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ORIGINAL ARTICLE

Surveillance for enteric pathogens in a case-control study of acute diarrhea in Western Kenya

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Background: Acute diarrhea remains a major public health problem in East African nations such as Kenya. Surveillance for a broad range of enteric pathogens is necessary to accurately predict the frequency of pathogens and potential changes in antibiotic resistance patterns.

Method: Stool samples were collected from September 2009 to September 2011; 193 and 239 samples, from age-matched cases and asymptomatic controls, were collected, respectively, from Kericho and Kisumu District Hospitals in western Kenya. Bacterial pathogens were identified by conventional microbiological methods; antibiotic susceptibility of bacterial isolates was ascertained using the MicroScan WalkAway 40 Plus. An enzyme immunoassay kit was used to detect rotavirus, and ova and parasite examination was conducted by microscopy and an enzyme immunoassay.

Results: Rotavirus (10.2% and 10.5%) and *Shigella* (11% and 8%) were isolated significantly more often in the cases than the controls from Kericho and Kisumu District Hospitals respectively. The diarrheagenic *Escherichia coli, Campylobacter jejuni* and *Salmonella* were found most often in the cases while *Giardia lamblia* and *Entamoeba histolytica/E. dispar* were found more often in the controls. Most pathogens were isolated from children under 5 years old. More than 50% of the *Shigella, Salmonella* and diarrheagenic *E. coli* isolates were multidrug resistant to ampicillin, tetracycline and trimethoprim/sulfamethoxazole with several enteroaggregative and enterotoxigenic *E. coli* isolates producing extended-spectrum beta-lactamases.

Conclusion: Accurate epidemiologic information on acute diarrheal illness in Kenya will be critical for augmenting existing diarrhea management policies in terms of treatment and to strengthen future community awareness and health promotion programs.

Keywords: Gastroenteritis, Protozoa, Rotavirus, *Shigella*, *Escherichia coli*, Antimicrobial resistance

Introduction

Diarrheal disease continues to be a major cause of morbidity and mortality worldwide, particularly in areas of poor sanitation and hygiene.[1] Acute diarrheal disease accounts for more than 1.8 million deaths annually, particularly in children under 5 years of age, and more than 40% of those deaths occur in Africa.[2] Globally, the most frequently identified pathogens of acute diarrhea are bacteria (diarrheagenic *Escherichia coli*, *Vibrio* spp., *Salmonella, Shigella*, and *Campylobacter*), viruses (rotaviruses and noroviruses) and parasites (*Entamoeba histolytica*, *Giardia lamblia*, and *Cryptospororidium parvum*).[3–5] The prevalence of these etiologic agents for diarrhea in any particular region is likely dependent on several factors, including sanitation infrastructure, social culture and personal hygiene practices.

Enteric surveillance studies conducted in Kenya over the past 20 years have been largely focused on certain bacterial causes, with a few individual studies reporting on parasitic and viral causes of diarrhea.[6–11] Additionally, a few enteric pathogen prevalence studies have been conducted in specific geographic regions of Kenya.[12,13] In many parts of Kenya, the laboratory capability to detect a broad spectrum of pathogenic agents is lacking; as a result there are insufficient data on the specific pathogens causing acute diarrhea in Kenya, particularly in rural areas. Empirical diagnosis of an enteropathogen by a healthcare provider without laboratory confirmation can result in missed diagnosis or the prescription of inappropriate treatment. To address this lack of data, a case-control acute diarrhea study was begun in September 2009 to determine the prevalence of enteric pathogens in patients with acute diarrhea.

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Stool samples were collected from patients seen in an outpatient setting in two district hospitals in western Kenya and processed for enteric pathogens at the microbiology laboratory of the US Army Medical Research Unit-Kenya, located in Kericho, Kenya. Here we report the enteric pathogen distribution and the antibiotic resistance patterns after 2 years of surveillance.

Materials and methods

Study sites

Stool samples were collected from September 2009 to September 2011 at Kericho District Hospital and Kisumu District Hospital. Kericho District Hospital is located in Kericho (lat 00°22′S, long 35°15′), in the South Rift Valley of Kenya. Kericho is a major area for tea production in Kenya, because of its high altitude and temperate climate. Kisumu District Hospital is in the sprawling city of Kisumu. Kisumu (lat 00°03′S, long 34°45′E, in Nyanza Province, is adjacent to Lake Victoria and is the third largest city in Kenya after Nairobi and Mombasa. Both facilities are public hospitals operated by the Kenya Ministry of Health and provide services to the local and outlying communities respectively.

Enrollment and specimen and data collection

Acute diarrhea was defined as three or more episodes of loose, bloody or watery stool in less than a 24-h period, but with these symptoms not lasting more than 14 days. Age-matched controls (reported for medical reasons other than acute diarrhea) were recruited from the same hospital in which the patients were seen and had no episodes of diarrhea within the 2 weeks prior to enrollment. All patients and controls were recruited from the outpatient population only. Informed consent was obtained directly from study participants aged 18 years and over, and from a parent or guardian of those below that age. After stool collection, demographic and historical clinical details were captured on questionnaires for both patients and controls; data collected included place of residence, age, sex, type of water source used daily (municipal, well, borehole and rainwater) and any overt clinical symptoms. A detailed physical examination was also conducted by a clinical officer at the time of enrollment to determine the study participants' nutritional status.

Specimen collection

A portion of the fresh stool specimen was aliquoted into Cary-Blair transport medium (Medical Chemical Corporation, Torrance, CA, USA), 10% formalin and a clean vial which was stored at −20°C at the site. All vials were transported in cool boxes to the microbiology laboratory in Kericho for processing.

Stool processing and analysis

Stool preserved in Carey-Blair transport medium was inoculated onto MacConkey agar (MAC), sheep blood agar (BAP), MacConkey-sorbitol agar (SMAC), Hektoen enteric agar (HE), cefsulodin-irgasan-novobiocin agar (CIN), cefoperazone-vancomycin-amphotericin agar (CVA), and thiosulfate-citrate-bile-sucrose agar (TCBS). Plates except for CVA were incubated overnight at 37 °C for 18–24 h. CVA plates were incubated at 42 °C for 48 h in a microaerophilic environment in a Campy pouch (Becton Dickinson, Franklin Lakes, NJ, USA). Subculture plates were inoculated and incubated as described above. Single colonies exhibiting the proper characteristics on the various media described above (except for *Campylobacter* spp. identification) were processed by the MicroScan WalkAway 40 automated bacterial identification and antibiotic susceptibility platform (Siemens, Erlangen, Germany). Briefly, cell suspensions of isolates at an optical density of 0.5 were inoculated into Microscan Gram-negative conventional (overnight) panels according to the manufacturer's instructions and incubated inside the platform overnight at 37 °C. Identification and antibiotic susceptibility results of the bacterial isolates were available the following day. Suspected *Campylobacter* isolates were characterized at the species level using confirmatory phenotypic analyses for *Campylobacter*.[14]

A multiplex PCR was used for the detection of enterotoxigenic *E. coli* (ETEC) and enteroaggregative *E. coli* (EAEC) according to previously described methods.[15] Five lactose-fermenting colonies of similar morphology were isolated from MAC plates and bacterial DNA was extracted by resuspending the colonies in 50 μl of sterile water, boiling the suspension for 5 min and centrifuging at 10 000 *g* for 1.5 min. The resulting supernatant was used as the DNA template for the PCR reaction. To detect ETEC, the following primers sets were used to amplify a 190 bp fragment of the heat-stable toxin and a 450 bp fragment of the heat labile toxin respectively respectively:

5'-ATTTTTCTTTCTGTATTGTCT-3'
5'-CACCCGGTACAAGCAGGATT-3'
5'-GGCGACAGATTATACCGTGC-3'
5'-CGGTCTCTATATTCCCTGTT-3'.

The following primers were used to amplify a 324 bp fragment of the *aggR* gene for the detection of EAEC:

5'-CTAATTGTACAATCGATGTA-3'
5'-CTGAAGTAATTCTTGAAT-3'.

Cycling conditions were as follows: 95°C for 45 s, 50°C for 45 s, 72°C for 45 s with a final elongation step of 72°C for 10 min. Amplicons were visualized using gel electrophoresis. American Type Culture Collection (ATCC) strains for ETEC and EAEC were used as positive controls in every PCR reaction.

The detection of *G. lamblia*, *E. histolytica/E. dispar*, and *C. parvum* was performed from September 2009 to September 2010, using the Triage Parasite Panel (Biosite Inc., San Diego, CA, USA), according to the manufacturer's instructions. From October 2010 to September 2011, routine ova and parasite examination was conducted via microscopy. A comparison of the two methods was conducted using more than 200 stool samples to ensure there were no major differences between the two methods.[16] For ova and parasite examination, stool was concentrated using the Mini Parasep SF kit (DiaSys, Wokingham, Berkshire, England) according to the manufacturer's instructions. To examine stool samples for ova and parasites, two wet mounts were prepared for each specimen (one stained with iodine) and analyzed by two laboratory technicians. For *C. parvum* identification, a modified acid-fast stain was used according to previously described methods.[17] For all ova and parasite examinations, control slides for *E. histolytica/E. dispar*, *G. lamblia* and *C. parvum* were prepared and used in real time along with the samples. Rotavirus was detected using the

Premier Rotaclone kit (Meridian Bioscience Europe) according to the manufacturer's instructions.

Antibiotic susceptibility testing

Antibiotic susceptibility testing for all bacterial isolates except for *Campylobacter* was conducted using the Microscan automated platform as described above. Each bacterial isolate's sensitivity was tested against 26 antibiotics in specific Gram-negative conventional (overnight) panels. Resistance was determined using standards as defined by the Clinical and Laboratory Standards Institute (CLSI, Wayne, PA, USA). E-tests (Biomerieux, Craponne, France) were used for antibiotic susceptibility testing for *Campylobacter* isolates. Antibiotics used were ampicillin, ciprofloxacin, tetracycline, trimethoprim/sulfamethoxazole and gentamicin. CLSI standards for Enterobacteriaceae were used to interpret results for *Campylobacter* antibiotic susceptibility.

Statistical analysis

Odds ratios and 95% confidence intervals were calculated using multivariate logistic regression for statistical analysis on case-control data. A χ^2 test was used to determine statistical significance of the numbers of enteric organisms in the different age groups. Data were analyzed using GraphPad Prism Version 5.01(GraphPad Software Inc., La Jolla, CA, USA). A p value of <0.05 was considered statistically significant.

Results

A total of 386 stool samples (193 from patients and 193 from controls) was collected from Kericho District Hospital from 25 September 2009 to 30 September 2011. The median ages for patients and controls from Kericho District Hospital were 2 years 5 months and 2 years 9 months respectively; 53% of the patients and 57% of the controls were male. The proportion of patients and controls in specific age groups were as follows: 0–1 year: 38%; 2–5 years: 38%; 6–19 years: 9%; ≥20 years: 15%. Physical examinations by clinical officers found that 2.5% (5/193) of the controls and 11% (21/193) of the patients were malnourished and severely dehydrated. The median age for these individuals was 1 year 6 months. Bloody diarrhea was observed in 6.8% (13/193) of the cases, the mean duration of diarrhea being 48 h.

A total of 478 stool samples (239 from patients and 239 from controls) was collected from Kisumu District Hospital from 27 November 2009 to 30 September 2011. The median ages for cases and controls for Kisumu District Hospital were 1 year 11 months and 2 years respectively; 43% of the cases and 53% of the controls were male. The proportion of cases and controls in specific age groups were as follows: 0–1 year: 49%; 2–5 years: 15%; 6–19 years: 7%; ≥20 years: 29%. Physical examinations by clinical officers found that 0.75% (2/239) of the controls and 4.6% (11/230) of the patients were malnourished and severely dehydrated. The median age for these individuals was 2 years. Bloody diarrhea was observed in 9.6% (23/239) of the patients, the mean duration of diarrhea being 48.5 h.

The most common organisms isolated from patients at Kericho District Hospital were *Shigella* (11%, 21/193), rotavirus (10.2%, 20/193) and *G. lamblia* (10%, 19/193) (Table 1). Of these, *Shigella* and rotavirus were isolated significantly more often from the patients (11% vs 1%, 10.2% vs 3% respectively) and both were associated with diarrhea (OR = 12.3, OR = 3.6 respectively; $p < 0.05$). Other organisms isolated more frequently from the patients, though differences were not statistically significant, were *C. jejnui*, ETEC, enterohemorrhagic *E. coli* (EHEC) and *E. histolytica*/*E. dispar*. Identical numbers of EAEC and *Salmonella* isolates were found in patients and controls, while *C. parvum* was detected more often in the controls (Table 1). Of the 22 *Shigella* isolates detected in the patients, 32% (7/22) were *S. sonnei*, 32% (7/22) were *S. flexneri* and 9% (2/22) were *S. dysenteriae*1-7A. Pathogens were detected significantly more often in the patients than in the controls (44%, 85/193 vs 17%, 33/193); OR = 3.8; $p < 0.0001$).

For Kisumu District Hospital, the most common organisms isolated more often from cases as compared to the controls were rotavirus (10.5% vs 0.08%) and *Shigella* (8% vs 0.08%) and were significantly associated with diarrhea (OR = 10.2, OR = 13.9 respectively; $p < 0.05$) (Table 1). Other organisms isolated more frequently from the cases, though differences were not statistically significant, were *C. jejuni*, the diarrheagenic *E. coli*, *Salmonella* and *C. parvum*. *G. lamblia* and *E. histolytica*/*E. dispar* (0.08% v 5.4%; $p < 0.05$) were detected more often in the controls (Table 1B). Of the 22 *Shigella* isolates detected in the cases, 32% (6/19) were *S. sonnei*, 47% (9/19) were *S. flexneri* and 5% (1/19) were *S. dysenteriae*1-7A. Pathogens were detected significantly more often in the patients than in the controls (48%, 115/239 vs 18%, 43/239; OR = 3.5; $p < 0.0001$).

The prevalence of age-specific pathogens for Kericho and Kisumu District Hospitals is shown in Table 2. Rotavirus isolates and *G. lamblia* were found significantly more often in children aged <5 years than in the other age groups collected from both Kericho and Kisumu District Hospitals ($p < 0.05$). Interestingly, 8.2% and 11% of the *Shigella* isolates from Kericho and Kisumu District Hospitals were detected in adults ≥20 years of age. There were no significant differences in the age-specific prevalence of the organisms detected at either site.

The antibiotic resistance pattern of bacterial pathogens isolated from patients and controls from Kericho and Kisumu District Hospitals is shown in Table 3. The antibiotic resistance data for patients and controls were combined for each hospital, as bacteria isolated from patients and those isolated from controls showed no significant difference in resistance pattern. Resistance to ampicillin (42–100%), tetracycline (50– 92%) and trimethoprim/sulfamethoxazole (83–100%) was found for all organisms except for *C. jejuni* isolated from patients and controls from Kericho District Hospital (Table 3). Resistance to ciprofloxacin was found in 42% of the EAEC and 8% of the *Shigella* isolates. Of the diarrheagenic *E. coli* isolates, five EAEC isolates and one ETEC isolate were identified as organisms producing extended-spectrum beta-lactamases (ESBLs) (data not shown).

Resistance to tetracycline (67–88%), ampicillin (52–100%) and trimethoprim/sulfamethoxazole (67–100%) was detected in all bacterial isolates except for *C. jejuni* from Kisumu District Hospital (Table 3). One of the 11 *C. jejuni* isolates was multidrug resistant to all of the antibiotics tested. Sensitivity to ciprofloxacin was found for all bacterial pathogens though there was resistance detected in the ETEC and EAEC isolates (27% and 50% respectively). Of the diarrheagenic *E. coli* isolates from

Table 1. Enteric pathogens in patients and controls from Kericho and Kisumu District Hospitals, Kenya

Kericho	Cases (n = 193)	Controls (n = 193)	OR (95% CI)	p value
Shigella	22 (11)	2 (1)	12.3 (2.8–53.1)	0.0001
Campylobacter	2 (1)	1 (0.5)	2.0 (0.1–22.14)	NS
EAEC	6 (3)	6 (3)	1.0 (0.3–3.1)	NS
EHEC	6 (3)	2 (1)	3.1 (0.6–15.4)	NS
ETEC	3 (1.5)	2 (1)	1.5 (0.2–9.1)	NS
Salmonella	1 (0.5)	1 (0.5)	1.0 (0.06–16.1)	NS
Rotavirus	20 (10.2)	6 (3)	3.6 (1.4–9.2)	0.007
Giardia lamblia	19 (10)	17 (9)	1.1 (0.5–2.4)	NS
Entamoeba histolytica/E. dispar	6 (3)	2 (1)	3.1 (0.6–15.4)	NS
Cryptospordium parvum	1 (0.5)	5 (2.6)	0.2 (0.01–1.7)	NS
Unidentified	107 (56)	159 (83)	3.8 (2.4–6.0)	0.0001
Kisumu	Cases (n = 239)	Controls (n = 239)	OR (95 CI)	p value
Shigella	19 (8)	2 (0.08)	10.2 (2.3–44.5)	0.0002
Campylobacter	5 (2.1)	4 (1.7)	1.2 (0.3–4.7)	NS
EAEC	6 (2.5)	2 (0.08)	3.1 (0.6–15.3)	NS
EHEC	2 (0.08)	1 (0.04)	2.0 (0.2–22.3)	NS
ETEC	11 (3.8)	4 (1.7)	2.8 (0.9–9.1)	NS
Salmonella	5 (2.1)	1 (0.04)	5.1 (0.6–43.9)	NS
Rotavirus	25 (10.5)	2 (0.08)	13.9 (3.3–59.4)	0.0001
Giardia lamblia	8 (2.7)	14 (6)	0.6 (0.2–1.4)	NS
Entamoeba histolytica/E. dispar	2 (0.08)	13 (5.4)	0.1 (0.03–0.7)	0.007
Cryptospordium parvum	2 (0.08)	1 (0.04)	2.0 (0.1–22.3)	NS
Unidentified	154 (52)	195 (82)	3.5 (2.3–5.4)	0.0001

Data are presented as no. (%).
OR and 95% confidence intervals calculated using multivariate logistic regression with a significant p value at <0.05.
NS: not significant. EAEC: enteroaggregative *Escherichia coli*; ETEC: enterotoxigenic *E. coli;* EHEC: enterohemorrhagic *E. coli.*

Kisumu District Hospital, one EAEC isolate and three ETEC isolates were identified as ESBL-producing organisms (data not shown).

Discussion

Diarrhea can be caused by a plethora of microbial pathogens and it is not unusual in diarrheal disease surveillance studies to find that in a large percentage of patients the etiology is unknown. Furthermore, enteric infections including *G. lamblia*, *Campylobacter* and the diarrheagenic *E. coli* have been detected in asymptomatic individuals in numerous studies. It is therefore difficult to define what is causing the disease without directly comparing symptomatic patients and asymptomatic controls.[18–21] In the current case-control study, the predominant pathogens associated with patients with acute diarrhea as compared to the asymptomatic controls from both Kericho and Kisumu District Hospital were rotavirus and *Shigella*. The most common *Shigella* species were *S. flexneri* and *S. sonnei*. The bacterial pathogens identified in this study are consistent with those found in previous studies conducted in western Kenya, although none of these studies conducted surveillance in Kericho directly and none was a case-control study.[8,22,23] In previous diarrhea case-control studies, ETEC, *Shigella* and rotavirus have been identified more frequently in symptomatic individuals than in controls.[24–26] Here, the diarrheagenic *E. coli* (ETEC, EAEC and EHEC) were also detected in more patients than controls but the differences were not significant. *G. lamblia* and *E. histolytica/E. dispar* were detected more often in the controls from Kisumu District Hospital than in the patients, and there was little difference in the number of EAEC, ETEC and *G. lamblia* isolates in patients and controls from Kericho District Hospital. Several studies have shown that asymptomatic individuals can have a high prevalence of *Giardia* and the diarrheagenic *E. coli* without displaying any overt symptoms.[27, 28] Additionally, ova and parasite examination via microscopy and the Triage Parasite Panel are unable to distinguish between the pathogenic *E. histolytica* and non-pathogenic *E. dispar*.[16,29, 30] Controls from Kisumu District Hospital could have been harboring the non-pathogenic *E. dispar* as opposed to *E. histolytica*.

Rotavirus was found more often in individuals <5 years of age at both sites. These results are consistent with previous rotavirus surveillance studies conducted in Kenya.[31, 32] The diarrheagenic *E.* coli, *G. lamblia, E. histolytica/E. dispar* and *C. jejuni* were found more often in children while *Shigella* was isolated more often from adults ≥20 years of age. Two previous studies conducted in western Kenya showed a similar trend, in which adults ≥20 years of age were infected more often with *Shigella*.[8, 22]

Sources of water used by both patients and controls were ascertained from the questionnaires completed at the time of study enrollment. However, a disproportionate amount of the

Table 2. Enteric organisms from combined patients and controls from Kericho and Kisumu District Hospitals, Kenya, according to age group

Kericho	0–1 year (n = 148)	2–5 years (n = 147)	6–19 years (n = 36)	20+ years (n = 55)
Campylobacter	1 (0.7)	0 (0)	0 (0)	1 (2)
Shigella	5 (3.5)	8 (5.7)	1 (3.3)	4 (8.2)
Salmonella	0 (0)	2 (1.4)	0 (0)	0 (0)
EHEC	3 (2.1)	4 (2.8)	1 (3.3)	2 (4.1)
ETEC	2 (1.4)	2 (1.4)	0 (0)	1 (2)
EAEC	2 (1.4)	2 (1.4)	1 (3.3)	3 (6.1)
Rotavirus	15 (10.6)	4 (2.8)	2 (6.6)	0 (0)
G. lamblia	12 (8.5)	22 (15.6)	2 (6.6)	2 (4.1)
E. histolytica/E. dispar	0 (0)	6 (4.3)	0 (0)	1 (2)
C. parvum	0 (0)	4 (2.8)	0 (0)	1 (2)
Kisumu	0–1 year (n = 263)	2–5 years (n = 77)	6–19 years (n = 39)	20+ years (n = 153)
Campylobacter	4 (1.5)	4 (5.2)	0 (0)	1 (0.6)
Shigella	4 (1.5)	4 (5.2)	1 (2.6)	11 (7.2)
Salmonella	3 (1.1)	1 (1.3)	0 (0)	2 (1.3)
EHEC	1 (0.4)	0 (0)	2 (5.2)	0 (0)
ETEC	8 (3)	3 (3.9)	0 (0)	3 (2)
EAEC	6 (2.3)	0 (0)	0 (0)	2 (1.3)
Rotavirus	23 (8.7)	2 (2.6)	0 (0)	2 (1.3)
G. lamblia	12 (4.6)	8 (10.4)	0 (0)	2 (1.3)
E. histolytica/E. dispar	8 (3)	2 (2.6)	1 (2.6)	4 (2.6)
C. parvum	2 (0.8)	1 (1.3)	0 (0)	0 (0)

Data are presented as no. (%).
EHEC: enterohemorrhagic *E. coli*; ETEC: enterotoxigenic *E. coli*; EAEC: enteroaggregative *E. coli*.

Table 3. Antibiotic resistance of enteric bacteria isolates from Kericho and Kisumu District Hospitals, Kenya

Kericho	*Shigella* (n = 24)	*Salmonella* (n = 2)	ETEC (n = 5)	EAEC (n = 12)	EHEC (n = 8)
Ampicillin	10 (42)	2 (100)	4 (80)	11 (92)	5 (63)
TMP-SXT	20 (83)	2 (100)	5 (100)	10 (83)	8 (100)
Tetracycline	17 (71)	1 (50)	3 (60)	11 (92)	6 (75)
Ciprofloxacin	2 (8)	0 (0)	0 (0)	5 (42)	0 (0)
Kisumu	*Shigella* (n = 21)	*Salmonella* (n = 6)	ETEC (n = 15)	EAEC (n = 8)	EHEC (n = 3)
Ampicillin	11 (52)	4 (67)	14 (93)	8 (100)	2 (67)
TMP-SXT	20 (95)	4 (67)	10 (67)	8 (100)	2 (67)
Tetracycline	15 (71)	4 (67)	11 (73)	7 (88)	2 (67)
Ciprofloxacin	0 (0)	0 (0)	4 (27)	4 (50)	0

Data are presented as no. (%).
TMP-SXT: trimethoprim/sulfamethoxazole); ETEC: enterotoxigenic *E. coli;* EAEC: enteroaggregative *E. coli;* EHEC: enterohemorrhagic *E.coli.*

patients and controls used municipal water so no significant correlations could be determined from this data. Several studies have examined the quality of water used in slums and rural areas and found significant levels of fecal coliforms and enteric viruses such as rotaviruses.[33–35] However, no water quality studies to date have been conducted in Kisumu and Kericho directly. No information on travel to rural villages and homes and water used while there was captured in the questionnaire used in this study.

More than half of the *Shigella, Salmonella,* ETEC, EAEC and EHEC isolates were multidrug resistant to ampicillin, tetracycline and trimethoprim/sulfamethoxazole. These antibiotics are commonly prescribed and readily available at local chemists in Kisumu and Kericho. The emergence of multidrug resistant bacteria is an ongoing problem in Kenya, as a result of ready access to the drugs, unwarranted use of the drugs in clinical settings without proper laboratory diagnosis, and the ease of genetic exchange of resistance genes among the bacterial pathogens.[36,37]

WHO guidelines state that antibiotic use is warranted only in cases of shigellosis.[38] Empiric diagnosis of the cause of acute diarrhea at these medical facilities could result in unnecessary prescription of antibiotics.

Bacterial resistance to ampicillin, tetracycline and trimethoprim/sulfamethoxazole has been well documented in Kenya and the results observed in this study are comparable to the antibiotic resistance observed in previous studies.[8,39-41] Most of the bacterial isolates were susceptible to ciprofloxacin; exceptions were several ETEC and EAEC isolates. These EAEC and ETEC isolates, classified by their antibiotic resistance pattern as extended-spectrum beta-lactamase producing organisms, were also resistant to aztreonam, levofloxacin, cefotaxime, ceftriaxone[CM1], imipenem and ceftazidine. Extended-spectrum beta-lactamase producing organisms are a major concern, with regard to treatment of diarrheal disease and other diseases of bacterial origin.[42,43] This emerging antibiotic resistance has been observed in bacterial pathogens in addition to EAEC and ETEC, including *Salmonella* and *Klebsiella* in Kenya.[44–46]

The laboratory surveillance data observed in this study reflects only the population in Kisumu and Kericho who presented at the district hospitals for care and treatment and were not admitted to the respective hospitals. Therefore the data may underestimate the true burden of acute diarrheal disease in Kisumu and Kericho. The laboratory testing did not include testing for noroviruses, astroviruses and adenoviruses, which all have been found to be causes of acute diarrhea in Kenya, particularly in children.[47–49] Primers for enteropathogenic *E. coli* (EPEC) and enteroinvasive *E. coli* (EIEC) were not included in the diarrheagenic *E. coli* PCR assay. As with the previously mentioned enteric viruses, these organisms have been found to cause acute diarrhea in Kenyan children.[12,50,51] These other organisms could have been responsible for the diarrhea in patients in which a pathogen was not detected.

The median age for all patients and controls from Kericho and Kisumu District Hospitals was less than 2 years, which reflects the greater burden of diarrheal illness in children than in the adult populations in Kisumu and Kericho. HIV status was not ascertained from the patients or controls from Kisumu and Kericho, so we were unable to compare enteric pathogens isolated from HIV-infected and uninfected individuals. A previous study conducted in Kisumu showed that HIV-infected children aged <2 years had higher rates of diarrhea when compared to HIV-uninfected children, though there were no significant correlations with any specific pathogen.[52] The increase in *Shigella* isolates detected in adults ≥ 20 years of age in both Kericho and Kisumu could be attributable to the increased HIV prevalence in this age group and to these individuals seeking care more often because of their status.[8,22,53] According to the Kenyan Demographic and Health Survey, 6.7% of Kenya's adult (15–49 years) population is infected with HIV, and HIV prevalence rates for the Rift Valley Province (Kericho) and Nyanza Province (Kisumu) are 5.3% and 15.1% respectively.[52,54]

Future investigations should include establishing additional surveillance sites in the rural areas of Kericho and Kisumu, to include those individuals who do not have the means to be seen at the district hospitals. Additionally, HIV status of the individuals should be ascertained in future studies. And for future studies the enteric pathogens testing menu should be expanded to include norovirus, astrovirus, adenovirus, EPEC and EIEC. With this data, intermediate diarrhea treatment strategies for outpatients at Kericho and Kisumu Dstrict hospital should adhere to the use of oral rehydration only, because antibiotics should be administered only in cases of known shigellosis. If antibiotics are to be prescribed, ciprofloxacin should be the drug of choice as most bacterial isolates were susceptible. Furthermore, education on proper hand washing and improved sanitation and hygiene should be emphasized to the patients.

Conclusion

The current study was a case-control study aimed at determining the etiology of acute diarrhea in an outpatient setting at two district hospitals in western Kenya. *Shigella* and rotavirus were isolated more often in the patients than in the controls from Kericho and Kisumu District Hospitals. Children <5 years old had the highest burden of acute diarrhea and most of the enteric pathogens detected were from this population. Significant numbers of asymptomatic controls, particularly those from Kisumu District Hospital, had higher numbers of *G. lamblia*, *E. histolytica/E. dispar* and *C. parvum*. Multidrug resistance to the most common antibiotics was evident in Kericho and Kisumu. Case-control studies of acute diarrhea using asymptomatic controls allow us to gain a much more accurate understanding of the disease burden and pathogen prevalence in a particular area. A more defined picture will allow for the development of community education programs and other interventions that could decrease the incidence of acute diarrhea.

Authors' contributions: EVO, MTW, CZ and JEL conceived the study and designed the study protocol. BES was the primary investigator. EAO, CPO, BES, EKC, RKK and JNN conducted the laboratory analysis. BES, MCK, EAO, CPO, EKC, RKK and JNN analysed the data. BES and EVO wrote the manuscript. All authors reviewed the manuscript, improved the content and approved the final version. BES is guarantor of the paper.

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