

SUPPLEMENTAL INFORMATION

CHRISTIAN MEDICAL COLLEGE HYGIENE SURVEY<sup>28</sup>

Questions	Response	Scoring
1. Do they have a devoted container to store drinking water?	0-no; 1-yes	0-0; 1-1
2. Is the container covered?	0-no; 1-yes	0-0; 1-1
3. Is there a dipper dedicated for drinking water?	0-no; 1-yes; 2-dipper not used	(0,2)-0; 1-1
4. Is the drinking water boiled?*	0-no; 1-yes, for someone; 2- yes for everybody	0-0; (1,2)-1
5. Is the food kept covered?	0-no; 1-yes	0-0; 1-1
6. Are the fruits washed before eating?	0-no; 1-yes	0-0; 1-1
7. Cleaning the breast before feeding	0-no; 1-yes; 9-NA	(0,9)-0; 1-1
8. Are the nails cut properly/without dirt in between the nails?	0-no; 1-yes	0-0; 1-1
9. Washing the hands before feeding	0-no; 1-yes	0-0; 1-1
10. Feeding device washed properly	0-no; 1-yes, by soap; 2-yes, by water; 9- NA	(0,9)-0; 1-1; 2-2
11. Water available in the toilet*	0-no tap; 1-tap present	0-0; 1-1
12. Handwashing after going to toilet*	0-no; 1-water only; 2- water and soap	0-0; 1-1; 2-2
13. Periodicity of bathing*	0-not daily; 1-once daily; 2- twice daily	0-0; (1,2)-1
14. Bathing soon after going to toilet*	0-no; 1-yes	0-0; 1-1
15. Place of defecation of household*	1-household; 2-public; 3-field; 4-backyard/surroundings	(3,4)-0; (1,2)-1
16. Where does the child defecate?*	1-household; 2-public; 3-field; 4-backyard/surroundings	(3,4)-0; (1,2)-1

Total number of criteria = 16. All the category scores were added up to obtain a final hygiene score. Maximum score = 18. All questions carried 1 score for positive response except "feeding device washed properly" and "handwashing after going to toilet" score 1 for washing with "water alone" and 2 for washing with "water and soap." Bolded values represent the score assigned for a given question.

\*These questions were asked to the respondent whereas the others were observations.

STOOL SPECIMEN PROCESSING

Collected specimens were analyzed for bacteria, helminths, protozoa, and viruses. Bacteria were identified using a variety of methods. Culture-based methods were used for the isolation and identification of *Salmonella*, *Shigella*, *Aeromonas*, *Vibrio*, *Plesiomonas*, *Yersinia*, and *Escherichia coli*, and *E. coli* isolates were further identified as pathogenic *E. coli* using PCR-based assays that were designed to detect Shiga toxin-producing *E. coli*, enterotoxigenic *E. coli*, enteropathogenic *E. coli*, enteroinvasive *E. coli*, and entero-aggregative *E. coli* (EAEC).<sup>29</sup> *Campylobacter* spp. were also analyzed using polymerase chain reaction (PCR)-based methods.<sup>30</sup> Protozoa (*Entamoeba histolytica*, *Cryptosporidium*, and *Giardia* spp.) and viruses (adenovirus, astrovirus, and rotavirus) were evaluated by enzyme-linked immunosorbent assays, with the exception of norovirus, which was identified

by reverse transcription PCR. Other ova and parasites, including soil-transmitted helminths, were identified by morphology using a wet prep and modified acid-fast stain.<sup>29</sup>

QUANTITATIVE REAL-TIME PCR

Total nucleic acids were extracted using the Qiagen Xtractor system (Qiagen Sciences, Germantown, MD), following manufacturer's instructions. EAEC was detected using primers and probes targeting the *aatA* gene.<sup>31</sup> GI norovirus was detected using genogroup-specific COG1 primers and RING1-TP probe, while GII norovirus was detected using the genogroup-specific COG2 primers and RING2-TP probe.<sup>32</sup> The standard curve for EAEC was generated from a plasmid containing the *aatA* gene. The standard curve for GI and GII noroviruses was generated from in vitro transcribed RNA.<sup>33</sup>

SUPPLEMENTAL TABLE 1

Analysis of *Escherichia coli* concentrations in child hand rinses by household- and cluster-level sanitation and FSM\*

	$\beta$ †	Standard error	P value
Household-level			
Household toilet	0.04	0.40	0.92
Toilet contains excreta onsite‡	0.04	0.41	0.92
Toilet discharges to drain‡	0.56	0.41	0.17
Open defecation (< 5-year-old)	0.52	0.39	0.19
Open defecation (adult)	0.34	0.43	0.44
Any public toilet use (adult)	-0.04	0.31	0.91
High public toilet use (> 10 times per month, adult)	-0.01	0.48	0.99
Most likely clusters			
High household toilet coverage	-0.44	0.44	0.32
Low household toilet coverage	-0.13	0.32	0.69
High coverage of poor FSM	0.05	0.51	0.93

\*Multivariate models are presented for each sanitation variable, adjusting for neighborhood and hygiene status ("poor" or "good", as discussed previously), and are separated by a blank row in the table. In all models, interaction terms between the sanitation variable and neighborhood were tested and are indicated with a "/" if significant at  $\alpha = 0.10$ , and  $N = 50$  samples.

†Estimates are in  $\log_{10}$  colony-forming units (CFU)/pair of hands.

‡Reference population is households without a toilet or those with "other" FSM practices.

SUPPLEMENTAL TABLE 2

Analysis of *Escherichia coli* concentrations in household swabs by household- and cluster-level sanitation and FSM\*

	$\beta$ †	Standard error	P value
Household level			
Household toilet	-0.14	0.49	0.78
Household toilet/Old Town	1.15	0.59	0.06
Toilet contains excreta onsite‡	0.33	0.38	0.39
Toilet discharges to drain‡	0.57	0.38	0.15
Open defecation (< 5-year-old)	0.24	0.37	0.52
Open defecation (adult)	0.10	0.49	0.84
Open defecation (adult)/neighborhood: Old Town	-1.15	0.59	0.06
Any public toilet use (adult)	0.41	0.29	0.16
High public toilet use (> 10 times per month, adult)	0.52	0.45	0.25
Most likely clusters			
High household toilet coverage	-0.27	0.42	0.52
Low household toilet coverage	0.43	0.39	0.27
Low cluster of household toilet coverage/Old Town	-1.38	0.56	0.02
High coverage of poor FSM	0.42	0.48	0.39

FSM = fecal sludge management.

\*Multivariate models are presented for each sanitation variable, adjusting for neighborhood and hygiene status ("poor" or "good", as discussed previously), and are separated by a blank row in the table. In all models, interaction terms between the sanitation variable and neighborhood were tested and are indicated with a "/" if significant at  $\alpha = 0.10$ , and  $N = 50$  samples.

†Estimates are in  $\log_{10}$  colony-forming units (CFU)/125  $\text{cm}^2$ .

‡Reference population is households without a toilet or those with "other" FSM practices.

SUPPLEMENTAL TABLE 3

Analysis of *Escherichia coli* concentrations in sentinel object rinses by household- and cluster-level sanitation and FSM\*

	$\beta$ †	Standard error	P value
Household level			
Household toilet	0.06	0.30	0.83
Toilet contains excreta onsite‡	0.03	0.30	0.91
Toilet discharges to drain‡	0.16	0.30	0.60
Open defecation (< 5-year-old)	0.37	0.29	0.20
Open defecation (adult)	-0.14	0.32	0.66
Any public toilet use (adult)	-0.14	0.23	0.54
High public toilet use (> 10 times per month, adult)	-0.08	0.35	0.82
Cluster level			
High household toilet coverage	-0.08	0.33	0.80
Low household toilet coverage	-0.01	0.24	0.97
High coverage of poor FSM	-0.14	0.37	0.71

FSM = fecal sludge management.

\*Multivariate models are presented for each sanitation variable, adjusting for neighborhood and hygiene status ("poor" or "good", as discussed previously), and are separated by a blank row in the table. In all models, interaction terms between the sanitation variable and neighborhood were tested and are indicated with a "/" if significant at  $\alpha = 0.10$ , and  $N = 49$  samples.

†Estimates are in  $\log_{10}$  colony-forming units (CFU)/100 mL.

‡Reference population is households without a toilet or those with "other" FSM practices.