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Enteric pathogen surveillance in a case-control study of acute diarrhoea in the town of Kisii, Kenya

Acute diarrhoea is a major cause of morbidity and mortality in Kenya, particularly in children younger than 5 years, and can be caused by a multitude of bacterial, viral and parasitic organisms (Beatty *et al.*, 2009; Santosham *et al.*, 2010). The capability of laboratories to detect a wide range of enteric pathogens varies considerably among medical facilities in Kenya, resulting in a significant proportion of acute diarrhoea disease cases with undetermined aetiologies. This lack of proper laboratory diagnosis can lead to missed treatments or the unwarranted use of antibiotics (Brooks *et al.*, 2006). An acute diarrhoea case-control study was initiated by the US Army Medical Research Unit – Kenya at several Kenya Ministry of Health facilities in western Kenya in September 2009 to account for this lack of data, and the results of 2 years of surveillance at two district hospitals located in Kisumu and Kericho were recently published (Swierczewski *et al.*, 2013). Here, we report the results of 1 year of surveillance at Kisii Level 5 Hospital in the town of Kisii, Kenya.

Stool samples were collected from 25 May 2011 to 31 May 2012 from outpatients with acute diarrhoea (cases) and age-matched controls seen at Kisii Level 5 Hospital. Acute diarrhoea cases were defined as those individuals having three or more episodes of loose, bloody or watery stool in less than a 24 h period and of no more than 14 days in duration. Asymptomatic age-matched controls were defined as having no episodes of diarrhoea within 2 weeks before enrolment. Informed consent was obtained from subjects older than 18 years and from a parent or guardian for individuals younger than 18 years.

Stool was aliquoted into 10 % formalin, Cary-Blair transport media (Medical Chemical Corporation) and a clean vial and transported in temperature-controlled coolboxes to the microbiology laboratory in Kericho. Stool was inoculated onto the following selective media: MacConkey, thiosulfate citrate bile sucrose, sheep blood, MacConkey-sorbitol, Hektoen, cefoperazone-vancomycin-amphotericin and cefsulodin-Irgasan-novobiocin. Bacterial identification and antibiotic susceptibility testing (except for *Campylobacter* spp. identification) were performed using MicroScan WalkAway40 (Siemens). Suspected *Campylobacter* isolates were identified at the species level and Etest (bioMérieux) was used to test antibiotic susceptibility (Davis & DiRita, 2008).

A multiplex PCR was used for the detection of enterotoxigenic *Escherichia coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC) and enteroaggregative *E. coli* (EAEC). To detect ETEC, the following primers were used to amplify a 508 bp fragment of the heat-labile toxin and a 147 bp fragment of the heat-stable toxin, respectively: 5′-CACACGGAGCTCCTCAGTC-3′ and 5′-CCCCCAGCCTAGCTTAGTTT-3′, and 5′-GCRAAACCAGTArGGTCT-3′ and 5′-CCCGGTACARGCAGGATTACAACA-3′. The following primers were used to amplify a 650 bp fragment of the *attA* gene for the detection of EAEC: 5′-CTG-GCGAAAGACTGTATCAT-3′ and 5′-CAATGTATAGAAATCCGCTGTT-3′. To detect EPEC, the following primers were used to detect a 350 bp fragment of the *bfpA* gene: 5′-GGAATCAGACGCAGAC-TGGTAGT-3′ and 5′-GGAATCAGACG-CAGACTGGTAGT-3′. The following primers were used to detect a 423 bp fragment of the *ipaH* gene to detect EIEC: 5′-TGGAAAAACTCAGTGCCTCT-3′ and 5′-CCAGTCCGTAAATTCATTCT. Cycling conditions were as follows: 95 °C for 45 s, 55 °C for 45 s and 72 °C for 45 s, with a final elongation step of 72 °C for 10 min. Amplicons were visualized using gel electrophoresis.

Ova and parasite examination was conducted by microscopy after stool concentration using the Mini Parasep SF kit (DiaSys) and analysed as previously described (Swierczewski *et al.*, 2013). Rotavirus was detected using an enzyme immunoassay kit (Premier Rotaclone, Meridian Bioscience). Multivariate logistic regression was used to calculate odds ratios (ORs) and 95 % confidence intervals (CIs). Data were analysed using GraphPad Prism Version 5.01 and $P<0.05$ was considered statistically significant.

A total of 312 total stool samples (156 cases and 156 controls) were collected and processed for enteric pathogens. The median ages for the cases and the controls were 61 and 60 months, respectively. The proportions of cases and controls in specific age groups were as follows: 0–1 year, 19 %; 2–5 years, 32 %; 6–19 years, 17 %; and ⩾20 years, 32 %. Rotavirus (OR 11.9; $P=0.0001$), *Shigella* (OR 18.9; $P=0.0001$) and *Campylobacter* (OR 9.4; $P=0.02$) were detected significantly more often in cases than in controls (Table 1). Similar numbers of the diarrhoeagenic *E. coli* and *Giardia lamblia* were found in both cases and controls. *Entamoeba histolytica/E. dispar* (OR 0.2; $P=0.002$) was detected significantly more often in controls than cases (Table 1). Enteric organisms were detected significantly more often in cases than controls (65 % versus 35 %; OR 3.5; $P<0.0001$). Rotavirus was found more often in the 0–11 months (18.3 %) and 2–5 years (9 %) age groups, while *E. histolytica/E. dispar* (14 %) and 9 % of *Shigella* isolates were detected in the age group ⩾20 years.

Overall, 56 %, 78 % and 89 % of the *Shigella* isolates and 40 % of *C. jejuni* isolates were resistant to ampicillin, tetracycline and trimethoprim/sulfamethoxazole (TMP-SXT), respectively. The ranges of antibiotic resistance to ampicillin, tetracycline and TMP-SXT for the diarrhoeagenic *E. coli*

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Table 1. Numbers and percentages of enteric pathogens in cases and controls from Kisii Level 5 Hospital

EAEC, enteroaggregative *E. coli*; EHEC, enterohaemorrhagic *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; NS, not significant.

Pathogen*	Cases, n (%) (n =156)	Controls, n (%) (n =156)	Odds ratio (95 % CI)	P value
Shigella	17 (10.9)	1 (0.6)	18.9 (2.5–144.4)	0.0001
Campylobacter	9 (5.8)	1 (0.6)	9.4 (1.2–75.6)	0.02
EPEC	8 (5.1)	4 (2.6)	2.1 (0.6–7.1)	NS
EAEC	11 (7.1)	13 (8.3)	0.8 (0.4–1.9)	NS
EHEC	2 (1.3)	2 (1.3)	1 (0.1–7.2)	NS
EIEC	12 (7.7)	5 (3.2)	2.5 (0.9–7.3)	NS
ETEC	5 (3.2)	2 (1.3)	2.5 (0.5–13.3)	NS
Salmonella	0 (0)	2 (1.3)	0.2 (0.0.01–4.1)	NS
Rotavirus	21 (13.5)	2 (1.3)	11.9 (2.8–52.1)	0.0001
Giardia lamblia	11 (7.1)	9 (5.8)	1.2 (0.5–3.1)	NS
Entamoeba histolytica/E. dispar	3 (1.9)	13 (8.3)	0.2 (0.06–0.8)	0.002
Campylobacter parvum	2 (1.3)	0 (0)	5.1 (0.2–10.4)	NS
Unidentified	55 (35)	102 (65)	3.5 (2.2–5.5)	0.0001

*P<0.05 for numbers of pathogens in cases versus controls.

were 75–94 %, 57–94 % and 75–100 %, respectively. Ciprofloxacin resistance was seen in 47 % of EIEC isolates, and 12.5 % of EAEC isolates and 11.8 % EIEC isolates were identified as producing extended-spectrum β-lactamases (ESBLs).

The present case-control study conducted at Kisii Level 5 Hospital is the first of its kind to be conducted at this facility. Rotavirus, *Shigella* and *Campylobacter* were detected significantly more often in cases than controls, and these findings are consistent with the data from the Kericho and Kisumu sites (Swierczewski *et al.*, 2013). *E. histolytica/E. dispar* was detected significantly more often in controls, as was also evident at the Kisumu site (Swierczewski *et al.*, 2013). Rotavirus, as expected, was most prevalent in children younger than 5 years and *Shigella* was found to be most prevalent in adults aged 20 years and older. These data are consistent with those from the Kericho and Kisumu sites.

The rotavirus results observed are comparable with data from the recently published Global Enteric Multicenter Study (GEMS), which included a site in Nyanza Province, Kenya (Kotloff *et al.*, 2013). The GEMS authors showed that in addition to rotavirus, *Shigella*, *C. parvum* and ETEC (heat-stable enterotoxin only or both heat-labile and heat-stable enterotoxins) were detected significantly more often in subjects with moderate to severe diarrhoea than in control subjects among children aged 0–59 months. In this and our previous study, *Shigella* was detected significantly more often in the cases, but in adults aged 20 years and older, while there was no difference in the detection of ETEC and *C. parvum* between cases and controls (Swierczewski *et al.*, 2013). Because GEMS only enrolled paediatric patients, it is uncertain whether *Shigella* would have been detected significantly more often in acute diarrhoea cases as opposed to controls in an adult population, as has been previously shown in Kenya (Brooks *et al.*, 2006; Swierczewski *et al.*, 2013). Furthermore, there could have been multiple GEMS surveillance sites in Nyanza Province in more remote, rural areas which would be in contrast to our single surveillance site in Nyanza Province located directly in the urban city of Kisumu. The surveillance sites in Kisii and Kericho were also located in the towns, where urban residents would be more apt and able to afford to seek treatment for acute diarrhoea, as opposed to those from more rural, remote locations. The inclusion of more rural areas with suspect water quality and possibly poorly treated water could account for the significant detection of ETEC and *C. parvum* in participants enrolled in GEMS.

A major concern from the current case-control study is the emergence of ciprofloxacin resistance in all bacterial pathogens and those EIEC and EAEC isolates identified as ESBLs. ETEC and EAEC ESBLs were isolated from the Kericho and Kisumu sites, but the percentage of ciprofloxacin resistance among the bacterial isolates was higher at the Kisii site (Swierczewski *et al.*, 2013). In contrast to the Kericho and Kisumu study, primers for EIEC and EPEC were added to the multiplex PCR used for the Kisii isolates, allowing us to identify these enteric pathogens and thus expanding the diagnostic capabilities from the previous study. Although there are several limitations to this study, including the lack of known HIV status among the participants and the limited viral diagnostic component, our findings will allow the development of better diarrhoea treatment and community intervention strategies to reduce acute diarrhoea in Kisii town and the surrounding areas.

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