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RESEARCH ARTICLE

Impact of integrated water, sanitation, hygiene, health and nutritional interventions on diarrhoea disease epidemiology and microbial quality of water in a resource-constrained setting in Kenya: A controlled intervention study

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Abstract

Objectives: We assessed the impact of water, hygiene and sanitation (WASH), maternal, new-born and child health (MNCH), nutrition and early childhood development (ECD) on diarrhoea and microbial quality of water in a resource-constrained rural setting in Kenya.

Methods: Through a controlled intervention study, we tested faecal and water samples collected from both the intervention and control sites before and after the interventions using microbiological, immunological and molecular assays to determine the prevalence of diarrhoeagenic agents and microbial quality of water. Data from the hospital registers were used to estimate all-cause diarrhoea prevalence.

Results: After the interventions, we observed a 58.2% (95% CI: 39.4–75.3) decline in all-cause diarrhoea in the intervention site versus a 22.2% (95% CI: 5.9–49.4) reduction of the same in the control site. Besides rotavirus and pathogenic *Escherichia coli,* the rate of isolation of other diarrhoea-causing bacteria declined substantially in the intervention site. The microbial quality of community and household water improved considerably in both the intervention (81.9%; 95% CI: 74.5%–87.8%) and control (72.5%; 95% CI: 64.2%–80.5%) sites with the relative improvements in the intervention site being slightly larger.

Conclusions: The integrated WASH, MNCH, nutrition and ECD interventions resulted in notable decline in all-cause diarrhoea and improvements in water quality in the rural resource-limited population in Kenya. This indicates a direct public health impact of the interventions and provides early evidence for public health policy makers to support the sustained implementation of these interventions.

KEYWORDS
diarrhoea, hygiene, impact, Kenya, nutrition, sanitation, water

INTRODUCTION

Diarrhoea is still an important public health problem globally despite the availability of simple and cost-effective interventions [1], with developing countries bearing the greatest

Sustainable Development Goals: Good Health and Wellbeing, Clean Water and Sanitation, Sustainable Cities and Communities

[Correction added on 12 August 2022, after first print and online publication: Author name Ryoichiro Mochizuki has been corrected in this version.]

burden [2]. Although mortality due to diarrhoea has declined substantially over the past decades, consistent reduction in the incidence of diarrhoea has hardly been achieved [3, 4], particularly in developing countries [5].

Narok County has among the worst health indicators of maternal, newborn and child health (MNCH) in Kenya [6]: under-five mortality 45 per 1000 live births; exclusive breastfeeding 40%; percentage of fully immunised children 54%; delivery at health facilities 18%; access to portable water 20%; latrine coverage 30%; under-five diarrhoea prevalence 40%; stunting 33%, wasting 2% and underweight 12%. The pastoral lifestyle, inadequate rainfall and low household food security (availability, accessibility and stability) undermine nutritional indicators for under-fives and pregnant women in Narok County [7]. Furthermore, water scarcity in this area hinders appropriate hygiene practices and insufficient latrine coverage increases susceptibility to diarrhoea [8].

Diarrhoea is caused by a range of pathogens including bacteria, viruses and parasites [9]. These diarrhoeal pathogens are predominantly transmitted via the faecal-oral route. After replication in the gastrointestinal tract, the pathogens are shed in high numbers in the faeces of infected humans and animals and may contaminate surface water, groundwater, drinking water and food, which in turn facilitates the water-borne transmission of these pathogens to humans and animals [10]. Thus, water, sanitation and hygiene (WASH) interventions bear a large potential of reducing diarrhoea mortality and morbidity [11–13] although they are difficult to implement especially in low-resource settings due to challenges in equitable access [14]. Outcomes of WASH interventions are also prone to confounding by baseline WASH conditions [15], scope, coverage and adherence [16, 17], and may be overwhelmed by increases in other risk factors. Consequently, mixed effects of WASH interventions on the occurrence of diarrhoea have been observed, suggesting the need for more comprehensive approaches of implementing WASH interventions and other diarrhoea interventions.

Besides being independent risk factors for diarrhoeal disease, contaminated water and poor sanitation and hygiene relate with nutritional status in a feedback loop that yields a cycle of diarrhoeal infection and malnutrition [16]. Particularly nutrition works in synergy with WASH to yield modest effect on prevention of enteric infections [15, 16]. Moreover, high diarrhoeal disease burden among infants has been linked with delayed school entry and poorer early childhood development (ECD) [18].

Therefore, considering the multifaceted risks for diarrhoeal disease, the World Vision Kenya, through the Ilaramatak Mother to Mother Support Project, implemented an integrated approach of WASH, MNCH, nutrition and ECD interventions in a resource-constrained area in Narok County, Kenya with the aim of improving maternal and child health and nutrition status and cognitive development of children in the area [19]. The conceptual framework of this approach is described in Figure 1. This article reports the impact of these interventions on the burden and aetiology of diarrhoeal disease and microbial quality of water in this area.

MATERIALS AND METHODS

Study design and setting

This was a controlled intervention study conducted in Narok County, Kenya between 2018 and 2021. Narok County is situated in the southern part of Kenya bordering the Republic of Tanzania. The County's population was 1130 million in 2018 [20]. The dominant tribe is Maasai. The main economic activities in the county include pastoralism, crop farming and tourism from the Maasai Mara Game Reserve. Elangata-Enterit, a resource-constrained sub-location in Narok County, was the intervention site whereas Maji-Moto sub-location which neighbours Elangata-Enterit

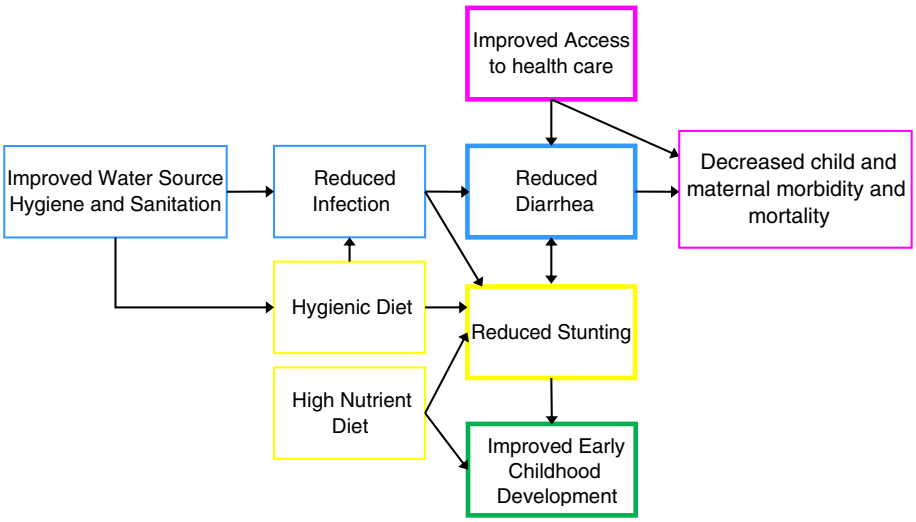

FIGURE 1 Conceptual framework of the project. The integrated approach of water, hygiene and sanitation (WASH), maternal, new-born and child health (MNCH), nutrition and early childhood development (ECD) interventions would improve maternal and child health and nutrition status and cognitive development of children thereby decreasing child and maternal morbidity and mortality

was the control site. The control site was selected based on its similarity with the intervention site in terms of socio-economic status, demography and geological formation. We conducted a baseline survey in both the intervention and control sites from January to April 2018 to establish the burden of diarrhoeal disease through an active hospital-based surveillance. The Elangata-Enterit Health Centre, which was the only health facility serving the residents of Elangata-Enterit sub-location, served as the intervention study facility while the Maji-Moto Dispensary, which was the only health facility serving the residents of Maji-Moto sub-location, served as the control study facility. After the implementation of the WASH, MNCH, nutrition and ECD interventions, we carried out an endline survey between January and May 2021 in both sites to evaluate the impact of these interventions.

Interventions

An approach of integrated intervention of WASH, MNCH, nutrition and ECD was implemented in Elangata-Enterit sub-location [19]. Briefly, to improve access to water, sanitation and hygiene, a mega borehole was sunk and water extended with pipelines to various water distribution points in the target community. Latrines were constructed by community members with the support of Community Health Volunteers (CHVs) and the project staff after extensive public sensitization in schools and within the community. Clean and hygienic practices were taught and promoted through delivery of key hygiene messages on hand washing, environmental hygiene and sanitation, and water and food safety to children and the general public.

To improve MNCH, the project promoted at least four antenatal clinic (ANC) visits by expectant mothers; delivery at a health facility; clean and hygienic birth practices for mothers, caregivers and birth attendants; early initiation of breast feeding and exclusive breastfeeding for 6 months; timely and complete immunisation; hand washing, environmental sanitation and water and food safety to children, mothers and caregivers.

Nutritional interventions included promotion of continued breastfeeding for up to 2 years and beyond; fresh hygienically prepared complementary food; clean and hygienic child-eating area and food preparation area; and provision of micronutrients, and supplementary or therapeutic food for malnourished children. Lastly, ECD focused on provision of age appropriate and environmental hygienic play spaces; and education on improved caregiver-child interactions.

Ethical considerations

This study was reviewed and approved by the Masino University Ethics Review Committee (*MSU/DRPI/MUERC/00492/17*). Informed written consent was sought from all the participating adults and from the caregivers of all participating children.

Collection of faecal samples

The study subjects for diarrhoeal disease surveillance were persons of all ages attending either Elangata-Enterit Health Centre or Maji-Moto Dispensary with acute gastroenteritis and having experienced an episode of 3 looser than normal or watery stools within a 24-h period for not more than 7 days with or without episodes of vomiting [21]. The patients came directly from the community. Decisions on treatment were at the discretion of the clinicians attending to the patients. Demographic and clinical data were collected from the study participants. After written informed consent had been granted, whole stool and/or anal swab samples were collected in clean sterile containers. Each sample was labelled with the date of collection and a sample number assigned. The samples were kept at 4°C at the health facilities before being transported to the Nagasaki University Institute of Tropical Medicine-Kenya Medical Research Institute (NUITM-KEMRI) laboratories in Nairobi for processing. A total of 416 faecal samples were collected during the baseline (Elangata-Enterit- 111; Maji-Moto- 148) and endline (Elangata-Enterit- 71; Maji-Moto- 86) survey.

Collection of water samples

To assess the microbial quality of water, water samples were collected from sources and at the point of use (i.e., in the houses) in Elangata-Enterit and Maji-Moto sub-locations once in each of the 4 months of the baseline survey (January–April 2018) and once in each of the 5 months of the follow-up survey (January–May 2021). The water samples were collected in sterile containers and ferried to the NUITM-KEMRI laboratories in Nairobi for processing. A total of 139 water samples were collected during the baseline (Elangata-Enterit- 24; Maji-Moto- 23) and endline (Elangata-Enterit- 46; Maji-Moto- 46) survey. Of the 46 water samples collected in Elangata-Enterit during the endline survey, 8 were obtained from the source (that is at the main borehole and water distribution points), whereas 38 were sampled from the point of use (i.e., from the houses utilising the borehole water).

Isolation and identification of diarrhoea-causing bacteria

The faecal specimens were cultured on appropriate media for primary isolation of the bacteria as described previously [22]. The media included xylose lysine deoxycholate (XLD) agar (Oxoid Ltd., Basingstoke, Hampshire, UK), deoxycholate hydrogen sulfide lactose (DHL) agar, bromothymol blue (BTB) agar, Salmonella-Shigella (SS) agar, thiosulfate-citrate-bile salt-sucrose (TCBS) agar (Eiken Chemical Company Ltd. Tochigi, Japan), alkaline peptone water and selenite broth (HiMedia Laboratories Pvt. Ltd., Mumbai, India). The plates and the broth were incubated at 37°C overnight. The isolated bacterial colonies were

identified by conventional biochemical identification methods and/or by the VITEK-2 automated analyser (bioMérieux, Inc., NC, USA).

Serological identification of *Escherichia coli*

Serologic identification of *Escherichia coli* isolates was performed by the slide agglutination technique with polyvalent and monovalent antisera for serotype identification of *E. coli* (Denka Seiken Co. Ltd.) according to the manufacturer's protocol.

Molecular identification of pathogenic *E. coli*

Pathogenic *E. coli* were identified using multiplex polymerase chain reaction (PCR) as described previously [23]. Briefly, bacterial genomic DNA was extracted from three colonies of each *E. coli* positive sample by the QIAGEN DNA extraction kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The genomic DNA was subjected to PCR using primers specific for *eae* gene for enteropathogenic *E. coli* (EPEC); *eae* and *stx* genes for enterohemorrhagic *E. coli* (EHEC); *est* and *elt* genes for enterotoxigenic *E. coli* (ETEC); *ipaH* gene for enteroinvasive *E. coli* (EIEC) and *Shigella* spp.; and *aggR*, CVD432 and *aspU* genes for enteroaggregative *E. coli* (EAEC). The PCR mixture was prepared with puReTaq Ready-To-Go PCR beads kit (GE Healthcare, UK) according to the manufacturer's instructions. The amplified product was analysed on a 2% agarose gel.

Detection and molecular characterisation of rotavirus

For rotavirus detection, about 1 ml of a 10% faecal suspension was prepared from each faecal sample and subjected to an enzyme-linked immunosorbent assay (ELISA), as described previously [24]. Rotavirus double-stranded RNA was extracted from the 10% faecal suspensions with ISOGEN-LS (Nippon Gene Co., Ltd., Toyama, Japan) according to the manufacturer's protocol. The RNA was reverse transcribed into the complementary DNA (cDNA) using a ReverTra Ace® qPCR RT Kit (Toyobo Biotechnology Co., Ltd., Japan). The cDNA was then amplified in a two-step multiplexed semi-nested reverse transcription-polymerase chain reaction (RT-PCR) to determine the G and P genotypes of the rotavirus strains using a KOD-Plus-Ver.2 high fidelity DNA polymerase kit (Toyobo Biotechnology Co., Ltd.), as described previously [25, 26]. The amplified product was then analysed on a 1.2% agarose gel.

Detection and identification of diarrhoea-causing parasites

The whole stool samples were examined microscopically for diarrhoea-causing parasites. Briefly, a drop of Lugol's iodine stain was mixed with a small amount of specimen on the microscope slide using a wire loop and examined under 10× and 40× objectives with the condenser iris closed sufficiently to give a good contrast. Motile trophozoites and egg cysts of the parasitic pathogens were targeted in these examinations.

Laboratory analysis of water samples

The microbial quality of water was examined using a special fluorescence-based ES Coli Blue Medium to selectively detect *E. coli* and total coliforms in water samples as described previously [27]. Briefly, 100 ml of each of the water samples were added into a bottle containing the ES Coli Blue Medium and incubated at 37°C overnight. This was followed by illumination in the dark using a mini fluorescent lamp. Only water samples contaminated with *E. coli* and other coliform organisms would fluoresce. All the contaminated water samples were cultured on appropriate media for primary isolation and identification of the water contaminant bacteria as described above for the faecal samples.

Data extraction for all-cause gastroenteritis

To evaluate the impact of the WASH, MNCH, nutrition and ECD interventions on trends in diarrhoeal disease burden, we reviewed hospital logbooks at Elangata-Enterit Health Centre and Maji-Moto Dispensary and recorded the daily all-cause hospitalizations and all-cause gastroenteritis at each of the health facilities before (January–April 2018) and after (January–May 2021) the interventions. Using these data, we compared trends in all-cause gastroenteritis at each facility between the baseline and endline survey.

Data analysis

Data were analysed using STATA Version 14 (StataCorp., 2015). Descriptive statistics were used to summarise data and calculate proportions. Isolation rates of diarrhoea-causing pathogens and hospital visits for all-cause diarrhoea before and after the interventions were compared using test of proportions. Differences in proportions between two groups were tested using the *t-test* where applicable. A p-value of <0.05 was considered to be significant. Percentage change was calculated to establish increase or decrease in parameters where necessary.

RESULTS

Impact of the interventions on diarrhoeal disease burden

Before the WASH, MNCH, nutrition and ECD interventions were implemented in Elangata-Enterit sub-location, a total of 145/527 patients seen at Elangata-Enterit Health Centre from

TABLE 1 Prevalence of all-cause diarrhoea among the study population in Elangata-Enterit and Maji-Moto before and after the water, sanitation, hygiene, health and nutritional interventions

Study period	Elangata-Enterit (intervention site)			Maji-Moto (control site)		
Study site	**Baseline survey**	**Endline survey**	***p*-value**	**Baseline survey**	**Endline survey**	***p*-value**
All-cause cases	527	1072		905	968	
Diarrhoea cases	145	123		122	102	
Diarrhoea prevalence (95% CI)	27.5% (23.8–31.5)	11.5% (9.7–13.5)	0.001	13.5% (11.4–15.8)	10.5% (8.7–12.6)	0.493
% Diarrhoea reduction	58.2% (39.4–75.3)			22.2% (5.9–49.4)		

Note: Baseline study period (before interventions, January–April 2018). Endline study period (after interventions, January–May 2021). CI, confidence interval. A *p*-value of <0.05 was considered to be significant.

TABLE 2 Prevalence and aetiological distribution of diarrhoea-causing pathogens in Elangata-Enterit and Maji-Moto before and after the interventions

	Elangata-Enterit			Maji-Moto		
Pathogen	**Baseline ($n = 111$) number (%)**	**Endline ($n = 71$) number (%)**	***p*-Value**	**Baseline ($n = 148$) number (%)**	**Endline ($n = 86$) number (%)**	***p*-Value**
Bacteria						
Pathogenic *Escherichia coli*	55/213[a] (25%)	36/109 (33%)	0.129	83/347[a] (24%)	73/162[a] (45%)	<0.001
EAEC	*32 (15%)*	*17 (15.6%)*	*0.887*	*66 (19%)*	*29 (19.1%)*	*0.979*
ETEC	*23 (10.8%)*	*18 (16.5%)*	*0.146*	*17 (4.9%)*	*38 (25%)*	*<0.001*
EHEC	*0*	*1 (0.9%)*	*0.166*	*0*	*3 (2%)*	*0.008*
STEC	*0*	*0*		*0*	*3 (2%)*	*0.008*
Aeromonas spp.	12 (11%)	1 (1.4%)	0.015	4 (3%)	0	0.105
Salmonella spp.	5 (4%)	1 (1.4%)	0.315	1 (0.6%)	2 (2.3%)	0.254
Shigella spp.	5 (4%)	2 (2.8%)	0.669	7 (5%)	5 (5.8%)	0.792
Plesiomonas shigelloides	1 (0.9%)	0	0.423	0	2 (2.3%)	0.064
Providencia alicafaciens	1 (0.9%)	3 (4.2%)	0.138	0	0	
Vibrio cholerae	0	0		0	0	
Vibrio parahaemolyticus	0	0		0	0	
Parasites						
Entamoeba coli	1 (0.9%)	Not done		0	Not done	
Entamoeba.histolytica	10 (9%)	Not done		4 (3%)	Not done	
Giardia lamblia	2 (2%)	Not done		3 (2%)	Not done	
Viruses						
Rotavirus	8 (7%)	6 (8%)	0.801	13 (9%)	1 (1.2%)	0.017
G1P [8]	*8 (100%)*	*0*		*6 (46%)*	*0*	
G3P [8]	*0*	*5 (83%)*		*0*	*0*	
G1P [4]	*0*	*0*		*4 (30%)*	*0*	
G12P [4]	*0*	*0*		*1 (7%)*	*0*	
Mixed	*0*	*0*		*2 (15%)*	*1 (100%)*	
GNTP [8]	*0*	*1 (16%)*		*0*	*0*	

Note: Elangata-Enterit (intervention site); Maji-Moto (control site); Baseline study period (before interventions, January–April 2018); Endline study period (after interventions, January–May 2021); A total of 111 and 71 faecal samples were collected and analysed in Elangata-Enterit during the baseline and endline survey, respectively; A total of 148 and 86 faecal samples were collected and analysed in Maji-Moto during the baseline and endline survey. The italics values represent values for the different pathotypes of *E. coli* and different genotypes of rotavirus.

[a]Three colonies of each *E. coli* positive sample were analysed for pathogenic *E. coli*, hence, the higher denominator than the respective *n*; Enteroaggregative *E. coli* (EAEC); Enterotoxigenic *E. coli* (ETEC); Enterohemorrhagic *E. coli*, (EHEC); Shiga toxin-producing *E. coli* (STEC). Mixed refers to rotavirus cases with more than one G and/or P genotypes. GNT refers to those strains whose G genotype could not be detected using the existing primer sets.

January to April 2018 presented with all-cause diarrhoea, representing a prevalence of 27.5% (95% CI: 23.8%–31.5%). During the same period, a total of 122/905 patients seen at Maji-Moto Dispensary presented with all-cause diarrhoea, thus, amounting to a prevalence of 13.5% (95% CI: 11.4%–15.8%). Following the interventions, a total of 123/1072 patients seen at Elangata-Enterit Health Centre from January to May 2021 presented with all-cause diarrhoea, representing a prevalence

TABLE 3 Prevalence of coliforms and distribution of potential diarrhoea-causing and other bacteria isolated from water samples in Elangata-Enterit and Maji-moto before and after the interventions

Pathogen	Elangata-Enterit (intervention site)			Maji-moto (control site)		
	Baseline ($n = 24$) Number (%)	Endline ($n = 46$[a]) Number (%)	p-Value	Baseline ($n = 23$) Number (%)	Endline ($n = 46$) Number (%)	p-Value
Escoli blue test						
Positive	23 (95.8%)	8 (17.3%)	<0.001	20 (87.0%)	11 (23.9%)	<0.001
Negative	1 (4.2%)	38 (82.7%)	<0.001	3 (13.0%)	35 (76.1%)	<0.001
% Coliform reduction (95% CI)	81.9% (74.5–87.8%)			72.5% (64.2–80.5%)		
Diarrhoea-causing bacteria						
Pathogenic Escherichia coli	18 (56%)	1 (5.8%)	*0.001*	18 (72%)	4 (13.8%)	<0.000
Aeromonas spp.	4 (13%)	0	*0.120*	1 (4%)	6 (20.6%)	*0.070*
Salmonella spp.	2 (6%)	0	*0.303*	0	0	
Serratia spp.	1 (3%)	3 (17.6%)	*0.083*	1 (4%)	4 (13.8%)	*0.215*
Vibrio metschnikovii	2 (6%)	0	*0.303*	0	0	
Providencia alicafaciens	0	1 (5.8%)	*0.169*	0	0	
Other bacteria						
Enterobacter spp.	1 (3%)	6 (35.3%)	*0.002*	1 (4%)	6 (20.6%)	*0.070*
Pseudomonas spp.	1 (3%)	3 (17.6%)	*0.083*	2 (8%)	3 (10.3%)	0.771
Klebsiella spp.	1 (3%)	1 (5.8%)	*0.633*	1 (4%)	2 (6.8%)	*0.653*
Citrobacter spp.	2 (6%)	2 (5.8%)	0.977	1 (4%)	3 (10.3%)	*0.377*
Edwadsiella spp.	0	0		0	1 (3.4%)	*0.352*
Total isolates	32	17		25	29	

Note: Baseline study period (before interventions, January–April 2018); Endline study period (after interventions, January–May 2021).
[a]Of the 46 water samples, 8 were obtained from the source (that is at the main borehole and water distribution points), whereas 38 were sampled from households utilising the improved (borehole) water source. Escoli blue test selectively detects *E. coli* and other coliform organisms in water using ES Coli Blue Medium, an enriched lauryl sulphate-aniline selective blue agar medium. A p-value of <0.05 was considered to be significant.

of 11.5% (95% CI: 9.7%–13.5%). As for Maji-Moto Dispensary, a total of 102/968 patients seen at the facility presented with diarrhoea of all cause, thus, translating into a prevalence of 10.5% (95% CI: 8.7%–12.6%). Thus, following the interventions, all-cause diarrhoea prevalence dropped in Elangata-Enterit (intervention site) by 58.2% (95% CI: 39.4–75.3) compared to the 22.2% (95% CI: 5.9–49.4) decline in Maji-Moto (control site) (Table 1).

Impact of the interventions on diarrhoeal disease aetiology

Of the bacterial causes of diarrhoea, pathogenic *E. coli* was most commonly isolated in both Elangata-Enterit (intervention site) and Maji-Moto (control site) before and after the interventions with a significantly higher rate in the control site following the interventions (24% vs. 45%; $p < 0.001$) (Table 2). Although EAEC predominated in both sites before interventions, this pathotype was displaced by ETEC following the interventions. The increase in ETEC prevalence was most significant in the control site (17% vs. 38%; $p < 0.001$).

The rate of isolation of other diarrhoea-causing bacteria such as *Aeromonas* spp., *Salmonella* spp. and *Shigella* spp., declined drastically in the intervention site from 11%, 4% and 4% before the interventions to 1%, 1% and 3%, respectively following the interventions. Other diarrhoea-causing bacteria included *Plesiomonas shigelloides* and *Providencia alcalifaciens* whose prevalence seemed to slightly increase in the control and intervention sites, respectively, following the interventions. *Vibrio cholerae* and *Vibrio parahaemolyticus* were not found during the study period.

Parasitic diarrhoea was mostly caused by *Entoamoeaba histolytica, Giardia lamblia* and *Entamoeba coli* in both sites. Elangata-Enterit recorded a higher prevalence and diversity of diarrhoea-causing parasites than Maji-Moto before the interventions. However, the parasitic causes of diarrhoea were not examined during the endline survey due to challenges in timely shipment of whole stool specimen from the field to the laboratory in Nairobi occasioned by the COVID-19 pandemic.

Whereas rotavirus prevalence declined significantly in Maji-Moto (9% vs. 1.2%; $p = 0.010$) after the interventions, its prevalence did not vary significantly in Elangata-Enterit (7% vs 8%; $p = 0.756$) during the same period. Rotavirus genotype G1P[8] was replaced with G3P[8] and mixed strains in dominance in Elangata-Enterit and Maji-Moto, respectively, after the interventions.

Impact of the interventions on microbial quality of water

During the baseline survey, 23/24 (95.8%; 95% CI: 81.1%–99.8%) of water sampled from sources and at the point of

use (i.e., in the houses) in Elangata-Enterit had coliforms. Following the interventions, the prevalence of coliforms in water reduced to 8/46 (17.4%; 95% CI: 8.4%–30.4%), indicating a significant improvement in microbial quality of water in this area (Table 3). None of the eight water samples from the source (that is at the main borehole and water distribution points) was found to be contaminated. Notably, only 8 of the 38 water samples (21.1%; 95% CI: 10.3%–36.1%) obtained at the point of use (i.e., in the houses) showed contamination, thereby signifying a significant improvement in microbiological quality of water at the household level as a result of the interventions. Furthermore, a reduced diversity of potential diarrhoea-causing bacteria was observed in the intervention site following the interventions, which could signify improved quality of water. During the same period, there was a significant improvement in microbial quality of community and household water in the control site with the level of contamination reducing by 72.5% (95% CI: 64.2%–80.5%). However, the relative improvement in water quality was a bit larger in the intervention site at 81.9% (95% CI: 74.5%–87.8%).

DISCUSSION

Diarrhoeal disease outcomes are objective measures of impact of WASH and nutritional interventions and help reduce the potential for recall bias [28]. Thus, with the imminent implementation of the WASH, MNCH, nutritional and ECD interventions in a resource-constrained rural area in Kenya, we conducted a controlled intervention study to evaluate the impact of these interventions in reducing diarrhoeal disease burden. We observed a significant drop in diarrhoea prevalence in the intervention site following the implementation of the interventions. This reduction was commensurate with increasing microbial quality of both community and household water and improved health indicators in this area such as knowledge, attitude and practices related to diarrhoea, handwashing, latrine coverage and usage, utilisation of health-care facilities, and child nutrition status and practices as assessed through a knowledge, attitude and practice (KAP) survey [Muriithi et al., in preparation] that was conducted concurrently with this clinical study. This reduction in diarrhoea infection may have been driven by the biologically plausible synergistic impact of the interventions when implemented together [15, 16]. The slight reduction in the incidence of diarrhoea in the control site observed over the same period could be partially driven by some informal improvements in water, sanitation and hygiene owing to the individual land tenure system in this area which has enabled many households to put up permanent residential structures and latrines while some have sunk boreholes and shallow wells [Muriithi et al., in preparation]. Taken together, the relatively higher reductions in diarrhoea incidence in the intervention site compared with the control site may strongly indicate a direct and better public health impact of the structured integrated WASH, MNCH, nutritional and ECD interventions than the informal water and sanitation improvements in the control site.

In this study, a substantially higher rate of pathogenic *E. coli* was recorded at the control site than at the intervention site following the interventions. Of the pathogenic *E. coli*, EAEC and ETEC were the leading pathotypes in both the intervention and control sites before and after the interventions. These findings are consistent with several other studies, including GEMS (Global Enteric Multicentre Study) and the MAL-ED (Malnutrition and Enteric Disease) study, which have found the two *E. coli* pathotypes to be among the pathogens contributing to most of the burden of diarrhoea in developing countries [5, 29–31]. Factors leading to the changing dominance in *E. coli* pathotype distribution as observed in this study require further investigation. Nonetheless, the high prevalence of these two pathotypes suggests that they should be prioritised for development and implementation of interventions to reduce the diarrhoeal disease burden. Our interventions had no statistically significant effects on rotavirus prevalence in the intervention arm of the study. These observations could be attributed to the effect of rotavirus vaccination, which reduces the rotavirus disease burden worldwide, including in resource-limited settings that are similar to our study [32, 33]. In addition, we observed a shift in strain dominance from G1P [8] to G3P [8] after the interventions. However, it is noteworthy that temporal fluctuations in rotavirus genotypes are common in Kenya [34–37] and an upsurge in G3 strains has been reported elsewhere in the country in the absence of WASH interventions [38]. Thus, it is possible that the changing dominance in rotavirus strain distribution in this study was due to natural fluctuations.

Both the structured WASH interventions in the intervention site and the informal water and sanitation improvements in the control site resulted in a similar degree of improved water quality, although the structured interventions resulted in better outcomes. Failure to detect any contaminations at the water source (i.e., at the main borehole and water distribution points) in the intervention site and the marked improvement in household water quality in both settings is encouraging as it shows improved water handling in such rural resource-limited settings. Nevertheless, there is still a need for continued public health education, focusing on the proper mode of storage and hygienic way of handling water to maintain its safety from collection at the source to consumption in the households.

Some limitations should be considered when interpreting the results of this study. First, enrolment was undertaken for only 4 months in the baseline survey and 5 months in the endline survey, thus, limiting the sample size and statistical power. Nevertheless, our controlled intervention study design involving both baseline and endline measurements allowed us to draw tentative conclusions about the relative efficacy of the interventions. Second, our estimates of all-cause diarrhoea incidence were dependent on hospital data availability, which we had no control on the quality as it could be affected by staffing at the health facilities and the

robustness of their reporting. An active community-based diarrhoea surveillance via regular home visits in each site would have strengthened this study. However, the fact that diarrhoea cases had dropped in both sites with the relative drop in the intervention site being much larger is suggestive of the potential public health impact of the interventions. Third, the observed reduction in all-cause diarrhoea did not take into account changes in health-seeking patterns, which may have impacted hospital visits for diarrhoea or other hygiene interventions, such as improvements in hand hygiene during the COVID-19 pandemic. However, from the hospital records, there were no abrupt changes in health-seeking patterns at the study facilities during the end-line survey. To our knowledge, the COVID-19 prevention measures such as hand hygiene were not widely adhered to such rural settings in Kenya as our study sites. Furthermore, one would assume that COVID-19-attributable hygiene interventions would consistently lead to a comparable decline in diarrhoea infection in both the intervention and control sites, rather than the more accelerated decline that we observed in the intervention site.

CONCLUSIONS

The significant reductions in all-cause diarrhoea and enteropathogen prevalence and improved microbial quality of water following the implementation of integrated WASH, MNCH, nutritional and ECD interventions in a rural resource-limited setting in Kenya provide evidence for better public health benefits of these structured interventions compared to the informal interventions in the control area. Hence, our data provide rationale for health stakeholders in the study area to support the sustained implementation of these interventions with emphasis on high adherence to help prevent and reduce diarrhoeal disease burden.

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