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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	All survey data collected during the original trial were collected on electronic tablets by SurveyCTO (Dobility, Cambridge, MA, USA) and securely uploaded to an online server.
Data analysis	All analyses were conducted in R v. 3.5.0 and R Studio v. 1.1.463. We used the following statistical packages (all available through GitHub or CRAN): corncob, metacoder, phyloseq, breakaway, ggplot2, tidyverse, Hmisc.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All raw reads (human sequences removed) were deposited in the Sequence Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra) under the project number PRJNA726052. Metadata are publicly available at the following link with no access restrictions: https://osf.io/gdnqv/

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed for this exploratory gut microbiome analysis. The number of samples we selected for short-read metagenomic sequencing was based on the funding that was available through the Thrasher Research Fund. Given that we observed significant differences in the relative abundance of over 20 bacterial genera between treatment and control children, we believe this sample size was sufficient.
Data exclusions	No samples were excluded from analysis, although we had preplanned exclusion criteria. We tabulated the number of raw reads for each sample and excluded from further analysis any sample with fewer reads than two standard deviations below the mean. Such a low read count indicates flaws at the time of sample collection or deteriorations in sample integrity during storage. No samples were excluded based on this criteria.
Replication	We did not reproduce our analyses. All data required to reproduce our analyses, including the metagenomic sequencing reads and associated metadata are publicly available without restrictions.
Randomization	In the original trial, randomisation was pair-matched by water point and stratified by study site. One investigator (AJP) sorted water points in descending order in each site by the number of children younger than 5 years of age residing and using the enrolled water point as their primary source of drinking water. Water points were then paired in descending order and, within pairs, assigned on a 1:1 allocation ratio to treatment or control status by means of a random number generator. For the present study which leveraged stool samples collected during the original trial, randomization was stratified by study site and three age groups that others have demonstrated to correspond to distinct phases of gut microbiome development (i.e., 6 -14 months, 15-30 months, 31 months and older).
Blinding	Study participants and outcome assessors were masked to treatment status.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	We examined fecal metagenomes from 130 children from the control (n=64) and treatment groups (n=66). Samples included in our final analysis were balanced between two study sites (Dhaka Uddan and Tongi) and three pre-specified age strata (6-14 months, 15-30 months, 31 months and older) corresponding to distinct phases of gut microbiome development. Characteristics known to impact the early-life gut microbiome were evenly distributed between the treatment and control groups, including child age, breastfeeding status, recent diarrhea, and recent antibiotic use (described in Table 1). Children were exposed to chlorine (treatment) and Vitamin C (active control suggested by the local human subjects protection board) doses for an average of 10.5 months (range due to open cohort study design = 1.7 to 14.4 months). Most children (89%) were exposed for at least 6 months
Recruitment	Households were visited in-person and screened for eligibility criteria (used the enrolled study water point as the primary drinking water source, and had at least one child under the age of five years), then invited to participate in the study. Written

informed consent was obtained.

Ethics oversight	The study protocol for the original trial was approved by the International Centre for Diarrhoeal Diseases Research, Bangladesh (icddr,b) scientific and ethical review committees (protocol number 14022) and the human subjects institutional review board at Stanford University (protocol number 30456). Field staff obtained informed written consent from the owner (ie, compound landlord) of each water point enrolled and all study participants.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	The original trial from which we leveraged stool samples for the present analysis is registered with ClinicalTrials.gov, number NCT02606981.
Study protocol	The full study protocol for the original trial is available on Open Science Framework: https://osf.io/9dh7k/
Data collection	The trial was implemented from July 2015 – December 2016 in two low-income communities in urban Bangladesh: Tongi, a community outside Dhaka city, and Dhaka Uddan, a community within Dhaka city.
Outcomes	We examined differences in bacterial taxa (primary outcome for this paper - not specified in the original trial), ARGs (secondary outcome - not specified in the original trial), and the occurrence of gastrointestinal pathogens (secondary outcome - pre-specified in the original trial) between treatment and control children using several approaches. For bacterial taxa, we used beta-binomial regression models that account for variable sequencing depth to identify taxa that were differentially abundant between treatment and control children with the R package corncob, while controlling for the child's age and study site. We also used corncob to identify differentially abundant ARGs between treatment and control children, while controlling for age and study site (secondary outcome). To examine differences in gastrointestinal pathogen burden between treatment and control children, we used Poisson regression models to examine associations between treatment status and the presence of any pathogen that was harbored by at least 5% of children, while controlling for child's age and study site (secondary outcome). Pathogen presence was ascertained using a qualitative multiplex assay.