Resistance of Common Circulating Enteric Bacterial Pathogens to Prescribed Antibiotics Among Under Five Years in Selected Public Hospitals in Kenya

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Summary

INTRODUCTION

Limited data on antimicrobial resistance (AMR) in *enteric* pathogens is available in Kenya yet diarrhea remains the main cause of morbidity and mortality among rural Kenyan children.

There was need to established antimicrobial susceptibility patterns of *enteric* bacterial *pathogens* to commonly prescribed antibiotics among children aged <5 years in selected cross-border Kenyan public hospitals between 2013 and 2016.

METHODOLOGY

This was a hospital based cross-sectional study. Single stool samples were collected from 1644 outpatients presenting with diarrhea at Busia, Kitale, Malindi, Wajir, and Machakos public-hospitals from June 2013 to June 2016 and shipped to Kenya Medical Research Institute (KEMRI). Pathogenic *E. coli, Shigella* and *Salmonella sp.* were isolated using standard microbiological methods. *Multiplex Polymerase Chain Reaction (PCR)* was used to characterize *E. coli* isolates. Antimicrobial susceptibility testing was done using Kirby Bauer disc diffusion method.

RESULTS

Out of the 1644 enrolled participants, bacterial pathogens identified were; Pathogenic *E. coli* in 232(14.1%), *Shigella* in 99 (5.4%) and *Salmonella* in 49(3.0%). High antimicrobial resistance (AMR) levels were observed. Highest AMR were observed for *Sulfamethoxazole* (93%) and Ampicillin **(88%) for *E. coli* isolates, *Sulfamethoxazole* (89%) and Ampicillin (88%) for *Shigella* isolates, *Sulfamethoxazole* (73%) and Ampicillin (86%) for *Salmonella i*solates.**

Emerging resistance at 15%(CI:10-19), 29%(CI:23-35), 1 4%(CI:9-18) for *E. coli*, 26% (CI:18-35), 55%(CI:45-64), 30%(CI:21-39) for Shigella and 31%(CI:18-44), 57%(CI:43-71) 29%(CI:16-41) for *Salmonella* were observed to Ciprofloxacin, Nalidixic acid and Gentamycin respectively.

CONCLUSION

Pathogenic *E. coli* were the most prevalent *enteric* bacterial pathogens. Highest AMR were observed to *sulfamethoxazole* and ampicillin then emerging resistance to 3rd line antibiotics is of a major concern.

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Introduction

Diarrhea is a significant health problem worldwide, especially in the developing world where adequate sanitation facilities are lacking [1].

Globally diarrhoeal diseases account for almost a fifth of all deaths in children below 5 years, with an annual estimate of 2.2 million deaths [2].

The cause of diarrhea includes an array of viruses, parasites and bacteria [3]. The widespread use of antimicrobial agents in medical practice and animal husbandry has led to serious problems of antimicrobial resistance world over [4].

Increasing antimicrobial resistance of *enteric* commensals and *pathogens* has been a major topic both in developed and developing nations for quite a while [1]. The emergence and spread of antibiotic resistance in *enteric pathogens* pose a serious problem in Kenya. This has heightened sense of awareness and interest in bacterial diarrheal illnesses [5, 6].

There is a concern on lack of effective treatment for drug resistant organisms. This concern has been brought about by the recent increase in enteric disease outbreaks in Eastern Africa region.

Among the *enteric pathogens,* major drug resistant *Shigellae sp., Salmonella typhi* strains and *Vibrio cholera spp.* have been reported in Kenya [7].

As a result, there is need to determine prevalence of these *pathogens* and their antimicrobial resistance profiles along the cross-border points of countries in the Eastern Africa region: Kenya, Uganda, and Tanzania.

This study aimed at determining the prevalence of common circulating *enteric* bacterial *pathogens* and their antimicrobial susceptibility patterns in select cross-border public hospitals in Kenya.

Methodology

Study Design

This was a hospital based cross-sectional study of all children aged five years and below who sought treatment for diarrheal illness from five (5) selected cross-border county hospitals in Kenya. These were; Busia, Kitale, Wajir, Malindi and Machakos who were willing to participate in the study.

Stool Sample Collection

The study was granted approval by the Scientific Ethics and Review Unit (SERU) at KEMRI. After obtaining consent, stool samples were collected between June 2013 and June 2016 from 1644 outpatient children presenting with diarrhea and were not on antibiotics in the last 72 hours.

Sample collection was done in sterile plastic containers. The specimens were transferred into Cary-Blair transport media (MML Diagnostics Inc. Troutdale, Oregon), labeled only with a unique study number, then packaged appropriately (Triple Packaging System) and transported to the Kenya Medical Research Institute (KEMRI), Nairobi Center for Microbiology Research (CMR) laboratories where they were processed.

Sample Processing

Bacterial Identification

All stool samples were plated onto MacConkey agar, Xylose-Lysine-Deoxycholate agar (XLD) and Sorbitol-MacConkey agar, (Oxoid Hampshire, England). The plates were incubated aerobically at 37ºC for 18 to 24 hours.

Thereafter, one to two suspect colonies each of *Shigella* and *Salmonella* and five to ten colonies with typical *E. coli morphology* were selected and characterized on the basis of their biochemical reactions [8].

Multiplex PCR

A multiplex PCR assay allowed the detection of eleven virulence markers in Pathogenic *E. coli.* Amplification parameters and primers for amplifying segments of genes of the *Shiga toxins (stx1, stx2,* and *eaeA*), *Cytotoxin necrotising* factors (*CNF*I and CNF2), attaching and effacing mechanisms (*eaeA*), *enteroaggregative* (EAEC), *enteroinvasive* mechanism (EIEC), and *enterotoxigenic* (ETEC) detection for heat-labile (LT) and heat stable (ST1 and ST2) toxins were used as described previously [9].

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were conducted using ***Kirby Bauer disc diffusion method*** [10].

The following were commonly used antimicrobial agents (Basingstoke Hampshire, England). They were tested on all the isolates as per CLS guidelines 2016 using *E. coli* reference strain **ATCC 25923** as a control. in hospitals:

Ampicillin,
Chloramphenicol,
Ciprofloxacin,
Tetracycline,
Nalidixic acid,
Furazolidine,
Gentamycin,
Cefotaxime,
Sulfamethoxazole

Results

A total of 1644 participants were enrolled from five Kenyan hospitals serving cross-border counties as follows:

Busia	n	=	291
Kitale	n	=	492
Machakos	n	=	70
Malindi	n	=	387
Wajir	n	=	404

Among the enrolled participants, *etiologies* of diarrheal were identified in 25.2% (n=415) cases as follows:

Pathogenic E. coli	14.1%	n	=	232
Shigella	5.4%	n	=	99
Salmonella	3.0%	n	=	49

Distribution of *E. coli pathotypes* was:

Busia	16.5%
Kitale	10%,
Machakos	4.3%,
Malindi	15%
Wajir	18.3%

while distribution of *Shigella* was:

7.6%, 8.9%, 5.7%, 5.2%, and 2.2%

and that of *salmonella* was:

1.4%, 3%, 0%, 4.9% and 2.7%

in the five hospitals respectively ***(Figure 1).***

Antimicrobial susceptibility testing was conducted on:

48, 49, 3, 58, and 74 *E. coli* isolates,
22, 44, 4, 20, and 9 *Shigella* isolates
4, 15, 19, 0 and 11 *salmonella* isolates

from Busia, Kitale, Machakos, Malindi and Wajir county cross-border hospitals respectively.

All the three *etiologies* of diarrhoeal showed resistance to the commonly prescribed antibiotics in Kenya though at varied levels.

Among the *E. coli* isolates, overall highest levels of resistance were observed in:

Sulfamethoxazole at 93% (95% CI: 90-96)
Ampicillin at 88% (95% CI: 83-92)

respectively ***(Table 1)***.

Among *Shigella* isolates the highest levels of resistance were also observed in:

Sulfamethoxazole at 89% (95% CI: 69-100) and ampicillin at 88% (95% CI: 81-94)

respectively ***(Table 1)***

while the highest resistance levels among the *Salmonella* isolates were in:

*ampicillin a*t 86% (95% CI: 76-96),
tetracycline and *sulfamethoxazole* both at 73% (95% CI: 61-86) (Table 1).

Disturbingly, emerging resistance to Nalidixic acid, Ciprofloxacin and Gentamycin was also observed among the three agents of diarrheal isolated and also at each of the cross border hospital.

Emerging resistance was observed as follows:

E. coli, at:
29%; (CI:23-35), 15%; (CI:10-19), 14%; (CI:9-18)
Shigella at:
55%;(CI:45-64), 26%; (CI:18-35), 30%;(CI:21-39)
Salmonella spp.
57%;(CI:43-71), 31%;(CI:18-44), 29%;(CI:16-41)
(Table 1).

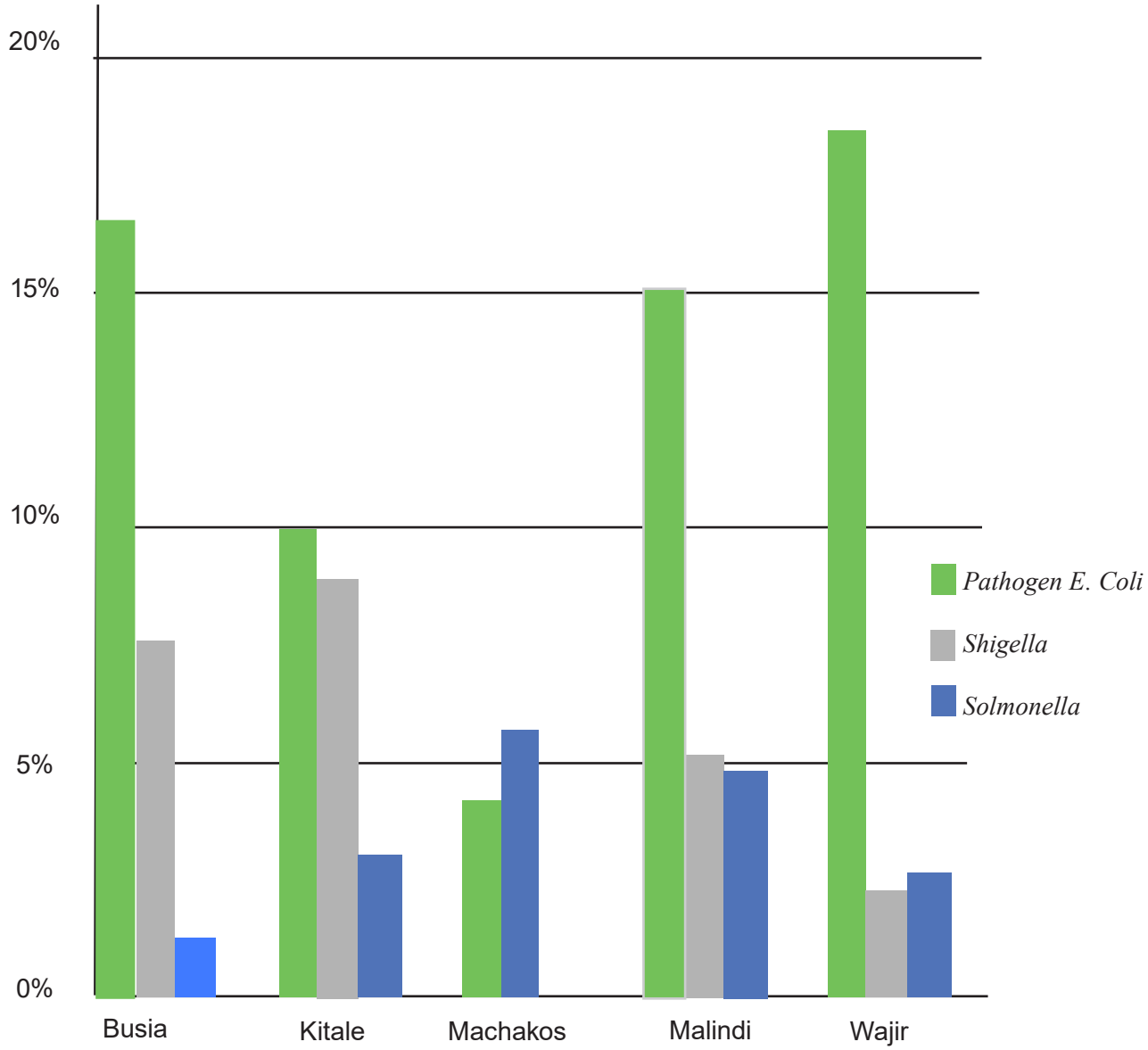
Table 1: *Resistance Profiles Of Enteric Bacterial Pathogens To Commonly Prescribed Antibiotics In The Selected County Hospitals In Kenya*

Bacterial Isolates from Sites	Amp	Chl	Cip	Te	Nal	Fur	Gen	Ctx	Stx
	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)
E.coli									
Busia (48)	92(84-100)	25(13-37)	10(2-18)	67(54-80	25(13-37)	21(9-33)	10(2-18)	8(0-16)	98(94-100)
Kitale (49)	92(84-100)	33(20-46)	6(0-13)	78(66-90)	31(18-44)	18(7-29)	12(3-21)	20(9-31)	94(87-100)
Machakos(3)*	100(n/a)	0(n/a)	33(0-86)	67(14-100)	33(0-86)	0(n/a)	0(n/a)	67(14-100)	100(n/a)
Malindi (58)	90(82-98)	31(19-43)	12(4-20)	79(69-89)	29(17-41)	9(2-16)	7(0-14)	10(2-18)	93(86-100)
Wajir (74)	80(71-89)	16(8-24)	24(14-34)	69(58-80)	30(20-40)	11(4-18)	23(13-33)	28(18-38)	89(82-96)
Total(232)	**88(83-92)**	**25(19-31)**	**15(10-19)**	**73(67-79)**	**29(23-35)**	**14(9-18)**	**14(9-18)**	**19(18-24)**	**93(90-96)**
Shigella									
Busia(22)	100(n/a)	27(75-33)	18(2-34)	73(54-92)	59(38-80)	18(2-3)	27(8-46)	18(2-34)	91(79-100)
Kitale(44)	82(71-93)	55(40-70)	25(12-38)	80(68-92)	50(35-65)	25(12-38)	34(20-48)	18(7-29)	84(73-95)
Machakos(4) *	75(33-100)	50(1-99)	50(1-99)	75(33-100)	75(33-100)	50(1-99)	75(33-100)	50(1-99)	100(n/a)
Malindi(20)	90(77-100)	35(14-56)	20(2-38)	85(69-100)	40(19-61)	15(0-31)	20(2-38)	10(0-23)	95(85-100)
Wajir(9)	89(69-100)	33(2-64)	44(12-76)	78(51-100)	89(69-100)	33(2-64)	11(0-31)	44(12-76)	89(69-100)
Total(99)	**88(81-94)**	**42(33-52)**	**26(18-35)**	**77(68-85)**	**55(45-64)**	**23(15-32)**	**30(21-39)**	**21(13-29)**	**89(83-95)**
Salmonella									
Busia(4) *	100 (n/a)	75(33-100)	75(33-100)	100(n/a)	100(n/a)	50(1-99)	75(33-100)	50(1-99)	75(33-100)
Kitale(15)	73(51-95)	13(0-30)	13(0-30)	53(28-78)	27(5-49)	13(0-30)	13(0-30)	27(5-49)	60(35-85)
Malindi(19)	95(85-100)	47(25-69)	26(6-46)	79(61-97)	68(47-89)	32(11-53)	26(6-46)	21(3-39)	84(68-100)
Wajir(11)	82(59-100)	64(36-92)	45(16-74)	82(59-100)	64(36-92)	45(16-74)	36(8-64)	55(26-84)	73(47-99)
Total(49)	**86(76-96)**	**43(29-57)**	**31(18-44)**	**73(61-86)**	**57(43-71)**	**31(18-44)**	**29(16-41)**	**33(20-46)**	**73(61-86)**

** Sample size inadquate to interpret resistance*

*Ampicillin **(Amp)**, Chloramphenicol **(Chl)**, Ciprofloxacin(**Cip**), Tetracycline(**Te**), Nalidixic acid**(Nal)**, Furazolidine(**Fur**), Gentamycin(**Gen**), Cefotaxime (**Ctx**), sulfamethoxazole(**Stx**).*

***Figure 1**: Distribution In Percentage(%) Of Common Circulating Enteric Bacterial Pathogens In Selected Kenyan Cross- Border Hospitals*



Discussion

Antimicrobial resistance (AMR) is a very critical healthcare, social and economic problem worldwide [11].

In Africa, it seems that same levels of antibiotic drug resistance are found in all regions and settings yet these regions are widely different in terms of Socio - Economic development. Based on published reports in Kenya antibiotic resistance has increased dramatically in the last 20 years [12].

Despite advances in diagnostic technology in some Hospitals in Kenya, many patients receive empiric treatment without evidence based laboratory identification of the causative agents.

Studies done in Kenya among the Maasai people fifteen years ago showed a high infection rate of *Pathogenic E. coli* especially in children with diarrhea who were under five years of age. Isolates from these children showed moderate resistance to antibiotics that were used than in treatment of diarrhea where it was necessary [13].

Moreover, the few recently reported studies about antimicrobial resistance among *diarrheagenic E. coli* and *Shigella* strains in children in Kenya show a high prevalence rate of resistance to commonly prescribed antibiotics for diarrheal illness [5, 14].

In this study, the susceptibilities of 232 diarrheagenic *E. coli*, 99 *Shigella*, 49 *Salmonella* isolates were tested for different antibiotics that are frequently prescribed at the study hospitals. In our study, the results showed that CIP, NAL, FUR, GEN and CTX had moderate activity amongst *Shigella* isolates.

However, previous studies in Kenya, especially in Malindi and Narok showed no resistant to these drugs [13]. In this case our results indicate an emerging resistance to *fluoroquinolone* (CIP), *quinolone* (NAL) and 3rd generation *cephalosporin* (CTX).

Therefore, it is of great concern while these antibiotics are used as the last option to treat infections, including traveler's diarrheal. The study showed a higher prevalence of resistance to STX, AMP, and TE in diarrheagenic *E. coli* compared to other studies elsewhere [14, 16].

Our study showed *pathogenic E. coli* as the most prevalent with high rates of resistance to AMP, STX and TE and moderate resistance was also noted to CHL, NAL and CTX, which are the commonly used drugs for treatment of diarrhea. Similar rates of resistance have been reported, [17, 18, 19].

On the contrary, a study by Vila et al. in a neighboring country, Tanzania, displayed high susceptibility of pathogenic E. coli to both NAL and CIP [20].

Shigella spp are becoming increasingly resistant to most antibiotics commonly used in the treatment of diarrhea [21, 22]. In the present study, the prevalence of resistance to STX and AMP were similar to those of other studies in developing countries [23, 24, 25].

In contrast, the study of Replogle et al. in United States showed lower prevalence of resistance of *Shigella,* with 59% resistant to STX and 63% resistant to AMP [26]

The lower resistance rates to these two antibiotics have also been reported by European Union in Slovania's summary report on antimicrobial resistance [27].

Many studies have reported multi-resistance in *Shigella* especially to STX and AMP, which are commonly used to treat *Shigellosis* [28, 29].

In our study, *Shigella* strains were resistant to:

STX at 89%,
AMP at 88% and
TE at 77%. Except for
CHL and
NAL

that showed moderate activity with *Shigella,* other antibiotics were showing decreased activity against this *pathogen.*

In this study the percentages of *Salmonella* resistance were:

AMP 86%, 73%
TE, 43%
CHL, and
STX 73%

which were quite variable in contrast, with previous studies in Kenya that showed the lowest resistance rates to:

AMP at 53%,
TET at 59%,
CHL at 36%, and
STX at 53% (5,30).

However, the lowest rates from Sri Lanka indicate a percentages ranging from 3% (resistance to CHL) to 10% (resistance to STX) [28].

Conclusion

In conclusion, the progressive increase in antibiotic resistance among enteric pathogens, particularly in developing countries, is becoming a sensitive concern especially in selecting the appropriate treatment for an effective, inexpensive antimicrobial agent that can be used safely for treatment of children with diarrhea.

The immediate concern is knowledge of the resistance patterns of *enteric pathogens* to commonly prescribed antibiotics in Kenya.

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