

# Emerging Antimicrobial Resistance Patterns of *Enteric Pathogens* Isolated from Children under 5 years in EAPHLNP Satellite Sites in Kenya

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The East Africa Public Health Laboratory Networking Project (EAPHLNP) is a regional project involving five East African countries, Namely: Burundi, Kenya, Rwanda, Uganda and Tanzania and it is supported by the World Bank.

### Summary

#### **INTRODUCTION**

The emergence of resistance to antimicrobial agents in bacterial *pathogens* is a worldwide problem that has been associated with inappropriate use in human and veterinary medicine. Epidemiological studies from several African countries by the year 2001 established that, diarrhoea was the most common illness reported by the United States military service members deployed to Africa for strategic training and contingency operations. Out of 15,000 US military personnel who participated, more than 500 service members were affected by acute diarrhoea [7].

#### **OBJECTIVE**

To determine the susceptibility of common circulating enteric bacterial pathogens to antimicrobials.

#### METHODOLOGY

Between 12<sup>th</sup> February 2013 and 30<sup>th</sup> July 2014, a total of 420 children under 5 years of age with diarrhea were analyzed for bacterial enteric *pathogens* of which *E. coli* isolates were characterized by Polymerase Chain Reaction for the presence of virulence genes.

#### RESULTS

Patients from whom bacterial enteric *pathogens* were isolated and identified from the 5 satellite sites were= 145, Wajir = 21, Malindi= 42, Kitale = 34, Machakos = 18 and Busia = 30 County Referral Hospitals. Antibiotic susceptibility testing was done on all isolates: *pathogenic E. coli* = 55, *Salmonella* =23 and *Shigella* =72 using disk-diffussion methods containing Ampicillin, Cefotaxime, Tetracycline, Erythromycin Gentamicin, Chloramphenicol, Trimethoprim / Sulphamethoxazole, Ciprofloxacin, Furasolidine and Nalidixic acid. *E. coli, Shigella* and *Salmonella* isolates showed up to 100% level of resistance to ampicillin, trimethoprin / sulphamethoxazole and erythromycin.

Furthermore, *pathogenic E. coli* revealed tetracycline resistance ranging from 67% to 76% in all sites. Emerging resistance to ciprofloxacin ranged from 14.3% in Wajir to 50.0% in



Machakos and gentamycin resistance ranged from 20% in Kitale to 100% in Wajir. *Salmonella* isolates showed levels of resistance ranging from 25% to 100% in Busia and 14% to 100% in Wajir for all the antimicrobials tested.

### CONCLUSION

Our findings on diarrhea due to enteric bacteria show that a high percentage is caused by antimicrobial-resistant strains, thus illustrating the effect of long-standing unregulated antimicrobial use. Most enteric pathogens easily share genes for antimicrobial resistance. There was emerging resistance to newly prescribed antibiotics. This may have policy implications on the use of antibiotics in Kenya.

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### Introduction

Diarrhoea was significantly a health problem worldwide. Particularly in the developing world where adequate sanitation facilities are lacking [1]. The disease account for almost a fifth of all deaths of children below five years of age, with an estimated 2.2 million deaths annually [2]. Epidemiological studies of diarrhoea have been reported from several African countries including South Africa, Gabon, Egypt and Kenya [3 - 6].

In the year 2001, diarrhoea was the most common illness reported by the United States military service members deployed to Africa for strategic training and contingency operations. Out of 15,000 US military personnel who participated, more than 500 service members were affected by acute diarrhoea [7]. The service members represented an immunologically naïve group to the various enteric pathogens and are likely to be at higher risks for contracting acute infectious diarrhoea.

The causes of diarrhoea include a wide array of viruses, parasites and bacteria. *Shigella, Salmonella, Cryptosporidium species* and *Giardia lamblia* are found throughout the world while *Campylobacter jejuni* and *cytotoxigenic Clostridium difficile* are seen with increasing frequency in developed countries [8].

The bacterial pathogen most commonly associated with childhood diarrhoea is *Escherichia coli* and at least six categories 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). The associated clinical pictures comprise childhood and traveller<sup>II</sup>s diarrhoea (ETEC), bloody diarrhoea and hemolytic uremic syndrome (EHEC), infantile diarrhoea (EPEC), and bacillary dysenterylike diarrhoea (EIEC). Enteroaggregative *E. coli* have been associated with acute and persistent diarrhoea in children and adults in industrial and developing countries in Europe, America, Asia and Africa [9].

New virulent enteric pathogens are emerging throughout the world, Africa included. A multidrug resistant enteroaggregative *E. coli*, O44, which is associated with acute and persistent diarrhoea, has been reported in Kenyan children [10]. Very recently, *E. coli* O157 was reported for the first time as the etiologic cause of a large dysentery outbreak in Swaziland [11].

Also, during a study on bacterial diarrhoeal diseases involving children below five years of age in Kenya, the KEMRI/JICA Research and Control of Infectious Diarrhea Project (between 1990 and 1995) reported the first confirmed case of *hemorrhagic colitis* due to *E. coli* serotype O157:H7 in Kenya [12]. This particular isolate produced only vero toxin II (VT2). In the same study, enterotoxigenic *E. coli* (ETEC) strains that elaborated at least one member of two defined groups of enterotoxins, heat-stable (ST) and heat-labile (LT) toxins, were isolated [6].

Antimicrobial resistance surveillance has been conducted only at the institutional levels (e.g., referral and private hospitals), with limited sharing of information and analysis of data. As a result, the actual scale of regional or national antimicrobial drug resistance is not well defined. This study identified the



bacterial causes of diarrhoea, the virulence properties associated with pathogenic *E. coli* isolates, and the antimicrobial susceptibility patterns of the enteric pathogens that were associated with diarrhoeal illnesses in children under five years of age from the selected study sites. These strains were tested for susceptibility to commonly used antimicrobials in Kenya for the management of diarrhoeal illness.

# Methodology

This study is part of the ongoing research funded by the World Bank under EAPHLNP and the protocol was approved by the KEMRI Scientific Steering Committee. Upon obtaining informed consent from either parent or guardian, stool samples were collected between 12th February 2013 and 30th July 2014 from a total of 420. The stool samples were analyzed by conventional biochemical methods, antimicrobial susceptibility and multiplex PCR diarrhoea who were under five years of age.

All the children enrolled gave assent and informed consent was obtained from parents. The enrolled children were drawn from the following geographically diversely distributed study sites: Malindi = 93, Wajir = 70, Machakos = 50, Busia = 115 and Kitale = 92.

Diarrhoea was defined as at least three loose stools in 24 hours, or any number of watery stools. Stool samples were collected on the day of presentation at the outpatient clinics, and were inoculated into Cary-Blair transport Media (MML Diagnostics Inc, Troutdale, Oregon, USA) and transported on ice bags to the laboratory at Kenya Medical Research Institute, Centre for Microbiology Research, and processed within 24 hours.

Enteric pathogens were cultured and identified by standard methods [13]. *E. coli* isolates were subjected to multiplex PCR for detection of virulence genes [14]. DNA standards were extracted from bacteria known to contain the relevant genes. Bacteria containing ATCC 35401 (LT/ST), pEWD299 (LT), pDAS100 (STp), pDAS101 (STh), ATCC 43893 (EIEC), ATCC43887 (BfpA/EAE), 933J (SLTI), 933W (SLTII), ATCC1175 negative control and pCVD432 (Eagg) were obtained from the Armed Forces Research Institute of Medical Sciences in Bangkok. These isolates were grown on MacConkey agar plates to check purity and later cultured on nutrient agar plates for PCR analysis. Antimicrobial susceptibilities of *pathogenic E. coli*, *Shigella* and *Salmonella* were determined by disc diffusion method of Bauer and coworkers [15].

The breakpoints used were those recommended by the NCCLS (National Committee for Clinical Laboratory Standards) on guidelines for susceptibility testing [16]. All these isolates {*Pathogenic E. coli* (55/149), *Salmonella sps* (23/149) and *Shigella sps* (72/149) were tested for resistance to the following antimicrobials: ampicillin, cefotaxime chloramphenicol, ciprofloxacin, erythromycin, furasolidine tetracycline, trimethoprin/sulphamethoxazole, nalidixic acid, and gentamycin. Standard *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 of known susceptibility were used as control organisms.

Statistical analysis was performed for the quantitative study data. Univariate descriptive and exploratory analyses were done by use of proportions. Bivariate analysis was performed using confidence interval (95%) to determine the difference in distribution of antimicrobial resistance by geographical region.

## Results

Bacterial diarrhea was present in 145 out of 420 children (34.5%). Among the isolates obtained from the children s stool samples,

*pathogenic E. coli* comprised 55/420 (13.1%), *Salmonella* 23/420 (5.5%) and *Shigella* 72/420 (17.1%).

Out of the isolated *E. coli*, multiplex PCR detected ETEC strains at 15/55 (27.3%),

EPEC 18/55 (32.7%), EIEC 4/55(7.3%), STEC 15/55 (27.3%) and Eagg 3/55 (5.5%).

EPEC strains were statistically significant between Wajir (1.2%, 95% CI; 0.0-3.5) and Malindi (5.5%, 95% CI; 4.6-17.4). *Table 1a,b* profile the distributions of the *E. coli* pathogens from various study sites.



Isolates	Geographical Region	No. of patients	Frequency	% Isolates	P - Value
<i>E. Coli</i> (55)	Busia	93	8	14.5	0.000549
	Kitale	98	15	27.3	
	Machakos	54	2	3.6	
	Malindi	91	23	41.8	
	Wajir	84	7	12.7	-
Salmonella (23)	Busia	93	8	34.8	0.120243
	Kitale	98	3	13	-
	Machakos	54	0	0	-
	Malindi	91	5	21.7	
	Wajir	84	7	30.4	

 Table 1a: Distribution of E. Coli, Salmonella Pathogens Per Study Site

Table 1 b: Distribution of Shigella pathogens per study site

Isolates	Geographical Region	No. of patients	Frequency	% Isolates	P - Value
<i>Shigella</i> spp. Busia (72)	93	14	19.4		
	Kitale	98	18	25.0	
Machakos	54	18	25.0	0.078516	
Malindi	91	15	20.8		
Wajir	84	7	9.7		

All 55 *E. coli* isolates, 72 *Shigella* isolates and 23 *Salmonella* isolates were tested for susceptibility to the commonly used antimicrobials. *E. coli, Shigella* and *Salmonella* isolates showed up to 100% level of resistance to ampicillin, trimethoprin/sulphamethoxazole and erythromycin. The results of antimicrobial susceptibility testing for pathogenic *E. coli* from the five study sites revealed, 49/55 (89.1%) were resistant to ampicillin, whereas 55/55 (100%) were resistant to trimethoprin/sulphamethoxazole, 55/55(100%) were resistant to erythromycin and tetracycline resistance ranged from 67% to 76% for the 3 pathogens.

Among *Shigella* isolates, high levels of resistance to ampicillin, trimethoprin/sulphamethoxazole and erythromcycin were noted in all the study sites. Furthermore, ciprofloxacin resistance was observed ranging from 14.3% in Wajir to 50.0% in Machakos. Gentamycin resistance ranged from 20% in Kitale to 100% in Wajir. *Salmonella* isolates showed levels of resistance ranging from 25% to 100% in Busia and 14% to 100% in Wajir for all the antimicrobials tested as shown in *Tables 2a, b* and *3a, b*.

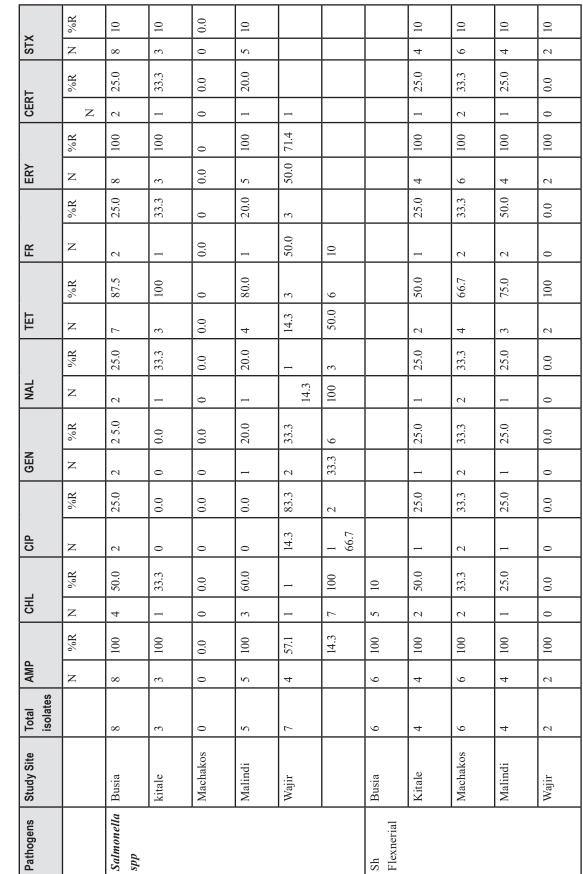


Table 2a: The antimicrobial susceptibility patterns of Shigella and Salmonella isolates per study site



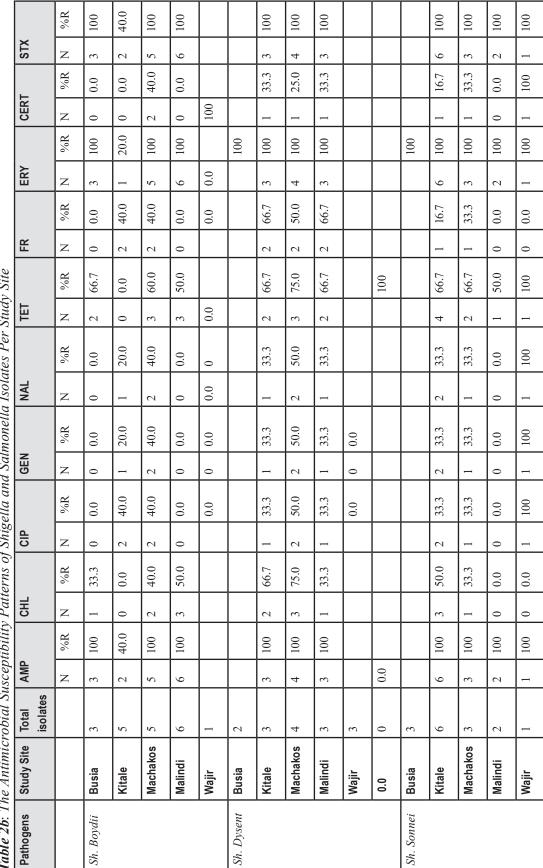


Table 2b: The Antimicrobial Susceptibility Patterns of Shigella and Salmonella Isolates Per Study Site

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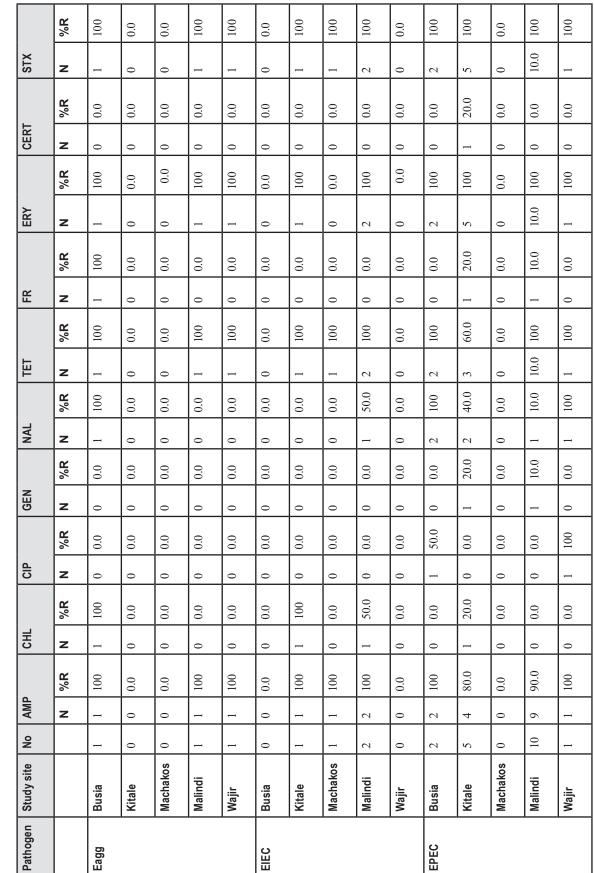


Table 3a: The Antimicrobial Susceptibility Testing of E. Coli Pathotypes Per Study Site



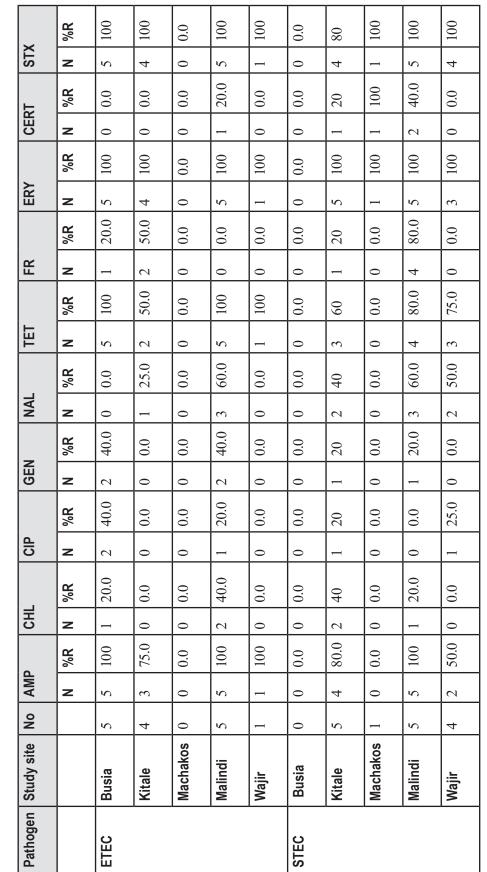


Table 3b: The Antimicrobial Susceptibility Testing of E. Coli Pathotypes Per Study Site





# Discussion

Previous studies in Kenya have documented the prevalence of antimicrobial resistance of some commonly recognized agents of diarrhoea [17-19]. The present study provides results of current prevalence of antimicrobial resistance patterns among enteric bacterial pathogens from children younger than five years, from five study sites. E. coli strains which were tested based on pertinent virulence factors associated with pathotypes such as shigatoxin, heat labile, and heat stable toxins were significantly distributed in the five study sites (P-value <0.05) which was not the case with Salmonella spp and Shigella spp as shown in *Table 1*.

Among the emerging pathogens in this study, EPEC was the most frequently identified potential pathogen representing 18/55 (32.7%) for *E. coli* isolates and 15/55 (27.3%) for ETEC. However, ETEC was more prevalent in Busia and Malindi as compared to other study sites. These results are not in agreement with previous studies in which ETEC was found to be the most common bacterial pathogens in childhood diarrhea [18].

Although the increase of resistance to fluoroquinolones and expanded-spectrum *cephalosporins* among *E. coli* isolates has been observed in several parts of the world [19], in Kenya, this phenomenon has not been reported before especially in areas where research on enteric pathogens has been done. Therefore, it is a public health threat both at community and hospital levels on the management of infections. Recently, intestinal colonization with fluoroquinolone-resistant or extended-spectrum  $\beta$ -lactamase-producing *E. coli* of nonhospitalized persons has been described as an emerging phenomenon [19]. In our study the reasons for the emergence that is noted for the *E. coli* strains that have developed resistance to newly prescribed antimicrobials in Kenya are not clear.

The widespread use of antimicrobial agents in the treatment of infections in the tropics has led to serious problems of antimicrobial resistance. The emergence and spread of antimicrobial resistance in bacteria of medical importance imposes serious constraints on the options available for treatment of many infections, and this raises a common concern among general practitioners and pediatricians in developing countries [20]. The resistance of enteric pathogens to currently used antimicrobial agents has increased the world over as a result of the widespread use of antimicrobials. There are several reports on multiple antimicrobial resistance among strains of *pathogenic E. coli* in Kenya [10, 21, 22]. All *E. coli* isolates from this study displayed resistance to one or more antimicrobials including gentamicin, ampicillin, chloramphenicol, tetracycline and trimethoprim/sulphamethoxazole.

The high levels of antimicrobial resistance among Shigella isolates have been observed in previous studies in Kenya [23, 24]. Experience in other parts of the world has confirmed that resistance to these agents arises rapidly when selective pressure is exerted through intensive use of quinolones and flouroquinolones [25]. However, this study points out a rising prevalence of antimicrobial resistance among enteric pathogens in the five study sites in the different geographic regions of Kenya.

Furthermore, it was noted, though at low levels, the emergence of E. coli resistance to nalidixic acid, and ciprofloxacin is a major concern. This has not been the case in the previous studies in Kenya [22, 26, 27].

Evidence from studies in other African countries demonstrates a high prevalence of multiple antimicrobial resistance in normal bowel flora, which suggests that they may act as a reservoir for resistance available to enteric pathogens.

A study of commensal gut flora of children in Sudan found that 39% of children had strains resistant to six antimicrobials and over 70% of the children had strains resistant to at least 4 out of 6 antimicrobials commonly used in the country [28].

In Kigali, Rwanda, resistance of *Shigella* species to nalidixic acid emerged in 1984 [29]. Four *Shigella dysenteriae* strains from this study showed resistance to nalidixic acid and also ciprofloxacin. The *Shigella*, *Salmonella* and *E. coli* isolates in this study revealed high levels of multidrug antimicrobial resistance to the antimicrobials most frequently used to treat diarrhoeal illnesses in Kenya, such as ampicillin, trimethoprim/ sulphamethoxazole and chloramphenicol.



The other entero-pathogens which could have caused diarrhoea such as *Yersinia enterocolitica*, *Aeromonas hydrophila*, *Entamoeba histolytica*, *Giardia lambia*, *Trichomonas hominis*, *Trichuris trichiura*, *Cryptosporidium* species and rotavirus were outside the scope of the study.

However our findings on diarrhea due to enteric bacteria show that a high percentage is caused by antimicrobial-resistant strains, thus illustrating the effect of long-standing unregulated antimicrobial use. Most enteric pathogens easily share genes for antimicrobial resistance, and the continuous selective pressure applied by the over-the-counter availability of these agents, as well as the prescription of those agents at most clinic visits, has potentially lethal consequences for a region plagued by epidemics of *Shigella dysenteriae* 1 (Sd1) and cholera. Judicious use of antimicrobial therapy requires education of health workers and the population, adequate laboratory diagnostic capabilities, and government regulations.

Antimicrobial susceptibilities must be monitored, to effectively treat pathogens such as Sd1 and Vibrio cholerae 01. Finally, emphasis should be placed on primary preventive measures such as ensuring sewerage management and safe drinking water in Kenya.

In conclusion there is emerging resistance to newly prescribed antibiotics. This may have policy implications on the use of antibiotics in Kenya.

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