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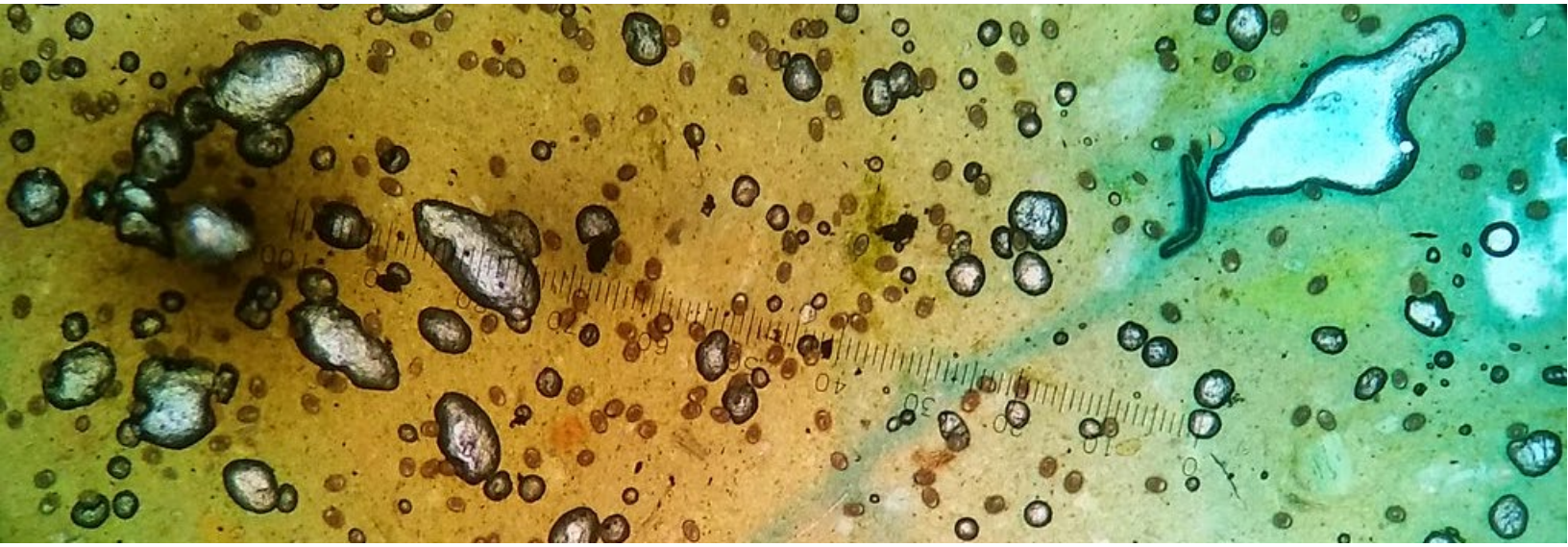

IMPACT EVALUATION OF THE CAMBODIA INTEGRATED NUTRITION, HYGIENE, AND SANITATION PROJECT

EVALUATION REPORT ADDENDUM

NOVEMBER 2021

This publication was produced for review by the United States Agency for International Development. It was prepared by Tetra Tech.

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COVER PHOTO: Detection of enteric pathogens in stool provides a direct indication of past exposures. This photo shows *Ascaris lumbricoides* ova in child stool. Ascariasis is a common sanitation-related infection. Credit: Trent Sumner.

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IMPACT EVALUATION OF THE CAMBODIA INTEGRATED NUTRITION, HYGIENE, AND SANITATION PROJECT

EVALUATION REPORT - ADDENDUM

November 2021

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ABSTRACT

This impact evaluation consists of a factorial randomized controlled trial to evaluate the impact of integrating sanitation and hygiene interventions along with nutrition programming on child growth in rural Cambodia. This addendum is supplemental to the Final Evaluation Report published in May 2020.

Following randomized assignment of communes (clusters) to intervention and control groups, a baseline survey, and intervention delivery, we enrolled 4,124 randomly selected children aged one to 28 months from the four study groups: (1) communes receiving a nutrition intervention to support caregivers to provide adequate nutrition for young children [n=817]; (2) communes receiving a sanitation intervention to encourage construction and use of latrines [n=792]; (3) communes receiving both the sanitation and the nutrition interventions [n=1,055]; and (4) control communes receiving no intervention [n=1,460]. Enrolled children were born after the delivery of interventions across the study area. The primary trial outcome was height-for-age z-score (HAZ), measured on a continuous scale. Secondary outcome measures included prevalence of caregiver-reported diarrhea and other growth measures: weight-for-height z-score (WHZ), weight-for-age z-score (WAZ), prevalence of stunting, all-cause mortality, and enteric infections measured in stool samples from children. Due to a delay in processing the stool samples, this addendum presents a separate discussion of enteric infections and prevalence of antimicrobial resistance genes in stool. All other outcomes were discussed in the Final Evaluation Report.

Neither the nutrition nor sanitation interventions (delivered independently or in combination) were shown to have a measurable impact on the prevalence, quantity, nor concentration of enteric pathogens and antimicrobial-resistant genes in child stools. These results are in accord with and supplemental to those laid out in the main report, namely: 1) meaningful gains in child growth were attributable to the nutrition intervention when delivered alone or in combination with sanitation programming; 2)the sanitation interventions did not significantly increase sanitation coverage over the strong secular trend in the control group and had no effect on child growth or diarrhea; and 3) we found no evidence that combining these sanitation and nutrition interventions resulted in increases in child growth over the nutrition programming alone.

ACRONYMS

AMR	Antimicrobial Resistance
aPR	Adjusted Prevalence Ratio
ARG	Antimicrobial Resistance Gene
aEPEC	Atypical Enteropathogenic *E.coli*
CHAMPS	Childhood Health and Mortality Prevention
COVID-19	Coronavirus Disease 2019
Cq	Quantification Cycle
E.Coli	Escherichia Coli
EAEC	Enteroaggregative *E.coli*
EIEC	Enteroinvasive *E.coli*
EPEC	Enteropathogenic *E.coli*
ESBL	Extended-spectrum ß-lactamase-producing
ETEC	Enterotoxigenic *E.coli*
GC	Gene Copies
HAZ	Height-for-age Z-score
IntI1	Integron-integrase
LOD	Limit-of-detection
LT	Heat-labile
PC	Positive Controls
PR	Prevalence Ratio
qPCR	Quantitative Polymerase Chain Reaction
RFS	Bureau for Resilience and Food Security
ST	Head-stable
STEC	Shiga-toxin Producing *E.coli*
STH	Soil-transmitted Helminths
TAC	TaqMan Array Card
tEPEC	Typical Enteropathogenic *E.coli*
WASH	Water, Sanitation, and Hygiene
WAZ	Weight-for-age Z-score
WHO	World Health Organization
WHZ	Weight-for-height Z-score
USAID	United States Agency for International Development

EXECUTIVE SUMMARY

The impact evaluation of the Cambodia Integrated Nutrition, Hygiene, and Sanitation NOURISH project was commissioned by the Center for Water Security, Sanitation and Hygiene in the United States Agency for International Development's Bureau for Resilience and Food Security (USAID/RFS). The evaluation incorporates a cluster randomized controlled trial (cRCT) with a factorial design to rigorously test the effectiveness of integrating sanitation programming with nutrition services to improve child linear growth and related child health outcomes, as well as whether this integrated approach is more effective than stand-alone nutrition or sanitation interventions.

The findings in the main Evaluation Report, published in May 2020, presented the full evaluation design and methodology and provided findings from household and anthropometry surveys. The nutrition interventions, when delivered alone or in combination with sanitation programming, improved child growth. The sanitation interventions did not significantly increase sanitation coverage over the strong secular (non-intervention) trend in the control group and had no effect on child growth or diarrhea. There is no evidence that combining these sanitation and nutrition interventions resulted in increases in child growth over the nutrition programming alone.

This addendum presents a separate discussion of enteric infections and prevalence of antimicrobial resistance genes (ARGs) in stool, which were not included in the Evaluation Report due to a delay in laboratory analysis associated with the Coronavirus Disease 2019 (COVID-19) pandemic. The evaluation team examined the impacts of the interventions on the prevalence of enteric infections (secondary measure) and antimicrobial resistance (AMR) in the target children. The team also examined risk factors related to enteric infection and AMR. Neither the nutrition nor sanitation interventions (delivered independently and in combination) were shown to have a measurable impact on the prevalence, quantity, nor concentration of enteric pathogens and antimicrobial-resistant genes in child stools. Small but measurable associations were found in the risk factor analysis: village-level sanitation coverage (measured as the percentage of households in the village with access to any sanitation facility), household-level sanitation, and finished floors were associated with lower prevalence of typical Enteropathogenic E.coli (tEPEC); and clean food preparation areas were associated with lower prevalence of *Shigella*/ Enteroinvasive *E.coli* (EIEC), consistent with the hypothesis that pathogen proliferation may be occurring via environmental pathways directly and indirectly related to sanitation facilities. Similarly, we found clean child play areas and clean food preparation surfaces to be associated with lower prevalence of colistin-related ARGs.

Overall, we detected high prevalence of enteric infection and ARG carriage across all treatment arms, suggesting that there were high levels of pathogen exposures during early age, despite interventions. Interruption of transmission will require more holistic and comprehensive interventions that include water, sanitation, and hygiene (WASH) improvements, as well as consideration of the living environment at both the household and community levels, which may include but are not limited to safely managed sanitation, drainage, separation of animals and animal feces, and hygiene. The findings of this study support a move to transformative WASH that can more effectively limit exposures in early childhood when the effects of enteric infections are greatest.

1.0 INTRODUCTION

This addendum to the evaluation report for the impact evaluation of the Cambodia Integrated Nutrition, Hygiene, and Sanitation NOURISH Project was commissioned by the Center for Water Security, Sanitation and Hygiene in the United States Agency for International Development's Bureau for Resilience and Food Security (USAID/RFS). It focuses on additional secondary outcomes not previously reported: enteric infections and antimicrobial resistance genes (ARGs), both measured in stool samples as proximal indicators of sanitation-related pathogen exposures. Annex A of the main report provides USAID's statement of work (SOW) for the evaluation.

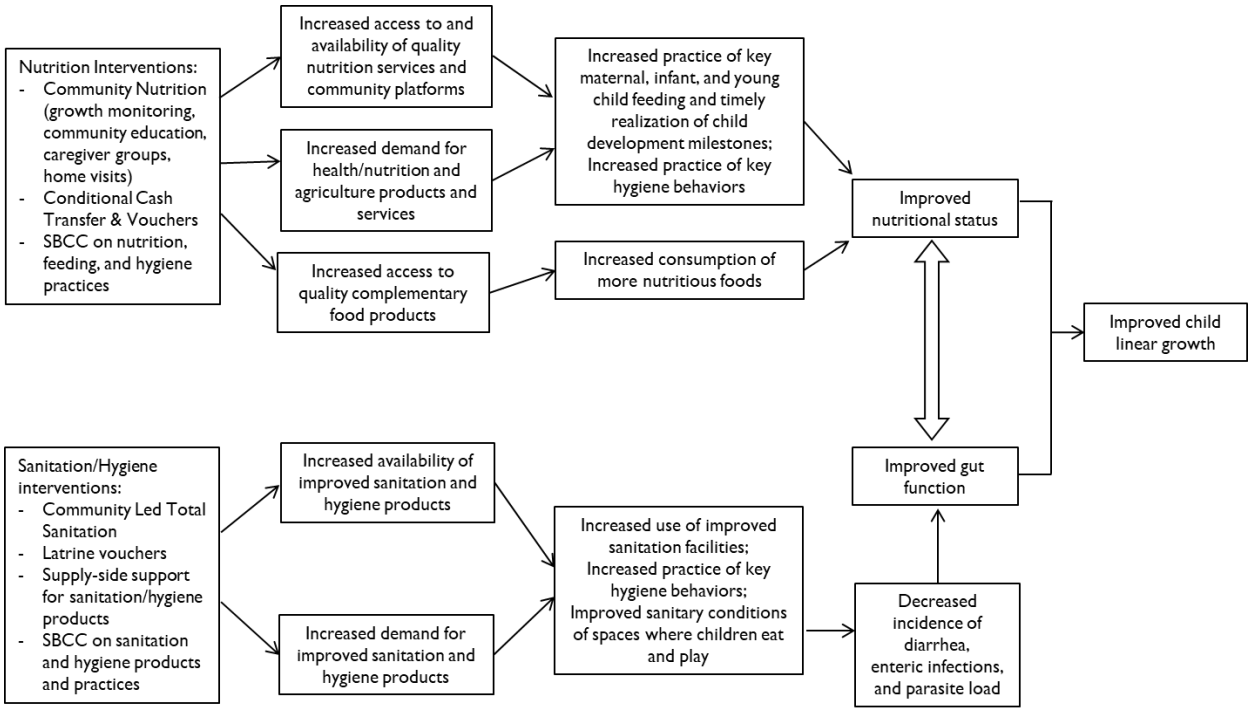
The NOURISH project promoted essential nutrition and sanitation behaviors with the aim of reducing stunting in children under two years old. The impact evaluation incorporated a cluster randomized controlled trial with a factorial design to test the effectiveness of integrating sanitation programming with nutrition services to improve child linear growth and related child health outcomes, as well as whether this integrated approach is more effective than stand-alone nutrition or sanitation interventions. The findings on the primary outcome (child linear growth) and related child health outcomes (stunting and other growth outcomes, self-reported diarrheal disease, and all-cause mortality) were presented in the Final Evaluation Report. The evaluation team also collected stool samples from children in the study area to assess the effects of the interventions on the prevalence of enteric infections and antibiotic resistance gene carriage. A supplemental risk factor analysis was performed to assess sanitiation and hygiene risk factors associated with carriage of enteric pathogen and antibiotic resistance genes. This report focuses on the findings for these secondary outcomes from the stool sample analyses and risk factor analysis.

2.0 NOURISH PROJECT BACKGROUND

Despite strong economic growth and rising living standards in the last two decades, high prevalence of undernutrition persists in Cambodia. Tackling childhood undernutrition requires a broad range of "nutrition specific" and "nutrition sensitive" interventions that act to ensure adequate dietary intake and address the multiple underlying or enabling determinants of child growth. However, when rigorously evaluated, interventions to improve dietary intake alone are not successful in reducing stunting. Acute and persistent infections associated with unsafe water, poor sanitation, and inadequate hygiene may impact gut health and therefore overall nutrition and growth. Thus, complementary water, sanitation, and hygiene (WASH) interventions aimed at reducing diarrheal disease and exposure to fecal bacteria may be an important means to securing optimal nutritional outcomes for children.

NOURISH promoted essential WASH and nutrition behaviors with the aim of reducing stunting in children under two years old and improving the nutritional status of mothers in the poorest households in rural areas. Save the Children implemented the project (in collaboration with two international organizations, SNV and the Manoff Group, as well as three local partners) in three provinces (Battambang, Pursat, and Siem Reap) from June 2014 to June 2020. NOURISH focused on women and children during the first 1,000 days, from the start of pregnancy until the child's second birthday. The WASH interventions consisted primarily of community-led total sanitation (CLTS), coupled with supply-side support for sanitation and hygiene products, and social and behavior change communication (SBCC). The nutrition interventions included complementary feeding activities and education through community-based growth promotion sessions, caregiver groups, and home visits, as well as conditional cash transfers (CCT) linked to the utilization of key health and nutrition services focusing on the first 1,000 days of a child's life. These interventions are the inputs of the project's theory of change laid out in Figure 1.

FIGURE 1: NOURISH THEORY OF CHANGE



3.0 EVALUATION PURPOSE, AUDIENCE, AND USES

This impact evaluation comes at an opportune time, as USAID strategies call for more integration of WASH and nutrition activities while also recognizing that additional research is needed to strengthen the evidence base for the nutrition linkages to WASH. While USAID and other actors in international development are exploring different approaches for integrating WASH and nutrition interventions on the basis of the emerging understanding of the link between enteric infections and nutrient uptake (Cumming & Cairncross, 2016),[1] limited evidence exists on the potential health impacts of combining improved WASH and nutrition interventions under real-world conditions. See Annex B of the Final Evaluation Report for a review of the existing evidence.

The primary audience for this evaluation is USAID, particularly the Center for Water Security, Sanitation, and Hygiene, as well as USAID/Cambodia, with secondary audiences in the Global Health Bureau and the Bureau for Resilience and Food Security. The evaluation also provides supporting evidence to the Government of Cambodia, given the overlap with its National Strategy for Food Security and Nutrition and its collaboration with NOURISH. Findings and lessons learned from this evaluation are also of interest to Save the Children, SNV, and other practitioners in these sectors who are seeking ways of accelerating health benefits by integrating cross-sectorial interventions. Finally, this impact evaluation serves the global audience by adding to the evidence base on the link between sanitation and undernutrition.

The findings from this impact evaluation will be used to further USAID's commitment to evidence-based programming in these sectors and will contribute to global knowledge on the nutrition and sanitation nexus.

[1] Cumming, O. & Cairncross, S. (2016). Can water, sanitation and hygiene help eliminate stunting? Current evidence and policy implications. *Maternal & Child Nutrition*, 12, no. S1.

4.0 EVALUATION QUESTIONS

Two groups of evaluation questions guide this evaluation. The first set of questions focuses on causality, or the attribution of detected effects to these specific nutrition and sanitation interventions on child growth outcomes. The second set of evaluation questions focuses on process, or whether project activities and the incentive schemes used by NOURISH resulted in the intended intermediate outcomes.

4.1 IMPACT QUESTIONS (CAUSAL LINKAGES)

USAID's central questions for this impact evaluation are:

1. Do nutrition interventions, as delivered at scale in the NOURISH program, lead to improved linear growth in children?
2. Does expanded access to sanitation, as delivered at scale in the NOURISH program, lead to improved linear growth in children?
3. Is the combined effect on linear growth in children of these sanitation and nutrition interventions delivered together greater than the effect of the two interventions delivered independently?

Rigorously examining these questions requires a "factorial design" whereby the intervention components can be assessed separately and together. Table 1 below describes the outcome measures for the evaluation.

4.2 PROJECT PROCESS QUESTIONS

Linked to the three impact questions are several subordinate questions that require the evaluation to look closely at the project implementation process (fidelity and uptake) and its intended results. These questions provide insights on the intermediate outcomes from the different causal pathways through which NOURISH aimed to increase child linear growth (see Figure 2). Given that the NOURISH interventions comprised multiple sanitation and nutrition components, these additional questions provide important insights into the relative contribution of these various components to improving child linear growth. The project process evaluation questions are:

1. Did sanitation interventions increase improved sanitation coverage and usage?
2. Did nutrition interventions increase uptake of nutrition and early childhood development services?
3. Did the nutrition and sanitation interventions change behavior related to nutrition, hygiene, and infant and young children feeding practices?
4. Did the sanitation interventions lead to more sanitary conditions of the home environment?

4.3 OUTCOME MEASURES

The outcome measures for the evaluation not only appropriately address the evaluation questions but also provide insights into the causal pathways through which children's health status can improve. The three impact questions are answered by collecting endline data on key health outcome measures, divided into primary and secondary outcomes. Including the secondary outcome measures adds explanatory value to the expected primary outcome (improved child linear growth). Table 1 shows outcome measures that are addressed in this addendum and in the Final Evaluation Report.

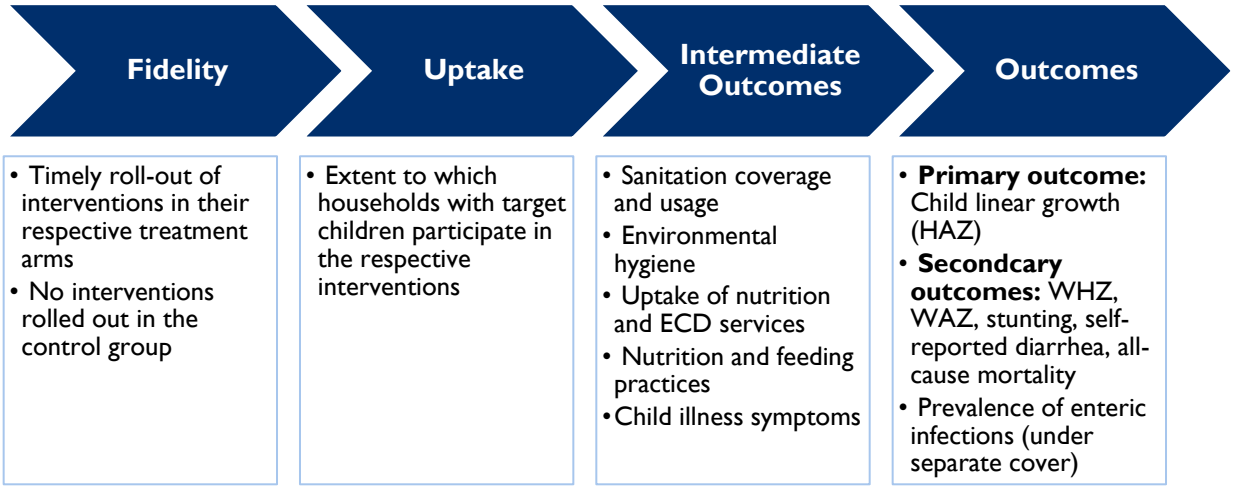
TABLE 1: KEY OUTCOME MEASURES

INDICATOR	MEASUREMENT	PRESENTED IN
Height-for-age z-score (HAZ)	Standardized measure of child's height for his/her age, as compared to the mean of the 2006 World Health Organization	Final Evaluation Report

INDICATOR	MEASUREMENT	PRESENTED IN
	(WHO) reference population. Mean of 0 and standard deviation of 1.	
Weight-for-height z-score (WHZ)	Standardized measure of child's weight for his/her height, as compared to the mean of the 2006 WHO reference population. Mean of 0 and standard deviation of 1.	Final Evaluation Report
Weight-for-age z-score (WAZ)	Composite index of HAZ and WHZ. Mean of 0 and standard deviation of 1.	Final Evaluation Report
Self-reported diarrheal disease	Caregiver reported child had diarrhea in seven days preceding the survey. Diarrhea is defined as three or more loose or liquid stools in a 24-hour period or any stool with blood. A visual aid card pointing to the two types of stool classified as diarrhea was used during each survey interview.	Final Evaluation Report
All-cause mortality	Caregiver reported child death from any cause.	Final Evaluation Report
Individual pathogen and ARG prevalence	Binary determination of presence or absence of pathogen, using known associated pathogen gene targets as a proxy for pathogen presence.	Addendum
Total pathogen and ARG prevalence	Mean difference in number of pathogen and antimicrobial resistance (AMR) genes detected.	Addendum
Individual pathogen concentration	Difference in quantitative estimation of pathogen concentration in child stool, measured by difference in quantitative cycle number (Cq) on PCR platform.	Addendum
Risk factors and enteric pathogen and ARG prevalence	Associations (measured in adjusted prevalence ratios [PRs]) of village-, household-, and child-level risk factors on individual pathogen and ARG prevalence (binary outcome).	Addendum

In addition, the evaluation included different aspects of intervention delivery (fidelity) and their use among the target population (uptake), as well as relevant intermediate outcomes which require changes in behavior. These measures provided insights into the causal mechanism through which change occurs and identified remaining barriers that need to be tackled to improve the implementation of integrated nutrition and sanitation interventions.

FIGURE 2: MEASURING IMPACT ALONG THE CAUSAL CHAIN



5.0 EVALUATION DESIGN

The NOURISH impact evaluation is based on the development hypothesis that integrated nutrition and sanitation interventions can lead to improved child linear growth that is greater than what is achieved when either intervention is delivered individually. In addition to the primary outcome (child linear growth) and secondary outcomes (stunting and other growth outcomes, self-reported diarrheal disease, and all-cause mortality), we also evaluated the impact of the interventions on the prevalence of enteric infections and ARGs in stool samples collected from randomly selected enrolled children, as proximal measures of exposure that are on the causal chain between WASH and distal health outcomes including growth and development.

We employed a cluster randomized controlled trial (cRCT) design layered on the NOURISH program, which offered interventions both as separate components and as an integrated program. The evaluation team randomly assigned 55 target communes to four groups—1) nutrition only, 2) sanitation only, 3) nutrition and sanitation, and 4) control—to allow the evaluation team to answer the evaluation questions. Randomization was applied at the commune level (clusters) to contain spillovers across villages and to prevent cross-group contamination. Please see the Final Evaluation Report for a full discussion on the evaluation design.

6.0 DATA COLLECTION

As part of the trial's primary analysis, the evaluation team conducted baseline data collection in September 2016, consisting of a survey of the primary caregivers of children under two years of age and anthropometric measures of their children in this age range. The NOURISH project then rolled out project activities in 36 communes over the course of two years, while the remaining 19 control communes stayed unexposed to the program. Endline measurement took place in August 2019, 28 months after the end of the roll-out period. During these 28 months, the evaluation team also collected implementation fidelity monitoring data to track the roll-out pace, uptake of core interventions, and intermediate outputs along the causal chain. Endline data collection consisted of surveys with the primary caregiver of children between one and 28 months, direct observation of certain household conditions, and anthropometry measures and stool samples from the children in this age range.

The evaluation team developed the survey questionnaire; the majority of questions are based on validated questions from the Cambodia Demographic and Health Survey (DHS) questionnaires. The team piloted and revised new questions added to the survey prior to the start of data collection. The study received approval from the National Ethics Committee for Health Research in the Cambodian Ministry of Health, Georgia Institute of Technology, and New England IRB. All instruments will be translated to Khmer and back translated for verification purposes. The endline sample size consisted of 4,015 households with at least one child aged one to 28 months and 4,124 total children in this age range. Please see the Final Evaluation Report for a more complete discussion of the household questionnaire and anthropometry survey data collection.

In total, 3,155 stool samples were collected from a subset of all enrolled children, chosen at random. At the end of the household survey, with consent from the caregiver, the enumerator left behind a sterile fecal collection container, instructing the primary caregiver to collect feces from the same evening or the following morning's defecation events. The container was labeled only with the child's unique ID number in order to locate the household for the pick-up of the stool sample containers the following day. Data was collected on electronic tablets and encrypted and uploaded to a password-protected server to which only study personnel had access. All personal identifying information was removed from the dataset for analysis. Specimens were stored, analyzed, and properly disposed of using international standardized operating procedures.

The field team picked up the filled container the following day. They mixed fecal specimens with a preservative, collected, and transported at room temperature. The study team assessed the capacity of several laboratories and determined that the analysis of stool samples could not be conducted in Cambodia. Following additional approval by the National Ethics Committee on Health Research in November 2019 and negotiation with shipping vendors, World Courier shipped the stool samples from Cambodia to the Brown Global WASH Lab in the School of Civil and Environmental Engineering at the Georgia Institute of Technology in the United States, where they were received by December 2019 and transferred to cold storage until they could be analyzed.

At the same time, the principal investigator confirmed that the originally proposed methodology for stool analysis would not be feasible[2] and developed several alternative options for analysis. In January 2020, the team decided to proceed with development of a new Taqman Array Card (TAC) to be manufactured by ThermoFisher, which was comparable technically and financially to the original

[2] The proposed supplier of TACs to be used for analysis indicated that they would not be publishing and, thus, not making public the detailed analytical methodology, thereby precluding publication in a peer-reviewed journal of any findings reliant on that methodology.

proposal. The evaluation team developed a custom TAC, which includes over 70 enteric pathogen and ARG targets, assembled from validated and published assays. These custom TACs were delivered to Georgia Tech in March 2020 and validated at the end of June. In response to the COVID-19 pandemic, Georgia Tech closed its campus and laboratory from March to mid-June 2020, when it partially reopened with limited capacity in mid-June. The laboratory resumed operation at full capacity in August.

7.0 METHODS

The following sections outline the methods used to process stool samples and the statistical analysis performed to generate results. The team utilized laboratory facilities at the Georgia Institute of Technology to perform nucleic acid extraction and analysis between June 2020 and June 2021. The team performed statistical analysis on laboratory results, in combination with data collected through household surveys, to comment on the impact of the NOURISH intervention and to assess risk factors for contraction of enteric pathogens.

7.1 NUCLEIC ACID EXTRACTION

We preserved stool samples 1:1 in Zymo DNA/RNA Shield buffer (Zymo Research, Irvine, CA) and stored them in -20C until used for extraction. Our extraction protocol was adapted from the xTAG Gastrointestinal Pathogen Panel (GPP; Luminex Molecular Diagnostics, Toronto, ON, Canada) protocol for pretreatment and the QIAamp 96 Virus QIAcube HT (Qiagen, Germany) protocol for remaining extraction procedure. Briefly, 200 mg solid (or 200 uL if liquid) stool was combined with 1,000 uL of stool lysis buffer (ASL, Qiagen, Germany) in an SK38 soil grinding tube (Bertin Corp., Rockville, MD), vortexed for five minutes (Vortex Genie 2, Scientific Industries, Bohemia, NY), incubated at room temperature for ten minutes, and centrifuged at 12,000 g for two minutes (Thermo Fisher Scientific, Waltham, MA). 200 uL of supernatant was used for automated DNA and RNA extraction following the QIAamp 96 Virus QIAcube HT protocol.

We tested preserved stools using a custom-developed TAC (ThermoFisher Scientific, Waltham, MA)—a compartmentalized probe-based quantitative polymerase chain reaction (qPCR) assay for enteropathogen genes and ARGs using individual assays validated on previously-published literature.[3]

qPCR cycling conditions were also adapted from previous work[4]. The team validated individual assays using synthetic nucleic acids (GeneArt, ThermoFisher Scientific) as positive controls (PCs). We combined PC material for each individual assay to a concentration of 10^7 gene copies (GC)/uL. We ran two serial dilutions on the custom TAC: a high-concentration 10-fold dilution series (10^7 GC/uL to 10^3 GC/uL) was used to determine range of the limit-of-detection (LOD) to order of magnitude; subsequently, a low-concentration two-fold dilution series (10^5 GC/uL to 1 GC/uL) diluted within the determined LOD range was used to estimate the delta-Rn threshold for each assay's upper LOD.

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[3] Diaz, M. H., Waller, J. L., Theodore, M. J., Patel, N., Wolff, B. J., Benitez, A. J., Morris, T., Raghunathan, P. L., Breiman, R. F., Whitney, C. G., Blau, D. M., & Winchell, J. M. (2019). Development and implementation of multiplex Taqman Array cards for specimen testing at Child Health and Mortality Prevention Surveillance Site Laboratories. *Clinical Infectious Diseases*, 69(Supplement_4).

Liu, J., Gratz, J., Amour, C., Nshama, R., Walongo, T., Maro, A., Mduma, E., Platts-Mills, J., Boisen, N., Nataro, J., Haverstick, D. M., Kabir, F., Lertsethtakarn, P., Silapong, S., Jeamwattanalert, P., Bodhidatta, L., Mason, C., Begum, S., Haque, R., … Houpt, E. R. (2016). Optimization of quantitative PCR methods for enteropathogen detection. *PLOS ONE*, *11*(6).

[4] Pholwat, S., Liu, J., Taniuchi, M., Chinli, R., Pongpan, T., Thaipisutikul, I., Ratanakorn, P., Platts-Mills, J. A., Fleece, M., Stroup, S., Gratz, J., Mduma, E., Mujaga, B., Walongo, T., Nshama, R., Kimathi, C., Foongladda, S., & Houpt, E. R. (2019). Genotypic antimicrobial resistance assays for use on E. coli isolates and stool specimens. *PLOS ONE*, 14(5).

Liu et al., 2016.

7.2 NUCLEIC ACID ANALYSIS

When the impact evaluation was originally conceived, the evaluation team proposed using the TAC designed for the Childhood Health and Mortality Prevention (CHAMPS) study led by the Center for Disease Control and Prevention (CDC), which included bacterial, viral, parasite, and fungal targets, and was optimized for testing respiratory swabs, cerebrospinal fluid, and whole blood specimens. However, the evaluation team eventually decided against using the CHAMPS TAC because full primer and probe sequences were not published,[5] and that would undermine our ability to be transparent about our methods. The team instead elected to create a custom TAC based on previously-validated TAC assays.[6] Our TAC was designed specifically for detection of enteric pathogens mediated by feces and fecal contamination. We also included assays for detection of key ARGs based on previously-validated TAC assays.[7] In summary, our TAC includes:

- Nine bacteria (*Campylobacter coli/jejuni*., *Clostridium difficile,* enteroaggregative *Escherichia coli* (EAEC), atypical or typical enteropathogenic *E.coli* (EPEC), heat-labile- (LT) or heat-stable- (ST) enterotoxigenic *E.coli* (ETEC), *Salmonella enterica*, *Shigella* spp./enteroinvasive *E.coli* (EIEC), shiga-toxin producing *E.coli* (STEC), and *Vibrio cholerae*);
- Six viruses (adenovirus, astrovirus, enterovirus, norovirus, rotavirus, and sapovirus);
- Four protozoa (*Cryptosporidium hominus, Crpytosporidium parvum, Entamoeba histolytica,* and *Giardia intestinalis*);
- Four soil-transmitted helminths (STH) (*Ascaris lumbricoides*, *Trichuris trichiura, Ancylostoma duodenale*, and *Necator americanus*); and
- Thirty-one ARGs associated with resistance against nine antibiotic families (aminoglycoside, ß-lactam, chloramphenicol, colistin, quinolone, macrolide, sulfonamide, tetracycline, and trimethoprim).

We tested samples from intervention groups and the control group simultaneously. Quantification cycles (C_q) are the PCR cycle values at which flourescence from amplification of the target gene segment exceeds background flourescence, inversely related to the quantity of the gene target. All AMR detections with a C_q greater than 32 were deemed negative,[8] and all other detections with a C_q greater than 35 were deemed negative. [9] All samples with a quantification cycle less than 32 for AMR or 35 for other were deemed a positive result and form the basis of statistical analysis desribed in the next section.

7.3 STATISTICAL ANALYSIS

The team performed statistical analysis on laboratory results, in combination with data collected through household surveys, to comment on the impact of the NOURISH intervention and to assess risk factors for contraction of enteric pathogens.

We conducted intention-to-treat analysis for intervention effects on enteropathogen infection. All analyses were carried out using Stata 16.1 (College Station, Texas, USA). The outcomes of interest were

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5 Diaz et al., 2019.

6 Pholwat et al., 2019; Liu et al., 2016.

7 Liu et al., 2016.

8 Pholwat et al., 2019.

9 Liu et al., 2016.

the intervention effects on enteric pathogen infection and AMR, measured by: prevalence (defined as presence or absence) of individual enteric pathogen genes and ARGs; mean difference in total number of pathogens, bacteria, viruses, protozoa, STH, and ARGs; and quantitative difference (measured by difference in gene copies) in pathogen targets.

To assess the impact of the interventions on enteropathogen prevalence, we employed log-linear Poisson regression to calculate PRs and adjusted prevalence ratios (aPRs), using generalized estimating equations with robust variance to account for clustering at the village-level. Enteropathogen infection outcomes were dichotomized, with positive detections defined by a quantification cycle (C_q) <35 for enteropathogens and Cq <32 for ARGs, which were previously determined to be the LODs. [10]

To assess impact on overall pathogen burden, we estimated the mean difference in number of pathogens (total and in subgroups by bacteria, viruses, protozoa, and STHs) and ARGs (total and in subgroups by antibiotic families) using zero-inflation Poisson regression, which models count data with an excess of zero counts.

We also assessed prevalence of enteric pathogen genes and ARGs with respect to household- and community-level risk factors. We estimated PRs and aPRs using log-linear Poisson regression of genes with prevalence greater than 10%; any genes with overall prevalence of <10% was not included in this analysis. Risk factors were identified at village, household, and child levels. The village-level risk factor includes village-level sanitation coverage (continuous, caregiver-reported with a random subset confirmed by field staff). Risk factors at the household- and child-level are described in Table 2.

TABLE 2: HOUSEHOLD- AND CHILD-LEVEL RISK FACTORS INCLUDED IN RISK FACTOR ANALYSIS

RISK FACTOR	VARIABLE DESCRIPTION
Access to any sanitation facility	Dichotomous; caregiver-reported with a random subset confirmed by field staff
Access to an improved sanitation facility	Dichotomous; caregiver-reported with a random subset confirmed by field staff
Access to a shared sanitation facility	Dichotomous; caregiver-reported with a random subset confirmed by field staff
Safe disposal of child stools	Dichotomous; caregiver-reported; defined by UNICEF as disposing child feces into any toilet or latrine facility[4]
Separation of animals from child play area	Dichotomous; observed by field staff
Child play area free of feces	Dichotomous; observed by field staff
Clean food preparation surface – i.e., for chopping, cutting, etc.	Dichotomous; observed by field staff
Elevated food preparation surface	Dichotomous; observed by field staff
Food preparation area free of dirt	Dichotomous; observed by field staff
Food preparation area free of flies	Dichotomous; observed by field staff
Handwashing station within 10 meters of food preparation area	Dichotomous; observed by field staff
Child level	
Breastfeeding status	Dichotomous; caregiver-report; based on whether child was being breastfed at time of survey
Diarrheal episode (24-hour recall)	Dichotomous; caregiver-report

[10] Pholwat et al., 2019; Liu et al., 2016.

8.0 RESULTS

8.1 PREVALENCE OF ENTERIC PATHOGENS

We analyzed 1,742 preserved stools using multiplex qPCR for presence of gene targets associated with key enteric pathogens (bacteria, viruses, protozoa, and STH) and AMR families. We omitted 125 samples due to lack of amplification in one or more of three controls (phHPV as DNA control; MS2 as RNA control; manufacturer internal PC) or due to unstable noise in amplification curves. The final dataset included 1,617 samples: 305 from the nutrition group, 330 from the sanitation group, 438 from the combined-intervention group, and 544 from the control group.

We detected at least one bacterial gene in 87% of all samples, at least one viral gene in 49% of all samples, at least one protozoan gene in 20% of all samples, at least one STH gene in 2% of all samples, and at least one AMR gene in 99% of all samples (Table 3; Annex A). Among positive samples, we detected a mean 2.5 bacterial genes (out of nine), 1.20 viral genes (out of six), 1.02 protozoan genes (out of foru), 1.16 STH genes (out of four), and 7.07 AMR genes (out of nine) (Table 3), with no measurable difference between treatment and control arms (Table 3).

Among enteric pathogen targets, enteroaggregative *E.coli* (EAEC), atypical enteropathogenic *E.coli* (aEPEC), enterovirus, *Campylobacter* spp., and enterotoxigenic *E.coli* (ETEC) were the most prevalent pathogens (Table 3). *Giardia* spp. was detected in 19% of our samples, and <1% of our samples had detectable quantities of non-*Giardia* protozoa (Cryptosporidium and Entamoeba) and STH (*A.lumbricoides*, *T.trichiura*, *A.duodenale, N.americanus*). Among ARG targets, we detected high levels of ARGs related to ß-lactam (98%), chloramphenicol (95%), quinolone (95%), macrolide (99%), sulfonamide (99%), tetracycline (98%), and trimethoprim (90%). There was moderate prevalence of the colistin-ARG (28%), and very low prevalence of aminoglycoside-ARGs (<1%) (Table 3).

Prevalence increased with age for many pathogens (aEPEC, ETEC, *Shigella*/EIEC (*ipah*), STEC, adenovirus, *Giardia*) and ARG-groups (ß-lactam, chloramphenicol, colistin, quinolone, tetracycline). Prevalence of the mobile integron gene *intI1* generally decreased with age. Prevalence peaked for children nine to 17 months for other pathogens (*Campylobacter* spp., *Clostrodium difficile*, EAEC, *Salmonella* spp.), although this trend was not observed for ARG groups (Annex A: Descriptive Statistics).

TABLE 3: PREVALENCE OF ENTERIC GENES AND ARGS, BY TREATMENT ARM

	ALL SAMPLES (N=1620)	NUTRITION-ONLY (N=305)	SANITATION-ONLY (N=333)	COMBINED (N=438)	CONTROL (N=544)
Bacteria					
Campylobacter spp.	551 (34%)	104 (34%)	114 (34%)	162 (37%)	171 (31%)
Clostridium difficile	139 (9%)	33 (11%)	25 (8%)	41 (9%)	40 (7%)
EAEC	1029 (64%)	204 (67%)	207 (62%)	281 (64%)	337 (62%)
EPEC	899 (55%)	172 (56%)	173 (52%)	234 (53%)	320 (59%)
aEPEC	703 (43%)	139 (46%)	137 (41%)	173 (39%)	254 (47%)
tEPEC	109 (7%)	15 (5%)	17 (5%)	32 (7%)	45 (8%)
ETEC	422 (26%)	86 (28%)	79 (24%)	114 (26%)	143 (26%)
ETEC-LT	342 (21%)	75 (25%)	68 (20%)	86 (20%)	113 (21%)
ETEC-ST	194 (12%)	39 (13%)	37 (11%)	63 (14%)	55 (10%)
ETEC-LT/ST	114 (7%)	28 (9%)	26 (8%)	35 (8%)	25 (5%)
Salmonella spp.	134 (8%)	28 (9%)	19 (6%)	39 (9%)	48 (9%)

	ALL SAMPLES (N=1620)	NUTRITION-ONLY (N=305)	SANITATION-ONLY (N=333)	COMBINED (N=438)	CONTROL (N=544)
Shigella spp.	186 (11%)	24 (8%)	39 (12%)	52 (12%)	71 (13%)
STEC	132 (8%)	27 (9%)	22 (7%)	45 (10%)	38 (7%)
Vibrio cholera	10 (1%)	1 (0%)	6 (2%)	1 (0%)	2 (0%)
Any bacterium	1410 (87%)	276 (90%)	282 (85%)	386 (88%)	466 (86%)
Mean number of bacteria[1]	2.48 (2.42, 2.55)	2.46 (2.32, 2.60)	2.43 (2.28, 2.57)	2.51 (2.39, 2.63)	2.51 (2.40, 2.62)
Viruses					
Adenovirus	287 (18%)	77 (25%)	59 (18%)	78 (18%)	73 (13%)
Astrovirus	7 (0%)	2 (1%)	1 (0%)	3 (1%)	1 (0%)
Enterovirus	558 (34%)	97 (32%)	105 (32%)	169 (39%)	187 (34%)
Norovirus	54 (3%)	8 (3%)	9 (3%)	20 (5%)	17 (3%)
Rotavirus	17 (1%)	2 (1%)	2 (1%)	6 (1%)	7 (1%)
Sapovirus	24 (1%)	4 (1%)	5 (2%)	9 (2%)	6 (1%)
Any virus	788 (49%)	152 (50%)	157 (47%)	231 (53%)	248 (46%)
Mean number of viruses[1]	1.20 (1.17, 1.23)	1.25 (1.17, 1.33)	1.15 (1.09, 1.21)	1.23 (1.17, 1.30)	1.17 (1.12, 1.23)
Protozoa					
Cryptosporidium	17 (1%)	4 (1%)	1 (0%)	6 (1%)	6 (1%)
Entamoeba	13 (1%)	1 (0%)	1 (0%)	8 (2%)	3 (1%)
Giardia	306 (19%)	52 (17%)	68 (20%)	84 (19%)	102 (19%)
Any protozoa	328 (20%)	56 (18%)	69 (21%)	93 (21%)	110 (20%)
Mean number of protozoa[1]	1.02 (1.01, 1.04)	1.02 (0.98, 1.05)	1.01 (0.99, 1.04)	1.05 (1.01, 1.10)	1.01 (0.99, 1.03)
STH					
Ascaris lumbricoides	3 (0%)	0 (0%)	0 (0%)	3 (1%)	0 (0%)
Trichuris trichiura	3 (0%)	1 (0%)	1 (0%)	0 (0%)	1 (0%)
Ancylostoma duodenale	17 (1%)	0 (0%)	4 (1%)	4 (1%)	9 (2%)
Necator americanus	20 (1%)	3 (1%)	6 (2%)	5 (1%)	6 (1%)
Any STH	37 (2%)	4 (1%)	10 (3%)	9 (2%)	14 (3%)
Mean number of STHs[1]	1.16 (1.04, 1.29)	1.00 (1.00, 1.00)	1.10 (0.90, 1.30)	1.33 (1.00, 1.67)	1.14 (0.95, 1.34)
Antibiotic families					
Aminoglycoside	10 (1%)	3 (1%)	1 (0%)	3 (1%)	3 (1%)
ß-lactam	1583 (98%)	299 (98%)	323 (97%)	428 (98%)	533 (98%)
Chloramphenicol	1533 (95%)	289 (95%)	313 (94%)	416 (95%)	515 (95%)
Colistin	458 (28%)	103 (34%)	78 (23%)	116 (26%)	161 (30%)
Quinolone	1536 (95%)	290 (95%)	317 (95%)	416 (95%)	513 (94%)
Macrolide	1600 (99%)	302 (99%)	331 (99%)	431 (98%)	536 (99%)
Sulfa	1605 (99%)	301 (99%)	331 (99%)	433 (99%)	540 (99%)
Tetracycline	1596 (99%)	300 (98%)	330 (99%)	429 (98%)	537 (99%)
Trimethoprim	1463 (90%)	276 (90%)	303 (91%)	392 (89%)	492 (90%)
Any ARG	1610 (99%)	304 (100%)	332 (100%)	434 (99%)	540 (99%)

	ALL SAMPLES (N=1620)	NUTRITION-ONLY (N=305)	SANITATION-ONLY (N=333)	COMBINED (N=438)	CONTROL (N=544)
Mean number of AMR groups[1,2]	7.07 (7.03, 7.11)	7.12 (7.01, 7.22)	7.01 (6.92, 7.10)	7.06 (6.98, 7.14)	7.09 (7.02, 7.16)
IntI1 (integron-integrase)	1478 (91%)	276 (90%)	310 (93%)	400 (91%)	492 (90%)

Atypical-EPEC (aEPEC) includes samples with detectable *EPEC_eae*. Typical-EPEC (tEPEC) includes samples with detectable *EPEC_bfpa* and *EPEC_eae*. Heat-labile ETEC (ETEC-LT) includes samples with detectable *ETEC_LT*. Heat-stable ETEC (ETEC-ST) includes samples with detectable *ETEC_sth* or *ETEC_stp*. ETEC-LT/ST includes samples with both heat-labile and -stable genes. ETEC includes samples with any detectable ETEC gene. All data is in count (percentage of total). [1]Data in count (95% CI). [2]Integorn-integrase gene *intI1* is not included in AMR groups.

8.2 INTERVENTION EFFECTS ON ENTEROPATHOGEN INFECTION AND ARG CARRIAGE

Overall, we found little effect of any intervention (nutrition, sanitation, or combined) on the prevalence of pathogens or ARGs (Table 3). We also found no effect of any intervention on the number of detectable pathogen genes or ARGs (Tables 4 and 5).

We detected fewer number of ARG groups in the sanitaiton-only arm compared to control arm (-0.10 mean difference, 95% CI -0.21 to 0.00); there were no other measurable differences in number of ARG groups, neither between treatment and control arms nor between individual and combined treatment arms. Comparing prevalence of individual ARG groups between arms, we found lower prevalence of the colistin ARG (*mcr-1*) in the sanitation-only arm compared to control. There were no other measurable differences in prevalence of ARG groups, neither between treatment and control arms nor between individual and combined treatment arms.

Differences in mean gene quantities were generally consistent with prevalence differences (Table 6). We detected lower concentrations of pathogen-associated genes in the nutrition-only and sanitation-only arms; children in the nutrition-only arm carried lower quantities of the *STEC1* gene (-1.46 log-copies compared to control, 95% CI -2.97-0.06) and *Giardia* gene (-1.73 log-copies, 95% CI -3.02- -0.44), and children in the sanitation-only arm carried lower quantities of *EPEC_eae* (-0.54 log-copies, 95% CI -1.17-0.09) and *STEC1* (-1.71 log-copies, 95% CI -3.07- -0.34). There was no measurable difference in mean gene quantities between the combined and control arm. Overall, with adjustment for multiple comparisons, there was no significant difference in quantity of pathogen genes between treatment arms.

FIGURE 3: IMPACT OF INTERVENTIONS ON ADJUSTED PREVALENCE RATIO OF INDIVIDUAL PATHOGENS

Point estimates and 95% confidence intervals were determined using generalized log-linear Poisson models adjusting for covariates associated with each pathogen outcome: child age, child sex, maternal age, maternal education, number of household members, and wealth quintile.

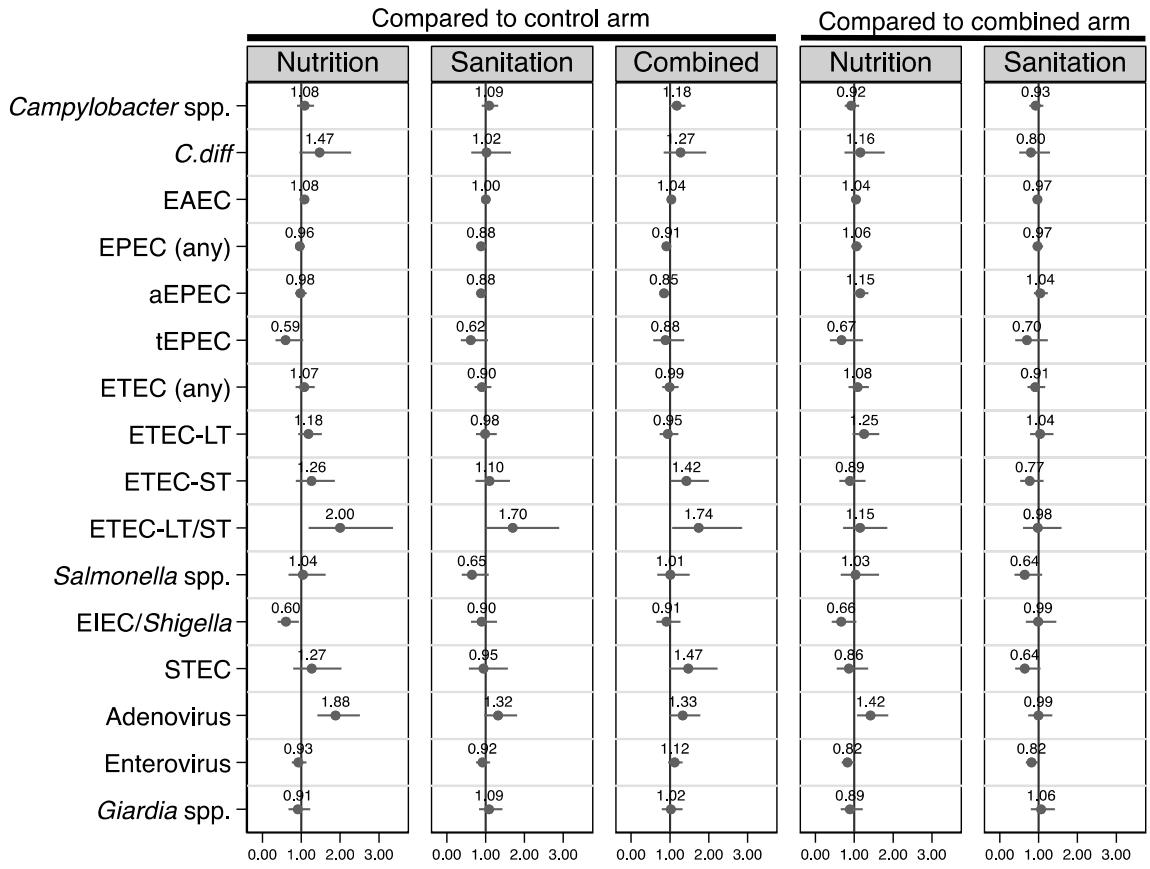


FIGURE 4: PRIMARY OUTCOME: IMPACT OF INTERVENTIONS ON ADJUSTED PREVALENCE RATIO OF INDIVIDUAL PATHOGENS

Point estimates and 95% confidence intervals were determined using generalized log-linear Poisson models adjusting for covariates associated with each pathogen outcome: child age, child sex, maternal age, maternal education, number of household members, wealth quintile.

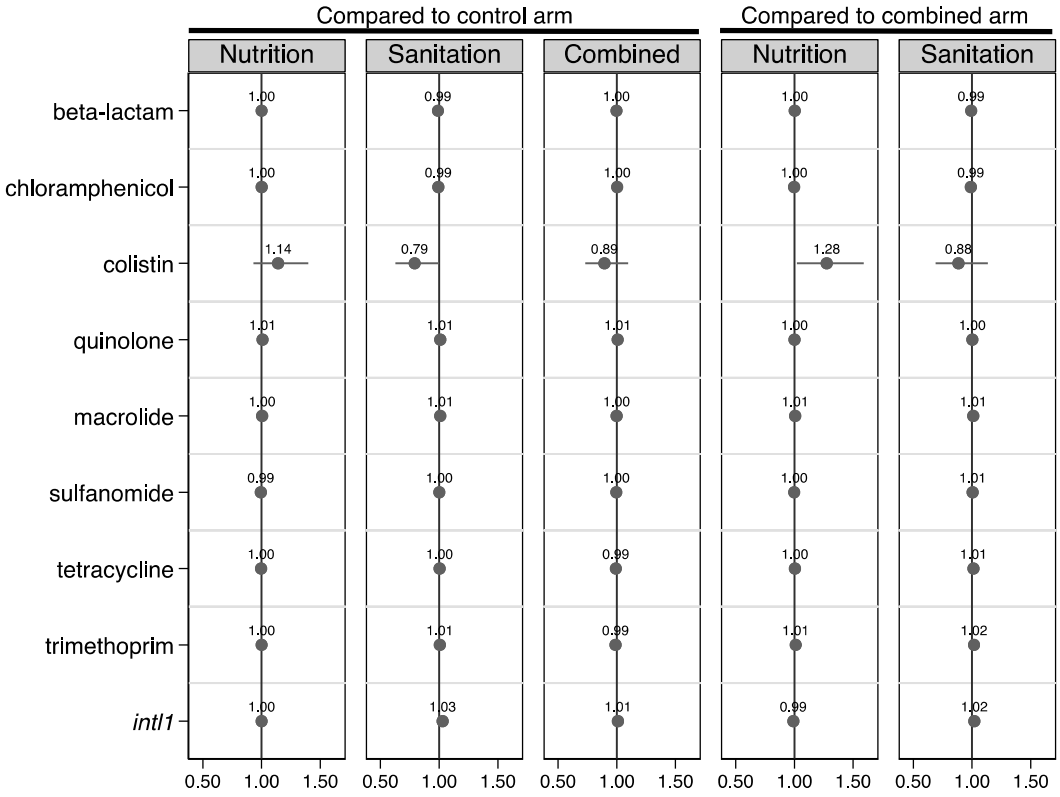


TABLE 4: ADJUSTED MEAN DIFFERENCE IN NUMBER OF DETECTS, COMPARED TO CONTROL

	NUTR	SAN	NUTR+SAN
Bacteria	0.00 (-0.20, 0.20)	-0.13 (-0.33, 0.07)	-0.01 (-0.19, 0.17)
Viruses	0.08 (-0.03, 0.18)	-0.05 (-0.13, 0.03)	0.07 (-0.02, 0.16)
Protozoa	0.02 (-0.03, 0.08)	0.01 (-0.03, 0.05)	0.06 (-0.01, 0.12)
STH	-0.02 (-0.42, 0.37)	-0.23 (-0.48, 0.01)	0.11 (-0.31, 0.53)
AMR families	0.02 (-0.10, 0.13)	-0.10 (-0.21, 0.00)*	-0.01 (-0.10, 0.09)

***p<0.001; **p<0.01; *p<0.05

TABLE 5: ADJUSTED MEAN DIFFERENCE IN NUMBER OF DETECTS, COMPARED TO COMBINED ARM

	NUTR	SAN
Bacteria	0.01 (-0.20, 0.22)	-0.12 (-0.33, 0.08)
Viruses	0.01 (-0.10, 0.12)	-0.12 (-0.21, -0.03)*
Protozoa	-0.04 (-0.11, 0.04)	-0.04 (-0.11, 0.02)
STH	-0.13 (-0.49, 0.22)	-0.34 (-0.73, 0.04)
AMR families	0.02 (-0.10, 0.14)	-0.10 (-0.21, 0.01)

***p<0.001; **p<0.01; *p<0.05

TABLE 6: UNADJUSTED MEAN DIFFERENCE IN LOG-TRANSFORMED GENE COPY ESTIMATES, COMPARING INTERVENTION ARMS TO CONTROL

	Compared to control arm			Compared to combined arm	
	NUTR	SAN	NUTR+SAN	NUTR	SAN
Bacteria					
CAMP	0.22 (-0.60 - 1.03)	-0.40 (-1.27 - 0.48)	-0.24 (-1.00 - 0.53)	0.45 (-0.37 - 1.28)	-0.16 (-1.05 - 0.73)
CDIF	0.31 (-1.14 - 1.75)	-0.37 (-1.92 - 1.19)	0.19 (-1.16 - 1.54)	0.11 (-1.29 - 1.52)	-0.56 (-2.08 - 0.96)
EAEC_aaic	-0.38 (-1.35 - 0.60)	0.35 (-0.52 - 1.22)	-0.28 (-1.08 - 0.52)	-0.10 (-1.11 - 0.92)	0.63 (-0.28 - 1.54)
EAEC_aata	-0.36 (-1.26 - 0.53)	-0.24 (-1.05 - 0.56)	0.12 (-0.69 - 0.93)	-0.48 (-1.43 - 0.47)	-0.36 (-1.24 - 0.51)
EPEC_bfpa	-1.08 (-3.47 - 1.31)	-1.13 (-3.16 - 0.89)	1.15 (-0.38 - 2.67)	-2.23 (-4.57 - 0.11)	-2.28 (-4.24 - - 0.32)
EPEC_eae	-0.12 (-0.80 - 0.56)	-0.54 (-1.17 - 0.09)	0.24 (-0.33 - 0.82)	-0.37 (-1.05 - 0.32)	-0.78 (-1.41 - - 0.15)
ETEC_LT	-0.62 (-1.64 - 0.40)	-0.47 (-1.56 - 0.61)	-0.03 (-1.06 - 1.00)	-0.59 (-1.65 - 0.47)	-0.44 (-1.57 - 0.68)
ETEC_stp	0.13 (-1.73 - 1.98)	1.29 (-0.61 - 3.20)	0.87 (-0.97 - 2.71)	-0.75 (-2.61 - 1.11)	0.42 (-1.49 - 2.33)
SALM	1.42 (-0.04 - 2.87)	0.27 (-0.97 - 1.52)	0.80 (-0.33 - 1.93)	0.62 (-0.93 - 2.17)	-0.53 (-1.89 - 0.83)

	Compared to control arm			Compared to combined arm	
	NUTR	SAN	NUTR+SAN	NUTR	SAN
IPAH	0.22 (-1.33 - 1.77)	-0.28 (-1.68 - 1.13)	1.17 (0.07 - 2.26)	-0.95 (-2.43 - 0.53)	-1.44 (-2.77 - -0.11)
STEC1	-1.46 (-2.97 - 0.06)	-1.71 (-3.07 - -0.34)	-0.00 (-1.49 - 1.49)	-1.46 (-2.84 - -0.07)	-1.70 (-2.92 - -0.49)
STEC2	-0.20 (-1.45 - 1.04)	0.09 (-1.20 - 1.37)	0.72 (-0.47 - 1.91)	-0.92 (-2.06 - 0.22)	-0.63 (-1.82 - 0.56)
Viruses					
ADEV	0.50 (-0.48 - 1.49)	0.67 (-0.32 - 1.67)	0.48 (-0.42 - 1.38)	0.03 (-0.96 - 1.02)	0.20 (-0.80 - 1.20)
ENTV	-0.40 (-1.09 - 0.28)	-0.35 (-0.97 - 0.26)	-0.26 (-0.77 - 0.24)	-0.14 (-0.84 - 0.56)	-0.09 (-0.72 - 0.54)
Protozoa					
GIAR	-1.73 (-3.02 - -0.44)	0.23 (-1.16 - 1.62)	-0.14 (-1.47 - 1.18)	-1.58 (-3.06 - -0.11)	0.37 (-1.19 - 1.93)
AMR - ß-lactam					
CTXM1	-0.28 (-0.73 - 0.18)	-0.64*** (-1.08 - -0.19)	-0.36* (-0.78 - 0.05)	0.09 (-0.39 - 0.56)	-0.27 (-0.74 - 0.19)
CTXM2M74	-0.07 (-0.95 - 0.80)	0.22 (-0.69 - 1.13)	0.61 (-0.53 - 1.74)	-0.68 (-1.91 - 0.56)	-0.39 (-1.65 - 0.87)
CTXM8M25	0.04 (-0.43 - 0.51)	0.05 (-0.43 - 0.54)	-0.06 (-0.45 - 0.34)	0.10 (-0.36 - 0.55)	0.11 (-0.35 - 0.57)
CTXM9	0.12 (-0.48 - 0.72)	-0.36 (-0.98 - 0.26)	-0.34 (-0.86 - 0.18)	0.46 (-0.15 - 1.07)	-0.02 (-0.66 - 0.61)
NDM	0.24 (-1.50 - 1.97)	-0.58 (-2.44 - 1.29)	0.44 (-1.46 - 2.34)	-0.20 (-2.28 - 1.87)	-1.01 (-3.20 - 1.17)
OXA1	0.26 (-0.33 - 0.85)	-0.54* (-1.12 - 0.05)	-0.00 (-0.56 - 0.56)	0.26 (-0.37 - 0.90)	-0.54* (-1.17 - 0.09)
SHV	0.12 (-0.27 - 0.50)	-0.19 (-0.59 - 0.20)	-0.33* (-0.68 - 0.02)	0.45** (0.06 - 0.84)	0.14 (-0.27 - 0.54)
AMR - chloramphenicol					
catA1	0.21 (-0.29 - 0.70)	-0.09 (-0.64 - 0.46)	-0.02 (-0.47 - 0.43)	0.23 (-0.27 - 0.73)	-0.07 (-0.63 - 0.48)
catB3	0.41 (-0.26 - 1.08)	-0.07 (-0.68 - 0.54)	0.15 (-0.45 - 0.76)	0.25 (-0.45 - 0.96)	-0.23 (-0.88 - 0.43)
cmlA	0.49* (-0.01 - 1.00)	-0.12 (-0.56 - 0.33)	0.19 (-0.23 - 0.60)	0.31 (-0.19 - 0.80)	-0.31 (-0.75 - 0.13)
floR	0.17 (-0.28 - 0.62)	-0.42** (-0.80 - -0.03)	-0.05 (-0.44 - 0.34)	0.22 (-0.26 - 0.70)	-0.37* (-0.78 - 0.05)
AMR - colistin					
mcr1	-0.02 (-0.49 - 0.45)	-0.12 (-0.69 - 0.45)	0.05 (-0.42 - 0.52)	-0.07 (-0.55 - 0.42)	-0.17 (-0.75 - 0.42)
AMR - macrolide					
ermB	0.27** (0.00 - 0.54)	-0.07 (-0.36 - 0.21)	-0.05 (-0.34 - 0.24)	0.32** (0.01 - 0.63)	-0.02 (-0.35 - 0.30)
mphA	0.31 (-0.06 - 0.69)	-0.28 (-0.70 - 0.14)	-0.10 (-0.46 - 0.26)	0.41** (0.02 - 0.81)	-0.18 (-0.62 - 0.26)
AMR - quinolone					
aac6Ib_104R	0.14 (-0.41 - 0.69)	-0.59** (-1.09 - -0.09)	-0.12 (-0.62 - 0.37)	0.27 (-0.32 - 0.86)	-0.46* (-1.00 - 0.08)

	Compared to control arm			Compared to combined arm	
	NUTR	SAN	NUTR+SAN	NUTR	SAN
aac6Ib_104W	0.07 (-0.92 - 1.06)	-0.17 (-0.98 - 0.65)	-0.42 (-1.24 - 0.40)	0.49 (-0.45 - 1.43)	0.26 (-0.50 - 1.01)
gyrA83L	-0.01 (-0.31 - 0.28)	-0.17 (-0.48 - 0.14)	0.02 (-0.25 - 0.28)	-0.03 (-0.34 - 0.29)	-0.18 (-0.51 - 0.14)
parC80I	0.15 (-0.49 - 0.79)	-0.03 (-0.64 - 0.58)	0.12 (-0.42 - 0.66)	0.03 (-0.60 - 0.66)	-0.15 (-0.74 - 0.45)
qnrA	0.01 (-1.26 - 1.27)	0.01 (-1.13 - 1.15)	0.38 (-0.98 - 1.74)	-0.37 (-1.68 - 0.94)	-0.37 (-1.56 - 0.83)
qnrB1	0.36 (-0.22 - 0.94)	-0.07 (-0.62 - 0.47)	-0.34 (-0.88 - 0.20)	0.70** (0.09 - 1.31)	0.27 (-0.31 - 0.84)
AMR - sulfonamide					
sul1	0.10 (-0.26 - 0.46)	-0.35* (-0.71 - 0.00)	-0.07 (-0.37 - 0.22)	0.17 (-0.19 - 0.53)	-0.28 (-0.64 - 0.08)
sul2	0.09 (-0.21 - 0.40)	-0.06 (-0.34 - 0.23)	0.04 (-0.24 - 0.32)	0.06 (-0.27 - 0.38)	-0.09 (-0.40 - 0.21)
AMR - tetracycline					
tetA	0.26* (-0.03 - 0.55)	0.01 (-0.29 - 0.31)	0.06 (-0.19 - 0.31)	0.20 (-0.10 - 0.49)	-0.05 (-0.35 - 0.25)
tetB	-0.15 (-0.55 - 0.24)	-0.73*** (-1.10 - -0.35)	-0.31 (-0.67 - 0.06)	0.15 (-0.24 - 0.54)	-0.42** (-0.79 - -0.05)
AMR - trimethoprim					
dfrA17	0.18 (-0.27 - 0.64)	-0.34 (-0.80 - 0.12)	0.01 (-0.36 - 0.38)	0.17 (-0.30 - 0.64)	-0.35 (-0.83 - 0.12)
Integron-integrase					
intI1	0.12 (-0.17 - 0.40)	-0.23 (-0.54 - 0.08)	-0.14 (-0.41 - 0.13)	0.26 (-0.06 - 0.58)	-0.08 (-0.42 - 0.26)

8.3 RISK FACTOR ANALYSIS

Genes with an overall prevalence >5% and <95% were included in a risk factor analysis; genes for *Vibrio cholera*, astrovirus, norovirus, rotavirus, sapovirus, *Cyrptosporidium* spp., entamoeba, all STHs, aminoglycoside-, ß-lactam-, macrolide-, sulfonamide-, and tetracycline-resistance were omitted.

The findings are summarized below:

- We measured village-level sanitation coverage as a community-level factor and found increased sanitation to be associated with lower prevalence of tEPEC (aPR 0.42, 95% CI 0.20 to 0.91).
- Among household-level factors, we found some to be protective of enteric pathogen infection in our adjusted analyses:
  - Household access to any sanitation facility was associated with lower prevalence of tEPEC (aPR 0.58, 95% CI 0.36 to 0.95);
  - Household access to an improved sanitation facility was associated with lower prevalence of ETEC (aPR 0.82, 95% CI 0.69 to 0.98);
  - An elevated food preparation surface was associated with lower prevalence of *Campylobacter* (aPR 0.87, 95% CI 0.74 to 1.01) and *Giardia* (aPR 0.76, 95% CI 0.57 to 1.01); and

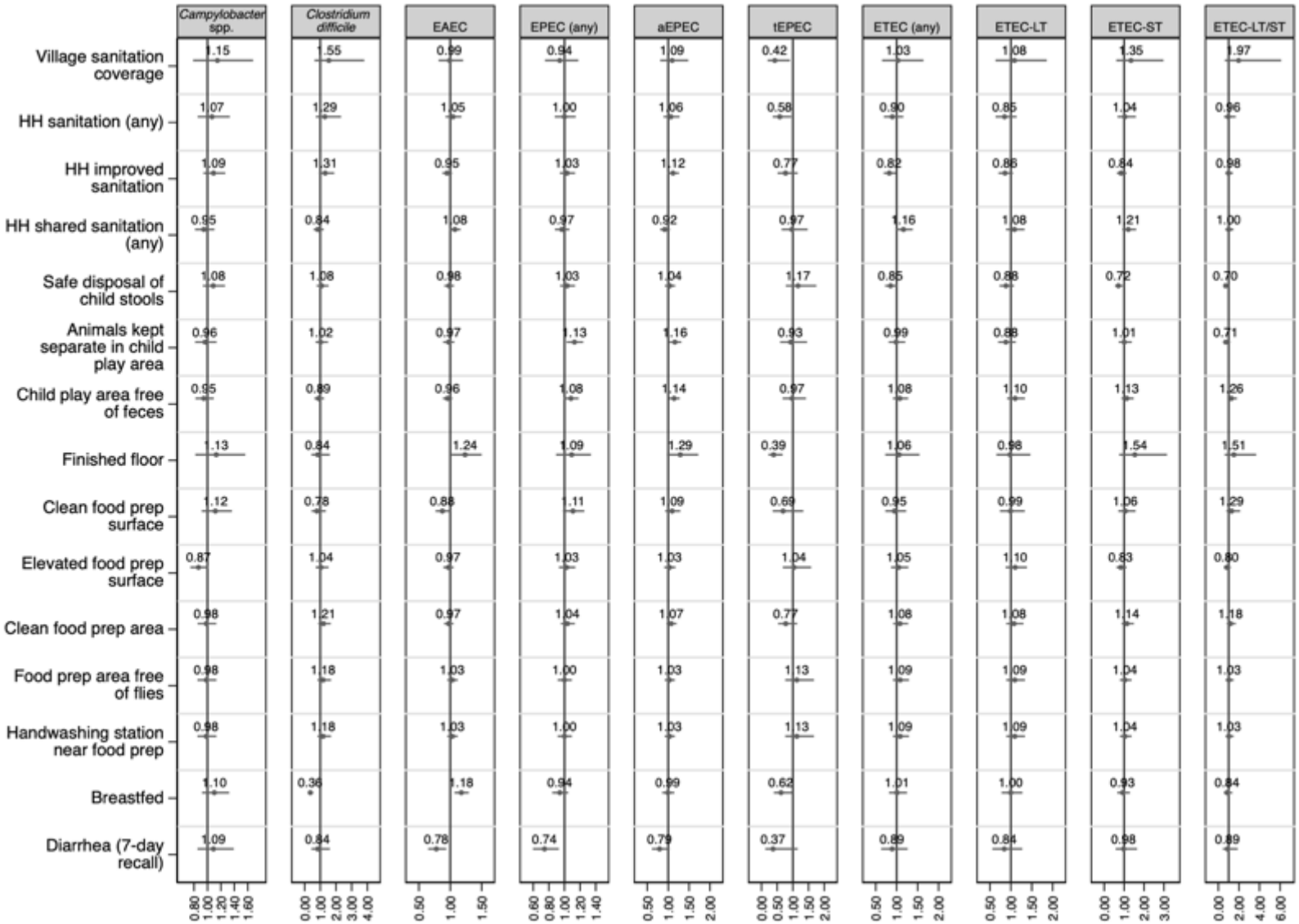
  - A clean food preparation area was associated with lower prevalence of *Shigella*/EIEC (aPR 0.70, 95% CI 0.52 to 0.93).
- We measured child breastfeeding status as a child-level factor and found current child breastfeeding to be associated with lower prevalence of *Clostridium difficile* (aPR 0.36, 95% CI 0.24 to 0.56) and tEPEC (aPR 0.62, 95% 0.39 to 0.99).
- Children in households with a play area that was visibly free of feces had a lower prevalence of colistin-related ARGs (aPR 0.85, 95% CI 0.72 to 1.00).

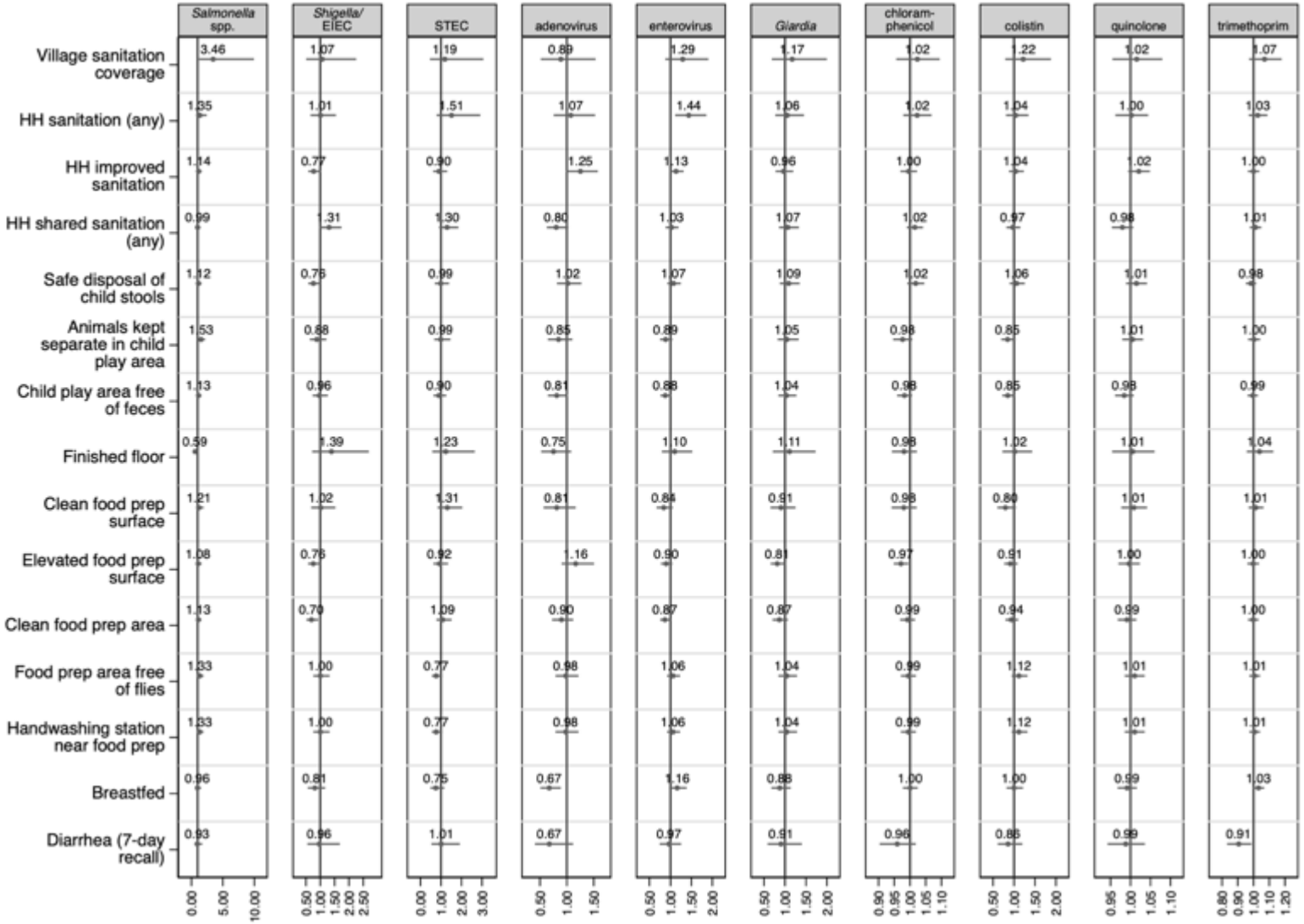
Conversely, we found some risk factors associated with higher prevalence of enteric pathogens:

- Village-level sanitation was associated with more *Salmonella* (aPR 3.46, 95% CI 1.20 to 9.94).
- Household-level sanitation was associated with more enterovirus (aPR 1.44, 95% CI 1.11 to 1.86).
- Child play area visibly free of feces was associated with more aEPEC (aPR 1.19, 95% CI 1.05 to 1.34).
- Breastfeeding was associated with more EAEC (aPR 1.18, 05% CI 1.07 to 1.30).
- Children in households where animals were kept separate from the living area had higher prevalence of EPEC (aPR 1.13, 95% CI 1.02 to 1.24) and aEPEC (aPR 1.16, 95% CI 1.03 to 1.32).
- Children in households where the play area was visibly free of feces had higher prevalence of aEPEC (aPR 1.14, 95% CI 1.02 to 1.28).
- Children in households with a food preparation area that was visibly free of flies and with a handwashing station within 10 meters of the food preparation area had higher prevalence of sulfonamide-related ARGs (both aPR 1.02, 95% CI 1.00 to 1.03).

FIGURE 5: ADJUSTED PREVALENCE RATIOS OF HOUSEHOLD RISK FACTORS ON ENTERIC INFECTION AND AMR GROUPS

With overall prevalence >5% and <95%, Adjusted for child age, child sex, maternal age, maternal education, number of household members, wealth quintile.




Salmonella spp.
Shigella/ EIEC
STEC
adenovirus
enterovirus
Giardia
chloram-phenicol
colistin
quinolone
trimethoprim
Village sanitation coverage
HH sanitation (any)
HH improved sanitation
HH shared sanitation (any)
Safe disposal of child stools
Animals kept separate in child play area
Child play area free of feces
Finished floor
Clean food prep surface
Elevated food prep surface
Clean food prep area
Food prep area free of flies
Handwashing station near food prep
Breastfed
Diarrhea (7-day recall)
3.46 1.07 1.19 0.89 1.29 1.17 1.02 1.22 1.02 1.07
1.35 1.01 1.51 1.07 1.44 1.06 1.02 1.04 1.00 1.03
1.14 0.77 0.90 1.25 1.13 0.96 1.00 1.04 1.02 1.00
0.99 1.31 1.30 0.80 1.03 1.07 1.02 0.97 0.98 1.01
1.12 0.76 0.99 1.02 1.07 1.09 1.02 1.06 1.01 0.98
1.53 0.88 0.99 0.85 0.89 1.05 0.98 0.85 1.01 1.00
1.13 0.96 0.90 0.81 0.88 1.04 0.98 0.85 0.98 0.99
0.59 1.39 1.23 0.75 1.10 1.11 0.98 1.02 1.01 1.04
1.21 1.02 1.31 0.81 0.84 0.91 0.98 0.89 1.01 1.01
1.08 0.76 0.92 1.16 0.90 0.81 0.97 0.91 1.00 1.00
1.13 0.79 1.09 0.90 0.87 0.87 0.99 0.94 0.99 1.00
1.33 1.00 0.77 0.98 1.06 1.04 0.99 1.12 1.01 1.01
1.33 1.00 0.77 0.98 1.06 1.04 0.99 1.12 1.01 1.01
0.96 0.81 0.75 0.67 1.16 0.88 1.00 1.00 0.99 1.03
0.93 0.96 1.01 0.67 0.97 0.91 0.96 0.85 0.99 0.91
0.00 5.00 10.00
0.50 1.00 1.50 2.00 2.50
0.00 1.00 2.00 3.00
0.50 1.00 1.50
0.50 1.00 1.50 2.00
0.50 1.00 1.50 2.00
0.90 0.95 1.00 1.05 1.10
0.50 1.00 1.50 2.00
0.95 1.00 1.05 1.10
0.80 0.90 1.00 1.10 1.20

9.0 CONCLUSIONS

9.1 ENTERIC INFECTION – THE EVIDENCE BASE

Few studies have evaluated the impact of interventions on enteric pathogen carriage, and among those that have, the evidence is mixed. In Bangladesh, children receiving WASH interventions (delivered at the household-level) had lower prevalence and quantity of enteric pathogens compared to control (-11% norovirus, 95% CI -5 to -17%; -9% sapovirus, 95% CI -3 to -15%; -9% adenovirus, 95% CI -2 to -15%), although there was no measurable difference in bacteria or parasites. In Zimbabwe, a supply-based WASH intervention (delivered at the household level) decreased the number of parasites detected compared to control (-0.07, 95% CI -0.14 to -0.02) but had no measurable effects on bacteria or viruses; the same trial found no effects of the nutrition intervention on any enteric pathogens.

One cross-sectional study in rural Ethiopia found presence of culturable *Campylobacter* spp. in child stools to be significantly associated with wasting, diarrhea, household ownership of livestock, and floor samples positive for *Campylobacter* spp. No association was found with household sanitation facility, signaling contaminated floors via animal contamination as an important pathway for fecal contamination and subsequent infant pathogen ingestion.[11] In rural Bangladesh and Kenya, finished floors were associated with lower prevalence of *Giardia* and STHs. Among the risk factors that were included in our pooled analysis, we found village-level sanitation coverage, household-level sanitation, clean child play area and food preparation areas, and elevated food surfaces to be linked to lower prevalence of enteric pathogens. While we are unable to assume causality through these associations, it is possible that these factors reduce fecal contamination in the living environment, which would support the causal pathway theory posited. Future trials may benefit from including molecular analysis of environmental samples as an intermediate outcome to elucidate gaps that sanitation interventions may not have addressed.

Enteric infections among children under two years in rural Cambodia were common, and our findings of high enteric infection are consistent with other studies examining early childhood infections in high-burden settings. One study detected high prevalence of *Campylobacter* spp., *Giardia* spp., EAEC, and norovirus (GII) in both diarrheal and non-diarrheal surveillance stools among children under two years of age in low- and middle-income countries.[12] Another study of diarrheal stools from multiple sites in Africa and Asia found high prevalence of *Shigella* spp., rotavirus, adenovirus 40/41, *Cryptosporidium* spp., and *Campylobacter* spp. that, combined, accounted for nearly 80% of all attributable diarrhea.[13] In urban Bangladesh, enteric pathogen prevalence was also high, particularly *Giardia* spp. (40%), *Salmonella enterica* (33%), ETEC (28%), and *Shigella* spp. (27%).[14] Similarly, in urban Mozambique, high prevalence of *Giardia*

[11] Budge, S., Barnett, M., Hutchings, P., Parker, A., Tyrrel, S., Hassard, F., Garbutt, C., Moges, M., Woldemedhin, F., & Jemal, M. (2020). Risk factors and transmission pathways associated with infant campylobacter spp. prevalence and malnutrition: A formative study in rural Ethiopia. *PLOS ONE*, *15*(5).

[12] Budge et al., 2020.

[13] Liu et al., 2016.

[14] Berendes, D., Capone, D., Knee, J., Holcomb, D., Sultana, S., Pickering, A. J., & Brown, J. (2020). Associations between Enteric Pathogen Carriage and height-for-age, weight-for-age and weight-for-height in children under 5 years old in urban Dhaka, Bangladesh. *Epidemiology and Infection*, *148*.

spp. (51%), *Shigella* spp. (44%), ETEC LT/ST (30%), and *Salmonella* (21%) were detected in stools of children under four years of age.[15]

9.2 ENTERIC INFECTION – THIS STUDY'S FINDINGS

We measured some differences in prevalence of enteric pathogens across treatment arms. tEPEC and ETEC-LT/ST were different between treatment arms, but there is inconsistent directionality with the trends, and these were present at low-prevalence (detected in 6% and 7%), increasing the likelihood of a spurious association. There was also measurable difference in prevalence of *Shigella*/EIEC in the nutrition-only arm (aPR 0.50, 95% CI 0.31 to 0.83), although this difference was not measured in the combined arm; the difference therefore cannot be unambiguously attributed to the nutrition program. Overall, we observe no strong or consistent relationships in enteric pathogen detection between treatment groups.

Infection of *Campylobacter* spp., *C. difficile*, EAEC, EPEC, ETEC, *Salmonella* spp., EIEC/*Shigella* spp., and STEC were strongly age dependent, suggesting that transmission pathways for each pathogen may differ and correspond to different behaviors (i.e., eating solid foods, teething, crawling, walking, etc.). Further investigation is needed to quantify risk of pathogen infection from child behaviors.

9.3 ANTIMICROBIAL RESISTANCE

Southeast Asia is at high risk of the emergence and spread of antibiotic resistance in humans. Misuse of antibiotics in humans and animals has been identified as the primary driver of the risk of AMR spread, while release of antibiotic residues, AMR-bacteria, and ARGs into the environment due to inadequate waste management has been identified as a moderate risk.[16] Presently, much of the evidence base pertinent to AMR in Cambodia is limited to the impacts of livestock production. One study found a high presence of *Salmonella* with ß-lactam resistance genes (>70%) and class 1 integrons (>60%) in pig and chicken samples in slaughterhouses and markets in Cambodia, many of which were located on conjugative plasmids.[17] Another study in rural Cambodia found moderate community carriage of extended-spectrum ß-lactamase-producing (ESBL) genes among human and livestock fecal samples (20% and 23%, respectively) that also expressed non-wild-type phenotypic resistance to sulfonamide, tetracycline, and chloramphenicol[18]; the same study detected no presence of colistin-resistance genes.

Among the eight ARG groups that we investigated for AMR, we found high prevalence of ARGs conferring resistance to six ARG groups. 98-99% of samples were found positive for ARGs conferring resistance to ß-lactam, chloramphenicol, quinolone, macrolide, sulfonamide, and tetracycline, 90% of samples had ARGs related to trimethoprim-resistance, 27% of samples had ARGs related to colistin-resistance, and <1% of samples had ARGs related to aminoglycoside-resistance. The presence of ESBL-

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[15] Knee, J., Sumner, T., Adriano, Z., Berendes, D., de Bruijn, E., Schmidt, W.-P., Nalá, R., Cumming, O., & Brown, J. (2018). Risk factors for childhood enteric infection in Urban Maputo, Mozambique: A cross-sectional study. *PLOS Neglected Tropical Diseases*, *12*(11).

[16] Chereau, F., Opatowski, L., Tourdjman, M., & Vong, S. (2017). Risk assessment for antibiotic resistance in South East Asia. *BMJ*.

[17] Trongjit, S., Angkititrakul, S., Tuttle, R. E., Poungseree, J., Padungtod, P., & Chuanchuen, R. (2017). Prevalence and antimicrobial resistance insalmonella entericaisolated from broiler chickens, pigs and meat products in Thailand-cambodia border provinces. *Microbiology and Immunology*, *61*(1), 23–33.

[18] Atterby, C., Osbjer, K., Tepper, V., Rajala, E., Hernandez, J., Seng, S., Holl, D., Bonnedahl, J., Börjesson, S., Magnusson, U., & Järhult, J. D. (2019). Carriage of carbapenemase- and extended-spectrum cephalosporinase-producing escherichia coli and klebsiella pneumoniae in humans and livestock in rural Cambodia; gender and age differences and detection of bla oxa-48 in humans. *Zoonoses and Public Health*, *66*(6), 603–617.

related ARGs (*CTX, NDM, OXA,* and *SHV*) is particularly concerning because carbapenems are often the last line of defense in combatting multidrug-resistant bacteria.[19]

Although highly prevalent detection of ESBL-related ARGs in our study merits concern, there are additional and substantial potential health implications associated with the frequent detection of colistin-resistance genes in this study. The presence of colistin-resistance genes (namely *mcr-1*) have been sparsely characterized throughout Asia and its characterization is nearly always with respect to proximity to chickens. In China, pathogenic *E. coli* was identified as the primary carrier of the *mcr-1* gene, with higher prevalence in food-animals than in humans, suggesting zoonotic transmission.[20] One study in Vietnam found that colistin was commonly used for chicken production, and the presence of *mcr-1* carrying-bacteria was strongly associated with exposure to *mcr-1* positive chickens.[21] In rural Cambodia, *mcr-1* was detected in <10% of human and livestock samples,[22] which makes our finding of *mcr-1* in 27% of our samples more striking and merits more surveillance of emerging AMR in rural Cambodia, particularly because colistin is often used as a last line of defense against carbapenem-resistant gram-negative bacteria.[23] We were unable to estimate associations between ARG prevalence and the presence of animals because the survey did not capture sufficient information about animals.

Among antibiotic groups, there was no discernable difference between treatment arms, presumably due to high overall prevalence. However, we observed small but measurable associations between some household risk factors and ARG carriage. Households where animals were kept separate from child play area, households with child play area visibly free of feces, and household with a clean food preparation surface were found to have lower prevalence of colistin-related ARGs (aPR 0.85, 95% CI 0.69 to 1.03; aPR 0.85, 95% CI 0.72 to 1.00; aPR 0.80, 95% CI 0.61 to 1.04), consistent with the hypothesis that AMR proliferation may be occurring via environmental pathways that are not directly related to sanitation facilities.

The class 1 integron-integrase gene (*intI1*) is a suitable proxy for AMR spread because it has been linked to ARGs, is found in a variety of bacteria (both pathogenic and commensal), is facilitated by horizontal gene transfer, and has been associated with anthropogenic AMR globally.[24] So, our finding of *intI1* in 91% of all samples is concerning and signals the potential of further AMR spread in this study setting.

9.4 LIMITATIONS

Several limitations constrain the current analysis. We collected only one stool sample at endline for each participating child, which cannot inform chronic shedding—a possible effect of infection—or intermittent

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[19] Papp-Wallace, K. M., Endimiani, A., Taracila, M. A., & Bonomo, R. A. (2011). Carbapenems: Past, present, and future. *Antimicrobial Agents and Chemotherapy*, *55*(11), 4943–4960.

[20] Elbediwi, M., Li, Y., Paudyal, N., Pan, H., Li, X., Xie, S., Rajkovic, A., Feng, Y., Fang, W., Rankin, S. C., & Yue, M. (2019). Global burden of Colistin-resistant bacteria: Mobilized Colistin Resistance Genes Study (1980–2018). *Microorganisms*, *7*(10), 461.

[21] Trung, N. V., Matamoros, S., Carrique-Mas, J. J., Nghia, N. H., Nhung, N. T., Chieu, T. T., Mai, H. H., van Rooijen, W., Campbell, J., Wagenaar, J. A., Hardon, A., Mai, N. T., Hieu, T. Q., Thwaites, G., de Jong, M. D., Schultsz, C., & Hoa, N. T. (2017). Zoonotic transmission of MCR-1 colistin resistance gene from small-scale poultry farms, Vietnam. *Emerging Infectious Diseases*, *23*(3), 529–532.

[22] Atterby et al., 2019.

[23] Nation, R. L., Garonzik, S. M., Thamlikitkul, V., Giamarellos-Bourboulis, E. J., Forrest, A., Paterson, D. L., Li, J., & Silveira, F. P. (2016). Dosing guidance for intravenous colistin in critically-ill patients. *Clinical Infectious Diseases*.

[24] Gillings, M. R., Gaze, W. H., Pruden, A., Smalla, K., Tiedje, J. M., & Zhu, Y.-G. (2014). Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *The ISME Journal*, *9*(6), 1269–1279.

shedding. Cross-sectional study designs limit causal inference in examining associations and do not allow for any assessment of directionality in associations. In multiple parallel hypothesis testing, the risk of incorrectly rejecting a null hypothesis (Type I error) increases with each additional hypothesis tested,[25] meaning that some associations could be expected to be spurious. There are also several molecular methodological limitations. The TAC format limits the number of genes we can detect. The assays used also limit our ability to detect low-abundance enteric pathogens and ARGs due to assay LODs. Finally, many of the factors considered in this study were obtained from self-report (see Table 2), and thus prone to self-reporting biases.

9.5 RECOMMENDATIONS

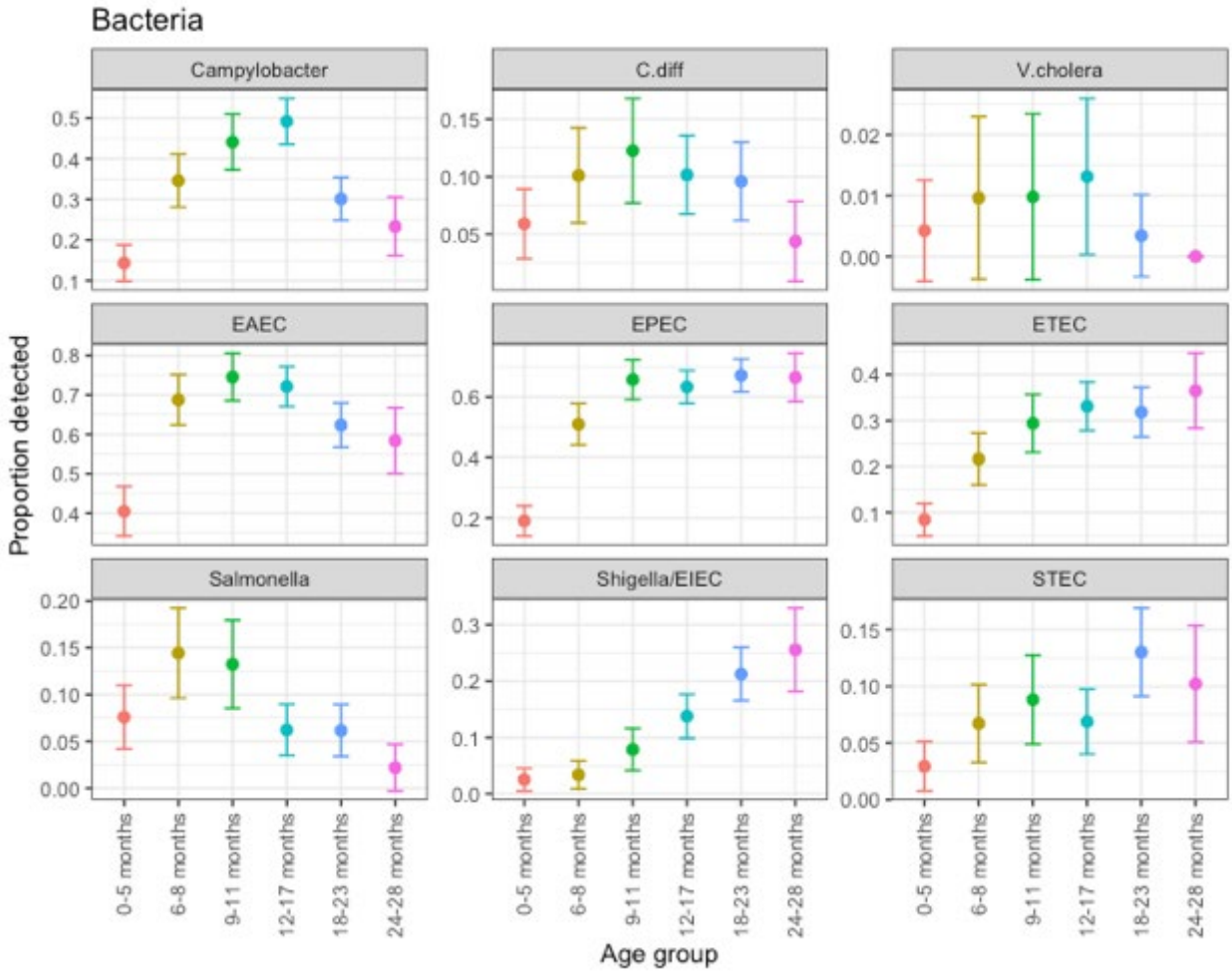
Overall, we detected high prevalence of enteric infection and ARG carriage across all treatment arms, suggesting that there were high levels of pathogen exposures during early age, despite interventions. Interruption of transmission will require more holistic and comprehensive interventions that include WASH improvements, as well as consideration of the living environment at both the household and community levels, which may include but are not limited to: safely managed sanitation, drainage, separation of animals and animal feces, and hygiene.

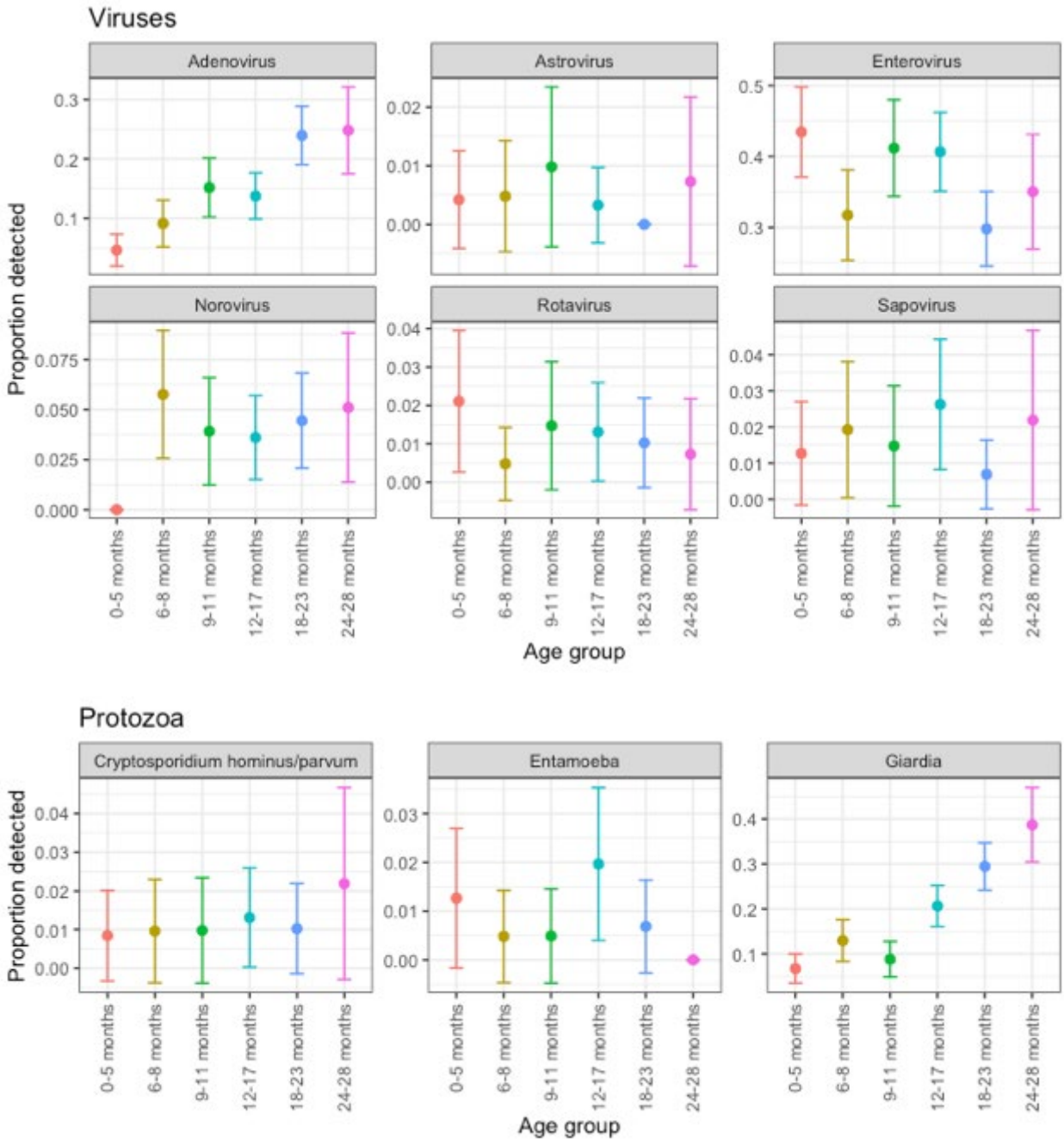
The findings of this study support a move to transformative WASH that can more effectively limit exposures in early childhood when the effects of enteric infections are greatest, beyond modest expansion of sanitation access as observed in this intervention program. Analysis presented in the Final Evaluation Report suggests that better targeting of sanitation interventions is needed to show health impacts. Taken together, the findings from this evaluation suggest that sanitation programming needs to achieve higher levels of coverage with more holistic strategies to interrupt the transmission of enteric pathogens to achieve measurable health impacts in high-burden settings. These findings are consistent with a growing number of other studies that have revealed a need for transformative approaches to WASH to impact global public health.[26]

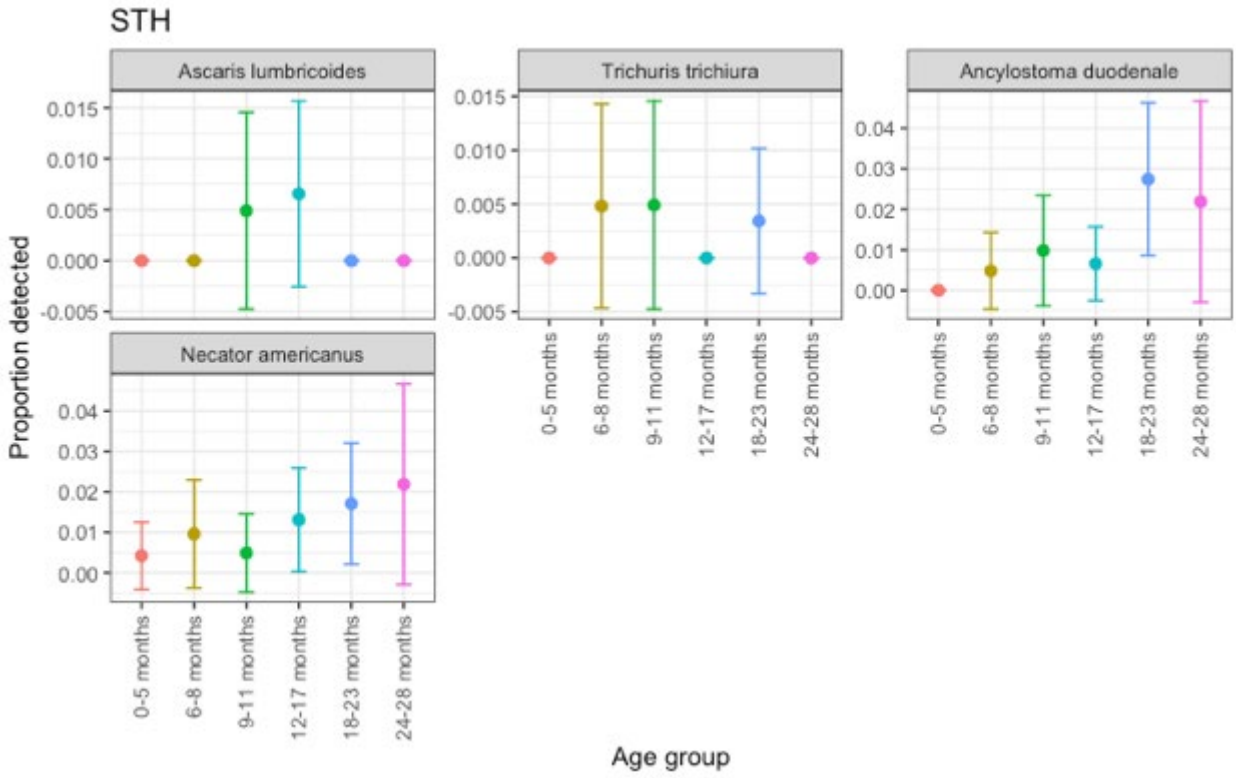
[25] Blakesley, R. E., Mazumdar, S., Dew, M. A., Houck, P. R., Tang, G., Reynolds, C. F., & Butters, M. A. (2009). Comparisons of methods for multiple hypothesis testing in neuropsychological research. *Neuropsychology*, *23*(2), 255–264.

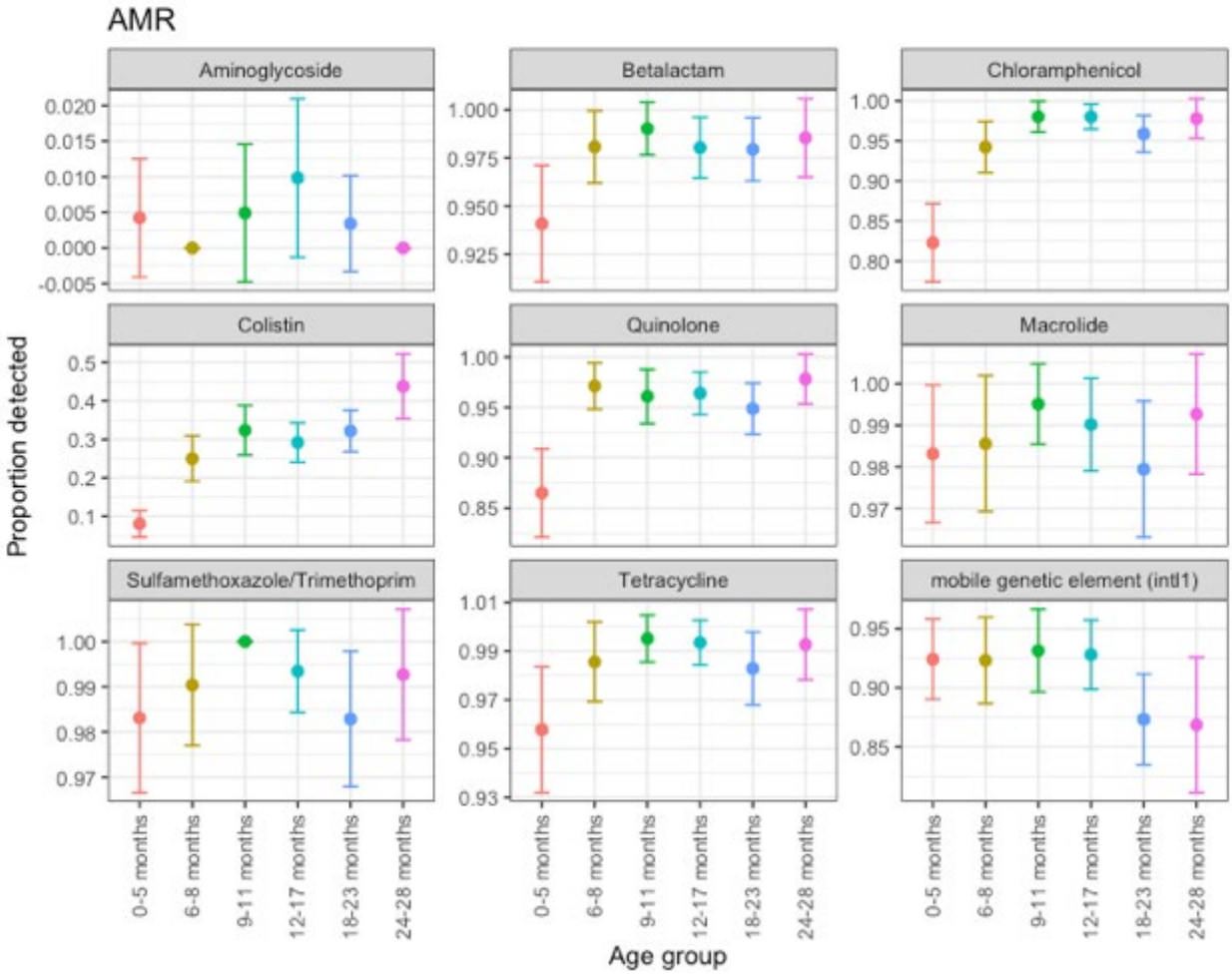
[26] Cumming, O., Arnold, B. F., Ban, R., Clasen, T., Esteves Mills, J., Freeman, M. C., Gordon, B., Guiteras, R., Howard, G., Hunter, P. R., Johnston, R. B., Pickering, A. J., Prendergast, A. J., Prüss-Ustün, A., Rosenboom, J. W., Spears, D., Sundberg, S., Wolf, J., Null, C., … Colford, J. M. (2019). The implications of three major new trials for the effect of water, sanitation and hygiene on childhood diarrhea and stunting: A consensus statement. *BMC Medicine*, *17*(1).

ANNEX A: DETECTS BY AGE STRATA

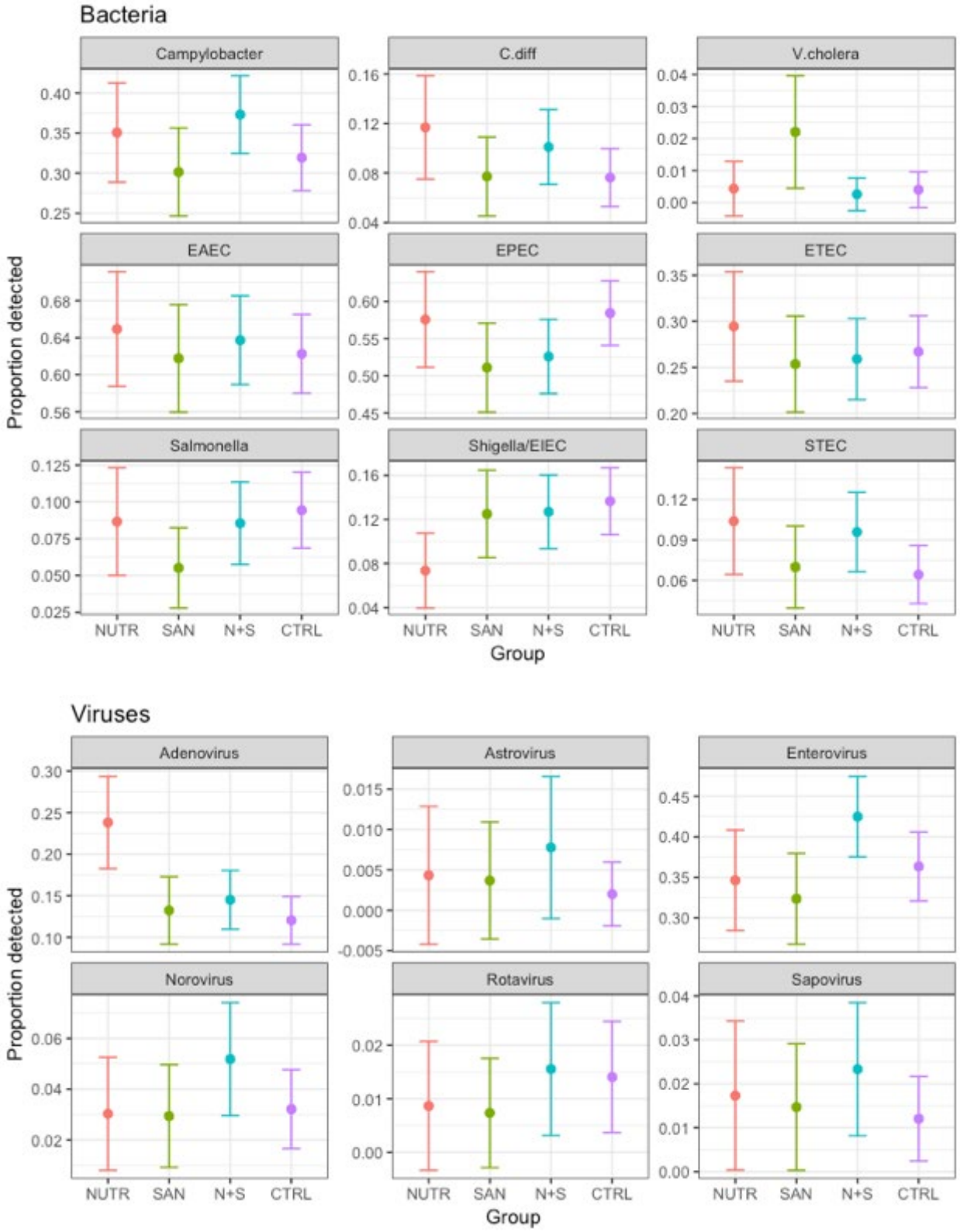


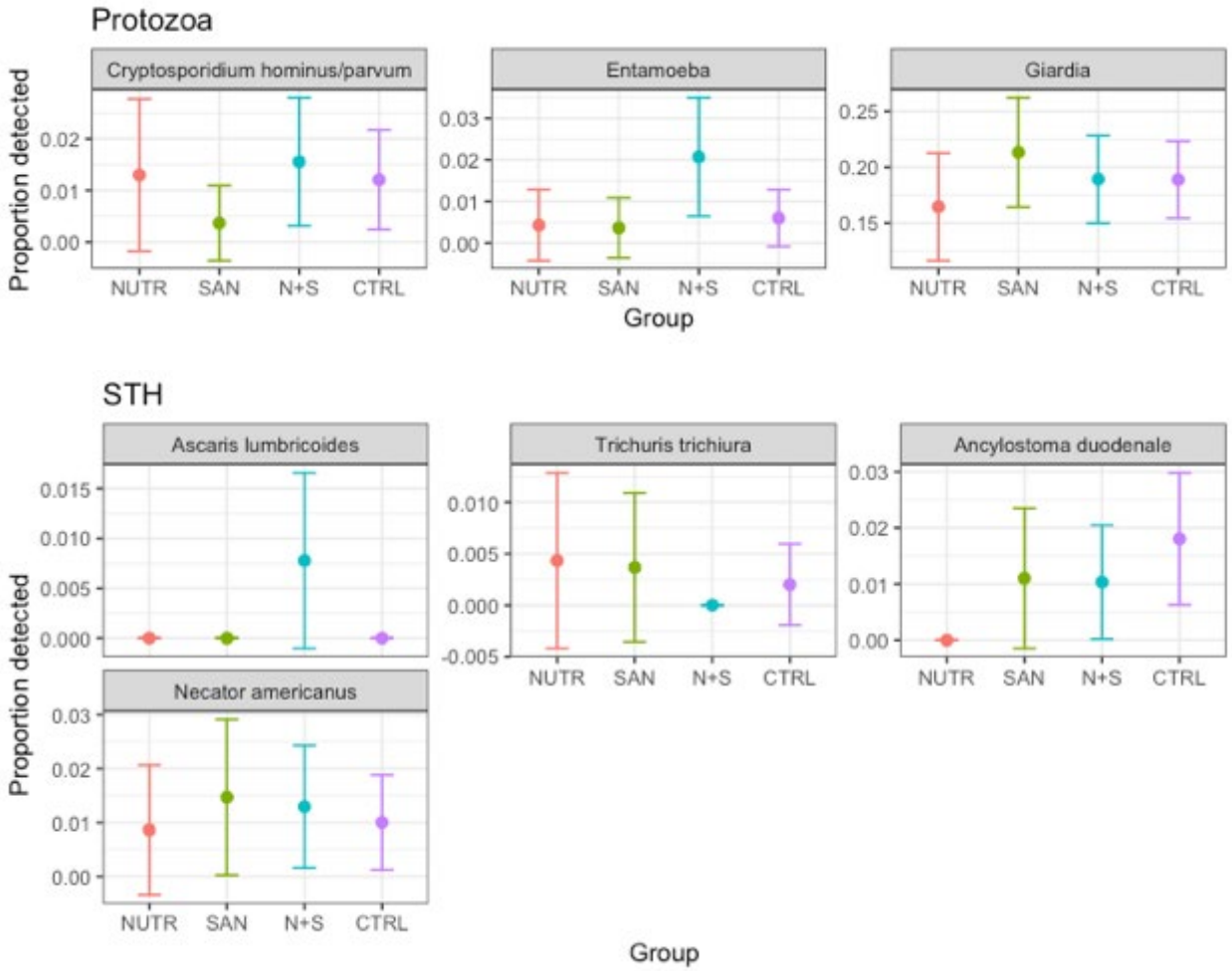

Viruses
Adenovirus
Astrovirus
Enterovirus
Norovirus
Rotavirus
Sapovirus
Proportion detected
0-5 months
6-8 months
9-11 months
12-17 months
18-23 months
24-28 months
Age group
Protozoa
Cryptosporidium hominus/parvum
Entamoeba
Giardia
Proportion detected
Age group


STH
Ascaris lumbricoides
Trichuris trichiura
Ancylostoma duodenale
Necator americanus
Proportion detected
0.015
0.010
0.005
0.000
-0.005
0.04
0.03
0.02
0.01
0.00
0-5 months
6-8 months
9-11 months
12-17 months
18-23 months
24-28 months
Age group


AMR
Aminoglycoside
Betalactam
Chloramphenicol
Colistin
Quinolone
Macrolide
Sulfamethoxazole/Trimethoprim
Tetracycline
mobile genetic element (intl1)
Proportion detected
0-5 months
6-8 months
9-11 months
12-17 months
18-23 months
24-28 months
Age group

ANNEX B: DETECTS BY TREATMENT ARM




Protozoa
Cryptosporidium hominus/parvum
Entamoeba
Giardia
Proportion detected
0.02
0.01
0.00
0.03
0.02
0.01
0.00
0.25
0.20
0.15
NUTR
SAN
N+S
CTRL
Group
STH
Ascaris lumbricoides
Trichuris trichiura
Ancylostoma duodenale
Necator americanus
Proportion detected
0.015
0.010
0.005
0.000
0.010
0.005
0.000
-0.005
0.03
0.02
0.01
0.00
NUTR
SAN
N+S
CTRL
Group

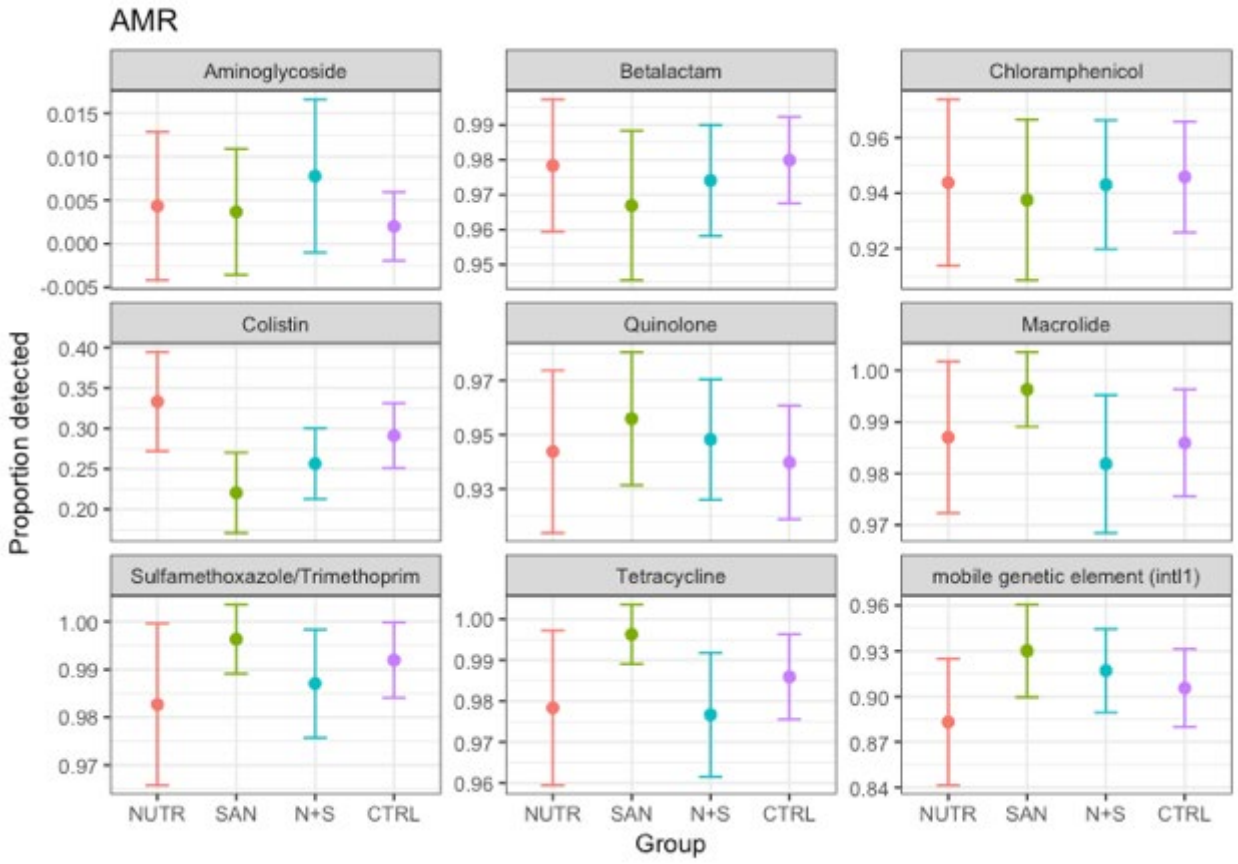


TABLE B-1: ENTERIC PATHOGEN AND AMR FAMILY PREVALENCE

	NUTR (N=305)	SAN (N=330)	NUTR+SAN (N=438)	CTRL (N=544)
Viruses				
Adenovirus	0.24 (0.18, 0.29)	0.13 (0.09, 0.17)	0.15 (0.11, 0.18)	0.12 (0.09, 0.15)
Astrovirus	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.01 (0.00, 0.02)	0.00 (0.00, 0.01)
Enterovirus	0.35 (0.28, 0.41)	0.32 (0.27, 0.38)	0.42 (0.38, 0.47)	0.36 (0.32, 0.41)
Norovirus	0.03 (0.01, 0.05)	0.03 (0.01, 0.05)	0.05 (0.03, 0.07)	0.03 (0.02, 0.05)
Rotavirus	0.01 (0.00, 0.02)	0.01 (0.00, 0.02)	0.02 (0.00, 0.03)	0.01 (0.00, 0.02)
Sapovirus	0.02 (0.00, 0.03)	0.01 (0.00, 0.03)	0.02 (0.01, 0.04)	0.01 (0.00, 0.02)
Bacteria				
Camploybacter spp.	0.35 (0.29, 0.41)	0.30 (0.25, 0.36)	0.37 (0.32, 0.42)	0.32 (0.28, 0.36)
Clostridium difficile	0.12 (0.08, 0.16)	0.08 (0.05, 0.11)	0.10 (0.07, 0.13)	0.08 (0.05, 0.10)
V.cholera	0.00 (0.00, 0.01)	0.02 (0.00, 0.04)	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)
EAEC	0.65 (0.59, 0.71)	0.62 (0.56, 0.68)	0.64 (0.59, 0.69)	0.62 (0.58, 0.67)
EPEC	0.58 (0.51, 0.64)	0.51 (0.45, 0.57)	0.53 (0.48, 0.58)	0.58 (0.54, 0.63)

	NUTR (N=305)	SAN (N=330)	NUTR+SAN (N=438)	CTRL (N=544)
ETEC	0.29 (0.24, 0.35)	0.25 (0.20, 0.31)	0.26 (0.22, 0.30)	0.27 (0.23, 0.31)
Salmonella spp.	0.09 (0.05, 0.12)	0.06 (0.03, 0.08)	0.09 (0.06, 0.11)	0.09 (0.07, 0.12)
Shigella spp.	0.07 (0.04, 0.11)	0.13 (0.09, 0.16)	0.13 (0.09, 0.16)	0.14 (0.11, 0.17)
STEC	0.10 (0.06, 0.14)	0.07 (0.04, 0.10)	0.10 (0.07, 0.13)	0.06 (0.04, 0.09)
Protozoa				
Cryptosporidium	0.01 (0.00, 0.03)	0.00 (0.00, 0.01)	0.02 (0.00, 0.03)	0.01 (0.00, 0.02)
Entamoeba	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.02 (0.01, 0.03)	0.01 (0.00, 0.01)
Giardia	0.16 (0.12, 0.21)	0.21 (0.16, 0.26)	0.19 (0.15, 0.23)	0.19 (0.15, 0.22)
STH				
Ascaris lumbricoides	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.01 (0.00, 0.02)	0.00 (0.00, 0.00)
Trichuris trichiura	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.00 (0.00, 0.00)	0.00 (0.00, 0.01)
Ancylostoma duodenale	0.00 (0.00, 0.00)	0.01 (0.00, 0.02)	0.01 (0.00, 0.02)	0.02 (0.01, 0.03)
Necator americanus	0.01 (0.00, 0.02)	0.01 (0.00, 0.03)	0.01 (0.00, 0.02)	0.01 (0.00, 0.02)
Antibiotic families				
Aminoglycoside	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.01 (0.00, 0.02)	0.00 (0.00, 0.01)
ß-lactam	0.98 (0.96, 1.00)	0.97 (0.95, 0.99)	0.97 (0.96, 0.99)	0.98 (0.97, 0.99)
Chloramphenicol	0.94 (0.91, 0.97)	0.94 (0.91, 0.97)	0.94 (0.92, 0.97)	0.95 (0.93, 0.97)
Colistin	0.33 (0.27, 0.39)	0.22 (0.17, 0.27)	0.26 (0.21, 0.30)	0.29 (0.25, 0.33)
Quinolone	0.94 (0.91, 0.97)	0.96 (0.93, 0.98)	0.95 (0.93, 0.97)	0.94 (0.92, 0.96)
Macrolide	0.99 (0.97, 1.00)	1.00 (0.99, 1.00)	0.98 (0.97, 1.00)	0.99 (0.98, 1.00)
IntI1 (MGE)	0.88 (0.84, 0.92)	0.93 (0.90, 0.96)	0.92 (0.89, 0.94)	0.91 (0.88, 0.93)
Sulfonamide	0.99 (0.97, 1.00)	0.99 (0.99, 1.00)	0.99 (0.98, 1.00)	0.99 (0.99, 1.00)
Tetracycline	0.98 (0.96, 1.00)	1.00 (0.99, 1.00)	0.98 (0.96, 0.99)	0.99 (0.98, 1.00)
Trimethoprim	0.90 (0.87, 0.94)	0.91 (0.88, 0.94)	0.89 (0.87, 0.92)	0.90 (0.88, 0.93)

TABLE B-2: ENTERIC PATHOGEN GENE AND ARG PREVALENCE

Target	Gene	NUTR (N=305)	SAN (N=333)	NUTR+SAN (N=438)	CTRL (N=544)	Total (N=1620)
Campylobacter spp.	*CAMP*	0.34 (0.29, 0.39)	0.34 (0.29, 0.39)	0.37 (0.32, 0.42)	0.31 (0.28, 0.35)	0.34 (0.32, 0.36)
Clostridium difficile	*CDIF*	0.11 (0.07, 0.14)	0.08 (0.05, 0.10)	0.09 (0.07, 0.12)	0.07 (0.05, 0.10)	0.09 (0.07, 0.10)

Target	Gene	NUTR (N=305)	SAN (N=333)	NUTR+SAN (N=438)	CTRL (N=544)	Total (N=1620)
EAEC	*EAEC_aaic*	0.40 (0.35, 0.46)	0.38 (0.33, 0.43)	0.41 (0.36, 0.45)	0.39 (0.35, 0.43)	0.40 (0.37, 0.42)
EAEC	*EAEC_aata*	0.55 (0.49, 0.61)	0.50 (0.44, 0.55)	0.52 (0.48, 0.57)	0.49 (0.45, 0.53)	0.51 (0.49, 0.54)
EPEC	*EPEC_bfpa*	0.05 (0.03, 0.08)	0.06 (0.04, 0.09)	0.08 (0.05, 0.10)	0.08 (0.06, 0.11)	0.07 (0.06, 0.08)
EPEC	*EPEC_eae*	0.56 (0.50, 0.62)	0.51 (0.45, 0.56)	0.53 (0.49, 0.58)	0.59 (0.55, 0.63)	0.55 (0.53, 0.58)
ETEC	*ETEC_LT*	0.25 (0.20, 0.29)	0.20 (0.16, 0.25)	0.20 (0.16, 0.23)	0.21 (0.17, 0.24)	0.21 (0.19, 0.23)
ETEC	*ETEC_sth*	0.01 (0.00, 0.03)	0.01 (0.00, 0.02)	0.04 (0.02, 0.06)	0.02 (0.01, 0.03)	0.02 (0.02, 0.03)
ETEC	*ETEC_stp*	0.11 (0.08, 0.15)	0.10 (0.07, 0.13)	0.11 (0.08, 0.14)	0.08 (0.06, 0.11)	0.10 (0.09, 0.11)
Shigella spp./EIEC	*IPAH*	0.08 (0.05, 0.11)	0.12 (0.08, 0.15)	0.12 (0.09, 0.15)	0.13 (0.10, 0.16)	0.11 (0.10, 0.13)
Salmonella spp.	*SALM*	0.09 (0.06, 0.12)	0.06 (0.03, 0.08)	0.09 (0.06, 0.12)	0.09 (0.06, 0.11)	0.08 (0.07, 0.10)
STEC	*STEC1*	0.06 (0.03, 0.08)	0.05 (0.02, 0.07)	0.06 (0.04, 0.09)	0.05 (0.03, 0.07)	0.05 (0.04, 0.06)
STEC	*STEC2*	0.08 (0.05, 0.11)	0.04 (0.02, 0.06)	0.07 (0.05, 0.10)	0.05 (0.03, 0.07)	0.06 (0.05, 0.07)
Vibrio cholera	*VTOX*	0.00 (0.00, 0.01)	0.02 (0.00, 0.03)	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.01 (0.00, 0.01)
Adenovirus	*ADEV*	0.35 (0.30, 0.41)	0.27 (0.22, 0.31)	0.23 (0.19, 0.27)	0.19 (0.16, 0.23)	0.25 (0.23, 0.27)
Astrovirus	*ASTR*	0.01 (0.00, 0.02)	0.00 (0.00, 0.01)	0.01 (0.00, 0.02)	0.00 (0.00, 0.01)	0.01 (0.00, 0.01)
Enterovirus	*ENTV*	0.32 (0.27, 0.37)	0.32 (0.27, 0.37)	0.39 (0.34, 0.43)	0.34 (0.30, 0.38)	0.34 (0.32, 0.37)
Norovirus	*NORO1*	0.01 (0.00, 0.02)	0.00 (0.00, 0.01)	0.01 (0.00, 0.02)	0.01 (0.00, 0.01)	0.01 (0.00, 0.01)
Norovirus	*NORO2*	0.02 (0.00, 0.03)	0.02 (0.01, 0.04)	0.03 (0.02, 0.05)	0.03 (0.01, 0.04)	0.03 (0.02, 0.03)
Rotavirus	*ROTA*	0.01 (0.00, 0.02)	0.01 (0.00, 0.01)	0.01 (0.00, 0.02)	0.01 (0.00, 0.02)	0.01 (0.01, 0.02)
Sapovirus	*SAP_I*	0.01 (0.00, 0.02)	0.01 (0.00, 0.02)	0.02 (0.01, 0.03)	0.01 (0.00, 0.02)	0.01 (0.01, 0.02)
Sapovirus	*SAP_IV*	0.00 (0.00, 0.01)	0.01 (0.00, 0.02)	0.00 (0.00, 0.01)	0.00 (0.00, 0.00)	0.00 (0.00, 0.01)
Cryptosporidium parvum	*CRYP_P*	0.01 (0.00, 0.02)	0.00 (0.00, 0.00)	0.01 (0.00, 0.01)	0.00 (0.00, 0.00)	0.00 (0.00, 0.01)
Cryptosporidium hominis	*CRYP_h*	0.01 (0.00, 0.02)	0.00 (0.00, 0.01)	0.01 (0.00, 0.01)	0.01 (0.00, 0.02)	0.01 (0.00, 0.01)
Entamoeba histolytica	*ENHI*	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.02 (0.01, 0.03)	0.01 (0.00, 0.01)	0.01 (0.00, 0.01)
Giardia spp.	*GIAR*	0.17 (0.13, 0.21)	0.20 (0.16, 0.25)	0.19 (0.15, 0.23)	0.19 (0.15, 0.22)	0.19 (0.17, 0.21)
Ancylostoma duodenale	*ANCY*	0.00 (0.00, 0.00)	0.01 (0.00, 0.02)	0.01 (0.00, 0.02)	0.02 (0.01, 0.03)	0.01 (0.01, 0.02)

Target	Gene	NUTR (N=305)	SAN (N=333)	NUTR+SAN (N=438)	CTRL (N=544)	Total (N=1620)
Ascaris lumbricoides	*ASLU*	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.01 (0.00, 0.01)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
Necator americanus	*NECA*	0.01 (0.00, 0.02)	0.02 (0.00, 0.03)	0.01 (0.00, 0.02)	0.01 (0.00, 0.02)	0.01 (0.01, 0.02)
Trichuris trichiura	*TRTR*	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.00 (0.00, 0.00)	0.00 (0.00, 0.01)	0.00 (0.00, 0.00)
Canine *Bacteroides*	*BacCan*	0.27 (0.22, 0.32)	0.27 (0.23, 0.32)	0.29 (0.25, 0.33)	0.28 (0.24, 0.32)	0.28 (0.26, 0.30)
Cow *Bacteroides*	*BacCow*	0.75 (0.70, 0.80)	0.75 (0.71, 0.80)	0.72 (0.68, 0.76)	0.72 (0.69, 0.76)	0.73 (0.71, 0.76)
Human *Bacteroides*	*BacHum*	0.82 (0.78, 0.87)	0.81 (0.77, 0.85)	0.82 (0.79, 0.86)	0.85 (0.82, 0.88)	0.83 (0.81, 0.85)
Human mitochondrial DNA	*mtDNA*	0.98 (0.96, 1.00)	0.99 (0.98, 1.00)	0.98 (0.96, 0.99)	0.97 (0.96, 0.99)	0.98 (0.97, 0.99)
Aminoglycoside resistance	*armA*	0.01 (0.00, 0.02)	0.00 (0.00, 0.01)	0.01 (0.00, 0.01)	0.01 (0.00, 0.01)	0.01 (0.00, 0.01)
ß-lactam resistance	*CTXM1*	0.88 (0.85, 0.92)	0.86 (0.82, 0.90)	0.88 (0.85, 0.91)	0.88 (0.85, 0.91)	0.87 (0.86, 0.89)
ß-lactam resistance	*CTXM2M74*	0.07 (0.04, 0.09)	0.07 (0.04, 0.10)	0.06 (0.04, 0.09)	0.08 (0.05, 0.10)	0.07 (0.06, 0.08)
ß-lactam resistance	*CTXM8M25*	0.46 (0.40, 0.52)	0.41 (0.36, 0.47)	0.40 (0.35, 0.44)	0.40 (0.36, 0.44)	0.41 (0.39, 0.44)
ß-lactam resistance	*CTXM9*	0.82 (0.78, 0.86)	0.80 (0.76, 0.84)	0.78 (0.74, 0.82)	0.81 (0.77, 0.84)	0.80 (0.78, 0.82)
ß-lactam resistance	*NDM*	0.13 (0.09, 0.17)	0.06 (0.04, 0.09)	0.07 (0.05, 0.09)	0.09 (0.07, 0.12)	0.09 (0.07, 0.10)
ß-lactam resistance	*OXA1*	0.56 (0.51, 0.62)	0.47 (0.42, 0.53)	0.49 (0.44, 0.54)	0.50 (0.46, 0.55)	0.51 (0.48, 0.53)
ß-lactam resistance	*OXA9*	0.00 (0.00, 0.01)	0.01 (0.00, 0.02)	0.01 (0.00, 0.02)	0.01 (0.00, 0.01)	0.01 (0.00, 0.01)
ß-lactam resistance	*SHV*	0.93 (0.90, 0.96)	0.90 (0.87, 0.93)	0.89 (0.86, 0.92)	0.93 (0.90, 0.95)	0.91 (0.90, 0.92)
ß-lactam resistance	*VIM*	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.00 (0.00, 0.00)
Chloramphenicol resistance	*catA1*	0.70 (0.65, 0.76)	0.67 (0.62, 0.72)	0.70 (0.65, 0.74)	0.70 (0.66, 0.74)	0.69 (0.67, 0.72)
Chloramphenicol resistance	*catB3*	0.35 (0.30, 0.41)	0.32 (0.27, 0.37)	0.30 (0.26, 0.35)	0.28 (0.24, 0.32)	0.31 (0.29, 0.33)
Chloramphenicol resistance	*cmlA*	0.82 (0.77, 0.86)	0.80 (0.75, 0.84)	0.78 (0.74, 0.82)	0.78 (0.74, 0.81)	0.79 (0.77, 0.81)
Chloramphenicol resistance	*floR*	0.86 (0.82, 0.90)	0.84 (0.80, 0.88)	0.87 (0.84, 0.90)	0.87 (0.84, 0.89)	0.86 (0.84, 0.88)
Colistin resistance	*mcr1*	0.34 (0.28, 0.39)	0.23 (0.19, 0.28)	0.26 (0.22, 0.31)	0.30 (0.26, 0.33)	0.28 (0.26, 0.30)
Macrolide resistance	*ermB*	0.92 (0.89, 0.95)	0.93 (0.90, 0.96)	0.92 (0.89, 0.94)	0.92 (0.89, 0.94)	0.92 (0.91, 0.93)
Macrolide resistance	*mphA*	0.96 (0.94, 0.98)	0.95 (0.93, 0.97)	0.93 (0.91, 0.95)	0.95 (0.94, 0.97)	0.95 (0.94, 0.96)

Target	Gene	NUTR (N=305)	SAN (N=333)	NUTR+SAN (N=438)	CTRL (N=544)	Total (N=1620)
Quinolone resistance	*aac6Ib_104R*	0.64 (0.59, 0.70)	0.59 (0.54, 0.65)	0.59 (0.55, 0.64)	0.63 (0.58, 0.67)	0.61 (0.59, 0.64)
Quinolone resistance	*aac6Ib_104W*	0.19 (0.15, 0.23)	0.20 (0.15, 0.24)	0.18 (0.15, 0.22)	0.14 (0.11, 0.17)	0.17 (0.15, 0.19)
Quinolone resistance	*gyrA83L*	0.86 (0.82, 0.90)	0.82 (0.78, 0.86)	0.84 (0.81, 0.88)	0.82 (0.79, 0.85)	0.83 (0.82, 0.85)
Quinolone resistance	*parC80I*	0.33 (0.28, 0.38)	0.26 (0.22, 0.31)	0.26 (0.22, 0.30)	0.27 (0.24, 0.31)	0.28 (0.26, 0.30)
Quinolone resistance	*qnrA*	0.06 (0.03, 0.08)	0.08 (0.05, 0.10)	0.05 (0.03, 0.07)	0.04 (0.03, 0.06)	0.05 (0.04, 0.07)
Quinolone resistance	*qnrB1*	0.53 (0.48, 0.59)	0.49 (0.44, 0.54)	0.46 (0.42, 0.51)	0.49 (0.45, 0.53)	0.49 (0.47, 0.52)
Sulfonamide resistance	*sul1*	0.95 (0.93, 0.98)	0.97 (0.95, 0.99)	0.96 (0.94, 0.98)	0.95 (0.93, 0.97)	0.96 (0.95, 0.97)
Sulfonamide resistance	*sul2*	0.98 (0.96, 1.00)	0.99 (0.98, 1.00)	0.98 (0.97, 1.00)	0.99 (0.98, 1.00)	0.99 (0.98, 0.99)
Tetracycline resistance	*tetA*	0.97 (0.95, 0.99)	0.98 (0.97, 1.00)	0.97 (0.95, 0.99)	0.96 (0.95, 0.98)	0.97 (0.96, 0.98)
Tetracycline resistance	*tetB*	0.88 (0.85, 0.92)	0.86 (0.82, 0.90)	0.85 (0.82, 0.89)	0.87 (0.84, 0.90)	0.87 (0.85, 0.88)
Trimethoprim resistance	*dfrA17*	0.90 (0.87, 0.94)	0.91 (0.88, 0.94)	0.89 (0.87, 0.92)	0.90 (0.88, 0.93)	0.90 (0.89, 0.92)
Integron-integrase	*intI1*	0.90 (0.86, 0.93)	0.92 (0.90, 0.95)	0.90 (0.87, 0.93)	0.90 (0.87, 0.92)	0.90 (0.89, 0.92)

ANNEX C: TABLES OF ADJUSTED PREVALENCE RATIOS

FIGURE C-1: PROPORTION OF DETECTED ARGS

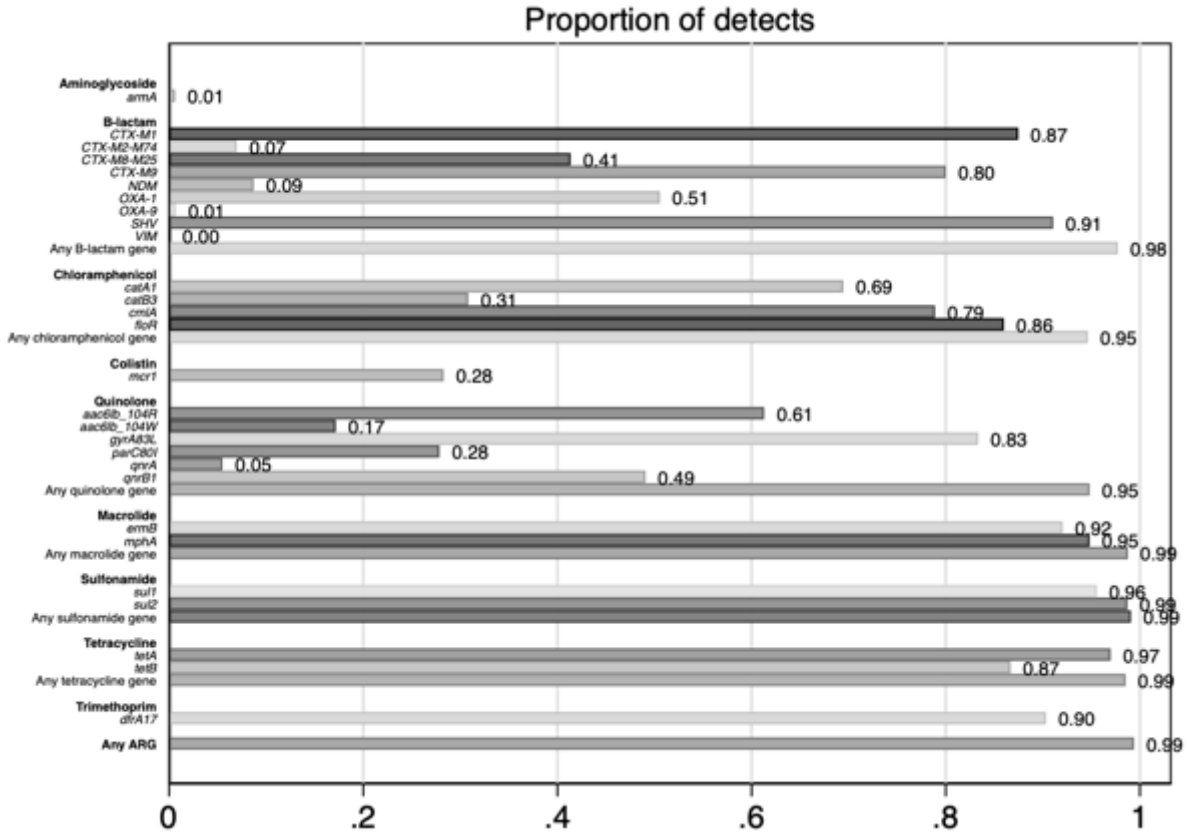


TABLE C-1: ADJUSTED PREVALENCE RATIOS, COMPARED TO CONTROL

	NUTR	SAN	NUTR+SAN
Bacteria			
Camploybacter spp.	1.13 (0.91, 1.41)	0.96 (0.77, 1.20)	1.20 (1.00, 1.45)*
Clostridium difficile	1.46 (0.91, 2.33)	0.96 (0.58, 1.61)	1.23 (0.80, 1.89)
EAEC	1.05 (0.93, 1.18)	0.99 (0.88, 1.11)	1.04 (0.93, 1.15)
EPEC	0.98 (0.86, 1.11)	0.86 (0.75, 0.99)*	0.93 (0.83, 1.05)
aEPEC	1.01 (0.86, 1.18)	0.89 (0.75, 1.05)	0.89 (0.76, 1.04)
tEPEC	0.42 (0.20, 0.87)*	0.45 (0.24, 0.87)*	0.74 (0.44, 1.22)
ETEC	1.11 (0.86, 1.42)	0.94 (0.73, 1.20)	1.00 (0.80, 1.25)
ETEC-LT	1.19 (0.90, 1.58)	1.01 (0.77, 1.34)	0.92 (0.71, 1.20)
ETEC-ST	1.29 (0.84, 1.98)	1.12 (0.73, 1.71)	1.43 (0.99, 2.07)
ETEC-LT/ST	1.89 (1.07, 3.33)*	1.67 (0.95, 2.96)	1.57 (0.92, 2.68)

	NUTR	SAN	NUTR+SAN
Salmonella spp.	0.96 (0.58, 1.59)	0.60 (0.34, 1.06)	0.87 (0.55, 1.37)
EIEC/*Shigella* spp.	0.50 (0.31, 0.83)**	0.88 (0.60, 1.27)	0.96 (0.69, 1.35)
STEC	1.60 (0.97, 2.63)	1.07 (0.62, 1.86)	1.51 (0.94, 2.41)
Viruses			
Adenovirus	1.88 (1.35, 2.63)***	1.09 (0.74, 1.59)	1.24 (0.88, 1.75)
Enterovirus	0.95 (0.77, 1.18)	0.91 (0.74, 1.12)	1.17 (0.99, 1.38)
Protozoa			
Giardia	0.87 (0.62, 1.23)	1.11 (0.84, 1.46)	1.10 (0.84, 1.43)
Antibiotic families			
ß-lactam	1.00 (0.97, 1.02)	0.99 (0.96, 1.01)	0.99 (0.97, 1.01)
Chloramphenicol	1.00 (0.96, 1.04)	0.99 (0.95, 1.03)	1.00 (0.97, 1.03)
Colistin	1.16 (0.92, 1.45)	0.76 (0.59, 0.98)*	0.92 (0.74, 1.14)
Quinolone	1.01 (0.97, 1.04)	1.02 (0.98, 1.05)	1.01 (0.98, 1.04)
Macrolide	1.00 (0.98, 1.02)	1.01 (1.00, 1.02)	1.00 (0.98, 1.01)
IntI1 (MGE)	0.97 (0.92, 1.03)	1.03 (0.98, 1.07)	1.01 (0.97, 1.05)
Sulfonamide			
Tetracycline	0.99 (0.97, 1.01)	1.01 (1.00, 1.02)	0.99 (0.97, 1.01)
Trimethoprim			

***p<0.001; **p<0.01; *p<0.05; targets with <5% prevalence were omitted from PR analyses: *V.cholera*, astrovirus, norovirus, rotavirus, sapovirus, *Cryptosporidium*, *Entamoeba,* all STHs (*Ascaris, Trichuris, Ancylostoma,* and *Necator*), and aminoglycoside-related ARGs.

TABLE C-2: ADJUSTED PREVALENCE RATIOS, COMPARED TO COMBINED ARM

	NUTR	SAN
Bacteria		
Camploybacter spp.	0.94 (0.75, 1.17)	0.80 (0.64, 1.00)*
Clostridium difficile	1.18 (0.74, 1.88)	0.78 (0.47, 1.29)
EAEC	1.01 (0.90, 1.14)	0.96 (0.85, 1.08)
EPEC	1.05 (0.92, 1.21)	0.93 (0.80, 1.07)
aEPEC	1.13 (0.95, 1.36)	1.00 (0.83, 1.21)
tEPEC	0.57 (0.26, 1.24)	0.62 (0.31, 1.24)
ETEC	1.11 (0.86, 1.44)	0.94 (0.72, 1.22)
ETEC-LT	1.30 (0.96, 1.76)	1.10 (0.82, 1.49)
ETEC-ST	0.90 (0.60, 1.36)	0.78 (0.51, 1.18)
ETEC-LT/ST	1.20 (0.70, 2.06)	1.07 (0.62, 1.83)

	NUTR	SAN
Salmonella spp.	1.10 (0.64, 1.89)	0.69 (0.38, 1.26)
EIEC/*Shigella* spp.	0.52 (0.31, 0.88)*	0.91 (0.61, 1.36)
STEC	1.06 (0.65, 1.73)	0.71 (0.41, 1.22)
Viruses		
Adenovirus	1.52 (1.09, 2.11)*	0.88 (0.60, 1.29)
Enterovirus	0.82 (0.66, 1.001)	0.78 (0.63, 0.96)*
Protozoa		
Giardia	0.80 (0.56, 1.13)	1.01 (0.75, 1.36)
Antibiotic families		
ß-lactam	1.00 (0.98, 1.03)	0.99 (0.96, 1.02)
Chloramphenicol	1.00 (0.96, 1.04)	0.99 (0.95, 1.03)
Colistin	1.26 (0.99, 1.61)	0.83 (0.63, 1.10)
Quinolone	1.00 (0.96, 1.03)	1.00 (0.97, 1.04)
Macrolide	1.00 (0.98, 1.02)	1.01 (1.00, 1.03)
IntI1 (MGE)	0.97 (0.91, 1.02)	1.02 (0.97, 1.07)
Sulfonamide	1.00 (0.98, 1.02)	1.00 (0.99, 1.00)
Tetracycline	1.00 (0.98, 1.02)	1.02 (1.00, 1.03)
Trimethoprim	1.00 (0.96, 1.05)	1.00 (0.96, 1.05)

TABLE C-3: ADJUSTED PREVALENCE RATIOS OF RISK FACTORS ON ENTERIC INFECTION AND ARG CARRIAGE (TABLE 1 OF 3)

	Village sanitation coverage	HH sanitation (any)	HH improved sanitation	HH shared sanitation (any)	Adequate disposal of child stool
Campylobacter coli/jejuni	1.15 (0.79, 1.68)	1.07 (0.85, 1.33)	1.09 (0.94, 1.26)	0.95 (0.81, 1.10)	0.95 (0.81, 1.10)
Clostridium difficile	1.55 (0.63, 3.80)	1.29 (0.72, 2.32)	1.31 (0.91, 1.89)	0.84 (0.58, 1.21)	0.84 (0.58, 1.21)
EAEC	0.99 (0.81, 1.21)	1.05 (0.93, 1.18)	0.95 (0.88, 1.03)	1.08 (0.99, 1.17)	1.08 (0.99, 1.17)
EPEC (any)	0.94 (0.75, 1.18)	1.00 (0.87, 1.14)	1.03 (0.94, 1.13)	0.97 (0.88, 1.06)	0.97 (0.88, 1.06)
aEPEC	1.09 (0.81, 1.48)	1.06 (0.89, 1.27)	1.12 (0.99, 1.26)	0.92 (0.81, 1.04)	0.92 (0.81, 1.04)
tEPEC	0.42 (0.20, 0.91)*	0.58 (0.36, 0.95)*	0.77 (0.51, 1.15)	0.97 (0.64, 1.47)	0.97 (0.64, 1.47)
ETEC (any)	1.03 (0.65, 1.64)	0.90 (0.69, 1.16)	0.82 (0.69, 0.98)*	1.16 (0.98, 1.39)	1.16 (0.98, 1.39)
ETEC-LT	1.08 (0.63, 1.86)	0.85 (0.63, 1.14)	0.86 (0.70, 1.05)	1.08 (0.88, 1.33)	1.08 (0.88, 1.33)
ETEC-ST	1.35 (0.61, 2.98)	1.04 (0.67, 1.59)	0.84 (0.64, 1.12)	1.21 (0.91, 1.61)	1.21 (0.91, 1.61)
ETEC-LT/ST	1.97 (0.63, 6.10)	0.96 (0.55, 1.68)	0.98 (0.68, 1.41)	1.00 (0.68, 1.47)	1.00 (0.68, 1.47)
Salmonella spp.	3.46 (1.20, 9.94)*	1.35 (0.74, 2.44)	1.14 (0.79, 1.64)	0.99 (0.69, 1.42)	0.99 (0.69, 1.42)
Shigella/EIEC	1.07 (0.51, 2.25)	1.01 (0.66, 1.55)	0.77 (0.58, 1.03)	1.31 (0.98, 1.74)	1.31 (0.98, 1.74)
STEC	1.19 (0.46, 3.06)	1.51 (0.79, 2.91)	0.90 (0.62, 1.31)	1.30 (0.91, 1.85)	1.30 (0.91, 1.85)
Adenovirus	0.89 (0.51, 1.53)	1.07 (0.75, 1.52)	1.25 (1.00, 1.58)	0.80 (0.63, 1.03)	0.80 (0.63, 1.03)
Enterovirus	1.29 (0.88, 1.90)	1.44 (1.11, 1.86)**	1.13 (0.98, 1.31)	1.03 (0.89, 1.19)	1.03 (0.89, 1.19)
Giardia	1.17 (0.69, 2.00)	1.06 (0.77, 1.45)	0.96 (0.78, 1.20)	1.07 (0.86, 1.33)	1.07 (0.86, 1.33)
Chloramphenicol	1.02 (0.96, 1.09)	1.02 (0.98, 1.07)	1.00 (0.97, 1.02)	1.02 (0.99, 1.04)	1.02 (0.99, 1.04)
Colistin	1.22 (0.79, 1.88)	1.04 (0.81, 1.35)	1.04 (0.88, 1.23)	0.97 (0.82, 1.15)	0.97 (0.82, 1.15)
Fluoroquinolone	1.02 (0.96, 1.08)	1.00 (0.96, 1.04)	1.02 (0.99, 1.05)	0.98 (0.95, 1.01)	0.98 (0.95, 1.01)
Trimethoprim	1.07 (0.97, 1.18)	1.03 (0.97, 1.09)	1.00 (0.96, 1.04)	1.01 (0.98, 1.05)	1.01 (0.98, 1.05)

***p<0.001; **p<0.01; *p<0.05; targets with <5% and >95% prevalence were omitted from PR analyses: astrovirus, norovirus, rotavirus, sapovirus, V.cholera, Cryptosporidium, Entamoeba, Ascaris, Trichuris, Ancylostoma, Necator, and aminoglycoside-resistant ARGs. Adjusted analyses controlled for the following covariates: child age, child sex, maternal age, maternal education, number of household members, wealth quintile, and treatment arm.

TABLE C-4: ADJUSTED PREVALENCE RATIOS OF RISK FACTORS ON ENTERIC INFECTION AND ARG CARRIAGE (TABLE 2 OF 3)

	Animals kept separate	Child play area visibly free of feces	Finished floor	Clean food prep surface	Elevated food prep surface
Campylobacter coli/jejuni	0.96 (0.81, 1.14)	0.95 (0.82, 1.09)	1.13 (0.82, 1.56)	1.12 (0.91, 1.36)	0.87 (0.74, 1.01)
Clostridium difficile	1.02 (0.70, 1.50)	0.89 (0.63, 1.24)	0.84 (0.44, 1.61)	0.78 (0.45, 1.36)	1.04 (0.71, 1.53)
EAEC	0.97 (0.89, 1.07)	0.96 (0.88, 1.03)	1.24 (1.02, 1.50)*	0.88 (0.77, 1.00)	0.97 (0.89, 1.05)
EPEC (any)	1.13 (1.02, 1.24)*	1.08 (0.99, 1.18)	1.09 (0.89, 1.34)	1.11 (0.98, 1.25)	1.03 (0.93, 1.14)
aEPEC	1.16 (1.03, 1.32)*	1.14 (1.02, 1.28)*	1.29 (0.97, 1.72)	1.09 (0.93, 1.29)	1.03 (0.91, 1.18)
tEPEC	0.93 (0.59, 1.45)	0.97 (0.67, 1.42)	0.39 (0.22, 0.67)***	0.69 (0.36, 1.33)	1.04 (0.68, 1.58)
ETEC (any)	0.99 (0.81, 1.21)	1.08 (0.91, 1.27)	1.06 (0.73, 1.55)	0.95 (0.73, 1.23)	1.05 (0.86, 1.28)
ETEC-LT	0.88 (0.70, 1.12)	1.10 (0.91, 1.34)	0.98 (0.65, 1.47)	0.99 (0.74, 1.33)	1.10 (0.88, 1.38)
ETEC-ST	1.01 (0.73, 1.39)	1.13 (0.87, 1.49)	1.54 (0.75, 3.15)	1.06 (0.71, 1.58)	0.83 (0.62, 1.12)
ETEC-LT/ST	0.71 (0.44, 1.15)	1.26 (0.88, 1.80)	1.51 (0.62, 3.68)	1.29 (0.78, 2.13)	0.80 (0.54, 1.20)
Salmonella spp.	1.53 (1.08, 2.18)*	1.13 (0.81, 1.59)	0.59 (0.33, 1.06)	1.21 (0.74, 1.95)	1.08 (0.73, 1.61)
Shigella/EIEC	0.88 (0.64, 1.21)	0.96 (0.73, 1.27)	1.39 (0.72, 2.70)	1.02 (0.68, 1.53)	0.76 (0.57, 1.01)
STEC	0.99 (0.67, 1.46)	0.90 (0.64, 1.26)	1.23 (0.57, 2.64)	1.31 (0.84, 2.04)	0.92 (0.63, 1.35)
Adenovirus	0.85 (0.65, 1.10)	0.81 (0.65, 1.02)	0.75 (0.52, 1.08)	0.81 (0.57, 1.16)	1.16 (0.90, 1.51)
Enterovirus	0.89 (0.75, 1.05)	0.88 (0.76, 1.01)	1.10 (0.80, 1.52)	0.84 (0.67, 1.06)	0.90 (0.77, 1.05)
Giardia	1.05 (0.83, 1.33)	1.04 (0.84, 1.27)	1.11 (0.71, 1.73)	0.91 (0.66, 1.25)	0.81 (0.66, 1.01)
Chloramphenicol	0.98 (0.95, 1.01)	0.98 (0.96, 1.01)	0.98 (0.94, 1.02)	0.98 (0.94, 1.02)	0.97 (0.95, 0.99)*
Colistin	0.85 (0.69, 1.03)	0.85 (0.72, 1.00)	1.02 (0.72, 1.43)	0.80 (0.61, 1.04)	0.91 (0.77, 1.09)
Fluoroquinolone	1.01 (0.98, 1.03)	0.98 (0.96, 1.01)	1.01 (0.95, 1.06)	1.01 (0.98, 1.04)	1.00 (0.97, 1.02)
Trimethoprim	1.00 (0.97, 1.04)	0.99 (0.96, 1.03)	1.04 (0.96, 1.13)	1.01 (0.97, 1.06)	1.00 (0.96, 1.04)

***p<0.001; **p<0.01; *p<0.05; targets with <5% and >95% prevalence were omitted from PR analyses: astrovirus, norovirus, rotavirus, sapovirus, V.cholera, Cryptosporidium, Entamoeba, Ascaris, Trichuris, Ancylostoma, Necator, and aminoglycoside-resistant ARGs. Adjusted analyses controlled for the following covariates: child age, child sex, maternal age, maternal education, number of household members, wealth quintile, and treatment arm.

TABLE C-5: ADJUSTED PREVALENCE RATIOS OF RISK FACTORS ON ENTERIC INFECTION AND ARG CARRIAGE (TABLE 3 OF 3)

	Clean food prep area	Food prep area free of flies	Handwashing station within 10m of food prep	Currently breastfed	Diarrheal episode
Campylobacter coli/jejuni	0.98 (0.85, 1.13)	0.98 (0.85, 1.13)	0.98 (0.85, 1.13)	1.10 (0.92, 1.32)	1.09 (0.85, 1.39)
Clostridium difficile	1.21 (0.87, 1.67)	1.18 (0.83, 1.69)	1.18 (0.83, 1.69)	0.36 (0.24, 0.56)***	0.84 (0.44, 1.61)
EAEC	0.97 (0.90, 1.05)	1.03 (0.95, 1.12)	1.03 (0.95, 1.12)	1.18 (1.07, 1.30)***	0.78 (0.65, 0.94)**
EPEC (any)	1.04 (0.95, 1.13)	1.00 (0.91, 1.09)	1.00 (0.91, 1.09)	0.94 (0.84, 1.05)	0.74 (0.60, 0.93)**
aEPEC	1.07 (0.96, 1.20)	1.03 (0.92, 1.16)	1.03 (0.92, 1.16)	0.99 (0.86, 1.15)	0.79 (0.61, 1.03)
tEPEC	0.77 (0.53, 1.14)	1.13 (0.76, 1.67)	1.13 (0.76, 1.67)	0.62 (0.39, 0.99)*	0.37 (0.12, 1.15)
ETEC (any)	1.08 (0.92, 1.27)	1.09 (0.91, 1.29)	1.09 (0.91, 1.29)	1.01 (0.82, 1.25)	0.89 (0.63, 1.26)
ETEC-LT	1.08 (0.89, 1.30)	1.09 (0.89, 1.34)	1.09 (0.89, 1.34)	1.00 (0.78, 1.28)	0.84 (0.56, 1.27)
ETEC-ST	1.14 (0.87, 1.49)	1.04 (0.79, 1.38)	1.04 (0.79, 1.38)	0.93 (0.66, 1.30)	0.98 (0.58, 1.65)
ETEC-LT/ST	1.18 (0.82, 1.69)	1.03 (0.71, 1.49)	1.03 (0.71, 1.49)	0.84 (0.52, 1.35)	0.89 (0.43, 1.88)
Salmonella spp.	1.13 (0.80, 1.58)	1.33 (0.91, 1.94)	1.33 (0.91, 1.94)	0.96 (0.60, 1.53)	0.93 (0.50, 1.73)
Shigella/EIEC	0.70 (0.52, 0.93)*	1.00 (0.76, 1.33)	1.00 (0.76, 1.33)	0.81 (0.56, 1.17)	0.96 (0.55, 1.68)
STEC	1.09 (0.78, 1.52)	0.77 (0.55, 1.06)	0.77 (0.55, 1.06)	0.75 (0.48, 1.16)	1.01 (0.53, 1.92)
Adenovirus	0.90 (0.72, 1.12)	0.98 (0.79, 1.22)	0.98 (0.79, 1.22)	0.67 (0.51, 0.89)**	0.67 (0.41, 1.12)
Enterovirus	0.87 (0.75, 1.00)	1.06 (0.92, 1.23)	1.06 (0.92, 1.23)	1.16 (0.97, 1.39)	0.97 (0.74, 1.26)
Giardia	0.87 (0.70, 1.07)	1.04 (0.85, 1.29)	1.04 (0.85, 1.29)	0.88 (0.68, 1.14)	0.91 (0.59, 1.40)
Chloramphenicol	0.99 (0.97, 1.02)	0.99 (0.97, 1.02)	0.99 (0.97, 1.02)	1.00 (0.98, 1.02)	0.96 (0.90, 1.02)
Colistin	0.94 (0.80, 1.10)	1.12 (0.94, 1.32)	1.12 (0.94, 1.32)	1.00 (0.82, 1.21)	0.86 (0.61, 1.20)
Fluoroquinolone	0.99 (0.97, 1.01)	1.01 (0.99, 1.04)	1.01 (0.99, 1.04)	0.99 (0.97, 1.02)	0.99 (0.94, 1.04)
Trimethoprim	1.00 (0.97, 1.03)	1.01 (0.97, 1.04)	1.01 (0.97, 1.04)	1.03 (1.00, 1.07)	0.91 (0.83, 0.99)*

***p<0.001; **p<0.01; *p<0.05; targets with <5% and >95% prevalence were omitted from PR analyses: astrovirus, norovirus, rotavirus, sapovirus, V.cholera, Cryptosporidium, Entamoeba, Ascaris, Trichuris, Ancylostoma, Necator, and aminoglycoside-resistant ARGs. Adjusted analyses controlled for the following covariates: child age, child sex, maternal age, maternal education, number of household members, wealth quintile, and treatment arm.

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