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Gut carriage of antimicrobial resistance genes among young children in urban Maputo, Mozambique: Associations with enteric pathogen carriage and environmental risk factors

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Abstract

Because poor sanitation is hypothesized as a major direct and indirect pathway of exposure to antimicrobial resistance genes (ARGs), we sought to determine a) the prevalence of and b) environmental risk factors for gut carriage of key ARGs in a pediatric cohort at high risk of enteric infections due to poor water, sanitation, and hygiene (WASH) conditions. We investigated ARGs in stool from young children in crowded, low-income settlements of Maputo, Mozambique, and explored potential associations with concurrent enteric pathogen carriage, diarrhea, and environmental risk factors, including WASH. We collected stool from 120 children <14 months old and tested specimens via quantal, multiplex molecular assays for common bacterial, viral, and protozoan enteric pathogens and 84 ARGs encoding potential resistance to 7 antibiotic classes. We estimated associations between ARG detection (number and diversity detected) and concurrently-measured enteric pathogen carriage, recently-reported diarrhea, and risk factors in the child's living environment. The most commonly-detected ARGs encoded resistance to macrolides, lincosamides, and streptogramins (100% of children); tetracyclines (98%); β-lactams (94%), aminoglycosides (84%); fluoroquinolones (48%); and vancomycin (38%). Neither concurrent diarrhea nor measured environmental (including WASH) conditions were associated with ARG detection in adjusted models. Enteric pathogen carriage and ARG detection were associated: on average, 18% more ARGs were detected in stool from children carrying bacterial pathogens than those without (adjusted risk ratio (RR): 1.18, 95% confidence interval (CI): 1.02, 1.37), with 16% fewer ARGs detected in children carrying parasitic pathogens (protozoans, adjusted RR: 0.84, 95% CI: 0.71, 0.99). We observed gut ARGs conferring potential resistance to a range of antibiotics in this at-risk cohort that had high rates of enteric infection, even among children <14 months-old. Gut ARGs did not appear closely correlated with WASH, though

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environmental conditions were generally poor. ARG carriage may be associated with concurrent carriage of bacterial enteric pathogens, suggesting indirect linkages to WASH that merit further investigation.

Introduction

Antimicrobial resistance (AR) is a major global health threat, with antimicrobial use and resistance concurrently growing worldwide. From 2000–2010, antimicrobial use increased by >30% globally, with the largest increases in low- and middle-income countries (LMICs), likely due to the high burden of infectious diseases and the increased availability and reduced cost of antimicrobials [1]. A recent birth cohort study of seven LMIC sites found 98% of infants were exposed to antimicrobials by 6 months of age [2]. Antimicrobial use in LMICs may be high due to both insufficient training or oversight into prescriber practices, ease of access and lack of regulation of antimicrobial agents to avoid use outside of pharmaceutical and clinical settings, and mass drug campaigns that include administration of antimicrobials to healthy children [1,3,4]. Thus, selective pressure from human use of antimicrobials in LMICs is increasing. However, evaluation and assessment of AR, and risk factors for antimicrobial-resistant infections, in LMICs remains limited because of poor surveillance and insufficient funding [1].

Emerging regional- and national-scale evidence suggests poor water, sanitation, and hygiene (WASH)—directly and indirectly—facilitates the development and environmental dispersion of AR organisms [5–8]. More directly, poor WASH conditions—especially poor containment and treatment of AR organisms and their genes through the sanitation chain—may facilitate the development of resistance and increase individual risks of exposure to AR genes (ARGs) or AR (vs. susceptible) pathogens [6,7,9,10]. Indirectly, poor WASH conditions result in children exposed to enteric pathogens more frequently and in potentially higher concentrations [11,12], increasing treatment with antibiotics or antimicrobials and thereby accelerating the development of AR [10].

However, the hypothesized environmental contributions to ARG exposures have not been directly measured on local scales, especially in LMICs where poor WASH infrastructure may be highly prevalent and of paramount concern [10,13]. Insufficient treatment of AR organisms and ARGs at sewage or fecal sludge treatment plants, and persistence of antibiotics in the fecal waste stream, put water sources and water-associated environmental transmission pathways at particular risk of contamination [6,14–18]. Furthermore, many LMIC communities lack consistent access to functional, safe management of human waste throughout the sanitation chain, leading to environmental discharge of untreated wastes [19–21]. More than half of the world's population and their feces (and almost two-thirds in LMICs [19]) are managed in onsite systems [22] and 60% of the world uses unsafely-managed sanitation [20], bringing exposure risks directly into the household or the peripheral environment. Carriage of bacterial enteric pathogens, due to exposure to poor WASH conditions [11], may co-occur with AR either because enteric bacteria carry ARGs or because ARGs may accompany diverse bacterial exposures.

Methodologically, challenges exist to assessing the fate, transport, and burden of AR from the environment to humans and its impact on human health. Culture-based methods remain the standard for assessing phenotypic resistance of AR organisms in the environmental and in the gut microbiome, and are widely used for surveillance and health impact assessments

[13,23]. Scaling these methods to large studies is constrained by human and financial resources [13]. Conversely, molecular approaches to ARG detection present the potential for high-throughput screening. However, interpretation of ARG carriage is challenging, given limited understanding of their background circulation and clinical implications without culture confirmation [23,24]. Molecular approaches are moving towards more accurate prediction of phenotypic resistance [25]. Additionally, there is a need to understand concurrent ARG prevalence in environmental media like sewage, wastewater, soil, drinking water, and recreational water, particularly in environments with high fecal contamination [9]. As a start, an in-depth understanding of the background prevalence and risk factors for ARGs in human hosts in these settings can characterize the epidemiology and potential for environmental measures to prevent ARG transmission.

In order to evaluate direct and indirect links between poor WASH (environmental risk factors) and ARG transmission, we evaluated ARG prevalence and associations between total number of ARGs detected (and diversity of ARGs detected) and 1) environmental risk factors (direct links: e.g. poor sanitation); and 2) enteric pathogen carriage (indirect links), in children born into a pathogen-rich, densely-populated urban environment in Maputo, Mozambique as part of a larger health impact trial of sanitation [26]. The goals of this study were to: a) evaluate the prevalence and diversity of ARGs in young children (<14 months old) in a low-income, urban environment; b) assess risk factors for ARGs in these children; and c) compare ARG outcome metrics of diversity and total genes from a qPCR array including pre-selected ARGs. Use of a qPCR array with pre-selected ARGs enabled comparison of relative AR profiles between children in varying environments. Results from this analysis can improve understanding of background circulation of ARGs in children in Mozambique and settings with similarly poor infrastructure and high burdens of enteric infections, and of links between environmentally-mediated risk factors and ARGs.

Methods

This study was nested within a subgroup of children enrolled from 2015–2017 in the Maputo Sanitation (MapSan) trial in Maputo, Mozambique [26]. Briefly, the MapSan trial tested the effects of a shared private sanitation intervention–pour-flush latrines with septic tanks and soakaway pits–on child health as compared to existing poor sanitation. In this setting, sanitation is shared at the compound level; compounds are groups of two or more houses that share outdoor living space (and sanitation). Thus, this study was a sub-analysis that the original MapSan study was not designed to explicitly evaluate. For this study, the first 60 stools (one per child) analyzed for the MapSan study that were from children <14 months old enrolled in MapSan control and intervention compounds in round 1 (February 2015 –February 2016, pre-intervention) and the first 60 meeting the same criteria (that is, among new children born into an intervention or control compound) in round 2 (March 2016-April 2017, 12 months post-intervention) were selected (n = 120 stools total). Selection was purposefully split evenly by group: 30 children per intervention or control group in each round. Enrollment age criteria ensured children were born into compounds following any changes in sanitation conditions associated with the intervention (if in that group). By design of this study and enrollment age criteria, no children could be enrolled multiple times, but a compound could be enrolled multiple times. Statistical models addressed potential within-compound level of clustering using random effects for the compound of the child. Enteric pathogens in stool specimens were assessed by a multiplex RT-PCR assay (Luminex xTAG Gastrointestinal Pathogen Panel (GPP)®, Luminex Corp, Austin, TX, USA). The GPP tests for 15 enteric pathogens: 9 bacteria (*Campylobacter*, *C. difficile*, enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli*

(STEC), *E. coli* O157, *Salmonella*, *Shigella*, *V. cholerae*, *Y. enterocolitica*), 3 viruses (adenovirus 40/41, norovirus GI/GII, rotavirus A), and 3 amoeba/protozoa ('parasites': *Giardia*, *Cryptosporidium*, *Entamoeba histolytica*). The GPP is a well-validated assay with high sensitivity and specificity that has been tested across multiple countries [27–33]. Child stool specimens were also assessed for 84 ARGs via a commercial qPCR ARG array (the Antibiotic Resistance Genes Microbial DNA qPCR array, Qiagen, Valencia, CA, USA, ARGs in S1 Table), which has been used previously to characterize ARGs in the human gut, environmental soil, and meat for consumption in high-income settings [34–38]. Ethical approvals for stool and survey data collection were obtained from the Comité Nacional de Bioética para a Saúde, Ministério da Saúde (333/CNBS/14), the Ethics Committee of the London School of Tropical Medicine and Hygiene (reference # 8345), and the Institutional Review Board of the Georgia Institute of Technology (protocol # H15160). Ethical committees approved the verbal consent procedures because most respondent were illiterate. All consents were recorded on the survey questionnaire or tablet prior to initiating surveys. The MapSan trial was registered at ClinicalTrials.gov (NCT02362932).

Study site

The study site comprised densely-populated, low-income neighborhoods of Maputo, Mozambique described elsewhere [26,39]. Briefly, children lived in households organized in compounds that generally shared a sanitation facility. Most households had access to a sanitation facility that contained waste onsite, while few lacked access to basic sanitation facilities [39]. Children from these compounds had to be $\geq$1 month old to be enrolled in MapSan [39].

Surveys and observation

Data were collected on child, household, and compound demographics; water, sanitation, and hygiene (WASH) practices; and household wealth by surveys and observation [39]. The child's mother was the target respondent for surveys about the child's health and the household. If the mother was unavailable, another parent or guardian was asked to respond to survey questions. The head of compound, or their spouse, was the target respondent for surveys about the compound itself. Observations of household conditions, WASH infrastructure, and the compound overall were also completed. Household wealth was measured by an asset-based index specific to and locally-validated in Mozambique [40].

Stool collection and analysis

Stool specimens were collected as described previously [39]. Briefly, caregivers were given diapers or containers for collecting children's stool with pre-labelled sample bags and to store collected stool in a cool, dry place in the household. Stool specimens were picked up from the household the following day, and transported on ice to the Mozambican Ministry of Health (MISAU/INS) within 6 hours for storage at -80˚C. Specimens were then shipped on dry ice, with temperatures monitored, to the Georgia Institute of Technology for storage at -80˚C until analysis.

Stool specimens were analyzed by the GPP according to manufacturer's instructions, using the QIAcube HT platform and QIAamp 96 Virus QIAcube HT Kit (Qiagen, Hilden, Germany). Extracted nucleic acids were stored at 4˚C and analyzed by GPP within 24 hours. Further details on stool analysis for enteric pathogens can be found in Knee et al. [39].

For ARG assessment, DNA from whole stool was extracted using the Qiagen PowerFecal Kit for Qiacube (Qiagen, Hilden, Germany). Extracted DNA was stored at -80˚C until use, and underwent $\leq$1 previous freeze-thaw cycle before analysis. Total reaction volumes, including

amount of Qiagen Microbial DNA Mastermix and target amount of DNA (500ng/array) were set according to manufacturer's instructions. Arrays were loaded and run on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) following manufacturer's instructions or stored at -20˚C for up to 7 days until the run. Quantification cycle (C_q) values of <34 indicated positive detects. Each sample had six pan-bacteria positive controls (to ensure sufficient quantities of bacterial DNA) and three plate controls (tests for inhibitors via artificial sequence and primers). Lower limits of quantification for most ARG targets in the assay (97%) were <100 gene copies per reaction.

Aggregate ARG indices

Beyond individual ARG prevalence, ARGs detected in each child's stool specimen were evaluated by three aggregate measures: total ARGs, Shannon Index, and Inverse Simpson's Index. Total ARGs were calculated as the total number of positive wells (targets) detected per specimen. In ecological literature, diversity indices (Shannon Index [41] and Inverse Simpson's Index [42]) measure the richness of individual species (generally: [number of species represented]:[number of individuals in each species], thus higher numbers indicate greater diversity) and each give different weights to dominant species. They have been used to quantify the diversity of mobile genetic elements and ARGs in infants in high-income settings [43] and of ARGs in environmental media (e.g. sewage sludge [44,45]). In this study, ARG groups (e.g. aminoglycoside resistance, tetracycline resistance; β-lactamase resistance subdivided into Ambler classes (class A-D [46])) represented the 'species' level, with number of targets detected within each ARG group representing the 'number of members of the species' in ecological terms.

Statistical analyses

All data were analyzed in R version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria [47]) using the 'lme4' package for generalized linear models with random effects [48] and the 'vegan' package for calculating Shannon and Inverse Simpson's indices [49]. The 'total ARG' outcome, the number of ARGs detected, was analyzed using mixed-effects Poisson regression, with a random effect for the child's compound. Diversity indices were analyzed using mixed-effects linear regression, with a random effect for the compound. Demographic, WASH, and wealth risk factors that were tested for associations with these outcomes were included as continuous or categorical variables. For categorical variables with >2 levels, dummy variables were created: e.g. for sanitation, children in compounds with a pit latrine were the referent group, compared with children in compounds with pour flush facilities compared to them. All analyses were adjusted for round of enrollment. Enteric pathogen risk factors (enteric pathogens detected in stool) were also adjusted for reported diarrhea to encompass symptomatic enteric infections that may cause caregivers to seek care for the child with antibiotics [2].

Results

Demographic characteristics and enteric infections

We assessed demographic characteristics and enteric pathogen carriage in study children by surveys and stool specimen analysis, respectively (Table 1). All children were <14 months old (average age of 8 months, standard deviation (SD): 3 months; Table 1a) and half (53%) were female. Most children (80%) were breastfed at the time of survey, but only 17% of those

Table 1. Demographic characteristics and enteric infections among children < 14 months old, Maputo, Mozambique[1].

a) Demographics	Mean (standard deviation (SD)) or N (%), Round 1	Mean (SD) or N (%), Round 2	Mean (SD) or N (%), all years
Age (months)	7.5 (3.2)	8.7 (2.8)	8.1 (3.0)
Female child[2]	33 (56%)	30 (50%)	63 (53%)
Child is breastfed	46 (77%)	50 (83%)	96 (80%)
Child is exclusively breastfed	12 (26%)	4 (8%)	16 (17%)
Wealth index (unitless, range: 0–1)	0.43 (0.13)	0.35 (0.14)	0.39 (0.14)
Type of toilet			
Pit latrine	59 (98%)	30 (50%)	89 (74%)
Pour flush to septic tank	0	27 (90%)	27 (23%)
Pour flush to other	1 (2%)	3 (10%)	4 (3.3%)
b) Reported diarrhea			
Diarrhea in last 7 days[3]	9 (18%)	8 (18%)	17 (18%)
Sought treatment for diarrhea	6 (67%)	3 (38%)	9 (53%)
c) Enteric pathogen detection (stool specimens)			
Any pathogen	51 (85%)	48 (80%)	99 (83%)
Number of pathogens			
0	9 (15%)	12 (20%)	21 (18%)
1	27 (45%)	20 (33%)	47 (39%)
2	15 (25%)	23 (38%)	38 (32%)
3	8 (13%)	4 (7%)	12 (10%)
4	0	0	0
5	1 (2%)	1 (2%)	2 (2%)
Bacterial pathogens[4]	43 (72%)	40 (67%)	83 (69%)
Number of bacterial pathogens			
0	17 (28%)	20 (33%)	37 (31%)
1	29 (48%)	23 (38%)	52 (43%)
2	13 (22%)	15 (25%)	28 (23%)
3	1 (2%)	1 (2%)	2 (2%)
4	0	1 (2%)	1 (1%)
Parasitic pathogens[5]	13 (22%)	10 (17%)	23 (19%)
Number of parasitic pathogens			
0	47 (78%)	50 (83%)	97 (81%)
1	11 (18%)	9 (15%)	20 (17%)
2	2 (3%)	1 (2%)	3 (3%)
Viral pathogens[6]	13 (22%)	11 (18%)	24 (20%)
Number of viral pathogens			
0	47 (78%)	49 (82%)	96 (80%)
1	13 (22%)	10 (17%)	23 (19%)
2	0	1 (2%)	1 (1%)

[1]n = 60 children enrolled in round 1 (February 2015-February 2016 enrollment) and 60 children enrolled in round 2 (March 2016-April 2017 enrollment);

[2]Sex of the child was not able to be ascertained for 1 child;

[3]94/120 children had dates of survey and stool collection within 7 days of each other, and thus were included in analyses for diarrhea;

[4]Bacterial pathogens included in Luminex Assay: *Campylobacter* spp., *C. difficile*, *V. cholerae*, enterotoxigenic *E. coli* (ETEC), *E. coli* O157, *Salmonella enterica*, *Shigella* spp., Shiga-toxin producing *E. coli* (STEC), *Y. pestis*;

[5]Parasitic pathogens included in Luminex Assay: *Cryptosporidium* spp., *Giardia* spp., *E. histolytica*;

[6]Viral pathogens included in Luminex Assay: adenovirus 40/41, norovirus, rotavirus

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breastfed were exclusively breastfed. Most households had access to a pit latrine (74%), and mean value for the (unitless, ranging from 0–1) wealth index was 0.39.

All analyses involving reported diarrhea were limited to the 94/120 children that had surveys of reported diarrhea collected within ±7 days of the stool specimen to ensure biological plausibility of concurrence of symptoms and pathogen carriage. About 18% of caregivers reported the child had diarrhea within the past week, with half reporting having sought care for the diarrhea (Table 1b). However, among all children, 83% had ≥1 pathogen detected in their stool, and 44% had multiple pathogens detected (Table 1c). Bacterial pathogens were most commonly detected (69%), followed by viruses (20%) and parasites (19%). The most commonly detected bacterial pathogens were *Salmonella enterica* (51%), ETEC (34%), and *C. difficile* (22%, S1 Table). *Giardia* spp. (78%) was the most commonly detected parasitic pathogen, while norovirus (79%) was the most common viral pathogen (S1 Table).

ARG outcomes in children

We assessed ARGs in stool specimens by group and using aggregate metrics for total genes and diversity (Table 2). Though ARGs were detected in all specimens, the most commonly detected ARGs conferred resistance to a) macrolides, lincosamides, and streptogramin b (MLS, 100%); b) tetracyclines (98%); c) β-lactams (94%); and d) aminoglycosides (84%) (Table 2a). On average, 3.1 of 5 MLS ARGs tested, 1.8 of 2 tetracycline ARGs tested, 4.6 of 55 β-lactam ARGs tested, and 1.1 of 5 aminoglycoside ARGs tested were detected in each sample. About half of children (48%) had ARGs conferring resistance to fluoroquinolones, and 38% had ARGs conferring resistance to vancomycin. ARGs conferring multidrug resistance were not common (<1%).

Prevalences of individual ARGs are shown in S2 Table. Of the 84 ARGs assessed per child, 9 (11%) were detected in >50% of children: 1 aminoglycoside ARG (*aadA1*); 3 β-lactamase ARGs (*SHV*, *SHV(156G)*, and *SHV(238G240E)*); 3 MLS ARGs (*ermA*, *ermB*, and *mefA*); and 2 tetracycline ARGs (*tetA* and *tetB*). Another 19 ARGs (23%) were detected in ≥10% of children: 1 aminoglycoside ARG (*aacC2*), 11 β-lactamase ARGs (*mecA*, *CTX-M-8*, *CTX-M-9*, *SHV*

Table 2. Antimicrobial Resistance Gene (ARG) detection by resistance group in children <14 months old, Maputo, Mozambique.

	All samples	Per sample	
a) Resistance group (# ARGs in group tested)	Samples positive for ≥ 1 ARG (%)	Avg. number of positive ARGs (standard deviation)	Range of ARGs detected
Aminoglycoside (5)	101 (84.2%)	1.13 (0.67)	0–3
β-lactamase (55)	113 (94.2%)	4.58 (2.83)	0–14
Class A β-lactamase (22)	96 (80.0%)	2.74 (1.75)	0–7
Class B β-lactamase (9)	33 (27.5%)	0.29 (0.49)	0–2
Class C β-lactamase (11)	75 (62.5%)	1.24 (1.32)	0–6
Class D β-lactamase (13)	29 (24.2%)	0.31 (0.65)	0–4
Erythromycin (1)	0 (0%)	0	0
Fluoroquinolone (7)	58 (48.3%)	0.87 (1.14)	0–4
Macrolide, lincosamide, streptogramin b (5)	120 (100%)	3.09 (1.08)	1–5
Multidrug (2)	1 (0.8%)	0.02 (0.18)	0–2
Tetracycline (2)	118 (98.3%)	1.78 (0.45)	0–2
Vancomycin (2)	45 (37.5%)	0.38 (0.49)	0–1
b) Aggregate metrics	Mean (SD)	Median (Range)	
Total ARGs	12.4 (4.5)	12 (4–28)	
Shannon Index	1.6 (0.3)	1.6 (0.6–2.0)	
Inverse Simpson's Index	4.5 (1.1)	4.5 (1.6–6.9)	

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(156D), *ccrA*, *ACT-5/7*, *ACT-1*, *DHA*, *LAT*, *MIR*, and *OXA-51* variants), 4 fluoroquinolone ARGs (*QnrB-1*, *QnrB-4*, *QnrB-8*, and *QnrS*), 2 MLS ARGs (*ermC* and *msrA*), and 1 vancomycin ARG (*vanC*).

On average, 12 ARGs were detected per child (range: 4–28, Table 2b). Average Shannon Index was 1.6 (range: 0.6–2), while average Inverse Simpson's Index was 4.5 (range: 1.6–6.9).

Unadjusted associations between demographic and enteric pathogen risk factors and ARG outcomes

We assessed associations between demographic or enteric pathogen risk factors and ARG outcomes by regression techniques with a random effect for the compound (Table 3). Stool specimens collected from children enrolled in round 2 had significantly more ARGs detected than from children enrolled in round 1 (risk ratio (RR): 1.21, 95% confidence interval (CI): 1.07, 1.38), but diversity indices did not vary significantly by round. Improved household wealth (10 percentage point change in the wealth index) was associated with fewer total ARGs (RR: 0.96, 95% CI: 0.92, 1.00). Children in households with septic tanks had moderately more ARGs detected than those in households with pit latrines (RR: 1.17, 95% CI: 1.00, 1.36).

Presence of $\geq$1 bacterial pathogen in stool was associated with 16% more ARGs detected (RR: 1.16, 95% CI: 1.02, 1.34). Each additional bacterial pathogen detected was associated with a 10% increase in ARGs (RR: 1.10, 95% CI: 1.01, 1.19). Conversely, parasitic pathogen detection was associated with 17% fewer ARGs (RR: 0.83, 95% CI: 0.70, 0.96). Pathogen detection was not associated with differences in diversity indices.

Table 3. Unadjusted associations between demographic and enteric pathogen-related characteristics and ARG outcomes among children < 14 months old, Maputo, Mozambique.

Risk factor	Total ARGs RR[1] (95% CI[2])	Shannon Index β[3] (95% CI[2])	Inverse Simpson's Index β[3] (95% CI[2])
Round (2 vs. 1 (ref.))	1.21 (1.07, 1.38)	-0.04 (-0.14, 0.07)	-0.06 (-0.48, 0.35)
Age (months)	1.01 (0.99, 1.04)	0.00 (-0.02, 0.02)	-0.01 (-0.0, 0.07)
Female child	0.98 (0.86, 1.09)	0.06 (-0.05, 0.17)	0.29 (-0.13, 0.71)
Child is breastfed	1.04 (0.88, 1.22)	-2.9×10^{-3} (-0.14, 0.13)	0.06 (-0.45, 0.57)
Wealth index[4]	0.96 (0.92, 1.00)	-0.01 (-0.05, 0.03)	-0.06 (-0.20, 0.08)
Type of sanitation			
Pit latrine	Ref.	Ref.	Ref.
Pour flush (to septic)	1.17 (1.00, 1.36)	-0.04 (-0.17, 0.09)	-0.12 (-0.65, 0.38)
Pour flush to (other)	1.05 (0.82, 1.30)	0.11 (-0.08, 0.31)	0.30 (-0.41, 1.02)
Reported diarrhea[4]	0.87 (0.71, 1.05)	0.01 (-0.13, 0.18)	-0.04 (-0.61, 0.57)
Any pathogen	1.07 (0.90, 1.25)	-0.01, (-0.14, 0.15)	-3.3×10^{-3} (-0.60, 0.50)
Number of pathogens	1.01 (0.94, 1.08)	9.2×10^{-4} (-4.9×10^{-2}, 0.05)	0.03 (-0.16, 0.23)
Any bacterial pathogen	1.16 (1.02, 1.34)	0.04 (-0.07, 0.16)	0.21 (-0.24, 0.66)
Number of bacterial pathogens	1.10 (1.01, 1.19)	8.2×10^{-3} (-0.06, 0.07)	0.07 (-0.20, 0.35)
Any parasitic pathogen	0.83 (0.70, 0.96)	0.04 (-0.11, 0.18)	0.12 (-0.43, 0.67)
Number of parasitic pathogens	0.83 (0.71, 0.95)	0.03 (-0.08, 0.14)	0.08 (-0.34, 0.46)
Any viral pathogen	0.88 (0.74, 1.04)	-0.08 (-0.20, 0.06)	-0.26 (-0.75, 0.27)
Number of viral pathogens	0.93 (0.80, 1.06)	-0.06 (-0.19, 0.05)	-0.21 (-0.68, 0.34)

[1]Risk ratio estimated by mixed effects Poisson regression, with a random effect for compound;

[2]Confidence interval;

[3]Estimate is from mixed effects linear regression for the diversity metric;

[4]Limited to children whose survey for self-reported diarrhea was $\leq$7 days from stool specimen collection (n = 94)

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Adjusted associations between demographic and enteric pathogen risk factors and ARG outcomes

We assessed associations between demographic or enteric pathogen carriage and ARG outcomes adjusted by round (Table 4). Adjustment for reported diarrhea for enteric pathogen risk factors—as initially designed—was instead conducted as a sensitivity analysis (S3 Table), given the 22% loss of observations from non-concurrent assessment of diarrhea and collection of stool. The wealth index was not significantly associated with total ARGs when adjusting for round (Table 4). Adjusting for round, detection of ≥1 bacterial pathogen in stool was associated with increased total ARGs (RR: 1.18, 95% CI: 1.02, 1.37), with an association of 9% more ARGs per bacterial pathogen detected (RR: 1.09, 95% CI: 1.01, 1.17). Detection of parasitic pathogens was associated with 16% fewer ARGs (RR: 0.84, 95% CI: 0.71, 0.99). Sensitivity analysis indicated no modeled estimates of enteric pathogen risk factors for total ARGs were meaningfully attenuated (maximum attenuation: 8% of the original effect estimate) when also adjusted for reported diarrhea (and therefore limited to the 94 children with surveys for reported diarrhea ≤7 days from stool specimen collection) and most were strengthened (S3 Table).

Discussion

We assessed the prevalence and diversity of ARGs as our outcome, and 1) poor sanitation and other environmental risk factors; and 2) enteric pathogen carriage; in children (<14 months

Table 4. Adjusted[1] associations between demographic and enteric pathogen-related characteristics and ARG outcomes among children < 14 months old, Maputo, Mozambique.

Risk factor	Total ARGs RR[2] (95% CI[3])	Shannon Index β[4] (95% CI[3])	Inverse Simpson's Index β[4] (95% CI[3])
Age (months)	1.01 (0.99, 1.03)	-3.8×10^{-4} (-0.02, 0.02)	4.7×10^{-3} (-0.08, 0.06)
Female child	0.99 (0.87, 1.11)	0.06 (-0.05, 0.16)	0.29 (-0.08, 0.74)
Child is breastfed	1.02 (0.87, 1.22)	8.6×10^{-4} (-0.13, 0.14)	0.07 (-0.51, 0.37)
Wealth index[5]	0.98 (0.93, 1.03)	-0.01 (-0.06, 0.02)	-0.07 (-0.23, 0.09)
Type of sanitation			
Pit latrine	Ref.	Ref.	Ref.
Pour flush (to septic)	1.04 (0.86, 1.24)	-0.02 (-0.17, 0.13)	-0.11 (-0.65, 0.55)
Pour flush (to other)	1.01 (0.79, 1.27)	0.11 (-0.06, 0.31)	0.30 (-0.39, 1.07)
Reported diarrhea[5]	0.88 (0.71, 1.08)	0.01 (-0.14, 0.16)	-0.04 (-0.66, 0.56)
Any pathogen	1.08 (0.92, 1.32)	-0.01 (-0.14, 0.13)	-0.01 (-0.60, 0.53)
Number of pathogens	1.01 (0.95, 1.08)	-4.7×10^{-4} (-0.06, 0.05)	0.03 (-0.17, 0.24)
Any bacterial pathogen	1.18 (1.02, 1.37)	0.03 (-0.08, 0.16)	0.21 (-0.31, 0.66)
Number of bacterial pathogens	1.09 (1.01, 1.17)	0.01 (-0.05, 0.07)	0.07 (-0.19, 0.31)
Any parasitic pathogen	0.84 (0.71, 0.99)	0.04 (-0.09, 0.17)	0.12 (-0.49, 0.67)
Number of parasitic pathogens	0.84 (0.72, 0.95)	0.03 (-0.09, 0.15)	0.08 (-0.41, 0.54)
Any viral pathogen	0.89 (0.75, 1.04)	-0.08 (-0.20, 0.05)	-0.26 (-0.83, 0.26)
Number of viral pathogens	0.93 (0.80, 1.07)	-0.07 (-0.19, 0.07)	-0.21 (-0.66, 0.27)

[1]All estimates adjusted for round;

[2]Risk ratio estimated by mixed effects Poisson regression, with a random effect for compound;

[3]Confidence interval;

[4]Estimate is from mixed effects linear regression for the diversity metric;

[5]Limited to children whose survey for self-reported diarrhea was ≤7 days from stool specimen collection (n = 94)

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old) in dense, low-income compounds in Maputo, Mozambique. Total ARGs detected from stool using an 84-target array varied from 4–28 per stool specimen. The most common ARGs detected were associated with resistance to MLS, tetracyclines, β-lactams, and aminoglycosides. We did not observed significant direct associations between sanitation facilities and ARGs, though sanitation conditions were poor overall and therefore the study's setting made it impossible to examine a wider range of WASH conditions. However, the total number of ARGs detected in a stool specimen was associated with the presence and number of bacterial pathogens detected in stool and inversely associated with presence of parasitic pathogens (mostly *Giardia* spp.), suggesting potential indirect links with WASH risk factors through enteric pathogen exposure. Differences in associations with risk factors by metric (total ARGs vs. diversity measures) may also warrant further investigation of these outcomes.

This study provides important evidence of the background carriage of ARGs in the developing gut of children in a low-income, urban environment of an LMIC and quantifies risk factors for ARG detection from the environment and concurrent enteric pathogen carriage. Although there are no studies—to our knowledge—focusing on children under 1 year of age in LMIC settings, these data are consistent with evidence from high-income settings of prevalent colonization of ARGs conferring resistance to aminoglycoside, tetracycline, or β-lactam antibiotics in the early infant gut [50–55].

In addition to the importance of ARG fate and transport in the environment and the child gut, the ARGs detected have potential clinical consequences in this setting. Most ARGs detected conferred resistance to MLS, tetracycline, β-lactam, or aminoglycoside antibiotics, with about 50% of children also having ARGs conferring resistance to fluoroquinolones. Mozambican guidelines for treatment of enteric infections in children <2 years old suggest ampicillin and gentamicin [3], which may have resistance conferred via β-lactamase [56] and aminoglycoside [57] ARGs, among others. Among children >2 years, chloramphenicol (a fluoroquinolone) is suggested [3]. Pediatric ARG surveillance is rare outside of clinical settings; however, our results indicate similarly high prevalence (≥85%) of tetracycline resistance genes (*tetA*, *tetB*) in children in this study as in previous clinical investigations of resistant *Shigella* and *Salmonella* in feces from children <5 with moderate-to-severe diarrhea in rural Mozambique [58]. Further, CTX-M gene prevalence (about 10%)—associated with extended-spectrum β-lactamase-producing *E. coli* and other coliforms—was lower in our study than in a recent assessment of *E. coli* and *Klebsiella* spp. in Mozambican university students (66%) [59]. However, given challenges in the interpretation of ARG detection as compared with phenotypic resistance [23,25], we underscore that molecular ARG detection in stool alone, even in the presence of an enteric pathogen, does not imply that the antimicrobial would not be useful for treatment of an enteric infection, nor that the pathogen is phenotypically resistant to it.

There are numerous potential sources of ARGs for the developing infant and child's gut. Studies in high-income settings suggest infants may acquire ARGs from their mother—both with and without recent maternal antibiotic use—before, during, and just after birth, especially tetracycline and β-lactamase ARGs [43,50,51,53]. Specifically, the type of delivery (e.g. increased detection of tetracycline ARGs from the mother's vagina in infants born vaginally vs. Cesarean section [53]), breast milk [43], and skin contact [43] may be important sources of mother-to-child ARG transmission. Longitudinal assessment of the infant gut resistome suggests environmental contributions to ARGs in the first year of life [60]. Notably, ARGs colonizing the gut may also be housed in commensal bacteria [61], suggesting both pathogenic and commensal bacterial exposures as sources.

Environmental exposure measures (e.g. type of sanitation) were not directly associated with pediatric ARG detection although emerging evidence suggests WASH facilitates the development and environmental dispersion of AR organisms at regional or national scales [5–8].

WASH conditions in study compounds were generally poor [39] and may not have improved sufficiently—even with the introduction of septic tanks—to observe or detect differences in ARGs. Given this evidence, local or site-specific sources, dynamics, and exposure pathways for AR organisms in these environments—especially with decentralized or onsite sanitation systems—are poorly understood. One study in rural Bangladeshi households with prevalent animal and human fecal contamination concluded that the physical and chemical characteristics of the environment (for example, water content of soil) were more important than household WASH characteristics in the detection of soil *E. coli*, many of which were pathogenic and carried clinically-relevant ARGs [62]. These findings suggest that other physical and chemical conditions of the household environment in Maputo, which we did not measure and could have changed with the introduction of water-based septic systems in round 2 in the MapSan trial [26], may also have modified WASH-AR organism relationships. Such change in environmental conditions with the introduction of septic systems may explain why significantly more ARGs were detected in round 2 children than those in round 1. Importantly, because septic systems were introduced for round 2, it was difficult to separate their associations with ARGs from the significantly higher detection of ARGs in round 2 alone, especially given small sample sizes. This could explain why septic tanks were associated with moderately higher ARG detection in unadjusted models but not in those adjusted for round.

Associations between total ARGs and carriage of enteric bacterial pathogens, including modest evidence of dose-response between total ARGs and number of bacterial pathogens, suggest these pathogens as potential vectors of ARGs and, subsuquently, indirect links with poor WASH. Data from clinical surveillance in Mozambique suggest phenotypic resistance to first- and second-line treatments for enteric infections is high among children <5 presenting with diarrhea due to enteric bacteria (e.g. *Salmonella enterica*, *Shigella* spp., ETEC [58,63]). AR in human and animal enteric pathogens is also increasing in the region [64]. Correspondingly, carriage of ARGs by enteric bacteria in the environment may concurrently increase, suggesting that continual high levels of exposure to such bacteria may transfer genotypic, and potentially phenotypic, resistance. Notably, the direction of association could also be reversed: that is, prevalent clinical treatment of pediatric diarrhea—irrespective of etiologic agent—with antibiotics and widespread non-prescription antimicrobial use in LMICs [2,3] could drive antimicrobial treatment, and subsequent selection pressure and ARG transfer. However, because we used molecular (and not culture-based) techniques and this was a cross-sectional assessment, we cannot ascertain whether the specific enteric pathogens detected in a child's stool were also carrying the specific ARGs that were concurrently detected. Pediatric exposure to antimicrobial treatment for other infections (e.g. respiratory infections), may also be significant: in Mozambique, invasive pneumococcal disease, which affects 416/100,000 child-years, is highest in children <2 and requires treatment with penicillin and chloramphenicol [3]. Thus, concurrent antimicrobial use that we did not assess in our survey may contribute to ARG carriage.

Inverse associations between parasites—primarily *Giardia* spp.—and total ARGs may indicate that previous evidence suggesting bacterial colonization of the gut is modified with concurrent *Giardia* spp. carriage extends to ARG transfer, though this finding would be the first of its kind and should be investigated further. Although the role of *Giardia* spp. in the incidence and severity of co-infecting agents is still being studied, *in vivo* mouse studies and *in vitro* studies of human cells suggest *Giardia* spp. may modify host immune response to bacterial pathogens, including reducing inflammatory responses and shortening bacterial attachment to gut lining [65–67]. These *Giardia*-bacteria interactions may reduce the incidence of symptoms in the host, and potentially could also—directly or indirectly—play a role in reducing colonization and subsequent ARG transfer via various molecular mechanisms in the human gut [68]. Conversely, if antibiotic exposure from treatment of diarrheal

infections drives ARG transfer in this environment, then the well-documented reduction in diarrhea associated with *Giardia* spp. infection [67] may also explain the reductions in ARGs detected.

Measures of gut ARG diversity were largely not associated with demographic or enteric pathogen risk factors, yet may be an important additional metric worth further study. The diversity of ARGs can provide important information about environmental exposures and changes in the gut, as in previous molecular and metagenomic analyses of gut bacteria and ARGs [43–45,69,70]. Thus, while we were not able to fully examine ARG diversity in this exploratory analysis, these metrics may warrant further assessment in future studies.

There are several limitations of this analysis. Importantly, given the absence of risk factor analyses for exposure to AR pathogens or ARGs in these settings, we used an exploratory approach that warrants caution and further investigation of significant associations detected using targeted, hypothesis-testing approaches. As noted, we were unable to quantify other potential environmental pathways of exposure to ARGs, such as food from animals treated with antimicrobials [71,72]. Although we adjusted for reported diarrhea as a proxy for recent antimicrobial treatment in a sensitivity analysis and did not observe evidence of confounding, we were unable to directly quantify the recent antibiotic or other clinical history of the child, an important potential risk factor [73]. Finally, we used a commercial array to assess pediatric AR profiles and test existing multi-target qPCR technologies for population-level ARG quantification in a low-income setting; however, the array may be more appropriate for clinically-relevant ARGs in high-income countries and therefore may not have comprised all ARGs of importance to children's guts in LMICs. Metagenomic approaches, though costly, may be a useful alternative with enhanced capacity (through access to ARG databases) to characterize the complete molecular 'resistome' and identify key ARGs of public health importance with environmental transmission [70,74–76].

We observed prevalent ARGs that could confer resistance to first-line drugs for multiple infections, including enteric infections, in the guts of young children (<14 months old) in a densely-populated, low-income, urban setting. Environmental risk factors—including poor sanitation—were not directly associated with ARG detection in childrens' guts; however, enteric bacterial pathogen carriage in stool was associated with increases in ARGs detected. This analysis provides important early data to begin elucidating the role that WASH—directly or indirectly—may play in transmission of AR in children in LMICs.

Supporting information

S1 Table. Specific enteric infections among children < 14 months old, Maputo, Mozambique. Table describing prevalence of specific enteropathogens in children's stool.
(DOCX)

S2 Table. List of ARGs and their prevalence in children < 14 months old, Maputo, Mozambique. Table describing prevalence of specific ARGs in children's stool.
(DOCX)

S3 Table. Associations between enteric pathogen-related characteristics and ARG outcomes among children < 14 months old, Maputo, Mozambique adjusted for round and reported diarrhea. Sensitivity analysis of associations between enteric pathogen detection and ARG outcomes among children with reported diarrhea outcomes.
(DOCX)

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References

1. CDDEP. State of the world's antibiotics, 2015. 2015.
2. Rogawski ET, Platts-Mills JA, Seidman JC, John S, Mahfuz M, Ulak M, et al. Use of antibiotics in children younger than two years in eight countries: a prospective cohort study. Bull World Health Organ. 2016; 95:49–61. https://doi.org/10.2471/BLT.16.176123 PMID: 28053364
3. Global Antibiotic Resistance Partnership, Mozambican Ministry of Health, Centro de investigacao em saude de manhica. Situation Analysis: Antibiotic Use and Resistance in Mozambique. 2015.
4. Keenan JD, Bailey RL, West SK, Arzika AM, Hart J, Weaver J, et al. Azithromycin to Reduce Childhood Mortality in Sub-Saharan Africa. N Engl J Med. 2018;1583–92.
5. Collignon P, Beggs JJ, Walsh TR, Gandra S, Laxminarayan R. Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. Lancet Planet Heal [Internet]. 2018; 2(9):e398–405. http://dx.doi.org/10.1016/S2542-5196(18)30186-4
6. Graham DW, Collignon P, Davies J, Larsson DGJ, Snape J. Underappreciated role of regionally poor water quality on globally increasing antibiotic resistance. Environ Sci Technol. 2014; 48(20):11746–7. https://doi.org/10.1021/es504206x PMID: 25330712
7. Graham DW, Giesen MJ, Bunce JT. Strategic approach for prioritising local and regional sanitation interventions for reducing global antibiotic resistance. Water (Switzerland). 2018; 11(1).

8. Talukdar PK, Rahman M, Rahman M, Nabi A, Islam Z, Hoque MM, et al. Antimicrobial Resistance, Virulence Factors and Genetic Diversity of Escherichia coli Isolates from Household Water Supply in Dhaka, Bangladesh. PLoS One. 2013; 8(4):1–8.
9. Ashbolt NJ, Amézquita A, Backhaus T, Borriello P, Brandt KK, Collignon P, et al. Human health risk assessment (HHRA) for environmental development and transfer of antibiotic resistance. Environ Health Perspect. 2013; 121(9):993–1001. https://doi.org/10.1289/ehp.1206316 PMID: 23838256
10. Fletcher S. Understanding the contribution of environmental factors in the spread of antimicrobial resistance. Environ Health Prev Med. 2015; 20(4):243–52. https://doi.org/10.1007/s12199-015-0468-0 PMID: 25921603
11. Berendes D, Leon J, Kirby A, Clennon J, Raj S, Yakubu H, et al. Household sanitation is associated with lower risk of bacterial and protozoal enteric infections, but not viral infections and diarrhea, in a cohort study in a low-income urban neighborhood in Vellore, India. Trop Med Int Heal [Internet]. 2017; 00(00):1–11. http://www.ncbi.nlm.nih.gov/pubmed/28653489%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/28653489
12. Berendes D, Kirby A, Clennon JA, Raj S, Yakubu H, Leon J, et al. The Influence of Household-and Community-Level Sanitation and Fecal Sludge Management on Urban Fecal Contamination in Households and Drains and Enteric Infection in Children. Am J Trop Med Hyg. 2017; 96(6):1404–14. https://doi.org/10.4269/ajtmh.16-0170 PMID: 28719269
13. Laxminarayan R, Duse A, Wattal C, Zaidi AKM, Wertheim HFL, Sumpradit N, et al. Antibiotic resistance-the need for global solutions. Lancet Infect Dis. 2013; 13(12):1057–98. https://doi.org/10.1016/S1473-3099(13)70318-9 PMID: 24252483
14. Vikesland PJ, Pruden A, Alvarez PJJ, Aga DS, Buergmann H, Li X, et al. Towards a Comprehensive Strategy to Mitigate Dissemination of Environmental Sources of Antibiotic Resistance. Environ Sci Technol [Internet]. 2017;acs.est.7b03623. http://www.ncbi.nlm.nih.gov/pubmed/28976743%0Ahttp://pubs.acs.org/doi/abs/10.1021/acs.est.7b03623
15. Calero-Cáceres W, Melgarejo A, Colomer-Lluch M, Stoll C, Lucena F, Jofre J, et al. Sludge as a potential important source of antibiotic resistance genes in both the bacterial and bacteriophage fractions. Environ Sci Technol. 2014; 48(13):7602–11. https://doi.org/10.1021/es501851s PMID: 24873655
16. Finley RL, Collignon P, Larsson DGJ, Mcewen SA, Li XZ, Gaze WH, et al. The scourge of antibiotic resistance: The important role of the environment. Clin Infect Dis. 2013; 57(5):704–10. https://doi.org/10.1093/cid/cit355 PMID: 23723195
17. Bouki C, Venieri D, Diamadopoulos E. Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: A review. Ecotoxicol Environ Saf [Internet]. 2013; 91:1–9. http://dx.doi.org/10.1016/j.ecoenv.2013.01.016 PMID: 23414720
18. Karkman A, Pärnänen K, Larsson DGJ. Fecal pollution explains antibiotic resistance gene abundances in anthropogenically impacted environments. Nat Commun [Internet]. 2018; 10(80):341487. https://www.biorxiv.org/content/early/2018/06/07/341487
19. Berendes DM, Sumner TA, Brown JM. Safely Managed Sanitation for All Means Fecal Sludge Management for At Least 1.8 Billion People in Low and Middle Income Countries. Environ Sci Technol. 2017; 51(5):3074–83. https://doi.org/10.1021/acs.est.6b06019 PMID: 28128924
20. UNICEF, WHO. Progress on Drinking Water, Sanitation and Hygiene [Internet]. 2017. http://apps.who.int/iris/bitstream/10665/258617/1/9789241512893-eng.pdf%0Ahttp://www.wipo.int/amc/en/%0Ahttp://www.wipo.int/amc/en/
21. Baum R, Luh J, Bartram J. Sanitation: A global estimate of sewerage connections without treatment and the resulting impact on MDG progress. Environ Sci Technol. 2013; 47(4):1994–2000. https://doi.org/10.1021/es304284f PMID: 23323809
22. Berendes DM, Yang PJ, Lai A, Hu D, Brown J. Estimation of global recoverable human and animal faecal biomass. Nat Sustain [Internet]. 2018 Nov 13 [cited 2018 Nov 13]; 1(11):679–85. http://www.nature.com/articles/s41893-018-0167-0
23. McLain JE, Cytryn E, Durso LM, Young S. Culture-based Methods for Detection of Antibiotic Resistance in Agroecosystems: Advantages, Challenges, and Gaps in Knowledge. J Environ Qual [Internet]. 2016; 0(0):0. https://dl.sciencesocieties.org/publications/jeq/abstracts/0/0/jeq2015.06.0317
24. Berendonk TU, Manaia CM, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, et al. Tackling antibiotic resistance: the environmental framework. Nat Rev Microbiol [Internet]. 2015; 13(5):310–7. http://www.nature.com/doifinder/10.1038/nrmicro3439 PMID: 25817583
25. Suzuki S, Horinouchi T, Furusawa C. Prediction of antibiotic resistance by gene expression profiles. Nat Commun [Internet]. 2014; 5:5792. http://www.nature.com/doifinder/10.1038/ncomms6792%5Cnhttp://dx.doi.org/10.1038/ncomms6792%5Cn10.1038/ncomms6792 PMID: 25517437
26. Brown J, Cumming O, Bartram J, Cairncross S, Ensink J, Holcomb D, et al. A controlled, before-and-after trial of an urban sanitation intervention to reduce enteric infections in children: research protocol

for the Maputo Sanitation (MapSan) study, Mozambique. BMJ Open [Internet]. 2015; 5(6):e008215. http://bmjopen.bmj.com/content/5/6/e008215.short https://doi.org/10.1136/bmjopen-2015-008215 PMID: 26088809

27. Perry MD, Corden SA, Howe RA. Evaluation of the luminex xTAG Gastrointestinal Pathogen Panel and the Savyon Diagnostics Gastrointestinal Infection Panel for the detection of enteric pathogens in clinical samples. J Med Microbiol. 2014; 63(2014):1419–26.

28. Navidad JF, Griswold DJ, Gradus MS, Bhattacharyya S. Evaluation of Luminex xTAG Gastrointestinal Pathogen Analyte-Specific Reagents for High-Throughput, Simultaneous Detection of Bacteria, Viruses, and Parasites of Clinical and Public Health Importance. J Clin Microbiol [Internet]. 2013; 51 (9):3018–24. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3754681/ https://doi.org/10.1128/JCM.00896-13 PMID: 23850948

29. Patel A, Navidad J, Bhattacharyya S. Site-specific clinical evaluation of the Luminex xTAG gastrointestinal pathogen panel for detection of infectious gastroenteritis in fecal specimens. J Clin Microbiol. 2014; 52(8):3068–71. https://doi.org/10.1128/JCM.01393-14 PMID: 24899032

30. Igbokwe H, Bhattacharyya S, Gradus S, Khubbar M, Griswold D, Navidad J, et al. Preponderance of toxigenic Escherichia coli in stool pathogens correlates with toxin detection in accessible drinking-water sources. Epidemiol Infect [Internet]. 2014;1–11. http://www.ncbi.nlm.nih.gov/pubmed/24787554

31. Deng J, Luo X, Wang R, Jiang L, Ding X, Hao W, et al. A comparison of Luminex xTAG® Gastrointestinal Pathogen Panel (xTAG GPP) and routine tests for the detection of enteropathogens circulating in Southern China. Diagn Microbiol Infect Dis [Internet]. 2015; 83(3):325–30. http://dx.doi.org/10.1016/j.diagmicrobio.2015.07.024 PMID: 26318973

32. Duong VT, Phat VV, Tuyen HT, Dung TTN, Trung PD, Van Minh P, et al. Evaluation of luminex xTAG gastrointestinal pathogen panel assay for detection of multiple diarrheal pathogens in fecal samples in Vietnam. J Clin Microbiol. 2016; 54(4):1094–100. https://doi.org/10.1128/JCM.03321-15 PMID: 26865681

33. Eibach D, Krumkamp R, Hahn A, Sarpong N, Adu-Sarkodie Y, Leva A, et al. Application of a multiplex PCR assay for the detection of gastrointestinal pathogens in a rural African setting. BMC Infect Dis [Internet]. 2016; 16(1):1–6. http://dx.doi.org/10.1186/s12879-016-1481-7

34. Jouhten H, Mattila E, Arkkila P, Satokari R. Reduction of Antibiotic Resistance Genes in Intestinal Microbiota of Patients With Recurrent Clostridium difficile Infection After Fecal Microbiota Transplantation. Clin Infect Dis. 2016; 63(5):710–1. https://doi.org/10.1093/cid/ciw390 PMID: 27317794

35. Hu H, Han X, Shi X, Wang J, Han L, Chen D, et al. Temporal changes of antibiotic-resistance genes and bacterial communities in two contrasting soils. FEMS Microbiol Ecol. 2016; 92(2):1–13.

36. Han XM, Hu HW, Shi XZ, Wang JT, Han LL, Chen D, et al. Impacts of reclaimed water irrigation on soil antibiotic resistome in urban parks of Victoria, Australia. Environ Pollut [Internet]. 2016; 211:48–57. http://dx.doi.org/10.1016/j.envpol.2015.12.033 PMID: 26736055

37. Vikram A, Rovira P, Agga GE, Arthur TM, Bosilevac JM, Wheeler TL, et al. Impact of "raised without antibiotics" beef cattle production practices on occurrences of antimicrobial resistance. Appl Environ Microbiol. 2017; 83(22):1–15.

38. Agga GE, Arthur TM, Durso LM, Harhay DM, Schmidt JW. Antimicrobial-resistant bacterial populations and antimicrobial resistance genes obtained from environments impacted by livestock and municipal waste. PLoS One [Internet]. 2015; 10(7):1–19. http://dx.doi.org/10.1371/journal.pone.0132586

39. Knee J, Sumner T, Adriano Z, Berendes D, de Bruijn E, Schmidt W-P, et al. Risk factors for childhood enteric infection in urban Maputo, Mozambique: A cross-sectional study. PLoS Negl Trop Dis [Internet]. 2018 Nov 12; 12(11):e0006956. https://doi.org/10.1371/journal.pntd.0006956 PMID: 30419034

40. Schreiner M. Simple Poverty Scorecard ® Poverty-Assessment Tool Mozambique. 2013.

41. Pielou EC. Shannon's Formula as a Measure of Specific Diversity: Its Use and Misuse. Am Nat. 1966; 100(914):463–5.

42. Simpson EH. Measurement of Diversity. Nature [Internet]. 1949 Apr 30; 163:688. https://doi.org/10.1038/163688a0

43. Pärnänen K, Karkman A, Hultman J, Lyra C, Bengtsson-Palme J, Larsson DGJ, et al. Maternal gut and breast milk microbiota affect infant gut antibiotic resistome and mobile genetic elements. Nat Commun [Internet]. 2018; 9(1):1–11. http://dx.doi.org/10.1038/s41467-018-06393-w

44. Fisher JC, Murat Eren A, Green HC, Shanks OC, Morrison HG, Vineis JH, et al. Comparison of sewage and animal fecal microbiomes by using oligotyping reveals potential human fecal indicators in multiple taxonomic groups. Appl Environ Microbiol. 2015; 81(20):7023–33. https://doi.org/10.1128/AEM.01524-15 PMID: 26231648

45. Ma Y, Wilson CA, Novak JT, Riffat R, Aynur S, Murthy S, et al. Effect of various sludge digestion conditions on sulfonamide, macrolide, and tetracycline resistance genes and class i integrons. Environ Sci Technol. 2011; 45(18):7855–61. https://doi.org/10.1021/es200827t PMID: 21815642

46. Jacoby GA, Munoz-price LS. The New Beta-Lactamases. N Engl J Med. 2005; 352(4):380–91. https://doi.org/10.1056/NEJMra041359 PMID: 15673804

47. R Core Team. R: A language and environment for statistical computing. [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2015. https://www.r-project.org/

48. Bates D, Maechler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw [Internet]. 2014; 67(1):1–48. http://arxiv.org/abs/1406.5823

49. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, et al. vegan: Community Ecology Package. R package version 2.3–0. 2015. R Found Stat Comput Vienna, Austria. 2015;

50. Gosalbes MJ, Vallès Y, Jiménez-Hernández N, Balle C, Riva P, Miravet-Verde S, et al. High frequencies of antibiotic resistance genes in infants' meconium and early fecal samples. J Dev Orig Health Dis [Internet]. 2016; 7(01):35–44. http://www.journals.cambridge.org/abstract_S2040174415001506

51. de Vries LE, Vallès Y, Agersø Y, Vaishampayan PA, García-Montaner A, Kuehl J V., et al. The gut as reservoir of antibiotic resistance: Microbial diversity of tetracycline resistance in mother and infant. PLoS One. 2011; 6(6).

52. Karami N, Nowrouzian F, Adlerberth I, Wold AE. Tetracycline Resistance in Escherichia coli and Persistence in the Infantile Colonic Microbiota Tetracycline Resistance in Escherichia coli and Persistence in the Infantile Colonic Microbiota. Antimicrob Agents Chemother. 2006; 50(1):156–61. https://doi.org/10.1128/AAC.50.1.156-161.2006 PMID: 16377681

53. Alicea-Serrano AM, Contreras M, Magris M, Hidalgo G, Dominguez-Bello MG. Tetracycline resistance genes acquired at birth. Arch Microbiol. 2013; 195(6):447–51. https://doi.org/10.1007/s00203-012-0864-4 PMID: 23483141

54. von Wintersdorff CJ, Wolffs PF, Savelkoul PH, Nijsen RR, Lau S, Gerhold K, et al. The gut resistome is highly dynamic during the first months of life. Future Microbiol [Internet]. 2016; 11:501–10. http://www.futuremedicine.com.proxy.library.uu.nl/doi/abs/10.2217/fmb.15.154 PMID: 27064174

55. Moore AM, Patel S, Forsberg KJ, Wang B, Bentley G, Razia Y, et al. Pediatric fecal microbiota harbor diverse and novel antibiotic resistance genes. PLoS One. 2013; 8(11).

56. Sutcliffe JG. Nucleotide sequence of the ampicillin resistance gene of Escherichia coli plasmid pBR322. Proc Natl Acad Sci [Internet]. 1978; 75(8):3737–41. http://www.pnas.org/cgi/doi/10.1073/pnas.75.8.3737 PMID: 358200

57. Kao SJ, You I, Clewell DB, Donabedian SM, Zervos MJ, Petrin J, et al. Detection of the high-level aminoglycoside resistance gene aph(2")-Ib in Enterococcus faecium. Antimicrob Agents Chemother. 2000; 44(10):2876–9. https://doi.org/10.1128/aac.44.10.2876-2879.2000 PMID: 10991878

58. Mandomando I, Jaintilal D, Pons MJ, Valles X, Espasa M, Mensa L, et al. Antimicrobial Susceptibility and Mechanisms of Resistance in Shigella and Salmonella Isolates from Children under Five Years of Age with Diarrhea in Rural Mozambique. Antimicrob Agents Chemother. 2009; 53(6):2450–4. PMID: 19332670

59. Chirindze LM, Zimba TF, Sekyere JO, Govinden U, Chenia HY, Sundsfjord A, et al. Faecal colonization of E. coli and Klebsiella spp. producing extended-spectrum beta-lactamases and plasmid-mediated AmpC in Mozambican university students. BMC Infect Dis. 2018; 18(1):1–8. https://doi.org/10.1186/s12879-017-2892-9

60. Moore AM, Ahmadi S, Patel S, Gibson MK, Wang B, Ndao IM, et al. Gut resistome development in healthy twin pairs in the first year of life. Microbiome [Internet]. 2015; 3(1):27. http://www.microbiomejournal.com/content/3/1/27

61. Rolain JM. Food and human gut as reservoirs of transferable antibiotic resistance encoding genes. Front Microbiol. 2013; 4(JUN):1–10.

62. Montealegre MC, Roy S, Böni F, Hossain MI, Navab-Daneshmand T, Caduff L, et al. Risk factors for detection, survival, and growth of antibiotic-resistant and pathogenic Escherichia coli in household soils in rural Bangladesh. Appl Environ Microbiol [Internet]. 2018;(October):AEM.01978-18. http://www.ncbi.nlm.nih.gov/pubmed/30315075

63. Mandomando I, Macete E, Ruiz J, Sanz S, Abacassamo F, Valles X, et al. Etiology of diarrhea in children younger than 5 years of age admitted in a rural hospital of southern Mozambique. Am J Trop Med Hyg. 2007; 76(3):522–7. PMID: 17360878

64. Mshana SE, Matee M, Rweyemamu M. Antimicrobial resistance in human and animal pathogens in Zambia, Democratic Republic of Congo, Mozambique and Tanzania : an urgent need of a sustainable surveillance system. Ann Clin Microbiol Antimicrob [Internet]. 2013; 12(1):1. Annals of Clinical Microbiology and Antimicrobials

65. Manko A, Motta JP, Cotton JA, Feener T, Oyeyemi A, Vallance BA, et al. Giardia co-infection promotes the secretion of antimicrobial peptides beta-defensin 2 and trefoil factor 3 and attenuates attaching and effacing bacteria-induced intestinal disease. PLoS One. 2017; 12(6):1–22.

66. Cotton JA, Motta JP, Schenck LP, Hirota SA, Beck PL, Buret AG. Giardia duodenalis infection reduces granulocyte infiltration in an in vivo model of bacterial toxin-induced colitis and attenuates inflammation in human intestinal tissue. PLoS One. 2014; 9(10):1–15.

67. Cotton J, Amat C, Buret A. Disruptions of Host Immunity and Inflammation by Giardia Duodenalis: Potential Consequences for Co-Infections in the Gastro-Intestinal Tract. Pathogens [Internet]. 2015; 4(4):764–92. http://www.mdpi.com/2076-0817/4/4/764/ https://doi.org/10.3390/pathogens4040764 PMID: 26569316

68. Huddleston JR. Horizontal gene transfer in the human gastrointestinal tract: Potential spread of antibiotic resistance genes. Infect Drug Resist. 2014; 7:167–76. https://doi.org/10.2147/IDR.S48820 PMID: 25018641

69. Wright GD. The antibiotic resistome: the nexus of chemical and genetic diversity. NatRevMicrobiol [Internet]. 2007; 5(1740–1534 (Electronic)):175–86. c:%5CKARSTEN%5CPDFs%5CAntibiotika-PDFs%5CAnti-2007%5CWright-The antibiotic resistome- the nexus of chemical and genetic diversity.pdf

70. Pal C, Bengtsson-Palme J, Kristiansson E, Larsson DGJ. The structure and diversity of human, animal and environmental resistomes. Microbiome [Internet]. 2016; 4(1):54. http://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-016-0199-5 PMID: 27717408

71. Silbergeld EK, Graham J, Price LB. Industrial food animal production, antimicrobial resistance, and human health. Annu Rev Public Health [Internet]. 2008; 29(March 2014):151–69. http://www.ncbi.nlm.nih.gov/pubmed/18348709

72. Sarmah AK, Meyer MT, Boxall ABA. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere. 2006; 65 (5):725–59. https://doi.org/10.1016/j.chemosphere.2006.03.026 PMID: 16677683

73. Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E. Fecal Colonization With Extended-spectrum Beta- lactamase–Producing Enterobacteriaceae and Risk Factors Among Healthy Individuals : A Systematic Review and Metaanalysis. Clin Infect Dis. 2016; 63(3):310–8. https://doi.org/10.1093/cid/ciw283 PMID: 27143671

74. Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MOA, Dantas G. The shared antibiotic resistome of soil bacteria and human pathogens. Science (80-). 2012; 337(6098):1107–11.

75. Gibson MK, Forsberg KJ, Dantas G. Improved annotation of antibiotic resistance determinants reveals microbial resistomes cluster by ecology. ISME J [Internet]. 2014; 9(1):1–10. http://www.nature.com/doifinder/10.1038/ismej.2014.106%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/25003965

76. Arango-Argoty GA, Garner E, Pruden A, Heath LS, Vikesland P, Zhang L. DeepARG : A deep learning approach for predicting antibiotic resistance genes from metagenomic data. bioRxiv. 2017;pre-print.