Check for updates

RESEARCH ARTICLE

Respiratory and diarrhoeal pathogens in Malawian children hospitalised with diarrhoea and association with short-term growth: A prospective cohort study [version 1; peer review: 1 approved with reservations]

Mphatso Chisala[1], Wongani Nyangulu[2], James Nyirenda[2], Pui-Ying Iroh Tam[2-4]

[1]Institute of Child Health; Department of Population, Policy and Practice, University College London Medical School, London, UK
[2]Paediatrics and Child Health Research Group, Malawi-Liverpool Wellcome Research Programme, Blantyre, Malawi
[3]Department of Paediatrics, Kamuzu University of Health Sciences, Blantyre, Malawi
[4]Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK

v1 **First published:** 22 Nov 2022, **6**:145
https://doi.org/10.12688/gatesopenres.14061.1
Latest published: 22 Nov 2022, **6**:145
https://doi.org/10.12688/gatesopenres.14061.1

Abstract

Background: Pneumonia and diarrhoea are the leading causes of childhood mortality and morbidity worldwide. The gut-lung axis is associated with disease, and these common infections, especially the parasite *Cryptosporidium,* are associated with malnutrition. We sought to evaluate the association of respiratory and gastrointestinal (GI) pathogens with short-term growth among children hospitalised with diarrhoeal disease.

Methods: In this sub-study, we followed 27 children (two-24 months) who tested positive for *Cryptosporidium* spp. for eight weeks with two weekly sampling of the respiratory and GI tract. Respiratory and stool pathogens were detected using quantitative molecular methods. Nutritional outcomes were assessed as length-for-age (LAZ), weight-for-length (WLZ) and weight-for-age (WAZ) z-scores. Changes over the study period were compared using repeated analysis of variance and mixed effects model analysis.

Results: In this period,104 sputum and stool samples were collected. All stool samples had at least one pathogen detected, with an average of 5.1 (SD 2.1) stool pathogens, compared to 84% of the sputum samples with an average 3.5 (SD 1.8). Diarrhoeagenic *E. coli* were the most common stool pathogens (89%), followed by *Cryptosporidium* (57.6%) and Adenovirus pan (41%). In sputum, *Streptococcus pneumoniae* was the most prevalent pathogen (84%), followed by hinovirus (56%) and *Moraxella catarrhalis* (50%). There was a significant change in WAZ over the follow-up period. Children who had ≥3 GI pathogens had significantly a lower LAZ mean score at enrolment (-1.8 [SD 1.4]) and across the follow-up period. No relationship between respiratory pathogens and short-term growth

Open Peer Review

Approval Status ?

	1
version 1 22 Nov 2022	? view

1. **Monira Sarmin**, International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh

Any reports and responses or comments on the article can be found at the end of the article.

was observed. Out of 49 sputum samples that had ≥3 pathogens, 42 (85%) concurrent stool samples had ≥3 GI pathogens.
Conclusions: Among young children hospitalised with diarrhoea, multiple GI and respiratory pathogens were prevalent over an eight-week follow-up period. The presence of more GI, but not respiratory, pathogens was significantly associated with reduced short-term growth.

Keywords
GI pathogens, respiratory pathogens, nutritional status, gut-lung axis

Corresponding author: Mphatso Chisala (mphatsochisala@gmail.com)

Author roles: Chisala M: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Nyangulu W**: Methodology, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing; **Nyirenda J**: Formal Analysis, Investigation, Validation, Writing – Review & Editing; **Iroh Tam PY**: Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported, in whole or in part, by the Bill and Melinda Gates Foundation [OPP1191165]. Under the grant conditions of the Foundation, a Creative Commons Attribution 4.0 Generic License has already been assigned to the Author Accepted Manuscript version that might arise from this submission. The funding body had no role in the design of the study; collection, analysis, and interpretation of data; and in writing the manuscript.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2022 Chisala M *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Chisala M, Nyangulu W, Nyirenda J and Iroh Tam PY. **Respiratory and diarrhoeal pathogens in Malawian children hospitalised with diarrhoea and association with short-term growth: A prospective cohort study [version 1; peer review: 1 approved with reservations]** Gates Open Research 2022, **6**:145 https://doi.org/10.12688/gatesopenres.14061.1

First published: 22 Nov 2022, **6**:145 https://doi.org/10.12688/gatesopenres.14061.1

Introduction

Lower respiratory infections (LRI) and diarrhoeal diseases are the top leading preventable causes of mortality and morbidity globally in children under five years of age, and are the annual cause of 12% (700,000) and 9% (446,000) of deaths, respectively[1–3]. Children with frequent and recurrent infections are at risk of malnutrition which also predisposes them to further infection[4]. Malnutrition is one of the most important risk factors for both diarrhoeal disease and LRI[3,5], and is associated with about half of all under-five deaths[6]. Reducing the burden of malnutrition could, therefore, concomitantly decrease respiratory and diarrhoeal disease amongst high-risk children. This was estimated in the "Global Burden of Disease" study to be a reduction of 9% of LRI and 12% of diarrhoeal disease over the past three decades[1,3].

Recently, large-scale studies including the "Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and Consequences for Child Health and Development" (MAL-ED) study and the "Global Enteric Multi-site" (GEMS) study explored the association between gastrointestinal (GI) pathogens, malnutrition and gut function with other long-term effects to understand the pathophysiology of malnutrition[7–9]. These studies noted that subclinical infections and quantity of pathogens are negatively associated with linear growth, and that this persists until two years of age[8–10]. Cumulative insults of infections like diarrhoeal on children likely cause failure of catch-up growth, resulting in growth faltering and decreased cognitive development[11–14]. However, the association between these pathogens and respiratory infections and short-term growth has not been explored.

The gut and lungs both have the same embryonic origin. Thus, while the mechanisms are not understood, studies have shown that the two sites interact in health and disease. Specifically, animal studies have demonstrated how the 'gut-lung axis' of host-associated gut and respiratory microbiota appear to influence local and systemic immunity[15–18]. However, while these studies have focused on how gut microbiota is broadly protective against respiratory infection[19], there has been less attention paid to the reverse relationship, *i.e.* respiratory microbiota and pathogens influencing the gut, and subsequent association with growth. Studies have indicated a link between respiratory infections and growth[20,21] although generally the pathways between growth, nutrition and infection are likely bidirectional.

Cryptosporidium infection is a common cause of diarrhoeal infection, malnutrition and excess mortality amongst children in developing countries[9,10]. The objective of this study was to describe detection of diarrhoeal and respiratory pathogens in children who were hospitalised with diarrhoeal disease (with detection of *Cryptosporidium)* at a tertiary hospital in Malawi, and to examine the association with short-term growth over an eight-week follow-up period.

Methods

Study design, setting, and participants

This is a secondary data analysis from a prospective longitudinal observational study evaluating respiratory cryptosporidiosis in paediatric diarrhoeal disease[22,23]. The main study recruited children presenting with primary gastrointestinal (GI) symptoms to Queen Elizabeth Central Hospital in Blantyre, Malawi, from March 2019 to April 2020. Children were eligible to participate if they were two-24 months of age and had at least three or more loose stools within the past 48 hours and lived 15km outside of the study[22,23]. Children were excluded if they had visible blood in loose stools, or dysentery[24]. Parents provided written informed consent. The study was approved by the University of Malawi College of Medicine Research Ethics Committee (P.07/18/2438) and the Liverpool School of Tropical Medicine Research Ethics Committee (18-066).

Clinical procedures

Participants positive for *Cryptosporidium* spp. in either respiratory or GI tract specimens at enrolment were followed up every two weeks until eight weeks post-enrolment. At each visit, history and physical exam were conducted, and induced sputum and stool were collected. The induced sputum procedure has been previously described in detail elsewhere[22,23]. In brief, sputum samples were obtained via oropharyngeal suctioning after nebulized 3% sodium chloride treatment and processed for microscopy and multiplex PCR testing.

For the main study, children with a positive PCR for *Cryptosporidium* in any of the collected samples were followed up, and they constitute the sample included in this sub-analysis[23]. At enrolment, *Cryptosporidium* spp. in stool/sputum/ NP were detected using PCR analysis specifically for *Cryptosporidium* spp. Diarrhoea was defined as ≥3 loose stools within the past 48 hours. A diarrhoeal episode was termed symptomatic if the participant reported any GI symptoms (to include abdominal pain/tenderness, dehydration, vomiting, and/or poor feeding) and the stool sample collected was PCR-positive for any pathogen, and asymptomatic if the participant did not report any GI symptoms but the stool was PCR-positive for any pathogen. Respiratory symptoms included cough, runny nose, difficulty in breathing, wheezing, chest indrawing/retractions, and/or crackles.

Nutrition indices were defined according to WHO growth standards[25]. We defined wasted, underweight or stunted, as weight-for-length z score (WLZ), weight-for-age z scores (WAZ) and length-for-age z score (LAZ) <-2, respectively.

Laboratory procedures

We extracted DNA from stool samples using a QIAamp Fast DNA Mini Kit (Qiagen, Hilden, Germany) with a procedure modified from that of the manufacturer as previously described[26]. Briefly, 200mg solid stool or 200µL liquid fecal samples were first mixed with InhibitEX buffer and glass beads before bead beating (Tissue Lyser II, Qiagen). Resulting lysates were heated at 95°C for 5 minutes prior to proceeding according to the manufacturer's protocol. Sputum samples for *Cryptosporidium* detection were extracted using QIAamp DNA mini kit. Briefly the 180µl ATL buffer and 20ul proteinase K was added to 300ul sample and incubated at 56°C for 1–3 hours with occasional vortexing during the incubation. This was followed by addition of 200µl Buffer AL and the sample was incubated at

70°C for 10 minutes. 200ul of absolute ethanol was added and this was followed by washing using 500µl buffer AW1 and 500µl buffer AW2. DNA was eluted using 200µl of elution buffer.

All samples were spiked with Phocine herpes virus (PhHV) and MS2 phage to be used as extraction controls. One extraction blank (200µL nuclease-free water as the sample) was included in each batch of extractions to monitor for contamination.

We performed qPCR as previously described[27]. These qPCRs were carried out using the ViiA7 or QuantStudio 7 Flex Real-Time PCR instruments (Thermo Fisher, Waltham, MA, USA). Primers (Crypto F primer: GGGTTGTATTTATTAGATAAAGAACCA, Crypto R primer: AGGCCAATACCCTACCGTCT) and probe (<FAM>GTGACATATCATTCAAGTTTCTGAC<BHQ1>). These were sourced from Integrated DNA Technologies (IDT, Coralville, Iowa, USA) and Sigma (Sigma-Aldrich, Haverhill, UK). All resulting qPCR data were analyzed using QuantStudio 6 and 7 Flex Real-Time PCR System Software, version 1.3 (Thermo Fisher). For initial denaturation and *Taq* activation we used one cycle at 95°C for 3 minutes, for amplification and subsequent target detection we used a total of 40 cycles (95°C for 10 seconds and 60°C for 1 minute). An analytical cutoff of 35 cycles was applied to the data (*i.e.* C_t values ≥35.0 were considered negative).

In sputum, multiplex testing detected bacteria (*S. pneumoniae, Staphylococcus aureus, M. catarrhalis, Bordetella pertussis, Haemophilus influenzae* and *H. influenzae* type b, *Chlamydia pneumoniae, Mycoplasma pneumoniae, Klebsiella pneumoniae, Legionella pneumophila,* and *Salmonella* species); viruses (influenza A/B/C, RSV A/B, parainfluenza virus types 1–4, coronaviruses NL63, 229E, OC43, and HKU1, human metapneumovirus A/B, rhinovirus, adenovirus, enterovirus, parechovirus, bocavirus, cytomegalovirus); and parasites (*Pneumocystis jirovecii*). A cycle threshold (Ct) of <38 was considered as a positive for any of these pathogens. *Cryptosporidium* spp. detection of respiratory specimens were measured using quantitative polymerase chain reaction (qPCR), with a positive result corresponding to a Ct <35.

In stool, GI pathogens were detected using qPCR in a TaqMan Array Card (Thermo Fisher, Waltham, MA) using a custom design developed at the Houpt Laboratory (Charlottesville, VA [25]). These were done at week 2 to week 8. Multiplex testing detected rotavirus, norovirus GII, adenovirus, astrovirus, sapovirus, enterotoxigenic *Escherichia coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), Shiga-toxigenic *E. coli* (STEC), Shigella/enteroinvasive *E. coli* (EIEC), *Salmonella, Campylobacter jejuni/coli, Vibrio cholerae, Clostridium difficile, Cryptosporidium* spp*, C. parvum, C. hominis, Giardia lamblia, Entamoeba histolytica, Ascaris lumbricoides,* and *Trichuris trichiura*). We considered a pathogen as present if Ct was <35 in all pathogens. For pathogens that were positive and are associated with diarrhoea in children under five years old[8,13], we calculated the prevalence of diarrhoeagenic Ct cut-offs (diarrhoea-associated Ct quantity) based on the GEMS study to estimate the prevalence of diarrhoeal samples in this population[27]. An increased pathogen load was defined as at least three pathogens present in each sample per participant per study visit to compare how these relate with other demographics[28].

Statistical analysis

At enrolment, categorical variables were compared using Pearson's X2 test or Fisher's exact test. Continuous variables were compared using Student's t-tests or nonparametric Mann-Whitney U tests where data were nonnormally distributed. We presented diarrhoeagenic quantitative cut-off based on the GEMS study to estimate the burden of diarrhoea attributed to the common causes of diarrhoeal pathogens in this population. These cut-off values are useful in studies that have no controls or that do not have diarrhoea data like our study. Comparison for different characteristics across the eight-week study period was done using one-way repeated measures analysis of variance and mixed-effect model analysis. Statistical significance was set at 0.05, characteristics that showed a significant change over the follow-up period were included in a mixed-effect model analysis as confounders. Statistical analysis was performed using Stata software, version 16 (StataCorp. 2019. College Station, TX, USA).

Results

Participants

From March 2019 to April 2020, 755 children were screened and 162 were recruited into the study. Of the 162 enrolled, 37 (23%) were positive for *Cryptosporidium* spp., 36 were entered into follow-up, and 27 children (75%) completed the 8-week follow-up, which was discontinued early due to COVID-19. Of these, the median age was 5.5 (IQR 2,14) months and 18 (64%) were male. Only 1 (3%) was HIV-infected, but HIV status was unknown in over half of the children (20/27). The enrolled study population is described elsewhere[29].

We tested 104 stool and sputum samples from the 27 participants that had completed follow-up from week 2 to week 8 post-enrolment. At least one pathogen was detected in all the 104 stool samples, while 87/104 (84%) of the sputum samples had at least one pathogen detected. Amongst the 104 stool samples collected, diarrhoeagenic *E. coli* was the most abundant pathogen (92/104 [89%]), of which EAEC was the most common subtype (88/92 [95%]) followed by typical EPEC (42/92 [45%]). *Cryptosporidium* spp. (60/104 [(57.6%]) were the second most common stool pathogen, of which a third were *C. hominis* (20/55), one sample was *C. parvum* and the rest were not identified. *Adenovirus* pan (44/104 [42%]),

Campylobacter pan (43/104 [41%]), *Campylobacter jejuni/coli* (34/104 [33%]), rotavirus (36/104 [34%] and norovirus (34/104 [32%]) were the other common pathogens identified. Over half (58/104 [56%]) of the follow-up stool samples with a pathogen associated with diarrhoea were below the diarrhoeagenic cut-offs as presented in the GEMS study (Figure 1). Out of the 104 induced sputum samples, *S. pneumoniae* was the most abundant pathogen 87/104 (84%) followed by Human

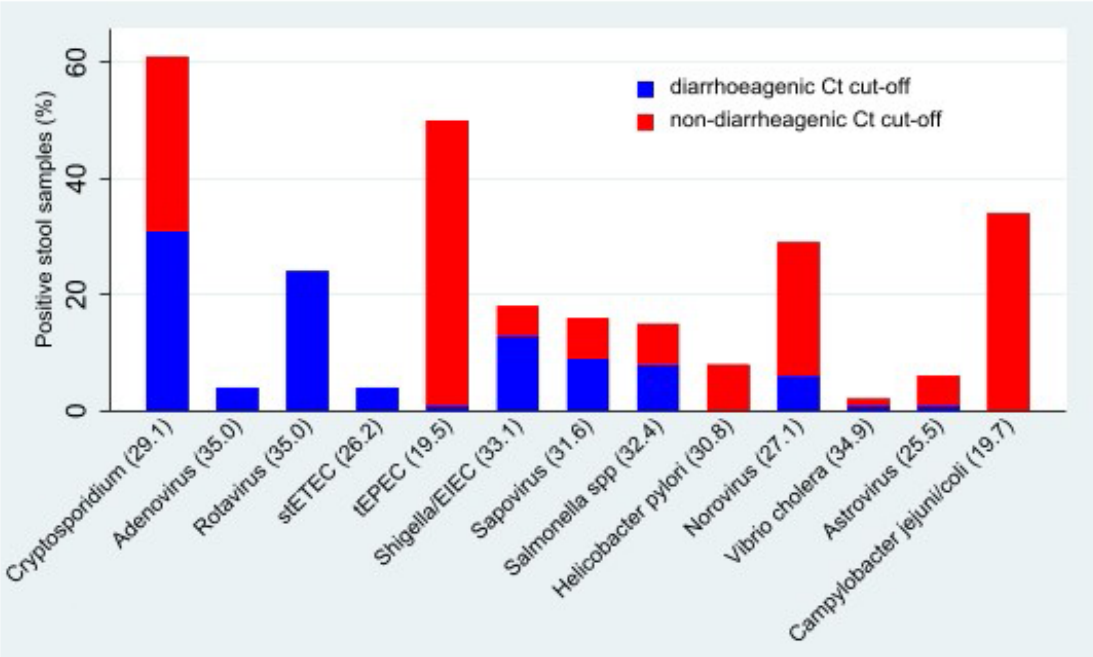

Figure 1. Positive stool samples with diarrhoeagenic cut-offs as described in the GEMS study. stETEC - heat-stable enterotoxin-producing E. coli; tEPEC – typical enteropathogenic E. coli; EIEC – enteroinvasive E. coli; H. pylori –Helicobacter pylori; V. cholera- Vibrio cholera.

Rhinovirus (58/104 (56%)), *M. catarrhalis* (52/104 [50%]), Adenovirus (29/104 [28%]) and *H. influenzae* (21/104 [20%]). The majority of stool samples (80/104 [77%]) and almost half of sputum samples (49/104 [47%]) had at least three pathogens detected (data not shown). Of the 49 sputum samples that had a high pathogen load, 42/49 (85%) simultaneously had ≥3 stools pathogens, although this was not statistically significant.

GI pathogens were detected in all stool samples from two weeks to eight weeks post-enrolment. In contrast, respiratory pathogens were detected in all sputum samples at 2 weeks and in 20/25 (80%) samples at the end of the eight weeks. On average, there were 5.1 (SD 2.1) stool pathogens detected per participant per study visit over the follow-up period, while an average of 3.5 (SD 1.8) sputum pathogens were detected per participant per study visit over the follow-up period (Table 1). There was an average of 3.1 (SD 1.6) bacteria compared to 1.0 (SD 0.8) parasites and 1.0 (SD 0.9) viruses in stool samples collected, and an average of 2.0 (SD 1.1) bacteria, 1.4 (SD 1.1) viruses and 0.3 (SD 0.5) parasites in sputum samples (Figure 2).

Figure 3A shows the changes in WAZ, WLZ and LAZ across the study period. Participants had low LAZ and WAZ at enrolment, and the average change in WAZ, LAZ, and WLZ scores over the eight-week period were 0.5 (0.6), 0.4 (1.4) and 0.4 (1.4), respectively. There was a significant change in WAZ across the follow-up period (p=0.002), but no significant changes were seen in LAZ and WLZ. Children with ≥3 GI pathogens in a sample had lower LAZ compared to those with <3 pathogens at two weeks (-2.0±1.1 vs 0.1±0.5), four weeks (-1.6±1.4 vs -0.5±0.8), six weeks (-1.8±1.4 vs -1.0±1.2) and eight weeks (-1.6±1.2 vs -0.6±0.9), and this was statistically significant (Figure 3B). This was not noted for WLZ and WAZ scores. No obvious changes in WLZ, WAZ and LAZ were noted with respiratory pathogen detection over the study period (Figure 3C). There was also no difference in any of the nutritional indices amongst children with ≥3 of both respiratory and GI pathogens compared to those with <3 (Figure 3D).

Discussion

This is the first description, to our knowledge, of both respiratory and GI pathogens in young children in a low- and middle-income country and association with short-term growth in the eight weeks after hospitalisation with diarrhoea. We found that our population had low anthropometric indices, and that these indices showed minimal change over the eight weeks after hospitalisation. A high average number of GI pathogens was detected throughout the eight weeks, and this was associated with GI symptoms. A high average of respiratory pathogens was also detected throughout the eight weeks, predominantly without associated respiratory symptoms. Significant changes were only noted in WAZ and not the other anthropometric measures over the eight-week follow-up period. Additionally, participants with ≥3 GI pathogens had a lower mean LAZ score at all

Table 1. Characteristics of and change in study population over 8 weeks.

Characteristics	Study period						p-value
	Enrolment*	2w	4w	6w	8w	Total	
Weight-for-age z score, mean (SD)	-1.2 (0.9)	-0.9 (1.0)	-0.8 (1.0)	-0.8 (0.9)	-0.8 (0.9)	-	0.002
Length-for-age z score, mean (SD)	-1.8 (1.4)	-1.7 (1.3)	-1.4 (1.4)	-1.5 (1.3)	-1.4 (1.1)	-	0.204
Weight-for-length z score, mean (SD)	-1.2 (0.9)	-1.2 (1.4)	-0.2 (1.2)	-0.0 (1.3)	-0.1 (1.2)	-	0.673
Total number of respiratory pathogens detected/participant/visit (mean, SD))	-	3.7 (1.6)	3.6 (1.8)	3.6 (1.8)	2.8 (1.8)	3.5(1.8)	0.115
Mean number of bacterial respiratory pathogens/visit (N=202)	-	2.2 (1.0)	1.8 (1.1)	1.7 (1.0)	2.1 (1.2)	2.0(1.1)	0.347
Mean number of viral pathogens per visit (N=141)	-	1.4 (1.1)	1.6 (1.0)	1 (1.0)	1.5 (1.1)	1.4(1.1)	0.148
Mean number of parasitic pathogens per participant/visit (N= 48)	-	0.5 (0.6)	0.5 (0.7)	0.3 (0.5)	0.3 (0.4)	0.3(0.5)	0.343
Visit to health centre with respiratory symptoms in the past 7 days (%)	-	10/27 (37%)	6/24 (25%)	5/27 (18%)	7/24 (29%)	-	0.107
Cough	-	5/10 (50%)	2/6 (33%)	2/5 (40%)	5/7 (71%)	-	0.147
Rhinorrhoea	-	7/10 (70%)	4/6 (66%)	1/4 (20%)	3/7 (43%)	-	0.347
Visit to a health facility for a diarrhoea episode in past 7 days		0/24 (0%)	2/ 24 (8%)	4/27 (15%)	3/24 (13%)		0.141
Total number of diarrhoea pathogens detected/participant/visit (mean, SD)	-	5.3 (2.0)	5.1 (2.1)	4.7 (2.1)	5.2 (2.5)	5.1 (2.1)	0.817
Number of bacterial GI pathogens detected/participant/visit (mean, SD)[d] (N=320)	-	2.8 (1.6)	3.1 (1.8)	3.1 (1.4)	3.4 (1.8)	3.1 (1.6)	0.375
Number of viral GI pathogens detected/participant/visit (mean, SD)[e] (N=10)	-	1.3 (1.0)	1.0 (1.0)	0.8 (1.0)	0.8 (1.0)	1.0 (0.8)	0.087
Number of parasitic GI pathogens detected/participant/visit (mean, SD)[f] (N=98)	-	1.2 (0.7)	1.0 (0.9)	0.7 (0.8)	1.0 (0.9)	1.0 (0.8)	0.099

GEMS, Global Enteric Multicenter Study; GI, gastrointestinal; SD, standard deviation; w, week

*TaqMan Array was only run at follow up visits

[a]*S. pneumoniae, S. aureus, M. catarrhalis, B. pertussis, H. influenzae* and *H. influenzae* type b, *C. pneumoniae, M. pneumoniae, K. pneumoniae, L. pneumophila,* and *Salmonella* species

[b]Influenza A/B/C, RSV A/B, parainfluenza virus types 1–4, coronaviruses NL63, 229E, OC43, and HKU1, human metapneumovirus A/B, rhinovirus, adenovirus, enterovirus, parechovirus, bocavirus, cytomegalovirus

[c]*Cryptosporidium, P. jirovecii*

[d]Enterotoxigenic *Escherichia coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), Shiga-toxigenic *E. coli* (STEC), Shigella/enteroinvasive *E. coli* (EIEC), *Salmonella, Campylobacter jejuni/C. coli, Vibrio cholerae, Clostridium difficile*

[e]Rotavirus, norovirus GII, adenovirus, astrovirus, sapovirus

[f]*Cryptosporidium, Giardia lamblia, Entamoeba histolytica, Ascaris lumbricoides,* and *Trichuris trichiura*

follow-up visits. Having a high number of both respiratory and GI pathogens was not associated with changes in nutritional indices over the follow-up period.

Previous studies that have evaluated GI pathogens in young children after hospitalisation and association with short-term growth have typically focused on a single pathogen[12,30–32]. However, co-infection with GI pathogens is common amongst children under two years and has significant effects on growth compared to single pathogens[33–36]. Short term growth after an infection in children under two years old is important because it allows for catch-up growth in this critical period for children to attain their optimal weight and height increment over time[11,37]. However, catch-up growth typically occurs in the

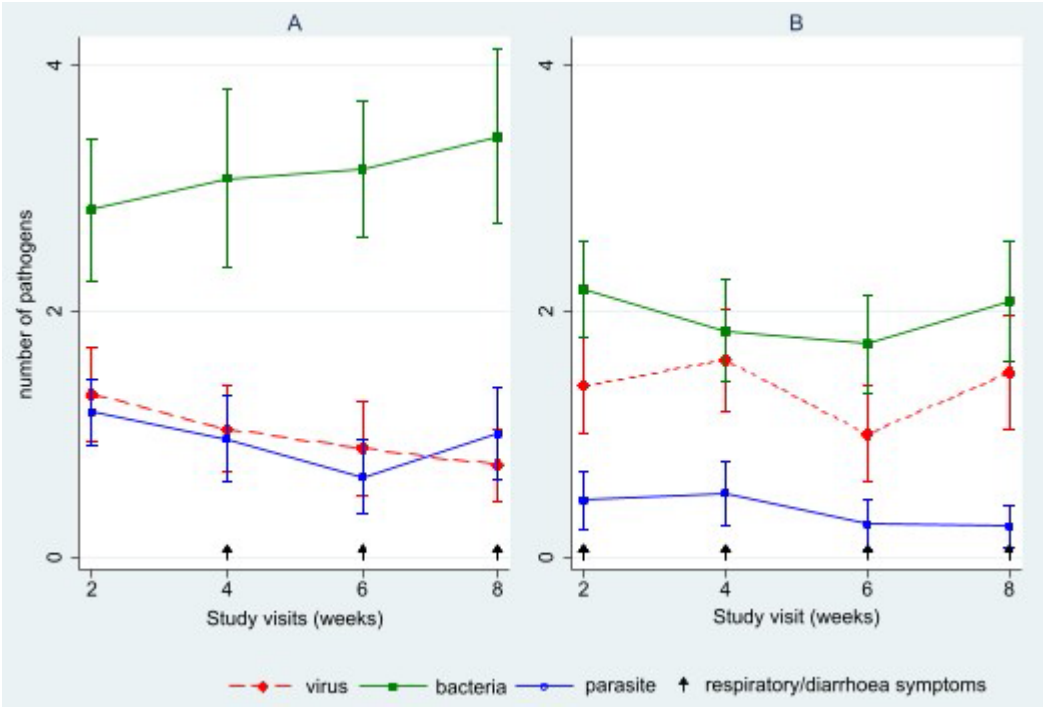

Figure 2. Average number of pathogens per study visit (mean, SD) over an eight-week period. A) Stool **B**) Sputum.

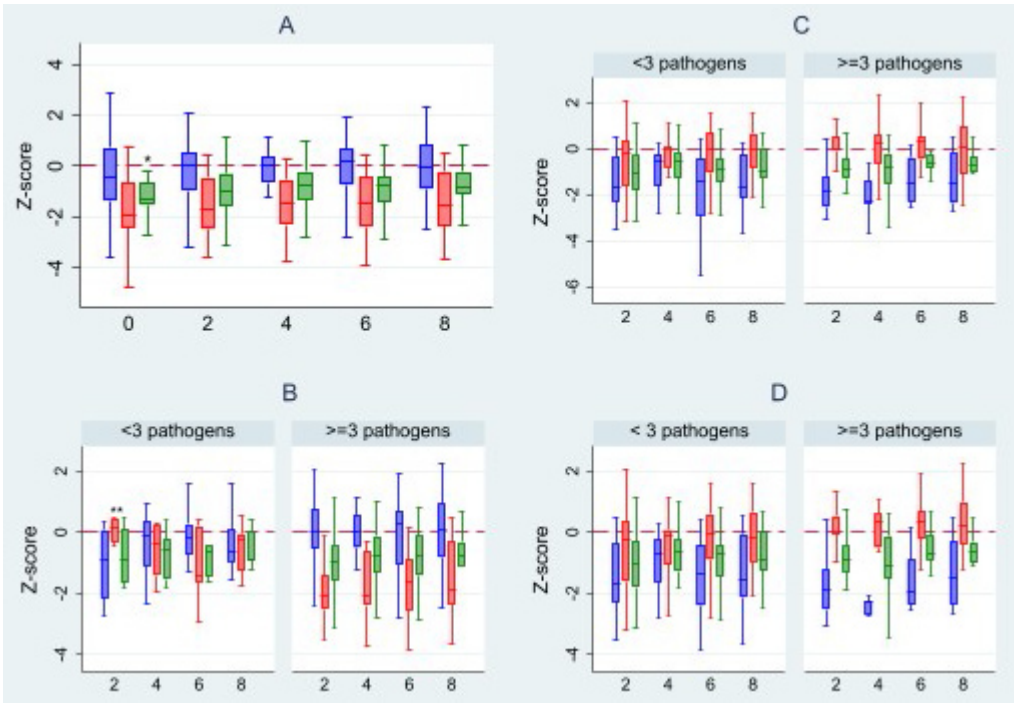

Figure 3. Change in mean WHZ, HAZ, LAZ scores over the 8-week study period. A) overall; **B**) in relation to average GI pathogens of i) <3 and ii) ≥3; **C**) in relation to average respiratory pathogens of i) <3 and ii) ≥3. **D**) in relation to respiratory and GI pathogens *p-value 0.002 **p-value 0.001 WLZ score LAZ score WAZ score

absence of diarrhoea, whether clinical or subclinical[14,38]. We noted that throughout the follow-up period, children who had a higher number of GI pathogens were more stunted than those with a lower number of GI pathogens. These changes may be due to environmental enteropathy, a poorly understood, chronic condition associated with enteropathogens, gut inflammation and a leaky gut seen in children from developing countries[39]. Diarrhoeal pathogens in the MAL-ED study, specifically EAEC, Campylobacter, and Shigella, were associated with a reduction in linear growth (LAZ) after three months[38]. Additionally, 95% of stool specimens in the MAL-ED study, from predominantly non-diarrhoeal episodes, detected at least one enteropathogen – which, if persistently present, can lead to altered growth[13,40].

The linkage between respiratory infection and growth is not well explored. In the Pneumonia Etiology Research for Child Health (PERCH) study, multiple respiratory pathogens were noted among children hospitalised with severe pneumonia, with an average of 3.8 (SD 1.5) pathogens/participant and 3·6 (SD 1·5) pathogens/participant among age and sex-matched controls[41] which is comparable to the average number of pathogens found in our study. Additionally, higher numbers of respiratory pathogens were consistently found in malnourished children, suggesting a possible link between respiratory pathogens and growth[42,43]. Our participants generally did not have respiratory symptoms, and we did not see an association between prevalence of respiratory pathogens with short-term growth. Colonisation is unlikely to affect short-term growth[41,44,45].

It is however worth noting that the complex interaction between nutrition, infection and immunity puts children at risk of persistent and recurrent colonisation and subsequent infections including those of the respiratory tract[16–19,44]. There was a high prevalence of stunting in this study population. At enrolment, over a third of our study population showed stunted growth and the mean LAZ was -1.8 (1.4)[23], similar to the national stunting prevalence amongst children <24 months[46]. There was a significant positive change in WAZ over the follow-up period, which would be explained by catch-up growth achieved from reduced incidence of diarrhoea/infection over the follow-up period[11,12,14]. We did not see any change in WLZ and WAZ. The majority of samples from this population that had a high number of stool pathogens also had a high number of pathogens in sputum. While an unhealthy gut microbiome composition is thought to be a risk factor for respiratory infections[16,19], we cannot make any inferences or conclusions from these data.

Our study has limitations. The sample size was small, and not powered to detect changes in short-term growth. This was a secondary analysis, and we did not have a comparison group of children with no diarrhoea/*Cryptosporidium* spp. infection at baseline. Stool or respiratory samples collected at the time of recruitment were not evaluated for pathogens beyond *Cryptosporidium*, and therefore we could not assess change in pathogens from enrolment. Although we collected HIV status, data were not available for 84% of the participants. However, the strengths of our study include the serial sampling, clinical, and anthropometry data collected over an eight-week period, the collection of induced sputum from the respiratory tract, and the high sensitivity and specificity of the molecular methods we used[26,45].

In summary, among young children hospitalised with diarrhoea, multiple gut and respiratory pathogens were prevalent in the participants over the following eight weeks, and the presence of more GI pathogens, but not respiratory pathogens, was associated with reduced short-term growth. Further study of larger cohorts is warranted, to delineate how gut and respiratory pathogens interact and contribute to linear deficits, during a period where insults that occur can impact long-term growth, developmental, and cognitive outcomes[26].

Consent

Written informed consent for publication of the participants' details was obtained from the participants.

Data availability

Underlying data

Figshare: Recruitment and follow-up data of participants recruited in the sub-analysis of the CryptoResp study, https://doi.org/10.6084/m9.figshare.21266142[47]

This project contains the following underlying data:

Data file 1: A dataset of participants presenting with diarrhoea and followed up over an 8-week period at Queen Elizabeth Central Hospital.

Reporting guidelines

Figshare: CONSORT checklist and flow chart for 'Respiratory and diarrhoeal pathogens in Malawian children hospitalised with diarrhoea and association with short-term growth' https://doi.org/10.6084/m9.figshare.21268572[48]

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

Acknowledgements

We thank the patients and parents for participating in this study. We thank the research clinical and laboratory staff for conducting the study. We thank David Moore and Tanja Adams with training the CryptoResp clinical and laboratory teams. We thank Neema Toto for interim study support, Thokozani Ganiza for assistance with data management, and Wes Van Voorhis for reviewing a draft of the manuscript and providing critical feedback.

References

1. Troeger C, Blacker BF, Khalil IA, *et al.*: **Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016.** *Lancet Infect Dis.* 2018; **18**(11): 1211–28.
PubMed Abstract | Publisher Full Text | Free Full Text

2. Kosek M, Bern C, Guerrant RL: **The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000.** *Bull World Health Organ.* 2003; **81**(3): 197–204.
PubMed Abstract | Free Full Text

3. Troeger C, Forouzanfar M, Rao PC, *et al.*: **Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015.** *Lancet Infect Dis.* 2017; **17**(11): 1133–61.
PubMed Abstract | Publisher Full Text | Free Full Text

4. Jones KD, Thitiri J, Ngari M, *et al.*: **Childhood malnutrition: Toward an understanding of infections, inflammation, and antimicrobials.** *Food Nutr Bull.* 2014; **35**(2 Suppl): S64–70.
PubMed Abstract | Publisher Full Text | Free Full Text

5. Marangu D, Zar HJ: **Childhood pneumonia in low-and-middle-income countries: An update.** *Paediatr Respir Rev.* 2019; **32**: 3–9.
PubMed Abstract | Publisher Full Text | Free Full Text

6. The Lancet: **Maternal and Child Nutrition-Executive Summary of The Lancet Maternal and Child Nutrition Series.** *The Lancet.* 2013; **5**(1): 1–11.
Reference Source

7. MAL-ED Network Investigators: **Relationship between growth and illness, enteropathogens and dietary intakes in the first 2 years of life: findings from the MAL-ED birth cohort study.** *BMJ Glob Health.* 2017; **2**(4): e000370.
PubMed Abstract | Publisher Full Text | Free Full Text

8. Kotloff KL, Nataro JP, Blackwelder WC, *et al.*: **Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): A prospective, case-control study.** *Lancet.* 2013; **382**(9888): 209–22.
PubMed Abstract | Publisher Full Text

9. Miller M, Acosta AM, Chavez CB, *et al.*: **The MAL-ED study: a multinational and multidisciplinary approach to understand the relationship between enteric pathogens, malnutrition, gut physiology, physical growth, cognitive development, and immune responses in infants and children up to 2 years of age in resource-poor environments.** *Clin Infect Dis.* 2014; **59 Suppl 4**: S193–206.
PubMed Abstract | Publisher Full Text

10. Kotloff KL, Nasrin D, Blackwelder WC, *et al.*: **The incidence, aetiology, and adverse clinical consequences of less severe diarrhoeal episodes among infants and children residing in low-income and middle-income countries: a 12-month case-control study as a follow-on to the Global Enteric Multicenter Study (GEMS).** *Lancet Glob Health.* 2019; **7**(5): e568–84.
PubMed Abstract | Publisher Full Text | Free Full Text

11. Checkley W, Gilman RH, Epstein LD, *et al.*: **Asymptomatic and symptomatic cryptosporidiosis: Their acute effect on weight gain in Peruvian children.** *Am J Epidemiol.* 1997; **145**(2): 156–63.
PubMed Abstract | Publisher Full Text

12. Richard SA, Black RE, Gilman RH, *et al.*: **Diarrhea in early childhood: Short-Term association with weight and long-Term association with length.** *Am J Epidemiol.* 2013; **178**(7): 1129–38.
PubMed Abstract | Publisher Full Text | Free Full Text

13. Platts-Mills JA, Babji S, Bodhidatta L, *et al.*: **Pathogen-specific burdens of community diarrhoea in developing countries: A multisite birth cohort study (MAL-ED).** *Lancet Glob Health.* 2015; **3**(9): e564–75.
PubMed Abstract | Publisher Full Text | Free Full Text

14. Richard SA, Black RE, Gilman RH, *et al.*: **Catch-up growth occurs when diarrhea burden is low in early childhood.** *American Journal of Tropical Medicine and Hygiene.* 2013; **89**(5): 429.

15. Brand MW, Wannemuehler MJ, Phillips GJ, *et al.*: **The Altered Schaedler Flora: Continued Applications of a Defined Murine Microbial Community.** *ILAR J.* 2015; **56**(2): 169–78.
PubMed Abstract | Publisher Full Text | Free Full Text

16. Budden KF, Gellatly SL, Wood DL, *et al.*: **Emerging pathogenic links between microbiota and the gut-lung axis.** *Nat Rev Microbiol.* Nature Publishing Group, 2017; **15**(1): 55–63.
PubMed Abstract | Publisher Full Text

17. Ninh Nguyen D, Chen CM, Nold C, *et al.*: **Intestinal Dysbiosis and the Developing Lung: The Role of Toll-Like Receptor 4 in the Gut-Lung Axis.** *Front Immunol.* 2020; **11**(357).

18. Wedgwood S, Warford C, Agvatisiri SR, *et al.*: **The developing gut-lung axis: postnatal growth restriction, intestinal dysbiosis, and pulmonary hypertension in a rodent model**. *Pediatr Res.* 2020; **87**(3): 472–479.
PubMed Abstract | Publisher Full Text | Free Full Text

19. He Y, Wen Q, Yao F, *et al.*: **Gut-lung axis: The microbial contributions and clinical implications.** *Crit Rev Microbiol.* 2017; **43**(1): 81–95.
PubMed Abstract | Publisher Full Text

20. Hadi H, Stoltzfus RJ, Moulton LH, *et al.*: **Respiratory infections reduce the growth response to vitamin A supplementation in a randomized controlled trial.** *Int J Epidemiol.* 1999; **28**(5): 874–81.
PubMed Abstract | Publisher Full Text

21. Mata LJ, Kromal RA, Urrutia JJ, *et al.*: **Effect of infection on food intake and the nutritional state: perspectives as viewed from the village.** *Am J Clin Nutr.* 1977; **30**(8): 1215–27.
PubMed Abstract | Publisher Full Text

22. Nyangulu W, Van Voorhis W, Iroh Tam PY: **Evaluating respiratory cryptosporidiosis in pediatric diarrheal disease: protocol for a prospective, observational study in Malawi.** *BMC Infect Dis.* 2019; **19**(1): 728.
PubMed Abstract | Publisher Full Text | Free Full Text

23. Tam PYI, Chisala M, Nyangulu W, *et al.*: **Respiratory cryptosporidiosis in Malawian children with diarrheal disease.** *medRxiv.* 2021; 2021.05.07.21256838.
Publisher Full Text

24. World Health Organization: **Pocket book of hospital care for children: guidelines for the management of common childhood illnesses**. Geneva: WHO Press; 2013; **2**.
Reference Source

25. World Health Organization: **WHO Child Growth Standards: methods and development**. France; 2006.

26. Liu J, Platts-Mills JA, Juma J, *et al.*: **Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study.** *Lancet.* 2016; **388**(10051): 1291–301.
PubMed Abstract | Publisher Full Text | Free Full Text

27. Operario DJ, Bristol LS, Liotta J, *et al.*: **Correlation between diarrhea severity and oocyst count via quantitative PCR or fluorescence microscopy in experimental cryptosporidiosis in calves.** *Am J Trop Med Hyg.* 2015; **92**(1): 45–9.
PubMed Abstract | Publisher Full Text | Free Full Text

28. Iturriza-Gómara M, Jere KC, Hungerford D, *et al.*: **Etiology of Diarrhea Among Hospitalized Children in Blantyre, Malawi, Following Rotavirus Vaccine Introduction: A Case-Control Study.** *J Infect Dis.* 2019; **220**(2): 213–8.
PubMed Abstract | Publisher Full Text | Free Full Text

29. Tam PYI, Chisala M, Nyangulu W, *et al.*: **Respiratory cryptosporidiosis in Malawian children with diarrheal disease.** *PLoS Negl Trop Dis.* 2021; **15**(7): e0009643.
PubMed Abstract | Publisher Full Text | Free Full Text

30. Brown KH, Black RE, Becker S: **Patterns of physical growth in a longitudinal study of young children in rural Bangladesh.** *Am J Clin Nutr.* 1982; **36**(2): 294–302.
PubMed Abstract | Publisher Full Text

31. Checkley W, Epstein LD, Gilman RH, *et al.*: **Effects of Cryptosporidium parvum infection in Peruvian children: Growth faltering and subsequent catch-up growth.** *Am J Epidemiol.* 1998; **148**(5): 497–506.
PubMed Abstract | Publisher Full Text

32. Black RE, Brown KH, Becker S: **Effects of diarrhea associated with specific enteropathogens on the growth of children in rural Bangladesh.** *Pediatrics.* 1984; **73**(6): 799–805.
PubMed Abstract | Publisher Full Text

33. Nath J, Hussain G, Singha B, *et al.*: **Burden of major diarrheagenic protozoan parasitic co-infection among amoebic dysentery cases from North East India: a case report.** *Parasitology.* 2015; **142**(10): 1318–25.
PubMed Abstract | Publisher Full Text

34. Squire SA, Ryan U: **Cryptosporidium and Giardia in Africa: current and future challenges.** *Parasit Vectors.* 2017; **10**(1): 195.
PubMed Abstract | Publisher Full Text | Free Full Text

35. Walson JL, Berkley JA: **The impact of malnutrition on childhood infections.** *Curr Opin Infect Dis.* 2018; **31**(3): 231–236.
PubMed Abstract | Publisher Full Text | Free Full Text

36. Zhang SX, Zhou YM, Xu W, *et al.*: **Impact of co-infections with enteric pathogens on children suffering from acute diarrhea in southwest China.** *Infect Dis Poverty.* 2016; **5**(1): 64.
PubMed Abstract | Publisher Full Text | Free Full Text

37. Ajjampur SSR, Sarkar R, Sankaran P, *et al.*: **Symptomatic and asymptomatic Cryptosporidium infections in children in a semi-urban slum community in southern India.** *Am J Trop Med Hyg.* 2010; **83**(5): 1110–5.
PubMed Abstract | Publisher Full Text | Free Full Text

38. Rogawski ET, Liu J, Platts-Mills JA, *et al.*: **Use of quantitative molecular diagnostic methods to investigate the effect of enteropathogen infections on linear growth in children in low-resource settings: longitudinal analysis of results from the MAL-ED cohort study.** *Lancet Glob Health.* 2018; **6**(12): e1319–28.
PubMed Abstract | Publisher Full Text | Free Full Text

39. Mayneris-Perxachs J, Swann JR: **Metabolic phenotyping of malnutrition during the first 1000 days of life.** *Eur J Nutr.* 2019; **58**(3): 909–30.
PubMed Abstract | Publisher Full Text | Free Full Text

40. Lima AAM, Soares AM, Filho JQS, *et al.*: **Enteroaggregative *Escherichia coli***

Subclinical Infection and Coinfections and Impaired Child Growth in the MAL-ED Cohort Study. *J Pediatr Gastroenterol Nutr.* 2018; **66**(2): 325–33.
PubMed Abstract | Publisher Full Text

41. Pneumonia Etiology Research for Child Health (PERCH) Study Group: **Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study.** *Lancet.* 2019; **394**(10200): 757–79.
PubMed Abstract | Publisher Full Text | Free Full Text

42. Mwananyanda L, Thea DM, Chipeta J, *et al.*: **The Etiology of Pneumonia in Zambian Children: Findings From the Pneumonia Etiology Research for Child Health (PERCH) Study.** *Pediatr Infect Dis J.* 2021; **40**(9S): S40–9.
PubMed Abstract | Publisher Full Text | Free Full Text

43. Tapia MD, Sylla M, Driscoll AJ, *et al.*: **The Etiology of Childhood Pneumonia in Mali: Findings From the Pneumonia Etiology Research for Child Health (PERCH) Study.** *Pediatr Infect Dis J.* 2021; **40**(9S): S18–28.
PubMed Abstract | Publisher Full Text | Free Full Text

44. Rodrí Guez L, Cervantes E, Ortiz R: **Malnutrition and Gastrointestinal and Respiratory Infections in Children: A Public Health Problem.** *Int J Environ Res Public Health.* 2011; **8**(4): 1174–205.
PubMed Abstract | Publisher Full Text | Free Full Text

45. Park DE, Baggett HC, Howie SRC, *et al.*: **Colonization Density of the Upper Respiratory Tract as a Predictor of Pneumonia—*Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, and *Pneumocystis jirovecii*.** *Clin Infect Dis.* 2017; **64**(suppl_3): S328–S336.
PubMed Abstract | Publisher Full Text | Free Full Text

46. **Global Nutrition Report Country Nutrition Profiles - Global Nutrition Report**. [cited 2021 Sep 8].
Reference Source

47. Chisala M: **Respiratory and diarrhoeal pathogens in Malawian children hospitalised with diarrhoea and association with short-term growth dataset**. *figshare*. [Dataset]. 2022.
http://www.doi.org/10.6084/m9.figshare.21266142.v2

48. Chisala M: **CONSORT checklist and flow chart for 'Respiratory and diarrhoeal pathogens in Malawian children hospitalised with diarrhoea and association with short-term growth'**. *figshare*. [Dataset]. 2022.
http://www.doi.org/10.6084/m9.figshare.21268572.v2

Open Peer Review

Current Peer Review Status: ?

Version 1

Reviewer Report 15 June 2023

https://doi.org/10.21956/gatesopenres.15361.r33317

© **2023 Sarmin M.** This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

?

Monira Sarmin
Dhaka Hospital, Nutrition Research Division, International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh

Well written and well-organized manuscript where the authors evaluated the association of GI and respiratory pathogen with short term growth. I have the following observations:

1. If this cohort was only derived from a lager cohort of children having crypto diarrhea at admission, then the title needs the reflection, it should be crypto diarrhea instead of diarrhea only as the primary cohort did not include children having diarrhea by other pathogens.

2. Did you use GI pathogen and diarrhea pathogen interchangeably? If yes, then please use one, sometime it is confusing.

3. How did the study achieve 104 samples; it is not clear from the description, 27 participants, 2 samples (GI and respiratory) from week 2 to week 8.

Introduction:
4. 1st para: Last sentence can be rephrased as "Global Burden of Disease" study reported an estimated reduction of 9% of LRI and 12% of diarrhoeal disease over the past three decades (1,3).

5. Last para: **The objective of this study was to describe detection of diarrhoeal and respiratory pathogens in children who were hospitalised with diarrhoeal disease (with detection of Cryptosporidium) at a tertiary hospital in Malawi, and to examine the association with short-term growth over an eight-week follow-up period.**
Is it diarrhea with Cryptosporidium only?

Methods:
6. Study design, setting, and participants - This is a secondary data analysis from a prospective longitudinal observational study evaluating respiratory cryptosporidiosis Was the primary study evaluating respiratory cryptosporidiosis?

7. qPCR- for respiratory pathogen Ct value <38 was considered as positive, is there any specific cause? Whereas in the main section it was told if Ct <35, it was positive.

Results:

8. Results: Figure 1: Did you mean symptomatic diarrhea by diarrhoeagenic Ct cut off? **Table 1:** Mean number of bacterial respiratory pathogens/visit (N=202), you can add respiratory viral pathogen and respiratory parasitic pathogen to remain similar with bacterial/viral/parasitic GI pathogens.

9. Table 1: Weight-for-age z score, mean (SD) at enrolment, -1.2 and at 8^{th} week, it was -0.8, (difference: 0.4) Weight-for-length z score, mean (SD) at enrolment, -1.2 and at 8^{th} week, it was -0.1, (difference: 1.1)
Though difference was more in WLZ then WAZ, P value is significant of WAZ. Please confirm the results.

Discussion:
10. Figure 2: Bacterial pathogens are increasing both in stool and sputum, why did it happen? Is there any explanation?

Other comments:
11. Did stool from symptomatic diarrhea have more pathogen then non-symptomatic diarrhea?
12. Did you stratified 3 pathogens or more according to symptomatic vs asymptomatic diarrhea and compare them?

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am physician-scientist. My clinical research involves diarrheal diseases,

malnutrition, sepsis and critical illnesses.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.