SUPPLEMENTAL MATERIALS

METHODS:

Study design

This study was nested within the CHoBI7 Baby WASH mobile health program conducted in urban Dhaka, Bangladesh. Environmental sampling was conducted between October to December 2018 in 26 households with a child <5 years living in slum areas of Dhaka through convenience sampling. Field Research Assistants (FRAs) conducted unannounced spot checks of the household compound for collection of food, soil, surfaces, and hand rinse samples. Unannounced spot checks occurred between 8 AM and 5 PM. Soil samples were collected from child play spaces areas where caregivers reported young children most frequently play. Food samples were collected from the children food given or to be given. Surface samples were collected from objects or surfaces that caregiver's reported children < 5 years most frequently put into their mouth. Hand-rinse samples were collected from caregivers and children < 5 years. Informed consent was obtained from a parent or guardian of all study participants, and study procedures were approved by the research ethical review committee of International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) and the Johns Hopkins Bloomberg School of Public Health.

Sample collection transport and processing:

All environmental samples were transported to the icddr,b Molecular Ecology and Metagenomic laboratory in cool boxes (2-8 ºC) and processed within 6 hours of collection. After initial processing samples were analyzed using IDEXX Quanti-Tray with Colilert-18 media (IDEXX laboratories, INC., Westbrook, ME), incubated at 37^{o}C for 18 h to enumerate *E. coli* with the

Most probable number (MPN) based on the number of fluorescent and yellow cells (1, 2). A detailed description of our laboratory methods are published elsewhere (3). This method is approved by the United States Environmental Protection Agency (EPA) as a Standard Method for examination of water and wastewater (APHA, 2012) (4).

Data analysis

The Quanti-Tray 2000 system with a wide detection range of 1-2419 MPN per tray was selected to accommodate variability within sample types. We replaced the *E. coli* MPN values where no contamination was detected with 0.5 (half the lower detection limit). One lab blank was run during each day of sample testing. We calculated the presence/absence and geometric means of *E. coli.*

Food Sampling

Forty-nine food samples were collected using a sterile spoon and placed in a Whirl-Pak bag. In the laboratory, 15 g of each food sample and 150 ml of phosphate buffered saline (PBS) were placed in a Whirl-Pak Sterile Filter bag (Weber Scientific), which contains a perforated layer of polyethylene to retain the solids while allowing bacteria to be eluted into the liquid media. The sample was then placed in a BagMixer 400 S Homogenizer (Interscience, France) for one minute and then left to settle for 5 minutes. For sample analysis, 10 ml of the soft food supernatant was pipetted into a Whirl-Pak bag containing 90 ml of PBS. To collect the dry weight and moisture content of each sample, approximately 5 g of food (±0.25 g) was placed on an aluminium weigh boat (Fisher Scientific) and dried in a high temperature incubator at 100–110°C for 72 h. Each

sample's moisture content was determined and used to report bacterial counts as MPN/1 g of dry weight.

Soil sampling

Forty-six soil samples were collected from the surface (<1 cm deep) of an area approximately 100 cm^2 measured using a plastic stencil that was disinfected by ethanol prior to sample collection. A sterile spoon was used to collect approximately 25 g of soil in a Whirl-Pak bag. In the laboratory for sample processing, 20 g of soil was mixed with 100 ml of PBS to elute the bacteria. A Whirl-Pak bag containing the soil and PBS was homogenized by hand for one minute and then left to settle for 5 minutes. From the supernatant, 25 ml of the homogenized sample was pipetted into a Whirl-Pak bag containing 76 ml of PBS, and mixed, for a ¼ dilution. This sample was further diluted by pipetting 1 ml of the mixture into a second Whirl-Pak bag containing 99 ml of PBS for a 1/400 dilution. To normalize the data, the moisture content of the soil was measured using the same protocol as described for food sampling and reported as MPN/g of dry weight.

Hand rinse sampling

Ninety-one hand rinse samples were collected. For each hand-rinse sample, the caregiver or child was asked to place first one hand, and then the other, into the same 500 ml Whirl-Pak bag containing 350 ml of PBS. Each participant dipped his or her hand into the PBS solution for one minute, which included 30 s of shaking followed by 30 s of research staff massaging the

participant's hand through the Whirl-Pak bag. For analysis, 100 ml of the sample was processed. Bacterial counts were multiplied by 3.5 (to account for the initial water volume) and reported as MPN/both hands.

Surface/object sampling

Sixty-six surface/object sampling was conducted to measure bacterial counts on surfaces/objects children commonly put in their mouths. For each surface, a sterile swab was first moistened with sterile PBS and then used to swab a surface area of approximately 100 cm^2 in size, within a plastic stencil that was disinfected by ethanol. For objects, the entire surface was swabbed. The swab was placed in a Whirl-Pak bag for transport. For laboratory analysis, the swab was placed in a Whirl-Pak bag containing 100 ml of PBS. For 30 s, the swab was massaged through the bag to elute bacteria into the PBS solution matrix. The 100 ml sample was analyzed, undiluted, and bacterial counts were reported as MPN/100 cm^2 or object.

References

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