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RESEARCH ARTICLE

Epidemiology of Antimicrobial Resistance among *Escherichia coli* Strains in Trans-Nzoia County, Kenya

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ABSTRACT

Objective: This study aimed to determine prevalence, antimicrobial susceptibility profile and the genetic basis to antimicrobial resistance, targeting bla_{TEM} gene expression of diarrheagenic *Escherichia coli* among patients suffering from gastroenteritis in Kitale County Referral Hospital.

Methods: A cross-sectional study design was adopted. A total of 103 fecal specimens were collected from participants ranging in age from two weeks to 82 years. *E. coli* was isolated and identified based on phenotypic and biochemical properties. Antimicrobial susceptibility was determined by Kirby-Bauer disk diffusion method. Polymerase chain reaction was used to detect the presence of bla_{TEM} gene.

Results: The prevalence of *E. coli* was 90.2% and age of the patient explained 53% of variation in prevalence. Isolates of diarrheagenic *E. coli* showed varied degree of susceptibility with sulfamethoxazole at 97%, co-trimoxazole 96%, ampicillin 84%, chloramphenicol 27%, tetracycline 16%, kanamycin 10% and streptomycin 9%. However, *E. coli* was highly sensitive to gentamicin at 96.8%. Approximately 42.2% of *E. coli* isolates were multidrug resistant to sulfamethoxazole, co-trimoxazole, ampicillin, chloramphenicol, tetracycline, kanamycin and streptomycin. All isolates that were resistant to ampicillin harbored bla_{TEM} gene suggesting genetic mediation.

Conclusion: The observed pattern of resistance to antibiotics points to the need to regulate their use and arrest buildup of resistant genes within the population. *J Microbiol Infect Dis 2016;6(3): 107-112*

Key words: Antimicrobial resistance, antimicrobial susceptibility, bla_{TEM} gene, *Escherichia coli*

INTRODUCTION

Gastroenteritis is the major cause of mortality worldwide and especially in Africa [1]. Most of the deaths occur in children under 5 years [2]. Gastroenteritis has a wide range of etiological agents that include viruses such as Rotavirus and Adenovirus that account for about 66.7% and 8.3% of acute gastroenteritis in children respectively [3]; protozoa such as *Entamoeba histolytica* and *Giardia intestinalis* play a major role in causing enteric associated infections [4]; and coliform bacilli that are associated with fatal diarrheagenic infections [5]. Of all these pathogens, *E. coli* accounts for about 23% of total gastroenteric infection [6]. *E. coli* has been associated with diarrhea among children in Chile [7] and India [8]. In Kenya, *E. coli* has also been associated with persistent drug resistant diarrhea among children. A survey across selected hospitals in Kenya reported that *E. coli* was responsible for 11.2% of all diarrhea cases [9].

Among the challenges, facing the efforts to control gastroenteritis is the emergence of multidrug resistance [9]. A surge in antibiotic resistance is generally attributed to the selective pressure exerted due to overuse of antibiotics both in the treatment of human diseases and at sub-therapeutic levels as additives in livestock feed [10]. *E. coli* is among the leading cause of resistance to ampicillin, trimethoprim/sulphamethoxazole and tetracycline in Kenya [9]. However it remains unknown whether *E. coli* contributes to prolonged periods of medication and potentially to drug resistance in Trans-Nzoia County in Kenya.

Furthermore, it has been shown that drug resistance is genetically instigated [11]. Although early studies showed that bacterial isolates had no genes

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for antimicrobial resistance [12,13], it has since been shown that the rate of *E. coli* adaptive mutation is in the order of 10^5 per genome per generation [14], a projection that is 1,000 times higher than previously inferred. In other words, pathogenic *E. coli* is developing drug resistance at a higher rate than the rate in which new antimicrobial agents are developed. Studies have demonstrated that pathogenic *E. coli* has developed resistance to a wide range of antibiotics [9,11]. The resistance to penicillin, monobactams and cephalosporin, members of β-lactam antibiotics, is mediated by extend spectrum *β*-Lactamase enzyme (ESBL), which is thought to harbor bla_{TEM} gene [15-17]. The bla_{TEM} gene is responsible for 90% of ampicillin resistance in *E. coli* [18].

In Trans-Nzoia County, a surge in the number of patients presenting with enteric infections who require prolonged periods of treatment with antibiotics points to emergence of drug resistance. However, the extent to which *E. coli* may be responsible for antimicrobial resistance in this population is not known. Moreover, it is not known whether bla_{TEM} gene, associated with antimicrobial resistance in other population, is responsible for antimicrobial in Trans-Nzoia. As such, this study set out to investigate *E. coli* drug resistant patterns and the role of bla_{TEM} gene in drug resistance in Trans-Nzoia County.

MATERIALS AND METHODS

This was a cross-sectional preliminary study involving recovery of clinical diarrheagenic *E. coli* isolates from patients treated at the Kitale County Referral Hospital (KCRH) in Trans-Nzoia County, Kenya, between February-April 2015. The fecal samples were obtained with informed consent from patients willing to participate in the study. Ethical clearance was issued by Maseno University Ethical Board reference number MSU/DRPI/MUERC/0243/15. Fecal samples were obtained and cultured immediately in enrichment media for subsequent determination of prevalence and antimicrobial susceptibility.

***Sample collection*:** Fresh fecal samples were cultured in Tryptone phosphate (TP) broth (Himedia pvt Ltd. Mumbai, India), an enrichment media, and incubated at 37°C for 20 hours. The enrichment broth was gently agitated and an aliquot streaked on MacConkey (MAC) agar (Himedia pvt Ltd. Mumbai, India) and incubated at 37°C for 24 hrs. Pure colony from the MAC agar was sub-cultured to Triple Sugar Iron (TSI) agar (Himedia pvt Ltd. Mumbai, India) and incubated for 48 hrs at 37 ^{0}C. The isolates from TSI agar that showed both acidic slant and butt were sub-cultured to Indole agar (Himedia pvt Ltd. Mumbai, India) and incubated for 48 hrs at 35°C. Then 3 to 5 drops of Kovac's reagent was added to test for Indole positive bacteria.

***Antimicrobial susceptibility test*:** The antibiotic susceptibility test was performed using the Kirby-Bauer disk diffusion method [19]. The drug susceptibility breakpoint standards used were the ones recommended by the Clinical and Laboratory Standard Institute (CLSI) [20]. The following antimicrobial agents were considered in this study; ampicillin, gentamicin, tetracycline, co-trimoxazole, streptomycin, sulfamethoxazole and chloramphenicol. *E. coli* ATCC 25922 was used as a control for potency of antibiotic discs. *E. coli* culture showing resistance was sub-cultured to Nutrient agar (Himedia pvt Ltd. Mumbai, India) for 18-24 hrs at 37°C to facilitate recuperation of stressed cells. Sweeps of the bacterial growth on Nutrient agar plates were then preserved at -20°C in Tryptic Soy broth (Himedia pvt Ltd. Mumbai, India) with 15% glycerol, for subsequent molecular analysis.

***DNA extraction and amplification*:** DNA extraction was done according to [21], with modification. *E. coli* isolates were allowed to thaw then suspended in 150 µL of PCR water in eppendorf tube (2.0 ml). The samples were heated at 100°C for 10 minutes in a Dri-Block 2D heating block then centrifuged at 10,000 rpm (Spectrafuge 16M, Labnut International) for five minutes at 4°C. The obtained supernatant was carefully aliquoted and stored at -20°C.

Amplification of bla_{TEM} gene was done using the forward primer ATGAGTATTCAACATTTCCG and reverse primer ACCAATGCTTAATCAGTGAG, performed in a final reaction volume of 25 µL according to protocol by Aarestrup et al. [22]. The following condition was used; denaturation at 94°C for four minutes followed by 35 cycles of 94°C for 30 seconds, annealing at 58°C for one minute, elongation at 72°C for one minute and final extension 72°C for seven minutes then 4°C to infinity. PCR amplification products were loaded onto ethidium bromide stained 1% agarose gel and electrophoresed at 100 volts for 35 minutes for visualization through Gel-Doc UV illuminator.

RESULTS

A total of 103 fecal samples were collected. Participants ranged in age from two weeks to 82 years,

with a mean of nine years and a standard deviation of 5.05. Out of this, *E. coli* infection accounted for 65.6% and 34.4% of cases in children <18 years and adults respectively. Overall 50.5% recorded cases were males while 49.5% were females. Using simple linear regression, it was found out that, age affects susceptibility to infection (R=0.53; p=0.01).

The prevalence of pathogenic *Escherichia coli*

The most prevalent *Enterobacteriaceae* was the diarrheagenic *E. coli* at 90.2%. The remaining percentage was shared among *Citrobacter freundii* (3.9%), *Shigella dysenteriae* (2.9%), *Salmonella* Typhi 1%, *Klebsiella* spp. 1% and *Yersinia enterocolitica* 1% (Table 1). Of all this, children were the most affected.

Table 1. Prevalence of pathogenic *Escherichia coli* in Trans-Nzoia, Kenya. Note that pathogenic *E. coli* was predominant, occurring in 90% of fecal samples.

Enteric pathogen	Number of isolates (%)
Pathogenic *E. coli*	93 (90.2)
Citrobacter freundii	4 (3.9)
Shigella dysenteriae	3 (2.9)
Salmonella Typhi	1 (1.0)
Klebsiella spp.	1 (1.0)
Yersinia enterocolitica	1 (1.0)
TOTAL	**103 (100)**

Prevalence of antimicrobial resistant diarrheagenic *Escherichia coli*

To determine the susceptibility of *E. coli* to antimicrobial agents, isolates from 93 patients were tested against the following eight commonly used antimicrobials: ampicillin, tetracycline, cotrimoxazole, streptomycin, kenamycin, gentamicin, sulfamethoxazole and chloramphenicol. The *E. coli* isolates showed varied degree of susceptibility. The isolates were highly resistant to sulfamethoxazole, cotrimoxazole and ampicillin (97%, 96% and 84% respectively). About 27% of isolates were resistant to chloramphenicol. Tetracycline, kanamycin and streptomycin had resistance levels of 16%, 10% and 9% respectively (Table 2).

E. coli isolates were then classified according to the number of antimicrobials that they were resistant to. Notably, up to 42.2% of all isolates were resistant to more than one antimicrobial. The isolates were highly susceptible to gentamicin (96.8%).

Presence of Beta-Lactam resistance genes, bla-$_{TEM}$, in Pathogenic *Escherichia coli*

Ten samples that showed high level of multidrug resistance based on Kirby-Bauer disk diffusion test were genetically analyzed by Polymerase Chain Reaction (PCR) for bla_{TEM} gene which codes for the enzyme that is responsible for ampicillin resistance. Isolates showing phenotypic resistance to ampicillin gave positive amplicons for bla_{TEM} gene (643 bp), which was consistent with phenotypic antimicrobial susceptibility results (Figure 1).

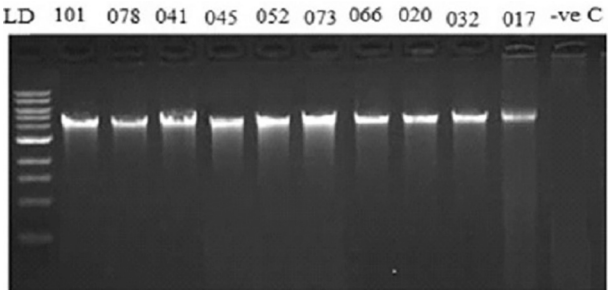

Figure 1. PCR gel showing positive bla_{TEM} gene from products of pathogenic *E. coli* isolates. LD: 100 bp DNA ladder, lane 1, lane 1-10 is isolates and lane 11 is the negative control.

Table 2. Antibiotic susceptibility of *Escherichia coli* by disc diffusion (n=93)

Antimicrobial Agents	% Resistance	% Intermediate	% Sensitive
Ampicillin (25 mcg)	83.9	3.2	12.9
Tetracycline (30 mcg)	16.1	18.3	65.6
Cotrimoxazole (25 mcg)	95.7	1.1	3.2
Streptomycin (25 mcg)	8.6	10.7	80.7
Kanamycin (30 mcg)	9.7	5.3	85
Gentamicin (10 mcg)	0	3.2	96.8
Sulfamethoxazole (25 mcg)	96.8	0	3.2
Chloramphenicol (25 mcg)	26.9	11.8	61.3

R= Resistance, I= Intermediate, S= Sensitive

DISCUSSION

The study demonstrated that majority of diarrhea cases at Kitale County Referral Hospital, serving western Kenya and North Rift are due to *Escherichia coli*. Interestingly, *Citrobacter freundii, Shigella dysenteriae, Salmonella* Typhi*, Klebsiella* spp. and *Yersinia enterocolitica* were also isolated but were together only responsible for less than 10% of diarrhea in patients. We also found substantial antimicrobial resistance to most of these pathogens. Furthermore, bla_{TEM} gene was demonstrated in most of the ampicillin-resistant isolates suggesting that the observed ampicillin resistance is genetically instigated.

This study reported a higher prevalence of pathogenic *E. coli,* 90.2%. In a study conducted by Sang et al. [9] in western and coastal Kenya reported that *E. coli* at 11.2% was the most prevalent enteric pathogen responsible for most enteric infections in the community. The rates of pathogenic *E. coli* were greatest among persons aged less than 6 years, 35.5% and decreased progressively with increasing age. These results are in line with the findings of prior reports by Brooks et al. [23] in their six years study done in rural western Kenya; they showed that pathogenic *E. coli* accounted for a total of 34% of the total infection in children. Also, Abba et al. [24] did a systematic study review of other studies done from different regions of the world, and found that pathogenic *E. coli* accounts for up to 63% of total infections in children less than 6 years.

The isolates from the present study showed varied degree of susceptibility with sulfamethoxazole, cotrimoxazole and ampicillin showing very high level of resistance. The varied resistance level is mostly associated with antimicrobial overuse leading to development of resistance by *E. coli* due to selective pressure and mutations [10]. Pathogenic *E. coli* was most susceptible to gentamycin. In this study, estimates of resistance of *E. coli* isolates to ampicillin and sulphamethoxazole were comparable with findings by Sang et al. [9] in coastal and western Kenya, which showed that resistant to ampicillin was 86% and trimethoprim/sulphamethoxazole was 87%. It was also reported that emergence of resistance, though still at low levels at the time was observed in gentamycin [9]. Also, in a study carried out in Narok and Kajiado regions of South Rift, Kenya, showed that 84% of total *E. coli* isolates were resistant to sulphamethoxazole [25].

Multidrug resistance (MDR) to commonly used antimicrobials is still a major challenge in management of microbial infection. In this study, the MDR phenotype of the pathogenic *E. coli* isolates was estimated at 42.2%; the isolates were mostly resistant to ampicillin, cotrimoxazole, sulfamethoxazole, chloramphenicol, tetracycline, kanamycin and streptomycin. The high level of MDR phenotype is similar to those in Khartoum, Sudan as recorded by Ibrahim et al. [26] isolates of pathogenic *E. coli* from urine samples where he found out that the MDR level was at 65.1%.

Bla_{TEM} (643 bp) gene was expressed by 100% of the 10 isolates analyzed for ampicillin resistance. Livermore [18] reported that bla_{TEM} gene expression is responsible for over 90% of ampicillin drug resistance. Results of our study are also consistent with Natarajan and Singaram [27], which showed that 90% of *E. coli* isolated from patients with urinary tract infection expressed bla_{TEM} gene.

The emergence of resistance to β-lactam antimicrobial agents does not only pose a challenge to management of microbial infections in a resource poor settings in Trans-Nzoia County, but also the widespread nature of bla_{TEM} gene in the *E. coli* isolates may increase the TEM gene pool in the population. Furthermore, the resistant strain may transfer these genes to other susceptible *Enterobacteriaceae* through conjugation and plasmid transfer. A point worth mentioning is that, since bla_{TEM} gene is one of the members of TEM gene family found in the same intergron with other ESBL genes, a slight mutation to it may lead to expression of other TEM genes that were previously not in Trans-Nzoia. Activation of such genes may ultimately lead to rapid development and spread of ESBL genes within the population, which may affect enteric pathogen susceptibility to other β-lactam antibiotics.

However, this study had three limitations. First, the study was hospital-based and thus only reflected the prevalence of diarrheal infection among persons seeking medical care. As such, the findings may not represent the true burden of the disease in the population. Secondly, the study concentrated on individuals with enteric infections, while clinical manifestation of pathogenic *E. coli* may include sub-clinical presentation, which may present without overt diarrhea. Lastly, the study did not identify the origin of the bla_{TEM} gene in the isolates. Knowing the origin of the resistance may help follow the mode of resistance gene transfer from one *Enterobacteriacea* to another and also to project how fast the resistance to antimicrobial may develop.

In conclusion, the presence of bla_{TEM} genes in pathogenic *E. coli* is discomforting because the gene may be transferred to other *Enterobacteriacea*, which are currently susceptible to most of the antibiotics in use. Transfer of resistance gene to other *Enterobacteriacea* will have a high economic impact in management of enteric diseases within the county. There's need to regulate the use of antibiotics and prescription patterns in order to preserve the utility of available antibiotics. In addition to epidemiological surveillance focusing on resistance to commonly used antimicrobials, the origin of the bla_{TEM} gene remains an open and important area of future research.

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Conflict of interest

We declare that there is no conflict of interest.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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