Characterization and Antimicrobial Susceptibility Pattern to Commonly Prescribed Antimicrobials of Diarrheagenic *Escherichia coli* in Patients attending Thika District Hospital – Kenya, 2014.

Kanyina E[1, 2, 3, 4*], Sang W[5], Kiiyukia C[4], Tonui J[1], Boru W[1, 3], Galgalo T[2, 6]

1. Ministry of Health,Nairobi, Kenya.
2. East Africa Public Health Laboratory Networking Project, Ministry of Health.
3. Field Epidemiology and Laboratory Training Program.
4. Institute of Tropical Medicine, Jomo Kenyatta University of Agriculture and Technology, College of Health Science.
5. Center for Microbiology Research Laboratory, Kenya Medical Research Institute, Nairobi, Kenya.
6. African Field Epidemiology Network, Kampala, Uganda

Corresponding Author: Evalyne Kanyina. E-mail: jkanyina@yahoo.com, Tel: +254 723 367 205

Summary

Background: Diarrheagenic *E. coli* (DEC) are associated with outbreaks of severe diarrhea and multiple drug resistance. We characterize DEC among diarrhoeal patients attending Thika Hospital and determine their antimicrobial susceptibility patterns.

Methods: A cross-sectional study was conducted among patients of all ages seeking diarrhea treatment. Stool samples were collected, inoculated on bacterial differential media for growth of enteric pathogens, characterized and antimicrobial susceptibility of DEC isolates determined.

Results: A total of 402 stool samples were cultured. *E. coli* was isolated from 269, of which 72 (27%) were DEC; 60 (83.3%) enteroaggregative *E. coli* (EAEC), 6 (8.3%) enteropathogenic *E. coli* (EPEC) and 6 (8.3%) enterotoxigenic *E. coli* (ETEC). Of the 72, 58% were female, median age was 8 (IQR: 2-28) years, 75% did not boil water and 100% did not treat water. Twenty five (35%) patients with DEC were under-five years. Drinking un-boiled water (OR: 2.51, 95% CI: 1.36-4.61) was associated with having DEC. All DEC isolates were sensitive to cefoxitin, meropenem, amikacin, gentamicin and ciprofloxacin. They were resistance to ampicillin (92%), trimethoprim-sulfamethoxazole (92%) and amoxicillin-clavulanic acid (85%).

Conclusion: The predominant DEC strain was EAEC. High resistant to ampicillin, trimethoprim-sulfamethoxazole and amoxicillin-clavulanic acid were observed. All isolates were sensitive to ciprofloxacin and gentamicin.

Key words: Diarrhea, *E. coli*, Diarrheagenic *E. coli*, Characterization, Kenya

[Afr J Health Sci. 2016; 29(1):25-35]

Introduction

Escherichia coli infection is transmitted through consumption of contaminated water or food [1]. Six categories of Diarrheagenic *Escherichia coli* (DEC) have been described: enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); enteroinvasive *E. coli*

(EIEC); enterohemorrhagic *E. coli* (EHEC), also known as shigatoxigenic *E. coli* (STEC); diffusely adherent *E. coli* (DAEC); and enteroaggregative *E. coli* (EAEC) [2]. *DEC* have been found to be responsible for outbreaks of severe diarrhea within Africa [3, 4]. In low income countries, D*EC* accounts for a third of all the diarrheal episodes among children under-five years [5, 6]. In Kenya, multiple antimicrobial resistance among strains of DEC have been documented [7, 8, 9, 10]. The distribution of strains of DEC, the emergence of new virulent enteric pathogen and the emerging antibiotic resistant threatens the effectiveness of successful treatment and management of diarrheal infections. These poses an emerging public health threat with local, national, and global dimensions which if not investigated and intervention initiated, can result to increased morbidity, disease burden, and mortality.

Baseline data from the rapid assessment of antimicrobial resistance in selected public health and clinical laboratories done in March 2013 reported that Thika District Hospital laboratory had the highest *E. coli* isolates from stool culture [11]. No further characterization was done to this isolates due to limited laboratory capacity. Our study set out to isolate, identify and characterize DEC among diarrhoeal patients in Thika District Hospital and determine their antimicrobial susceptibility pattern.

Materials and methods

A hospital based cross-sectional study was conducted in Thika District Hospital located in Thika town, Kiambu County, Kenya. The hospital catchment area covers both urban and rural population. To calculate the sample size, we used a prevalence of 50% estimated by Fisher and Van Belle (2004) as no previous study had been done in the area.

Study participants were consenting individuals of all ages from both in and out patient departments seeking treatment for diarrhoea at the hospital from April to July 2014 who had not taken antibiotics within 72 hours of diarrhea onset. Diarrhoea was defined as at least three loose stools in 24 hours, or any number of watery stools. Non-consenting patients and those who had taken antibiotics within 72 hours of diarrhea onset were excluded in the study. All diarrhea patients meeting the case definition were enrolled until the sample size was attained.

Data collection

Interviews were conducted using a structured questionnaire to collect clinical and epidemiologic information.

Laboratory Methods

All fecal samples were collected in a sterile stool container, inoculated overnight into Selenite F. broth at 37 ^{0}C, plated onto Xylose-Lysine-deoxycholate (XLD) media for isolation of *Salmonella* and *Shigella spp* at 37^{o}C for 18–24 hours. The samples were also directly plated onto Thiosulfate Citrate Bile salts Sucrose (TCBS) media and MacConkey media and incubated aerobically at 37^{o}C for 18 – 24 hours for the isolation of *Vibrio cholera* and *E. coli,* respectively. After overnight growth, five to ten single colonies with typical *E. coli* morphology were selected and characterized on the basis of their biochemical reactions. These colonies were further tested using the VITEK© 2 Compact, identification card for Gram-Negative Bacilli (ID-GNB cards) (bio-Me´rieux, Marcy L'Etoile, France), automated microbiology analyzer for identification of *E. coli* [12] with *E. coli* ATCC 25922 strain being used as the test standard. DEC nucleic acid extraction was

done as guided by Qiacube® automated nucleic acid extraction system [13].

E. coli isolates screening for detection of virulence genes was conducted using multiplex RT Polymerase Chain Reaction (PCR). The PCR targets were: ETEC *elt* (heat labile enterotoxins) and *est* (heat stable enterotoxin), EHEC *stx* (Shiga toxins), EAEC *aggR* (activator aggregative adherence regulator) and *aspU (EAEC*-secreated protein U gene), EIEC *ipaH* (invasion plasmid antigen) and EPEC *eae* (intimin) (Table 1) [14-20]. The reaction was carried out in 0.2 ml thin-walled PCR tubes using a 25 ul reaction mixture containing: puReTaq™ Ready-To-Go™ Polymerase Chain Reaction (PCR) Beads (GE Healthcare, Buckinghamshire, HP7 9NA UK), 20 ul of double distilled water, 2 ul of extracted *E. coli* DNA templates and 3 ul of primers mixer (Table 1). PCR cycle parameters were: initial denaturation at 95°C for 5 minutes, followed by 30 cycles of 95°C for 30 seconds, 54°C for 45 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 7 minutes. Positive controls containing targeted virulence genes; EAEC strain 17-2, ETEC ATCC 35401 (*eltB*, *estA*), EHEC ATCC 43890 (*vt1*, *eaeA*), EHEC ATCC 43889 (*vt2*, *eaeA*), EPEC ATCC 43887 (*eaeA*, *bfpA*), EIEC ATCC 43893 (*ial*) and negative control without virulence genes were used in every amplification round. The PCR products were electrophoresed on 2% L03 agarose (TaKaRa Bio Inc.) with ethidium bromide and visualized on an ultraviolet trans-illuminator, the Gel Doc™ EZ Imager (BioRad Laboratories).

Table 1: Sequences for Diarrheagenic E. coli PCR primers and their product size

	Sequence (5' to 3')	Target Gene	Amplicon size (bp)	reference
EPEC	CCCGAATTCGGCACAAGCATAAGC CCCGGATCCGTCTCGCCAGTATTCG	*eae*	881	Oswald *et al.,* 2000
EHEC	GAGCGAAATAATTTATATGTG TGATGATGGCAATTCAGTAT	*stx*	518	Yamasaki *et al.,* 1996
ETEC	TTAATAGCACCCGGTACAAGCAGG CCTGACTCTTCAAAAGAGAAAATTAC	*est*	147	Hornes *et al.,* 1991
ETEC	TCTCTATGTGCATACGGAGC CCATACTGATTGCCGCAAT	*elt*	322	Tamanai *et al.,* 1994
EIEC	GTTCCTTGACCGCCTTTCCGATACCGTC GCCGGTCAGCCACCCTCTGAGAGTAC	*ipaH*	619	Sethabutr *et al.,* 1993
EAEC	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	*aggR*	254	Ratchtrachenchai *et al.,* 1997
EAEC	GCCTTTGCGGGTGGTAGCGG AACCCATTCGGTTAGAGCAC	*aspU*	282	Claudia *et al.,* 2003

Antimicrobial susceptibility pattern for diarrheagenic *E. coli* were determined using Gram-Negative VITEK 2 compact card (bio-Me´rieux, Marcy L'Etoile, France) whose breaking point are guided by Clinical and Laboratory Standards Institute guidelines [21]. The following antibacterial agents: ampicillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, piperacillin/tazobactam, cefazolin, cefuroxime, cefuroxime axetil, cefoxitin, cefotaxime, ceftazidime, ceftriazone, cefepime, aztreonam, meropenem, amikacin, gentamicin, ciprofloxacin, nitrofurantoin and trimethoprim/sulfamethoxazole were used.

Data analysis

Data generated on clinical and epidemiologic information as well as laboratory analysis was entered in Microsoft Excel 2010 and analyzed using Epi info version 3.5.1. Univariate analyses were determined for both the dependent and independent variables. Antimicrobial susceptibility frequencies were also determined. The association between socio-demographic characteristics of diarrhea patients infected with DEC and diarrhea patients not infected with DEC was determined using chi-square, Fisher exact test and logistic regression.

Ethical approval

The protocol was approved by Kenyatta National Hospital and Thika District Hospital research committees before the commencement of the study. All study participants above 18 years gave an informed consent before participation. Consent for participants below 18 years was given by their guardians. The participant' data was stored properly and accessed by only authorized persons through a password to avoid a breach in confidentiality.

Results

During the study period, 402 participants were enrolled; 222 (55%) female with a median age of 14 (IQR: 3-31) years. One hundred and thirty three (33%) samples were from children under-five years and 269 (67%) were from person aged above-five years. Of the total 402 stool cultures performed, 101 (25%) obtained no pathogenic growth. A total of 269 (89.4%) *E. coli*, 13 (4.3%) *Pseudomonas spp*, 12 (4.0%) *Citrobacter spp*, 6 (2.0%) *Klebsiella spp* and 1 (0.3%) *Salmonella spp* were isolated. Of the *E. coli* isolates, 72 (27%) were DEC; 60 (83.3%) EAEC, 6 (8.3%) EPEC and 6 (8.3%) ETEC. *Entamoeba histolytica* was identified in 12 (17%) patients with DEC; 6 (50%) with EAEC and 6 (50%) with EPEC. Of the 60 EAEC strains, 24 (40%) EAEC strains were positive for both aggR and aspU genes, while 36 (60%) were positive for *aspU* gene only. All EPEC and ETEC strains were positive for their target genes, EPEC *eae* (100%, n=6) and ETEC *elt* (100%, n=6).

Of the 72 patients with DEC infection, 42 (58%) were female with a median age of 8 (IQR: 2-28) years. Twenty five (35%) patients with DEC infection were under-five years, 54 (75%) had secondary education, 39 (54%) had water piped inside their houses, 48 (67%) stored water in jerry can containers, 54 (75%) did not boil water, 72 (100%) did not treat water and 48 (67%) shared toilets (Table 2). The interval between onset of symptoms and collection of specimens was 1-3 days for all patients where ETEC and EPEC were isolated.

However, longer duration were presented by patients infected with EAEC; 48 (80%) patients presenting within 1–3 days, 6 (10%) within 4–6 days and 6 (10%) after 6 or more days.

Table 2: Socio-demographic characteristics of patients with Diarrheagenic *E. coli*

Variable	Participants	N*= 72 (%)
Age	< 5 years	25 (34.7)
	> 5 years	47 (65.3)
Gender	Male	30 (41.7)
	Female	42 (58.3)
Level of education	No formal education	0 (0)
	Primary	12 (16.7)
	Secondary	54 (75)
	Post-secondary	6 (8.3)
Source of drinking water	Piped to house	39 (54.2)
	Borehole	19 (26.4)
	Piped to common point outside the house	14 (19.4)
Water storage container	Jerry can	48 (66.7)
	Bucket	18 (25)
	Tank	6 (8.3)
Boil water	No	54 (75)
	Yes	18 (25)
Treat water	No	72 (100)
	Yes	00 (00)
Toilet description	Serving a plot	48 (66.7)
	Built into the house	18 (25)
	Pit latrine	6 (8.3)

* N = Number of diarrhea patients with DEC

The odds of DEC infection was one time higher among the under-five years (OR: 1.13, 95% CI: 0.64-2.00), female (OR: 1.23, 95% CI: 0.71-2.12), participants drinking water piped into a common point (OR: 1.08, 95% CI: 0.71-2.12) and those who stored water in buckets (OR: 1.27, 95% CI: 0.67-2.39), jerry can (OR: 1.01, 95% CI: 0.57-1.79) and tanks (OR: 1.03, 95% CI: 0.39-2.74) as well as those who used communal toilets (OR: 1.01, 95% CI: 0.57-1.79) but the association in this study was not significant at the 95% level of confidence. No significant association with DEC infection was found with age, gender, water storage container and

toilet description on the study participants. Drinking borehole (OR: 2.26, 95% CI: 1.16–4.38), un–treated (P<0.05) and un–boiled (OR: 2.58, 95% CI: 1.41 – 4.70) water was associated with DEC infection (Table 3). After adjusting for age, sex, level of education, type of water storage container and type of toilets, drinking un–boiled water (OR: 2.51, 95% CI: 1.36–4.61) and lack of post–secondary education (OR: 3.32, 95% CI: 1.34–8.22) were independently associated with DEC infection (Table 4).

Table 3: Factors associated with Diarrheagenic *E. coli* among study participants

Socio demographic characteristics	Odds Ratio (OR)	Confidence Interval (CI)	Two–tailed P–value at 95% CI	Test
Education Level				
No post–secondary	3.45	1.40 – 8.46	0.0078	Chi squared
Source of drinking water				
Borehole	2.26	1.16 – 4.38	0.0236	Chi squared
Boiling water				
Drinking un–boiled water	2.58	1.41 – 4.70	0.0028	Chi squared
Treating water				
Drinking un–treated water			0.0003	Fishers exact

Table 4: Adjusted relationship between factors associated with Diarrheagenic *E. coli* among study participants

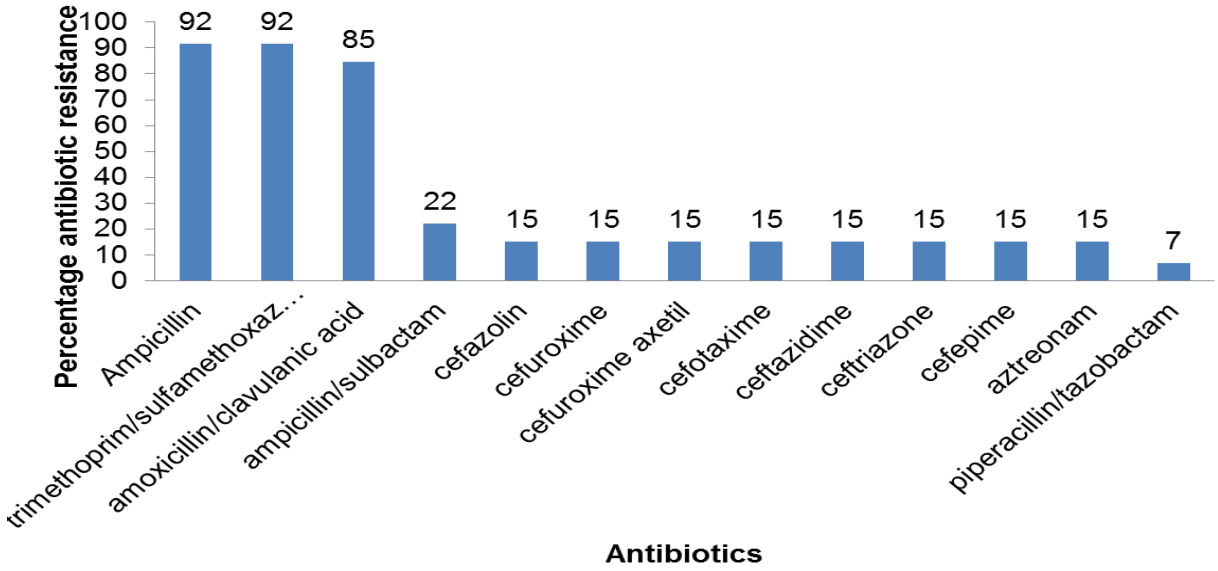
Variable	Odds Ratio	95% C.I*	Coefficient	S.E**	Z-Statistic	P-Value
Drinking un-boiling water	2.51	1.36-4.61	0.92	0.31	2.953	0.0032
No post-secondary	3.32	1.34-8.22	1.20	0.46	2.593	0.0095

*95 % C.I = Confidence Interval **SE = Standard Error

All the DEC isolates were sensitive to cefoxitin, meropenem, amikacin, gentamicin and ciprofloxacin. High resistance was observed in ampicillin (92%), trimethoprim /sulfamethoxazole (92%) and amoxicillin/clavulanic acid (85%). However, low resistance trends were observed among other antimicrobials; piperacillin/tazobactam (7%), cefuroxime, cefuroxime axetil, cefotaxime, ceftazidime, ceftriazone, cefepime and aztreonam (15%), cefazolin (22%) and nitrofurantoin (24%) (Figure 1). The predominant resistance profile was ampicillin and co–

trimoxazole (63%). This was followed by ampicillin- co-trimoxazole-amoxicillin/clavulanic acid and ampicillin/sulbactam both at 14%.

Figure 1: Antimicrobial resistance patterns of diarrheagenic *E. coli* isolates from diarrhea patients in Thika Level 5 Hospital, April to July 2014



Discussion

This study documents DEC infection among patient seeking diarrhea treatment in Thika District Hospital between April to July 2014. Three classes of DEC were isolated. High resistance was observed among commonly prescribed antimicrobials; ampicillin, trimethoprim /sulfamethoxazole and amoxicillin/clavulanic acid. All DEC isolates were sensitive to aminoglycosides and quinolones.

All ETEC and EPEC infected persons were over-five years in our study. This is inconsistent with other studies where ETEC has been largely associated ETEC with childhood and traveler's diarrhea while EPEC has been associated with watery diarrhea of infants [22, 10]. The predominant bacterial cause of diarrhea was EAEC in our study. These results differ with Sang *et al* [10] findings where EPEC was reported as the common bacterial pathogen in diarrhea in four provinces in Kenya. This could be attributed to the effectiveness of introduction of zinc supplementation plus oral rehydration salts to improve diarrhea management in children [23] as well as the advocacy of 0-6 months exclusive breastfeeding which have been reported to decreases morbidity from gastrointestinal diseases [24].

The interval between onset of symptoms and collection of specimens was 1-3 days for all patients where ETEC and EPEC were isolated. This is in agreement with other studies where ETEC and EPEC infections have been

reported to have an abrupt onset with a short incubation period; 14 to 50 hours for ETEC and 2.9 hours for EPEC [25, 2]. This leads to rapid search for medical intervention.

Almost a half (41%) of EAEC isolates were from children under-five years. Longer duration between onset of symptoms and collection of specimens were presented among patients suffering from EAEC infection; 48 (80%) patients presenting within 1-3 days, 6 (10%) within 4-6 days and 6 (10%) after 6 or more days. This is in agreement with other studies where EAEC has been associated with acute and persistent diarrhea in children and adults [22, 10].

High prevalence of resistance to commonly used antimicrobials such as ampicillin and trimethoprim /sulfamethoxazole was observed (Figure 1). This is consistent with other studies in different parts of Kenya which have reported high ampicillin and trimethoprim /sulfamethoxazole resistance among enteric bacterial pathogens [26, 10]. However, the resistance levels observed in this study are much higher than those that have been reported from other areas in Kenya. These could be associated with increased non-laboratory evidence based prescription as well as poor adherence to these drugs which are also easily accessed over the counter without prescription in Kenya. All DEC isolates were sensitive to ciprofloxacin and gentamicin. Similar findings were reported among the Maasai community in Kenya [26]. However, the emergence of resistance among these antimicrobials to enteric pathogens has been reported across the country though at low levels [10]. These calls for actual scale up of antimicrobial resistance surveillance not only at institutional levels (e.g. referral and private hospitals) but also at regional or national levels to inform optimal selection of antimicrobial in patient management and ascertain antimicrobial resistant patterns for antimicrobial stewardship.

Limitations of the study

This study was a hospital based study. This theoretically limits representativeness and generalizability. It was a descriptive study and thus did not include a control group to determine the relationship between the risk factor and diarrhea disease. Despite this limitation, the study documents the existence of DEC among patients seeking diarrhea treatment in Thika District Hospital.

Conclusion

Molecular characterization of *E. coli* isolates is necessary to establish their pathotypes. Three DEC strains were isolated. High levels of resistant to ampicillin, trimethoprim-sulfamethoxazole and amoxicillin-clavulanic acid were observed. On the contrary, all isolates had absolute sensitivity to ciprofloxacin and gentamicin.

Acknowledgement

This work was funded by African Field Epidemiology Network (AFENET). We acknowledge Field Epidemiology and Laboratory Training Program (FELTP) Kenya for their technical advice during data collection and scientific writing. We also acknowledge the management and staff of Thika District Hospital for supporting the investigation.

References

1. World Health Organization. Enterohaemorrhagic *Escherichia coli* (EHEC). Fact sheet 2011; N°125, Reviewed December. www.who.int/mediacentre/factsheets/fs125/en/

2. Nataro JP and Kaper JB. Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews*. 1998; **11**:142–201.

3. Effler E, Isaäcson M, Arntzen L, Heenan R, Canter P, Barrett T, Lee L, Mambo C, Levine W, Zaidi A and Griffin PM. Factors contributing to the emergence of *Escherichia coli* O157 in Africa. *Emerging Infectious Diseases*. 2001; **7**: 812–819.

4. Bundi M, Ochi S, Miringu G, Huka S, Galata A, Ager V, Abubakar M, Karama M, Tsuji T and Ichinose Y. Enteroaggregative *Escherichia coli* associated with a massive outbreak of gastrointestinal illness in Mandera, Kenya. *The African Journal of Health Science*. 2013; 782.

5. Clarke SC. (2001). Diarrhoeagenic *Escherichia coli*– an emerging problem? *Diagnostic Microbiology and Infectious Disease.* 2001; **41**: 93–98.

6. O'Ryan M, Prado V and Pickering LK. A millennium update on pediatric diarrheal illness in the developing world. *Seminars in Pediatric Infectious Diseases.* 2005; **16**: 125–136.

7. Sang WK, Oundo JO, Mwituria JK, Waiyaki PG, Yoh M, Iida T and Honda T. Multidrug- resistant enteroaggregative *Escherichia coli* associated with persistent diarrhea in Kenyan children. *Emerging Infectious Diseases*. 1997; 3: 373–374.

8. Bii CC, Taguchi H, Ouko TT, Muita LW, Wamae N and Kamiya S. Detection of virulence-related genes by multiplex PCR in multidrug-resistant diarrheagenic Escherichia coli isolates from Kenya and Japan. *Epidemiology of Infections*. 2005; **133**: 627–633.

9. Brooks JT, Ochieng JB, Kumar L, Okoth G, Shapiro RL, Wells JG, Bird M, Bopp C, Chege W, Beatty ME, Chiller T, Vulule JM, Mintz E and Slutsker L. Surveillance for bacterial diarrhea and antimicrobial resistance in rural western Kenya. *Clinical Infections and Diseases.* 2006; **43**: 393–401.

10. Sang KW, Boga IH, Waiyaki GP, Schnabel D, Wamae CN and Kariuki MS. Prevalence and antibiotic resistance of bacterial pathogens isolated from childhood diarrhea in four provinces of Kenya. *Journal of Infection in Developing Countries*. 2012; **6**: 572 – 578.

11. Odhiambo F, Tura G, Arvelo W, Maina O, Kanyina E, Tonui J, Amwayi S and Waqo B. Antimicrobial resistance: capacity and practices among clinical laboratories in Kenya, 2013. *Pan African Medical Journal*. 2014; 19:332.

12. VITEK 2 Compact. Standard Operating Procedure for VITEK 2 Compact: Use, Maintenance and Quality Control Procedures. SOP Number: QC-22-00. Revised: 02-22-2006

13. Qiagen protocol. QIAamp® DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook pg. 52, 02/2003.

14. Oswald E, Schmidt H, Morabito S, Karch H, Marche`s O and Caprioli A. Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *Escherichia coli*: characterization of a new intimin variant. *Infection and Immunity.* 2000; **68**:64-71.

15. Yamasaki S, Lin Z, Shirai H, Terai A, Oku Y, Ito H, Ohmura M, Karasawa T, Tsukamoto T, Kurazono H and Takeda Y. Typing of verotoxins by DNA colony hybridization with poly- and oligonucleotide probes, a bead-enzyme-linked immunosorbent assay, and polymerase chain reaction. *Microbiology and Immunology.*1996; **40**:345-352.

16. Hornes E, Wasteson Y and Olsvik Ø. Detection of *Escherichia coli* heat-stable enterotoxin genes in pig stool specimens by an immobilized, colorimetric, nested polymerase chain reaction. *Journal of Clinical Microbiology.*1991; **29**:2375-2379.

17. Tamanai SZ and Jolivet GA. Detection of enterotoxigenic *Escherichia coli* in water by polymerase chain reaction amplification and hybridization. *Canadian Journal of Microbiology.* 1994; **40**:243-249.

18. Sethabutr O, Venkatesan M, Murphy GS, Eampokalap B, Hoge CW and Echeverria P. Detection of Shigellae and enteroinvasive *Escherichia coli* by amplification of the invasion plasmid antigen H DNA sequence in patients with dysentery. *Journal of Infectious Diseases.*1993; **167**:458-461.

19. Ratchtrachenchai OA, Subpasu S and Ito K. Investigation on enteroaggregative *Escherichia coli* infection by multiplex PCR. *Bulletin of the Department of Medical Sciences.* 1997; **39**:211-220.

20. Claudia T, Yan L, Naomi H, Noboru N, Isabel C, Ariela B, Marta R and Masaaki I. Multiplex PCR Assay for Identification of Human Diarrheagenic *Escherichia coli. Journal of Clinical Microbiology.* 2003; **41 (6)**: 2669-2671.

21. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Seventeenth Informational Supplement. CLSI document M100-S17 Vol 27 No. 1 [ISBN 1-56238-625-5] 2007.

22. Mamun KZ, Shears P and Hart CA. The prevalence and genetics of resistance to commonly used antimicrobial agents in faecal *Enterobacteriacea* from children in Bangladesh. *Epidemiology of Infections*. 1993; **110**: 447-458.

23. World Health Organization. Technical updates of the guidelines on the Integrated Management of Childhood Illness (IMCI): evidence and recommendations for further adaptation. World Health Organization press, 2005. ISBN 92 4 159348 2.

24. Bernardo LH and Cesar GV. Short-term effects of breastfeeding: a systematic review on the benefits of breastfeeding on diarrhoea and pneumonia mortality.

World Health Organization press, 2013. ISBN 978 92 4 150612 0

25. Donnenberg MS, Tacket CO, James SP, Losonsky G, Nataro JP, Wasserman SS, Kaper JB and Levine MM. Role of the eaeA gene in experimental enteropathogenic Escherichia coli infection. Journal of Clinical Investigation. 1993; **92**:1412–1417.

26. Sang WK, Kariuki SM, Schnabel D, Boga HI, Waiyaki PG and Wamae CN. Antibiotic susceptibility of Enteric pathogens from the Maasai community, Narok and Kajiado Districts, Kenya. *African Journal* of *Health Sciences*. 2011; **19**:78-83