Fecal Sampling of Soil, Food, Hand, and Surface Samples from Households in Urban Slums of Dhaka, Bangladesh: An Evidence-Based Development of Baby Water, Sanitation, and Hygiene Interventions

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Abstract. The aim of this study was to identify the exposure pathways of fecal pathogens for a pediatric population living in the urban slums of Bangladesh. A total of 252 soil, food, surface, and hand rinse samples were collected from the pilot households with children less than 5 years of age. All samples were analyzed using the IDEXX Quanti-Tray System (Colilert-18) to enumerate fecal indicator bacteria *Escherichia coli*. *Escherichia coli* was detected in all soil samples collected from children play spaces (N = 46), 35% of objects and surfaces children frequently put in their mouths, and 31% of child food samples. Thirty-three percent of hand samples from the child and 46% of hand samples from the caregiver had detectable *E. coli*. These findings showed high fecal contamination of soil, food, and on hands and surfaces in households with young children and demonstrate the need for interventions reducing these exposure pathways for susceptible pediatric populations.

INTRODUCTION

Diarrhea is the second leading cause of death in young children globally, causing an estimated 500,000 deaths annually.¹ Previous studies have found that fomites, inanimate objects, and surfaces that are capable of transmitting microorganisms, are often vectors for the transmission of enteric pathogens to young children^{2,3} as the young children constantly put objects and surfaces in their mouths during the first 2 years of life during their exploratory development phase.⁴ Soil is a fomite that has been found to contain pathogenic E.coli in setting with high fecal contamination from the animal and human feces.⁵ Previous studies have also found E. coli on fomites such as child toys and plates, bottles, and utensils that often serve as toys for children in low resource settings.⁶ Previous studies have found that children mouthing soil and contaminated objects were associated with diarrhea and environmental enteropathy.^{7–9} However, most studies focus on rural areas and on food, water, and hands.¹⁰

Building on these previous studies, the objective of this study was to investigate multiple exposure pathways for fecal pathogens for young children in urban Dhaka, Bangladesh. This study was conducted to build evidence on exposure routes to fecal pathogens for the development of our recent CHoBI7 Baby WASH (water, sanitation, and hygiene) mobile health program in urban Dhaka, Bangladesh.¹¹

METHODS

Environmental sampling was conducted between October and December 2018 in 26 households with a child < 5 years living in slum areas of Dhaka through convenience sampling. Households were selected to be the representative of slum areas across Dhaka, Bangladesh, and were selected based on the locations where diarrhea patients were recruited in our recently completed randomized controlled trial of the CHoBI7 mHealth Program.¹² Field research assistants (FRAs) conducted unannounced spot checks of the household compound for the collection of food, soil, surfaces, and hand rinse samples. Unannounced spot checks occurred between 8 a.m. and 5 p.m. Soil samples were collected from the child play spaces areas where caregivers reported young children most frequently playing. Food samples were collected from the children's food given or to be given. Surface samples were collected from objects or surfaces that caregivers reported children < 5 years most frequently put into their mouths. Hand-rinse samples were collected from caregivers and children < 5 years. Multiple hand samples (obtained from multiple visits) were collected from 30 children in the enrolled households (N = 26) during months 1, 2, and 3 follow-up visits. Sixty-three samples were collected during these visits, because some households were absent during follow-up visits. Caregiver hand samples (N = 28) were collected throughout the 3-month study period on their availability during follow-up visits. Two samples were collected from one caregiver in months 1 and 3, and two samples from another in months 2 and 3. Therefore, we collected 28 hand rinse samples in total. 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 the International Center for Diarrheal Disease Research, Bangladesh (icddr,b), and the Johns Hopkins Bloomberg School of Public Health.

All the environmental samples were transported to the Molecular Ecology and Metagenomic laboratory, icddr,b, in cool boxes (2–8°C) 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°C for 18 hours to enumerate *E. coli* with the most probable number (MPN) based on the number of fluorescent and yellow cells.^{13,14}

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A detailed description of our laboratory methods is published elsewhere,⁶ we have also included a supplementary file with these methods. One laboratory blank was run during each day of sample testing. The Quanti-Tray 2000 system with a wide detection range of 1–2,419 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).

RESULTS AND DISCUSSION

A total of 30 children under 5 years were enrolled. The mean age of the children was 1 year \pm 0.8, (0.08–3) (mean \pm SD, range). Thirty percent of children (9/30) were female, and the average number of individuals living in a household was 4 ± 1 (2–8). The mean age of the caregiver was 27 years ± 7 (19–46) and 89% (23/26) of caregivers could read and write. Seventy-three percent of households (19/26) owned a television, 54% (14/26) a radio, and 35% (9/26) a refrigerator (Table 1). Eighty-one percent of households (21/26) had only one room for sleeping. Thirty-five percent of households (9/26) did not have soap presence in the bathroom during spot checks.

A total of 252 soil, food, surface, and hand rinse samples were collected. All soil samples (N = 46) had detectable *E. coli* with a geometric mean of 89,473 MPN *E. coli* per gram dry weight (Table 2). Thirty-five percent of surface samples had detectable *E. coli* (23/66). Balls (geometric mean: 181 *E. coli* MPN/100 cm²) and plastic bottles (geometric mean: 281 *E. coli* MPN/100 cm²) were the items with the highest *E. coli* concentrations. All baby bottles had detectable *E. coli* (N = 3). Other items with detectable *E. coli* included sticks, glasses, bowls, dolls, toy cars, and shoes. Thirty-one percent of food samples (15/49) had detectable *E. coli* per gram dry weight. Rice was the food with the high *E. coli*

TABLE 1 Baseline characteristics of study children

Criteria	Ν	%
Children $<$ 5 years of age	30	_
Baseline age (year)		
Mean \pm SD (Min–Max)	1.3 ± 0.78 (0.08, 2.75)	-
Gender		
% Female (children)	09	30
Caregiver	26	-
Age (year) Mean ± SD (Min–Max)	27.4 \pm 7.02 (19, 46)	-
Household roof type		
Tin	23	89
Other	3	12
Household floor type		
Concrete	24	92
Other	02	8
Household wall type	10	~~
Concrete	18	69
Tin	07	27
Caregiver literacy	23	89
Total household member mean ± SD (Min–Max)	4.2 ± 1.2 (2, 8)	-
One room for sleeping	21	81
Television	19	73
Refrigerator	9	35
Radio	14	54
No soap in bathroom	9	35
Household soap	26	100

concentration (geometric mean: 38 MPN E. coli/g dry weight). Other foods with detectable E. coli included khichuri, noodles, bananas, and suji (semolina). Forty-six percent of caregivers (13/28) had detectable E. coli on their hands (geometric mean: 88 E. coli MPN/100 mL rinse water for two hands), and 33% of children under 5 years of age (21/63) (geometric mean: 149 E. coli MPN/100 mL rinse water for two hands). To compare sample types, E. coli MPN were log-transformed and linear regression models were performed using general estimating equations (GEE) to account for clustering within households. Comparing the E. coli values among four different groups of samples, there was a significantly higher E. coli level in soil (estimated difference in log MPN: 4.4, P < 0.001; 95% CI: 3.73, 5.075) compared with food samples but no significant difference was found between objects and hand rinse E. coli level.

The *E. coli* concentrations were also compared between child and caregiver hand rinse samples. No significant differences were observed (estimated difference in log MPN: 0.1, P = 0.74, 95% Cl: -0.51, 0.72). There was also no significant difference in *E. coli* levels when comparing rice and other food samples (estimated difference in log MPN: -0.08, P = 0.89, 95% Cl: -1.16, 1.00) or between balls versus other objects (estimated difference in log MPN: -0.22, P = 0.74, 95% Cl: -1.35, 0.91).

In this study, we found fecal contamination along multiple environmental pathways in the urban slums of Dhaka, Bangladesh. Our results indicate the dominant exposure pathway of fecal contamination is soil and the caregiver's hand. The soil in the child's play spaces is an important contributor of fecal pathogens to young children. In an earlier study in rural Bangladesh, 97% of households had detectable *E.coli* in soil,⁵ and 69% of soil samples in a recent study in the Democratic Republic of the Congo (DRC).⁶ Contaminated soil in child play spaces is associated with elevated fecal markers of environmental enteropathy in children, diarrhea and linear growth faltering in young children.^{5,15} Despite this growing evidence, there are a handful of studies focused on reducing fecal exposure through mouthing of soil.^{11,16,17}

Child hand contamination has been associated with pediatric diarrhea in several studies in rural Bangladesh, DRC, Zimbabwe, and Tanzania.^{10,15,18,19} Consistent with our findings, a high proportion of child hand rinse samples were found to have fecal contamination in rural Bangladesh (43%).⁸ As young children have frequent hand-to-mouth and hand-to-fomite contacts, their hands represent an important pathway for fecal contamination^{7,9}. Caregiver hand hygiene is also important for gut health of young children. In an earlier study in rural Bangladesh, caregiver's hands having visible dirt were associated with the fecal markers of environmental enteropathy.²⁰ Future research is needed in urban settings such as slum areas of Dhaka, Bangladesh, to investigate the association between child and caregiver hand contamination and diarrheal diseases in young children.

Thirty-five percent of fomites and food samples had fecal contamination found in the current study. This is consistent with previous studies. In Peru, 43% of dinner plates had *E. coli*² and in Zimbabwe 23% of child cups and spoons had *E. coli*.²¹ In DRC, 44% of balls, toys, and kitchen utensils had *E. coli* in households of young children.⁶ In Bangladesh and DRC, 58% and 54% of child food, respectively, had *E. coli*.^{6,8} There are limited interventions targeting reducing

TABLE 2
Escherichia coli concentration in food, soil, surface, and hand rinse samples ($N = 252$).

			% Of E. coli					
	N	n	Positive	Geometric Mean	Median	SD	Min	Max
All soil samples <i>E. coli</i> (MPN/g dry weight)	46	46	100%	89,473	22,235	124,692	111	540,537
All food samples E. coli (MPN/g dry weight)	49	17	35%	29	0	170	0	1,186
Rice	37	10	27%	38	0	195	0	1,186
Khichuri	4	2	50%	0.5	0.4	0.67	0	1.36
Porridge	3	0	0	-	-	-	-	-
Mixed food	2	0	0	-	-	-	-	-
Noodles	1	1	1	1.2	1.2	-	1.2	1.2
Suji	1	1	100%	17	16.85	-	17	17
Bananas	1	1	100%	8	8	-	8	8
All object/surface samples <i>E. coli</i> (MPN per object/100 cm ² surface area)	66	23	35%	92	0	432	0	2,500
Ball	14	6	43%	181	0	667	0	2,500
Doll	4	1	25%	2.15	Ō	4.3	Ō	8.6
Car	14	1	7%	0.14	Ō	0.53	Ō	2
Spoon	1	0	0	_	_	_	_	_
Plate	1	0	0	_	_	-	_	_
Bowl	4	2	50%	51	1	100	0	201.4
Glass	1	1	100%	206.4	206	_	206.4	206.4
Fish toy	1	0	0	_	_	-	_	_
Mobile	4	0	0	-	-	-	-	-
Plastic item	10	4	40%	281	0	785	0	2,500
Stick	1	1	100%	1	1	-	1	[′] 1
Baby feeder bottle	3	3	100%	17	4.1	25	1	46
Muditem	1	1	100%	52	52	-	52	52
Other	7	3	43%	25	0	62	0	166.4
All hand rinse samples <i>E. coli</i> (MPN/100 mL rinse water for both Hands)	91	34	37%	130	0	941	0	8,750
Caregivers	28	13	46%	88	0	405	0	2,146
Children < 5 years	63	21	33%	149	0	1101	0	8,750

MPN = most probable number. 2,500 MPN E. coli was the detection limit for water, food, surface, and object samples.

fecal pathogens on objects and surfaces children frequently put in their mouths.^{11,16} This is an area where further research is needed.

The laboratory findings from this study build the evidence for development of the CHoBI7 Baby WASH mobile health (mHealth) program.¹¹ This study is giving us a snapshot about the household environment and generated primary data on fecal pathogen transmission for planning and developing an intervention. We took an evidence-based and theory-driven approach to develop this intervention using the Integrated Behavioral Model for Water, Sanitation, and Hygiene.^{11,22} The CHoBI7 Baby WASH mHealth program targets food hygiene through promotion safe food storage and caregiver hand hygiene, child mouthing of soil, hands, and contaminated surfaces through promotion of frequent cleaning of objects and surfaces children frequently come into contact with and child hands and use of a play mat for young children, and child safe feces disposal to reduce fecal contamination in households with young children in slum areas of Bangladesh. Formative research activities conducted to develop this intervention included 31 semi-structured interviews, 5 group discussions, 6 mHealth workshops, and a 3-phase iterative pilot study among 102 households. High user acceptability and uptake of the intervention was found during our pilot study of CHoBI7 Baby WASH mHealth program.

This study has some limitations. First, our small sample size that prevents us from investigating the association between diarrhea and the presence of *E. coli* in the environment. Second, we tested only *E. coli* as fecal indicator bacteria and did not quantify viral and protozoan pathogens

responsible for pediatric diarrhea or identify whether *E. coli* strains were pathogenic. This study also had strengths. First is the inclusion of object and surface samples, which is currently a severely understudied area in the WASH field. Second is our urban study setting, most previous studies investigating transmission routes for fecal pathogens for young children have been in rural areas.

These findings demonstrate an evidence-informed approach to develop a WASH intervention targeting food hygiene, reducing child contact with contaminated fomites, and child hand hygiene.

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