani-scoop used to remove animal feces while the other half of participating households did not receive sanitation treatment.

Environmental samples collected from Mymensingh households include hand-rinse samples from children (N=360), hand-rinse samples from mothers (N=720), stored water samples (N=720), and soil samples (N=720). Upon sample collection, the IDEXX-Colilert test was used to simultaneously quantify total coliforms and *E. coli.* To investigate the presence of pathogenic *E. coli* (PEC) in samples, DNA was extracted from the cells from the positive wells of the IDEXX tray using the Qiagen DNeasy Blood and Tissue Kit. One extraction blank was prepared for each set of samples extracted. A multiplex polymerase chain reaction (PCR) assay was used to amplify extracted DNA. Gel electrophoresis was used to visualize results and detect pathogenic *E. coli*. Positive and no-template controls were run on each gel.

| Environmental Sample | WASH Benefits Group Assignment | Percent Positive for at least one *E. coli* pathotype |
|---|---|---|
| Soil | Control | 71% (N = 52) |
| | Sanitation | 76% (N = 55) |
| Mother Hands | Control | 51% (N = 57) |
| | Sanitation | 53% (N = 58) |
| Child Hands | Control | 71% (N = 51) |
| | Sanitation | 68% (N = 51) |
| Stored Water | Control | 53% (N = 62) |
| | Sanitation | 54% (N = 63) |

**Table 1.** *E. coli* pathotypes detected in various environmental samples in control group and sanitation arm of WASH Benefits study.

Flow cytometry was used to determine the lower-detection limit of the culture-PCR method used to detect pathogenic *E. coli* in environmental samples. To date, no studies have investigated the lower-detection limit of the IDEXX-Colilert test followed by DNA extraction and PCR for pathogenic *E. coli*. Various concentrations (1,000 cells of ETEC/mL-1 cell of ETEC/mL) were prepared via flow cytometry. Then, the IDEXX-Colilert test was conducted, followed by DNA extraction, PCR, and gel electrophoresis.

Results from the lower-detection limit experiment indicate that as few as 2.5 cells/mL ETEC can be detected using the culture-PCR method. However, each Bangladeshi environmental sample has numerous other bacteria present, which may compete for growth media nutrients and possibly increase the detection threshold.

Hands, soil, and water could all be key sources of exposure to pathogenic *E. coli*. Further analysis is needed to determine which source of exposure contributes the most to diarrheal disease transmission in children. The most important reservoirs of pathogens are the ones that children come in contact with most often. Overall, the WASH Benefits sanitation intervention seems to have a limited effect on the presence of pathogenic *E. coli* in hands, soil, and water.

Future work includes continuing to analyze remaining mother hand, child hand, soil, and stored water samples, determining if fecal contamination is from human or animal sources, and modeling which source of exposure children have most contact with.

References

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