Antibiotic susceptibility of Enteric pathogens from the Maasai community, Narok and Kajiado Districts, Kenya.

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SUMMARY

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. Between August 2004 and July 2005 stool samples from 380 patients were cultured for enteric pathogens and characterized by Polymerase Chain Reaction for the presence of virulence properties. Patients were from Narok and Kajiado Districts of Kenya, mainly populated by the Maasai community majority of who practice traditional medicine. 218 patients were from Narok District Hospital and 62 from Entosopia Clinic in Kajiado. A total of 107 *E. coli* and 35 *Shigella* isolates were tested.

Antibiotic susceptibility testing was done using the E-test strips containing Tetracycline, Gentamicin, Chloramphenicol, Fosfomycin, Amoxicillin/Clavulanic acid, Trimethoprim/Sulphamethoxazole, Ticarcillin/ Clavulanic acid and Ciprofloxacin. The resistance frequencies did not differ significantly between other *E. coli* and Shiga toxigenic *E. coli*, respectively; Gentamicin (3% vs. 3%), Chloramphenicol, (24% vs. 23%) and ampicillin (25% vs. 23%), Tetracycline (63% vs. 68%), Fosfomycin (44% vs. 54%) and Trimethoprim/Sulphamethoxazole (84% vs. 84%). Overall antibiotic resistance levels were at much lower levels than those reported from the rest of Kenya, possibly due to the lower levels of exposure and usage of antimicrobials among the Maasai community.

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Introduction

Diarrhoea is a significant health problem worldwide, especially in the developing world where adequate health facilities and proper sanitation are lacking [1]. Globally diarrheal diseases account for an estimated 2.2 million deaths of children below 5 years annually, [2,3]. The progressive increase in antimicrobial resistance among enteric pathogens particularly *Shigella, Vibrio cholerae,* Enteropathogenic *E. coli, Salmonella* Typhi and *S.* Enteriditis species is becoming a critical concern worldwide, particularly in the developing world where there are high rates of diarrheal diseases which are associated with mortality. This also affects travelers to these regions. Antimicrobial resistance in developing world is most likely related to the frequent unrestricted use of over-the-counter drugs without medical supervision [4].

The emergence and spread of antibiotic resistance in bacteria is of medical importance and poses serious constraints on the options available for the treatment of many infections. This problem has been brought into prominence by the recent widespread outbreaks of enteric diseases caused by drug resistant organisms [5, 6]. Among enteric pathogens, major epidemics of infection with antibiotic resistant *Shigella* have occurred in Latin America, Asia and Africa [7, 8, 9]. In Kenya, diarrheal illnesses ranked 4th after Malaria, upper respiratory tract infections and skin infection [10]. A major outbreak of multi-drug resistant *Shigella dysenteriae* I, occurring concurrently with *Vibrio cholerae* serovar O1 Ogawa was reported along the coastline of Kenya [11]. This strain of S. *dysenteriae*

was found to be resistant to ampicillin, Tetracycline, Chloramphenicol, and cotrimaxazole but sensitive to gentamycin, nalidixic acid and kanamycin, apart from one strain that was resistant to kanamycin. However most *V. cholerae* strains were sensitive to Gentamicin, nalidixic acid, kanamycin, Tetracycline, Chloramphenicol and a few were resistant to ampicillin. Also multi-drug resistant enteroaggregative *E .coli* serotype O44 associated with acute and persistent diarrhea was reported in Kenyan children [12*]*. A number of studies done at a public teaching hospital in Kenya in 1991 and 1992 [13, 14] recorded a prevalence of over 50% to ampicillin and 80% to 100% resistance to Tetracycline among *Salmonella* and *Shigella* isolates causing nosocomial infections during the period 1986-1990. A more recent study in Kenya further attests to this trend in which about 70% of the *E. coli* isolated was resistant to Tetracycline, ampicillin and sulphamethoxazole-trimethoprim [15].

Materials and Methods

After obtaining informed consent, stool samples from 380 outpatients with diarrhea (318 from Narok District Hospital and 62 from Entosopia Clinic) were collected in sterile plastic containers. The specimens were transferred into Cary-Blair transport media (MML Diagnostics Inc, Troutdale, Oregon, USA), labeled only with a unique study number and then placed in an insulated box with ice packs and transported to the Kenya Medical Research Institute Centre for Microbiology laboratory where they were processed within 6 hours of collection.

All stool samples were plated onto MacConkey agar, Xylose-Lysine-deoxycholate agar XLD), Sorbital-MacConkey agar (for detection of O157 STEC) and *Campylobacter* blood-free agar. Initially selenite broth was used for enrichment purposes. The plates were incubated aerobically at 37^{o}C for 18 – 24 hrs, with the exception of *Campylobacter* plates, which were incubated at 42^{o}C in microaerophilic conditions for 48 hrs [16].

After overnight growth at 37^{o}C one to two suspect colonies each of *Shigella* and *Salmonella* and five to ten single colonies with typical *E. coli* morphology were selected and characterized on the basis of their biochemical reactions using BBL Enterotubes™ II. (Becton Dickson Microbiology Systems Sparks, Maryland USA)

The strains identified as *Salmonella, Shigella* or *E. coli* by their colonial morphology and biochemical properties were further serotyped using O-antigen and H-antigen antisera (Denka Seiken Co LTD, Tokyo-Japan) by slide agglutination assays as previously described [17].

Antibiotic Susceptibility Testing by MIC method

A total of 107 *E. coli* isolates of which 31 were STEC and 76 isolates from other pathogenic *E. coli (* ETECs, Eagg, Einv and EPEC, including 35 isolates of S*higella* spp). were tested for susceptibility.

The E-test method (AB Biodisk) was used to screen for the antibiotic susceptibility patterns. The minimum inhibitory concentration (MIC) susceptibility test was determined in accordance with the manufacturer's guidelines (AB Biodisk, Sweden). The 0.5 McFarland standards isolates were inoculated onto Mueller Hinton agar plates by swabbing evenly in three directions. The E-test strip (obtained from the refrigerator at 4^{o}C) was applied to each plate with sterile forceps with lowest concentration toward the center of the agar plate. The plates were then incubated at 30 to 35 °C for 24 hours. The E-test MIC values were read directly from the E-test strip MIC scale. The following antibacterial agents: Fosfomycin (Fm), Ciprofloxacin (Cip), Cefotaxime (Ctx), Ticarcillin/Clavulanic acid (Ctl), Gentamicin (Gn), trimethoprim-sulfamethoxazole (Sxt), ampicillin (Am), Chloramphenicol (Chl) and Tetracycline (Te) were used as guided by CLS (2005) criteria. The concentration gradient of each antimicrobial agent on the E-test strips was 0.016 to 256µg/ml with the exception of Ciprofloxacin and co-trimoxazole for which the gradient ranged from 0.002 to 32µg/ml.

Results

Diarrheagenic *E.coli*

The antibiotic susceptibility testing data are shown in Table 1. Of the 76 diarrheagenic *E.coli* isolates (31 ETEC*,* 20 EPEC*,* 14 Eagg and 11 Ein*v*), 84% were resistant to SXT, 63% were resistant to TC, 44% were resistant to FM and 24% were resistant to CHL. Lower levels of resistance were observed for gentamycin and ampicilin 3% and 5% respectively. All these isolates were fully sensitive to CIP, CTX and CTL. The traditional antibiotics, including CHL, TC and STX showed low activity against these *E.coli* strains (MIC at which 90% of these isolates tested are inhibited [MIC_{90}] of 64 mg/liter for CHL and MIC_{90} of 1024 mg/liter.

Table 1: Antibiotic susceptibilities of diarrheagenic *E.coli* and *Shigella* strains.

Organism and agent	N.o of isolates	% Resistance	MIC_{50}	MIC_{90}	Range
Diarrheagenic *E.coli*	76				
CIP		0	0.012	0.012	0.002-0.75
GEN		3	1	1.5	0.125-256
AM		5	4	8	1.5-256
CHL		24	4	64	0.016-256
TC		63	192	256	1-256
FM		44	48	1024	0.125-1024
SXT		84	32	32	0.023-32
CTX		0	0.064	0.064	0.016-32
CTL		0	0.26	0.26	0.25-0.26
STEC	31				
CIP		0	0.008	0.016	0.006-0.75
GEN		3	1	1.5	0.25-24
AM		23	6	12	1.5-256
CHL		23	4	256	1-256
TC		68	256	256	1.5-256
FM		54	192	1024	0.125-1024
SXT		84	32	32	0.023-32
CTX		0	0.064	0.064	0.016-1
CTL		0	0.26	0.26	0.25-0.26
Shigella sp.	35				
CIP		0	0.012	0.047	0.006-35
GEN		0	0.75	1	0.25-2
AM		34	6	12	2.0-48
CHL		29	4	32	0.75-256
TC		77	64	192	1-256
FM		29	6	96	1-1024
STX		91	32	32	0.094-32

Out of 35 STEC strains, 31 were tested for all the antibiotics. The resistance frequencies did not differ significantly between other *E. coli* pathotypes and STEC respectively ($p>0.05$), that is gentamycin (3% vs. 3%), and Chloramphenicol, (24% vs. 23%) with the exception of ampicillin (5% vs. 23%). The frequencies were slightly higher for Tetracycline (63% vs. 68%), Fosfomycin (44% vs. 54%) and Trimethoprim/Sulphamethoxazole (84% vs. 84%).

Shigella

Thirty five *Shigella* strains tested showed that high levels of resistance for TC and SXT 77% and 91% respectively. TC and SXT showed very low activity against *Shigella* strains with MIC_{90}'s of 192 and 32 mg/liter, respectively. All *Shigella* strains were 100% sensitive to CIP and Gen. All *Shigella* strains were not tested for two antibiotics CTX and CTL.

Discussion

There are several reports on the multiple antimicrobial resistance among strains of pathogenic *E. coli* in Kenya [12, 18, 15]. However, the resistance rates observed in this study are much lower than those that have been reported from the rest of Kenya. As compared to our study which showed lower prevalence of resistance to AM, CHL, TC for STEC and non STEC strains, a study by Kariuki et al in 1997 recorded multiple resistance rates in *E. coli* as high as 86% to Tetracycline and 89% resistance to ampicillin [18]. Study results showed moderate activity against *E.coli* strains for AM and CHL. The overall occurrence of STEC drug resistant isolates range from 3% resistance to Gentamycin to 84% for Trimethoprim/Sulphamethoxazole for both STEC and none STEC. The MIC of STEC isolates showed almost similar patterns as those of non STEC with an exception of Ampicilin drug where non-STEC showed lower levels of resistance at 3% as compared to 23% for STEC.

Variation in resistance frequencies of STEC and non STEC strains agree with findings of a similar study on antibiotic resistance (8% vs. 26%), [19]. The observation that all STEC and non STEC strains were fully sensitive to Ciprofloxacin is important considering that fluoroquinolones are used to treat a range of *E. coli* infections in humans. Other researchers in the developed countries found low levels of resistance of *E. coli* isolates to Ciprofloxacin ranging from 1% to 5% [20, 21]. The use of antibiotics to treat patients with STEC infections has been quite controversial. Most clinicians experienced in the management of STEC infections in the United States and Canada have found that antimicrobial agents at best have little clinical effect and at worst causes harm, for example increase the chances of acquiring Hemolytic Uremic Syndrome (HUS) [22, 23]

All *Shigella* isolates were fully sensitive to Ciprofloxacin and Gentamycin. Moreover, as compared to *E.coli* isolates the resistance of S*higella* isolates was much higher to antimicrobials which ranged from 29% resistant to chloramphenical and to 91% resistance to trimethoprim-sulphamethoxazole. The increase in resistance of *Shigella* species to trimethoprim- sulphamethoxazole and to ampicillin has been reported world wide [21, 24]. Also in Kenya, a study by Kariuki et al reported the resistance rate of *Shigella* spp. to trimethoprim/sulphamethoxazole and ampicillin to be as high as 100% for both drugs [25]. In this study, resistance rates of *Shigella* spp. to these antibiotics were not as high as those reported in Burundi [26], Zimbabwe [27] and in the coastal province of Kenya [11].

The level of antimicrobial resistance were much lower compared with those reported elsewhere [28, 29, 30]. The results of STEC strains from our study could be different in that the Maasai people have not been grossly exposed to antibiotics compared to other communities.

Conclusion

We observed low levels of antibiotic resistance among the Maasai communities who may not have been grossly exposed to antibiotics and still practice the use of traditional medicine. The antibiotics that have developed resistance in the rest of Kenya can still be administered to the patients in the Maasai communities.

The overall antibiotic resistance levels were at much lower levels than those reported from the rest of Kenya, possibly due to the lower levels of exposure and usage of antimicrobials among the Maasai people.

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