



Prevalence, virulence genes and Antimicrobial Resistance of Shiga-toxigenic *E.coli* in diarrhoea patients from Kitale, Kenya

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Summary

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are among the most important causes of food-borne diseases. They cause illnesses ranging from mild diarrhea to more severe conditions that may progress to hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). The burden of STEC in patients with diarrheal illness in Kitale county referral hospital, Trans-Nzoia County had not been established.

Objectives: To determine the prevalence of STEC, its associated virulence genes and antimicrobial resistance among patients seeking treatment for diarrhoeal illness at Kitale County Referral Hospital.

Methods: Stool samples from patients seeking treatment for diarrheal illness and had consented to participate in the study were collected and cultured for enteric bacteria.

Suspect *E.coli* isolates were further identified using conventional biochemical methods. Conventional multiplex PCR targeting Shiga toxins (*stx*₁, *stx*₂, *hlyA* and attaching and effacing mechanisms (*eaeA*) were used to detect STEC virulence markers responsible for the Pathogenicity of STEC infection among other *E.coli* pathotypes.

Results: A total of 295 participants were enrolled; median age 120 months (IQR: 36–312). 39 % (115) were children aged <5years of whom 54% (160) were females. The prevalence of pathogenic *E.coli* was 19% 56/295 and STEC was the most prevalent among *E.coli* pathotypes at 5.4% 16/295. The *Stx*₂ gene and the *Stx*₁/*Stx*₂/*hlyA* combination were the most prevalent in the STEC strains. The virulence genes (*Stx*₁, *Stx*₂, *eaeA** and *HlyA**) were observed in 13, 19, 9 and 14 in STEC isolates respectively. The most common gene was *Stx*₂ and combinations of (*Stx*₁+*Stx*₂+*hlyA*) genes. Antimicrobial resistance to commonly prescribed antibiotics: chloramphenicol, ampicillin 10µg, erythromycin 15µg, gentamicin 10µg, ciprofloxacin 5µg, tetracycline 30µg, Trimethoprim/Sulfamethoxazole 25 µg, Cefotaxime 30 µg, furazolidine (8µg) and nalidixic acid 30 µg. were



observed for all *E.coli* isolates except one (1.8%; 95% CI=0.1–9.6%). No isolates among STEC showed resistance to Furazolidine drug. However, Trimethoprim / Sulphurmethoxazole) was the drug which exhibited the highest resistance at (94%, 95% CI 70 to 99%).

Conclusion and recommendation: Prevalence of STEC was 5.4%, (*Stx₁/Stx₂/hlyA*) virulence genes combination was the most common. High resistance to commonly prescribed antibiotics were observed in *E.coli* isolates and may be an existing problem that needs to be further research investigation.

KEY words: Shiga–Toxigenic *Escherichia coli* (STEC), antimicrobial resistance, Kitale County referral hospital.

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Introduction

Diarrhoea is a significant health problem worldwide, especially in the developing world where adequate sanitation facilities are lacking [1]. Globally diarrheal diseases account for almost a fifth of all deaths of children below 5 years of age; with an estimated 2.2 million deaths annually [2]. Shigatoxin–producing *Escherichia coli* (STEC) are rare pathogens but potentially fatal cause of gastroenteritis. They are associated with a wide spectrum of diseases ranging from mild to bloody diarrhoea, haemorrhagic colitis and a life threatening condition that progress to haemolytic uremic syndrome (HUS) that can be fatal [3]. Among several sero–groups of STEC, O157:H7 is the most pathogenic serotype associated with food–borne diseases [3, 4]. Domestic ruminants especially cattle are recognised as predominant reservoir of STEC [5, 6], although this pathogen can be transmitted to humans directly through consumption of several foods of non–bovine origin including unpasteurised juices, salami, cheese, raw(unpasteurised) milk contaminated food and water or indirectly through their environments [4, 7]. There is little information about epidemiology of STEC in humans in Kenya, however some information on possible reservoirs for STEC have been described, including food products, healthy and diarrhoeagenic

cattle, camels, and her ruminants [8–10]. Gastroenteritis caused by STEC strains has the capacity to produce cytotoxins similar to that produced by *Shigella dysenteriae* type 1a study carried out among the Maasai community, in Narok and Kajiado districts showed STEC as one of the major causes of bloody diarrhoea at infection rate of 24.1% [12].

The primary STEC virulence factor responsible for the most serious outcomes of human infection is Shiga toxin (*Stx*), an AB5 toxin that targets cells expressing the Glycolipid–globo–triaosylceramide (Gb3) by disrupting host protein synthesis and causing apoptotic cell death [2]. Although the pathogenicity mechanisms of STEC are not yet fully understood, Shiga toxin genes 1 and 2 (*Stx₁* and *Stx₂*) are the main factors for virulence properties in STEC pathogenesis and development of clinical symptoms [12]

Other virulence factors may play significant roles in the pathogenicity of this pathogen such as intimin which is encoded by the chromosomal gene *eaeA* and is responsible for intimate attachment of STEC to intestinal epithelial cells [13]. Since cows have been incriminated as the primary hosts of STEC and especially serotype O157:H7, Trans –Nzoia County has a potential of STEC presence due to both dairy and beef farming



which entails human contacts with animals thus poses a threat on outbreaks of human illnesses due to STEC. Recently, several reports have documented a significant increase in antimicrobial resistance in STEC strains [14, 15]. Inappropriate usages of antibiotics for treating human and plant diseases and for promoting food–animal growth have been proposed to contribute to antimicrobial resistance among bacteria population [16, 17]. Moreover, the use of antimicrobials agents to treat STEC infections is contravalsals since they can induce Shigatoxin (Stx) production resulting in HUS in human [18, 19]. STEC strains have been recovered from a variety of animals, and cattle are considered the major reservoir for STEC strains [20–22]. Small domestic ruminants are also relevant STEC reservoirs that could potentially be transmitted to humans, thru direct or indirect contact, or via the food chain [23]. In addition, the present study examined antimicrobial resistance patterns of all pathogenic *E.coli* isolated from the diarrheal patients from the study site in order to gauge the effectiveness of commonly available antimicrobials. Therefore, due to the importance of STEC in human health, this study sought to address the prevalence of STEC, its virulence gene profiles and antimicrobial resistance among patients treated with diarrhoea illness in Kitale County referral hospital, Trans–Nzoia County.

Materials and Methods

Study design: This was hospital based cross–sectional study carried out between August 2015 and June 2016 that aimed to provide prevalence of STEC its associated virulence genes and antimicrobial susceptibility from patients of all ages that sought treatment for diarrhoea illness at Kitale County Referral Hospital, Trans–Nzoia County.

Study site and population: The study was carried out at Kitale County hospital situated in Trans–Nzoia County which is located in the North Rift bordering West Pokot County to the North, Elgeyo – Marakwet County to the East, Bungoma County to the West and Uasin–Gishu County to South. The hospital was selected because it has recorded high rates of childhood morbidity and mortality due to bloody diarrhea (hospital records). Kitale hospital serves a population of about 106,187 according to the Census report in 2009 and of who most are livestock farmers and milk vendors and being a cosmopolitan town. This is an ideal target population that favors transmission and infection for STEC. The target population included patients of all ages seeking treatment in Kitale County hospital, presenting with acute diarrheal illness (3 or more passages of loose or watery stool within 24 hrs) and willing to participate were recruited and enrolled into the study.

Inclusion criteria: All out patients of all ages who sought treatment for diarrheal illness at Kitale County hospital and were willing to consent/assent for children to participate in the study were recruited.

Exclusion criteria: Patients who had taken antibiotics within 72hrs of symptom onset and those who were unwilling to participate or give stool samples. Those who declined to participate in the study were also excluded.

Collection of stool sample

A total of 295 Stool samples were collected from patients with diarrheal illness in sterile containers and transferred into Cary Blair transport medium at room temperature and packaged following the triple packaging system [24] and sent to KEMRI Centre for microbiology for processing. All the samples were plated onto differential media, Sorbital–MacConkey for detection of



O157 (STEC) and MacConkey to screen for other *E. coli*. Thereafter, plates were then incubated at 35°C – 37°C for 18–24 hours.

After an overnight incubation approximately five to ten pink colonies with a darker centre from MacConkey and colourless colonies from Sorbital– MacConkey were picked and subjected to biochemical test using standard method. Confirmed *E. coli* colonies were further cultured onto nutrient agar and incubated for 18–24 hours at 37°C for PCR analysis [25].

Control and clinical *E. coli* strains

Control *E. coli* strain O157:H7 which harbours (*Vt₁*, *Vt₂*, *hlyA* and intimin genes (*eaeA*) was used as a standard and clinical *E. coli* isolates isolated from stool were used for preparation of DNA templates for PCR. Single colony from each plate was picked using sterile applicator sticks and mixed with 25µl of distilled water in a micro

centrifuge tube. Only 2.0 µl of this suspension was used as template for the PCR [26].

Primers

Primer set for amplifying segments of the Shiga toxins (*Vt₁*, *Vt₂*, *hlyA* and intimin genes (*eaeA*) of attaching and effacing mechanisms), cytotoxic necrotising factors 1&2 (*CNF1*) and (*CNF2*) Enteroaggregative mechanism (*Eagg*), enteroinvasive mechanism (*Einves*), and heat-labile (*LT*) and heat-stable (*ST1* ad *ST2*) toxins was tested using the method described [26]. Multiplex PCR primer sequence and the size of the PCR products of each set of primers used in this study are listed in (table 1). The primers were selected based on the specificity and sensitivity and also based on the PCR product size distinguishable by the agarose gel electrophoresis in multiplex PCR systems from different studies.

Table 1. Primers used for PCR and DNA sequencing described.

DEC Group	Virulence Factor	Primer sequence 5–3'	Product size
STEC	<i>Vt₁</i>	fp:5'– ACGTTACAGCGTGTTCRGGGATC–3' rp:5'–TTGCCACAGACTGCGTCAGTRAGG–3'	121 [26]
	<i>Vt₂</i>	fp:5'–TGTGTCTGGGTTCGTTAATACGGC–3' rp:5'–TCCGTTGTCATGGAACCGTTGTC–3'	102[26]
	<i>hlyA</i>	fp:5'–AGCTGCAAGTGCGGGTCTG–3' rp:5' TACGGGTTATGCCTGCAAGTTCAC –3'	569 [27]
EPEC	<i>eaeA</i>	fp:5'–TGAGCGGCTGGCATGAGTCATAC–3' rp:5' TCGATCCCCATCGTCACCAGAGG–3'	482 [28]
EAGG	<i>Eagg</i>	fp:5'–AGACTCTGGCGAAAGACTGTATC–3' rp:5'–ATGGCTGTCTGTAATAGATGAGAAC–3'	194 [26]
EIEC	<i>Einvs</i>	fp:5'–TGGAAAACTCAGTGCCTCTGCGG–3' rp:5'–TTCTGATGCCTGATGGACCAGGAG–3'	140 [26]
ETEC	<i>ST₁</i>	fp:5' TTTCCCCTCTTTTAGTCACTCAACTG3' rp:5'–GGCAGGATTACAACAAAGTTCACAG–3'	160 [26]
	<i>ST₂</i>	fp:5'–CCCCCTCTCTTTTGCCTTCTTTCC–3' rp:5'–TGCTCCAGCAGTACCATCTAACCC–3'	423 [26]
	<i>Lt₁</i>	fp:5'–TGGATTCATCATGCACCACAAGG–3' rp:5'–CCATTTCTCTTTTGCCTGCCATC–3'	360 [26]



Colony PCR, Single and multiplex

Amplification reactions were performed in a reaction volume of 25µl. The DNA template of 2µl of bacterial suspension was added to a master mix containing 2.5 µL of 10× PCR buffer, 0.5mM MgCl₂, 250 Mm dNTPs, 1 µM of each primer and 0.5 µL Dream Taq DNA polymerase. The thermo cycling conditions for all the PCRs were as follows: 94 °C for 5 min for initial denaturation and 30 cycles of 95 °C for 30 s (denaturation), 52 °C for 1 min and 72 °C for 1 min 30 s (extension), followed by 72 °C for 10 min (final extension) and all the PCRs were performed in 0.2ml Eppendorf tubes in the MJ Research PTC-200 Thermal Cycler.

Amplified PCR products were separated and visualised by 100µL of 1% agarose gel electrophoresis in the final mixture Tris-acetate-EDTA buffer containing 0.5 mg EtBr staining. To determine molecular weight, 100–1,000 bp DNA ladder (Fermentas) was used.

Antibiotic Susceptibility Testing

Antimicrobial resistance patterns of *E. coli* pathotypes were done using the disk diffusion technique of Kirby-Bauer determination of the antimicrobial resistance profile; the isolated colonies from overnight growth on nutrient agar medium were emulsified in 3ml of sterile distilled water to achieve the correct turbidity that matched that of 0.5 McFarland standards and was used within 15 minutes. The drug susceptibility was done against the following Antibiotics: chloramphenicol (30 µg), ampicillin 10µg, erythromycin 15µg, gentamicin 10µg, ciprofloxacin 5µg, tetracycline 30µg, Trimethoprim/Sulfamethoxazole 25 µg, Cefotaxime 30 µg, furazolidine 6µg and nalidixic acid 30 µg. The zones' inhibition was interpreted using CLS standards guidelines [29] using *E. coli* (ATCC 25922) as a quality control strain for drug susceptibility testing.

Statistical analysis

With a prevalence of STEC of 24.1% [12], level of significance of 0.05, a sample size of at least 277 participants was adequate for the study [30]. Prevalence of STEC was computed as proportion of all the study participants, its associated virulence genes and antimicrobial resistance were computed as proportion of participants with an isolate with 95% binomial exact confidence intervals. Age of the participants was grouped into 3 categories; <5 (pre-school going age), 5–18 (school going age and adolescents) and ≥18 years (adults) as is commonly stratified.

Ethical considerations

This study was approved by KEMRI ethical review committee (KEMRI/SERU/CMR/P0016/3094) and also by the University Board. All study participants gave written consent and assent before inclusion.

Results

Demographic characteristics of the participants

Participants age range between 1–804 months and (medial=120, IQR=36–312) months with majority (39%; n=115/295) being children < 5 years (pre-school going age), 18% were aged between 5–18 years (school going age and adolescents) and 43% of the participants were above 18 years (adults) respectively. One hundred and sixty (54%) of all the participants were female. Distribution of participants in gender groups was fairly random (Table 1).



Table 1. Distribution of *E. coli* pathotypes based on Gender and Age

No. Of participants N=295 (%)	Any <i>E. coli</i> Pathotypes (N=56)	STEC Pathotypes (n=20)
Gender		
Male	24 (43)	8 (40)
Female	32 (57)	12 (60)
p-value	0.62	0.68
Age		
<5years	24 (43)	9 (45)
5 to 18 years	11 (20)	3 (15)
≥ 18years	21 (38)	8 (40)
p-value	0.70	0.84

STEC– Shigatoxigenic *Escherichia coli*

Prevalence of Pathogenic *E. coli*.

The overall prevalence of Pathogenic *E. coli* was 19% (n=56; 95% CI=15 – 24%). From a total of 20 *E. coli* isolates STEC was identified as follows; 16 STEC only, 3 STEC/Eagg and 1 STEC/ETEC combination. The prevalence of only STEC, STEC/Eagg and STEC/ETEC infections were 5.4% (95% CI= 3.1–8.7%), 1.3%, (95%

CI= 0.2 – 2.9%) and 0.3%, (95% CI=0.006 – 1.9%) respectively (*Figure 1*).

The overall distribution of the Pathogenic *E. coli* did not differ by gender (P=0.62) or age (0.34) groups similar to the distribution of STEC Pathotypes (p=0.59 and P= 0.68 respectively).

Figure.1 Prevalence of various *E. coli* pathotypes isolated from diarrheal patients.

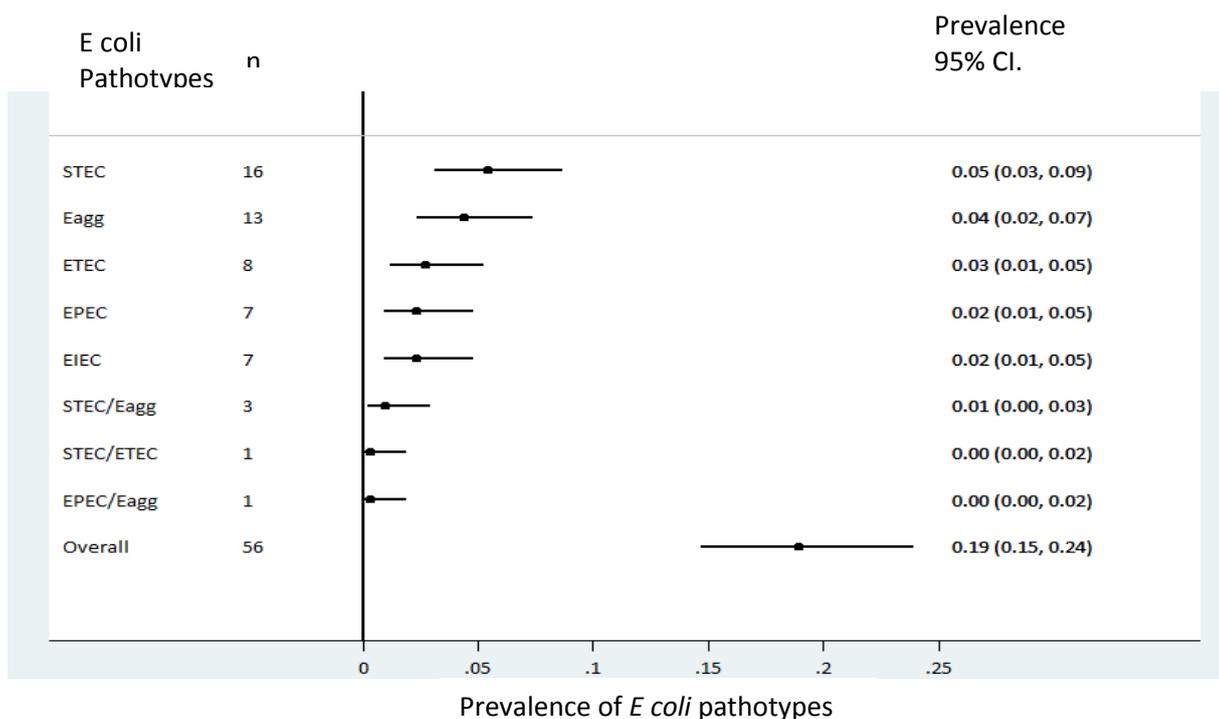




Table legend figure 1.

Lower limit Prevalence Upper limit

At 95% Confidence Interval.

Distribution of virulence genes indifferent *E.coli* pathotypes

Virulence genes were distributed among 56 isolates of pathogenic *E.coli*, identified in various isolates as follows; Thirty four isolates (12) had one, 6 (2.0) two 12 (4.1) three, 3 (1.0) four and 1 (0.3) five virulence genes respectively. *Stx₂* gene was the most common gene at 19% (95 CI= 12–28%) while *Lt* was the least common gene observed at 8.1% 95% CI=3.6–15).

Of the 20 STEC isolates, a total of 55 virulence genes were identified and different virulence genes combinations of STEC were as follows; *Stx₁/Stx₂/hlyA* (n=7), was the most common combination followed by *Stx₁/Stx₂/ hlyA/ eaeA* (n=3). *Stx₁/ Stx₂/ eaeA*, *Stx₂/ hlyA*, *Stx₂/ eaeA* and *Stx₂/hlyA/ eaeA* combinations were each observed in 2 isolates, Only *Stx₁* or *Stx₂* were each observed in one isolates while *Stx₁/ hlyA*, *Stx₁/ eaeA*, *Stx₁/ hlyA/ eaeA* and *Stx₁/ Stx₂* combinations were not observed in any isolates (Table 2).

Table 2: Shiga-toxin producing *Escherichia coli* isolate virulence gene profiles

Virulence genes combinations observed	No. Of STEC isolates N=20 (%)
<i>Stx₁</i>	1 (5)
<i>Stx₁/ eaeA</i> *	0
<i>Stx₁/ hlyA</i> *	0
<i>Stx₁/ hlyA / eaeA</i>	0
<i>Stx₂</i>	1 (5)
<i>Stx₂/ eaeA</i>	2 (10)
<i>Stx₂/ hlyA</i>	2 (10)
<i>Stx₂/ hlyA / eaeA</i>	2 (10)
<i>Stx₁/ Stx₂</i>	0
<i>Stx₁/ Stx₂/ eaeA</i>	2 (10)
<i>Stx₁/Stx₂/hlyA</i>	7 (35)
<i>Stx₁/Stx₂/ hlyA / eaeA</i>	3 (15)

*The detection of these virulence factors was carried out only in samples positive for *Stx₁*, *Stx₂* or both *Stx₁* and *Stx₂*

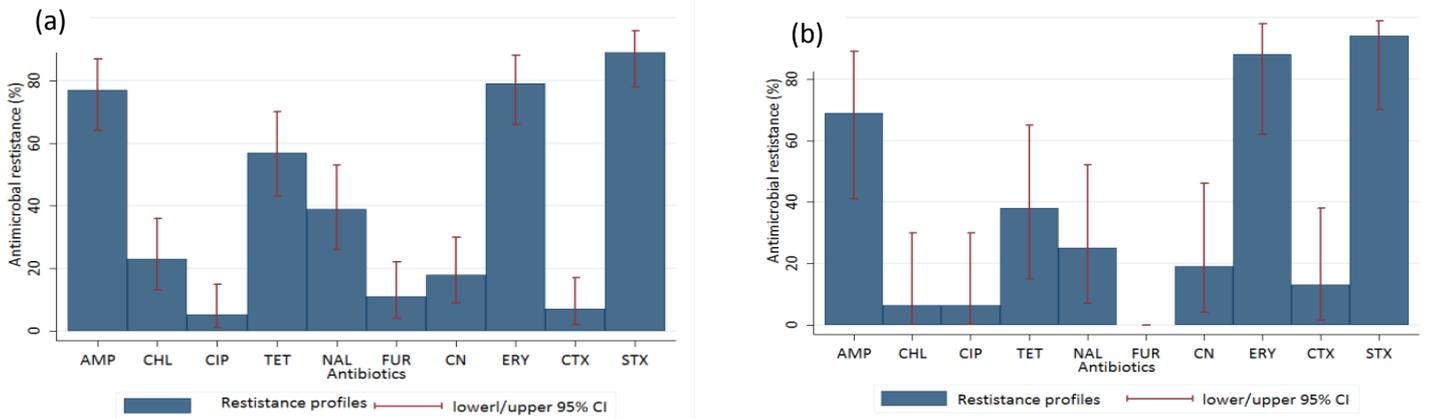


Antimicrobial resistance patterns of pathogenic *E.coli* isolates

A total of 56 isolates were tested for antimicrobial resistance against 10 drugs and analysis showed that pathogenic *E.coli* had highest resistance to STX at 89% of over 75% to Erythromycin (ERY) and trimethoprim/Sulphurmethoxazole (STX). Among these isolates only 1/56 (1.8%, 95% CI 0.1 to 9.6%) was susceptible and the remaining 55 isolates showed resistance to all the 10 antibiotics tested. Of these

36/55 (65%, 95% CI= 51 to 78%) isolates were resistant to at least one drug, 19/55 isolates had resistance to <5 drugs. with one isolate showing high levels of resistance to 9 drugs tested. Among the STEC isolates, most resistance was observed to Trimethoprim/ Sulphurmethoxazole antibiotics 15/16 (94%, 95% CI 70 to 99%) while none showed resistance to Furazolidine (Figure 2b). Overall, trimethoprim/ Sulphurmethoxazole was the most resistance drug 50/56 (89%, 95% CI 78 to 96%) while Ciprofloxacin showed the least resistance 3/56 (5.3%, 95% CI 1.1 to 15%) (Figure2a)

Figure 2: Antimicrobial resistance patterns to commonly prescribed antibiotics (a) Overall resistance in all *E coli* pathotypes isolated (b) Over resistance in STEC isolates.



***KEY:** (AMP–Ampicillin, CHL–Chloramphenicol, CIP–Ciprofloxacin, TET–Tetracycline, NAL Nalidixic acid, FUR– Furazolidine, CN– Gentamicin, ERY– Erythromycin, TX–Cefotaxime, STX– Trimethoprim/Sulphurmethoxazole)

Discussion

The present study shows the prevalence associated virulence genes and antimicrobial resistance patterns of STEC from patients of all ages seeking treatment for diarrheal illness, in Trans–Nzoia County, Kenya. Virulence factors associated with *E. coli* pathotypes in the County have also been highlighted.

In the 295 participants, the overall prevalence of Pathogenic *E.coli* was 19% (n=56; 95% CI=15–24%). STEC was the most frequently (5.4%) identified potential *E. coli* pathogen and a major cause of acute illness and cases of HUS as compared to other pathogens of a food–borne transmission component. EAEC at 4% (n=13) was the second most prevalent cause of diarrhoea followed by ETEC at 2.7% (n=8) while EPEC and EIEC both at (2.4%) were the least prevalent *E. coli* pathotypes in our study (figure 1). Although STEC outcome range from mild intestinal discomfort, to haemolytic Uremic syndrome (HUS), end–stage renal disease and death [31, 32] although the



reported incidences is often low, larger outbreaks with serious consequences occur [33]. These are severe sequelae that must be accounted for when prioritizing food borne pathogens for control efforts. As expected we observed a relatively higher STEC prevalence than those which have been reported in many developed countries including Switzerland 1% [34], Germany 1% [35], Belgium 0.2% [36] and 3% [37] France, and urban parts of Kenya such as Nairobi where a prevalence of 0.97% [38] and 0.8% [11] have been reported. Kitale is relatively rural area and the inhabitants mainly obtain most of its water from River Nzoia, which crosses densely populated farmlands of Trans Nzoia possibly carrying along large amounts of agricultural and domestic waste, which is presumably checked by the treatment process at Nzoia Water treatment plant also rely on untreated water from boreholes, springs wells and river for domestic use, bacterial contamination in Kitale is 77.3% [39]. However the STEC prevalence in in this study was lower than reports from Maasai land; where STEC prevalence of up to 24.1% has been reported [12]. The Maasai community are pastoralists famous for consumption of raw meat and milk whereas STEC strains have been recovered from a variety of animals, and cattle are considered the major reservoir for STEC strains [20, 21]. Recent evidence has indicated that small domestic ruminants are also relevant STEC reservoirs [22, 23].

Though there was no significant difference in distribution of STEC by age ($P=0.34$), the prevalence was highest (39%) in children < 5years (table 1). STEC has been reported to be more frequent and its impact more severe in young children from developed countries [40–43]. In less developed countries where childhood diarrhoeal diseases are more endemic, studies typically focus solely on children but have also identified STEC as a pathogen of concern in this age group [44–47], although

our study cuts across all age groups majority of the study participants being children. The overall distribution of the Pathogenic *E.coli* did not differ by gender ($P=0.62$) or age groups ($p= 0.34$) similar to the distribution of STEC Pathotypes ($p=0.59$ and $P= 0.68$) this shows that both age and gender are not risk factors for *E.coli* infection in Kitale.

Studies from different geographical areas show different pattern of *E coli* pathotypes. In Kenya Sang reported EAEC (8.9%), as the most frequent followed by ETEC (1.2%) and EIEC (0.6%) among the Maasai community [12] while among the Maasai community ETEC (29.8%) was the most prevalent followed by STEC (24%), EAEC (14.2%) then EPEC (3.5%) [1]. From an urban community in Nairobi, [38] identified EPEC (19.3%) as the most prevalent followed by ETEC (7.25%); EAEC (3.86%) and STEC (0.97%) while [38] observed EAEC as the most frequent *E coli* pathotype (36.6%) in diarrhoeagenic *Escherichia coli* isolates from children < 5 years from Kenya and Japan. In Tanzania country, EAEC (64.1%) was the most detected followed by EPEC (20.3%) then ETEC (15.6%) [48]. These differences in prevalence of *E coli* pathotypes in different geographical areas could be explained by the different social behavioural, life styles and cultural practises in the communities. For instance the Maasai community in the savannah of Narok where major national parks: Maasai Mara and Amboseli are located these communities in savannah areas practice open field defecation and also share same water points with their domestic animals and wild animals. [12, 49] they all observed EAEC as the most prevalent *E.coli* pathotype. Multiple infections with STEC/EAEC and STEC/ETEC were observed in 3 and 1 isolates respectively. This was quite unique for our isolates as no study in Kenya has reported multiple infections of STEC and other *E.coli* pathotypes in diarrhoea isolates. The varying



combination of multiple virulence genes harboured by the STEC/ EAEC is a rarer pathotypes that harbours the phage-mediated Shiga toxin determinant with an Enteroaggregative gene while the STEC/EPEC isolates showed that the strains were a mixture of several different *E. coli* pathogroup-associated properties. In addition, these strains (STEC/EAEC and STEC/EPEC) harboured other virulence genes, some of which have been associated with EPEC, STEC, and EAEC.

In this study, the most observed virulence genes in different *E. coli* pathotypes was *Stx*₂ gene at 19% while *Lt* gene was the least common gene at 8.1%. Among the 20 STEC isolates, *stx*₂ gene was observed most at 6.4% and the most common virulence genes combination observed was *Stx*₁/*Stx*₂/*hlyA* at 35%.3 (15%) of the isolates were positive for all the four virulence genes (*stx*₁+*stx*₂+*hlyA*+*eaeA*) that are responsible for STEC infection. An accessory genes (*eaeA* n=9 and *hlyA* n=14) were detected only in isolates positive for *Stx*₁, *Stx*₂ or both *Stx*₁ and *Stx*₂ (table 2). These finding concur with other studies done elsewhere. Epidemiological studies have revealed that *stx*₂ is more associated with severe human disease (HC and HUS) than *stx*₁ [50, 5].

However other studies observe a slight difference in gene combination. In Kenya, in 2012 Sang observed *stx*₁+*stx*₂ and *stx*₁ as the most prevalent, while [51] in India i an overall uneven distribution of all the three virulence genes in the STEC strains isolated from food samples, indicating the most isolated genes as *stx*₁ and *stx*₁+*stx*₂ combination. Virulence genes make *E. coli* pathogens capable of causing disease and Shigatoxin-producing *E. coli* (STEC or EHEC) with shiga-like toxin production by the *Stx*₁, *Stx*₂ and *eaeA*, genes are considered the most virulent [52, 53]. Worldwide epidemiological studies show that not all the strains producing Shigatoxin are pathogenic; it is believed that

accessory genes may be responsible for or contribute to human pathogenic state [54–5]).

Although Shigatoxin production is essential but not sufficient for STEC virulence, dual existence of multiple virulence genes in combination viz-a-viz single gene in this study raises questions as to whether or not these genes act in synergy to produce to induce acute disease. In this study most isolates exhibited multiple virulent gene combination demonstrating that STEC is among significant causes of gastroenteritis in the region. Enterohaemolysin liberates hemoglobin from the RBCs and is linked to the severity of the disease. It is widely accepted that *E. coli* strains harboring both *stx* and *hlyA* may pose a higher risk to human health [5].

The study also investigated the prevalence and Antimicrobial resistance profiles of pathogenic *E. coli* and the results identified an overall resistance of over 75% to commonly prescribed drugs (AMP), (ERY) and (STX). A total of 36/55 (65%), isolates showed resistance to at least one of the commonly prescribed antibiotics, 19/55 isolates had resistance to <5 drugs and only one isolate showed highest resistance to all the 9 commonly prescribed antibiotics in the country tested in this study (figure 3a). Overall, Trimethoprim/ Sulphamethoxazole was the most resistant drug 50/56 (89%, 95% CI 78 to 96%) while Ciprofloxacin was least resistant drug 3/56 (5.3%, 95% CI 1.1 to 15%). Most studies have confirmed emergence of antibiotics resistance in Bacterial organisms. A study on commensal gut flora from children in Sudan found that 39% of children had strains resistant to six antimicrobial agents and over 70% of the children had strains resistant to at least 4 of 6 antimicrobial agents commonly prescribed in the country [58]. Multiple antimicrobial resistances among strains of pathogenic *E. coli* have also been reported in Kenya [48, 59, 60]. This is most likely to have been attributed by increased in the widespread use of



antimicrobial agents diminishes the efficacy of affordable and available drugs [61]. The emergence and spread of antimicrobial resistance in bacteria of medical importance imposes serious constraints on the options available for treatment of many infections.

Among the STEC isolates, Trimethoprim/Sulphamethoxazole was the most resistant drug 15/16 (94%, 95% CI 70 to 99%) however, none of the STEC isolates showed resistant to Furazolidine (figure 3b). Generally, the resistance rates observed in this study were much lower for Ampicillin, Tetracycline and Chloramphenicol. Although the results from this study indicated that all isolates were susceptible to furazolidine while Cefotaxime and ciprofloxacin had low resistance levels, chromosomal resistance to these agents could be easily transferred. Although previous studies showed moderate activity against STEC *E. coli* strains for Tetracycline and Chloramphenicol [59]. This study points out a rising prevalence of antimicrobial resistance among pathogenic *E. coli* isolates from Trans Nzoia County. Emerging resistance to third generation cephalosporin's (Gentamicin at 18% and ciprofloxacin at 5.4%) and quinolones (nalidixic acid at 39%) was observed in this study highlighting the significance of antimicrobial resistance. This rapid emergence of antimicrobial resistance could be attributable to change in life style, introduction of new activities in the region, climatic changes and increased human traffic.

Conclusion and recommendation

Although STEC infection has not attracted much attention as a medical problem in Kenya, this study finding demonstrates the presence of the STEC at the rate of 5.4% in Kitale. The most common Virulence genes combination associated with the isolates was *Stx₁/Stx₂/hlyA* and *Stx₁/Stx₂/hlyA/eaEA* the combinations responsible for enterohaemorrhagic *E. coli*

infections. High antimicrobial resistance of up to 75% to commonly prescribed antibiotics including emerging resistance to 3rd generation cephalosporin's and quinolones were observed.

We highlight STEC infection not only an existing problem in Kitale but also an escalated problem considering the high/emerging antimicrobial and resistance observed. These necessitate further and immediate investigation to identify reservoirs and potential sources of STEC in order to establish control and prevention strategies for STEC associated diseases in humans. Early recognition of the problem, as well as appropriate treatment and improvement of hygiene may be beneficial in increasing survival rates.

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