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TREATMENT OF *SHIGELLA* INFECTIONS: WHY SULFAMETHOXAZOLE-TRIMETHOPRIM, TETRACYCLINES AND AMPICILLIN SHOULD NO LONGER BE USED
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ABSTRACT

***Background*: Bloody diarrhoea results in high morbidity and mortality especially in developing countries with shigellosis being the main cause of acute bloody diarrhoea. The use of appropriate antimicrobial agents in the treatment of acute diarrheal disease shortens the duration of illness and bacterial shedding leading to a reduction in morbidity and mortality. Treatment options for many infections are becoming limited due to globally emerging antibiotic resistance. Globally, resistance of *shigella* species to trimethoprim-sulfamethoxazole (TMP-SMX), tetracyclines and ampicillin has been reported with subsequent recommendations of not using these antimicrobial drugs for empirical therapy of acute bloody diarrhoea.**

***Objective*: To establish the antimicrobial susceptibility patterns and antimicrobial drug use for treatment of *shigella* species in patients with acute bloody diarrhoea.**

***Design*: A hospital based case control study.**

***Setting*: Six health facilities, three in Kilifi County and three in Nairobi County.**

***Subject*: A total of 284 stool specimens were collected from patients who fitted the standard cases definition for acute bloody diarrhoea.**

***Results*: Eighty (28.2%) bacterial isolates were recovered from 284 stool samples collected from cases presenting with acute bloody diarrhoea of which 67 (83.8%) were *Shigella* species, nine (11.3%) were Enteroinvassive *Escherichia coli* isolates, three (3.8%) were *Salmonella* Typhi and one (1.3%) were *Yersinia enterocolitica*. *Shigella* isolates had high resistance to sulfamethoxazole-trimethoprim (97%), tetracycline (83.6%) ampicillin (58.2%) and chloramphenicol (20.9%). The isolates showed low resistance to nalidixic (4.5%) and ciprofloxacin (3.0%) while there was no resistance to ceftriaxone. The most common multidrug resistance pattern detected in *Shigella* strains combined sulfamethoxazole-trimethoprim, amoxicillin/ampicillin and tetracyclines.Antibiotic prescriptions were given to 243(85.6%) of the patients presenting with acute bloody diarrhoea. Among these, 94 (38.7%) were given prescriptions for ciprofloxacin, 53 (21.8%) for sulfamethaxazole-trimethiprin and 36(14.8%) for Tetracyclines. Chloramphenicol, amoxicillin/ampicillin, nalidixic acid and ceftriaxone were prescribed to 10.7 %, 3.7%, 2.9% and 0.4% of the patients respectively. A total of 123 (51%) received antibiotics which were ranked to have high resistance (sulfamethoxazole-trimethoprim, tetracyclines ampicillin and chloramphenicol).**

***Conclusion*: The high rates of antimicrobial resistance among the commonly prescribed antimicrobials such as sulfamethoxazole-trimethoprim, tetracycline, ampicillin and chloramphenicol is of major concern. Despite recommendations discouraging the empirical use of sulfamethoxazole-trimethoprim, tetracycline, ampicillin and chloramphenicol for treatment of acute bloody diarrhoea, more than half of the patients with acute bloody diarrhoea were still treated with these antibiotics.There is need to train health care workers on the proper management of acute bloody diarrhoea and the importance of adhering to the clinical guidelines.**

INTRODUCTION

Bloody diarrhoea results in high morbidity and mortality especially in developing countries (1). Shigellosis is a major cause of diarrhoea-related morbidity and mortality with an estimated annual incidence of about 165 million cases and over 1 million deaths (2, 3). Ninety-nine percent of infections caused by *Shigella* occur in developing countries (4).

The World Health Organisation's first global report on antimicrobial resistance, focusing on antibiotic resistance, provides the most comprehensive picture of resistance to date, with data provided by 114 countries (3). It reveals that resistance to important antibiotics for treating common life-threatening infections has spread to all regions of the world. The report also shows that key tools to tackle antibiotic resistance such as basic systems to track and monitor the problem have major gaps or do not exist in many countries at all.

Appropriate antimicrobial treatment can shorten the duration and severity of illness, decrease morbidity and mortality, and reduce the duration of bacterial shedding (5, 6). However, antimicrobial resistance among the major bacterial causes of bloody diarrhoea is increasing worldwide (7). Resistance causes people to be sick for longer, increases the risk of death, increases the cost of health care due to lengthier stays in hospital and requirement for more intensive care (3).The emergence of multidrug resistance has exacerbated the problem and made the availability of effective antimicrobial therapy more difficult (6).The aim of our study was to establish the antimicrobial susceptibility patterns associated with enteric pathogens causing acute bloody diarrhoea and assess antimicrobial drug use for patients with acute bloody diarrhoea.

MATERIALS AND METHODS

The study was conducted at six sites; Kilifi County hospital, Bamba sub county Hospital and Vipingo health centre in Kilifi County and in AMREF Kibera health centre, Langata health centre and Riruta health centre in Nairobi County. A hospital based matched case control study was conducted with neighbourhood controls between January and December 2012. The ratio of cases to controls was 1:2 and the calculated minimum sample size was 198 cases and 396 controls. Patients presenting with acute bloody diarrhoea from the catchment areas of the six study sites were recruited in the study between January and December 2012. A case was defined as a person of any age who attended outpatient clinic at the selected health facilities in Kilifi and Nairobi West Sub-Counties between January and December 2012 with acute diarrhoea and visible blood in the stool. Cases with concomitant infection and those with persistent diarrhoea (lasting ≥14 days) were excluded from the study.

Stool swabs and specimens were inoculated onto the surface of MacConkey agar (MAC) and eosin methylene blue agars and streaked for colony isolation. The culture plates were incubated at 37°C for 24h., the non-lactose fermenting colonies (NLFs) were picked and subjected to a Gram stain. Subsequently, all the Gram –ve colonies were picked from the respective plates and prepared for biochemical identification using the semi-automated bacterial identification systems, the Vitek 2®. The colonies were briefly emulsified into 0.45% normal saline solution for the system to attain a 0.5-0.63 McFarland standard strength. The Gram -ve identification cassette was inserted into the respective tubes and then into the system. After 18-24h.of incubation the biochemical reactions were obtained through a print-out from the machine.

Bacterial isolates recovered from stool samples of study participants were investigated. Antimicrobial susceptibility testing was done by disk diffusion on Muller Hinton agar using Kirby-Bauer technique (8).Commercial discs were used according to the manufacturer's instructions (Becton, Dickson and company, Maryland USA). Susceptibility to penicillin antibiotics was tested using ampicillin (10 μg) while susceptibility to cephalosporin was determined using ceftriaxone (30ug). Ciprofloxacin (5 μg), norfloxacin(10 μg), and nalidixic acid (30 μg) were used for testing susceptibility to the quinolones. Tetracycline antibiotics included doxycycline (30 μg) and tetracycline (30 μg). Other antibiotics included were chloramphenicol (30μg), sulphamethoxazole (23.75 μg)-trimethoprim (1.25 μg) and amoxicillin-clavulanic acid (30 μg).Minimum inhibitory concentration was determined by broth dilution techniques as per the Clinical and Laboratory Standards Institute (9). Multidrug resistance was defined as resistance of a bacterial isolate to 3 or more antibiotics whereas high resistance was defined as resistance of more than 20%(8, 10,11).

Data were collected using two different forms; a case report form and laboratory surveillance form. The case report form was filled for all the cases and the information collected included;demographic data, vital signs, signs and symptoms, disease history and treatment information. The laboratory based surveillance form accompanied the stool samples to the laboratory.

Ethical approval and ethical considerations: Approval to carry out this study was obtained from the Ministry of Health and Kenya Medical Research Institute (KEMRI) Scientific Steering Committee (SSC No. 2177) and Ethical Review Committee (ERC).At the time of enrolment, written informed consent was obtained from participants who were 16 years and above and

from parents / guardians of children who were under the age of 16 years.

RESULTS

A total of 805 participants were enrolled (284 cases and 521 controls) into the study between January and December 2012. The mean age of the cases was 24.4 years with a range of 1month to 73 years while the mean age of controls was 27.5 years with a range of 3 months to 73 years. The proportion of females was 56% among cases and 67% among controls. About 61% of the cases and 63% of the controls resided in the rural areas. We report on the antimicrobial susceptibility aspect of the study.

Eighty(28.2%) bacterial isolates were recovered from 284 stool samples collected from cases presenting with acute bloody diarrhoea. The bacterial isolates comprised of; Sixty seven (67)*Shigella* species (40 *S. flexneri*, 8 *S.boydii*, 8 *S.sonnei* and 11 *S.dysentriae*), Nine (9) Enteroinvassive *Escherichia coli* isolates, three (3) *Salmonella enterica* serovar Typhi and one (1) *Yersinia enterocolitica*. The isolates were assessed for their antimicrobial susceptibility;high resistance to sulfamethoxazole-trimethoprim (86.3%), tetracycline (73.8%) and ampicillin (63.8%) was observed. The isolates had low resistance to nalidixic acid (3.8%) and ciprofloxacin (2.5%) while there was no resistance to ceftriaxone (Figure 1).

Shigella isolates had high resistance to sulfamethoxazole-trimethoprim (97%), tetracycline (83.6%) ampicillin (58.2%) and chloramphenicol (20.9%). The isolates showed low resistance to nalidixic (4.5%) and ciprofloxacin (3.0%) while there was no resistance to ceftriaxone (Table 1). Analysis of the resistance patterns by strains shows that; *S. dysentriae, S. sonnei* and *S. boydii* were 100% resistant to sulphamethoxazole-trimethoprim while *S. flexneri* was 95% resistant. There was no resistance to ceftriaxone across all the four species. Resistance to tetracycline ranged between 75% and 87.5% across all the *Shigella* spp. The resistance to ampicillin was higher for *S.boydii* (87.5%) than for *S.flexneri* (52.5%) and *S. dysentriae* (54.5%). *Shigellaf lexneri* had a 5% resistance to ciprofloxacin while the other 3 strains did not show any resistance to ciprofloxacin. *Shigella sonnei* was the most resistant to chloramphenicol (37.5%) while *S. boydii* was the least resistant (12.5%) (Table 1).

High levels of antimicrobial resistance were observed among the Enteroinvassive *Escherichia coli* and *Salmonella* typhi isolates. All the nine Enteroinvassive *Escherichia coli* isolates were highly resistant to: sulfamethaxazole-trimethiprine (88.9%), chloramphenicol (77.8%), ampicillin (66.7%), tetracycline (55.5%) and nalidixic acid (33.3%). The isolates were found to be 100% sensitive to ciprofloxacin but showed emerging resistance to ceftriaxone (11.1%) and amoxicillin-clavulanic acid (11.1%)(Figure 2). All the three *Salmonella* typhi isolates were highly resistant to; sulfamethaxazole-trimethiprine (100%), tetracycline (66.7%), chloramphenicol (66.7%) (MIC≥20) and ampicillin (66.7%) (MIC≥32) but 100% sensitive to ciprofloxacin (MIC≥1.0), nalidixic acid and ceftriaxone (MIC≥1.0).

Out of the 80 isolates, 12 (15%) were resistant to one drug, 13 (16.3%) to 2 drugs, 26 (32.5%) to 3 drugs and 29 (36.3%) were resistant to 4 drugs (Figure 3). The overall prevalence of multidrug resistance for the bacterial pathogens was therefore 68.8%.Out of the 67 *Shigella* isolates, 36 (53.7%) had multidrug resistance. Eight (88.9%) out of the nine Enteroinvassive *Escherichia coli* isolates were multidrug resistant. Only one *E.coli*isolate had no resistance to any antibiotic while three isolates were resistant to three drugs, two isolates were resistant to four drugs while 3 isolates were resistant to five drugs. The level of multidrug resistance varied among the four *Shigella* strains. *Shigella boydii* had the highest (62.5%) while *S. Sonnei* had the least (50.0%). Of the 3 S*almonella* typhi isolates, 1 (33.3%) was resistant to one drug while 2 (66.7%) were resistant to 4 drugs. Antibiotic prescriptions were given to 243(85.6%) of the patients presenting with acute bloody diarrhoea. Among these, 94 (38.7%) were given prescriptions for ciprofloxacin, 53 (21.8%) for sulfamethaxazole-trimethiprine while 26 (10.7%) for chloramphenicol. Tetracycline / doxycycline, amoxicillin / ampicillin, nalidixic acid and ceftriaxone were prescribed to 14.8%, 3.7%, 2.9% and 0.4% of the patients respectively. A total of 123 (51%) received antibiotics which were ranked to have high resistance (figure 4).

Figure 1
Overall drug susceptibility to positive bacterial isolates

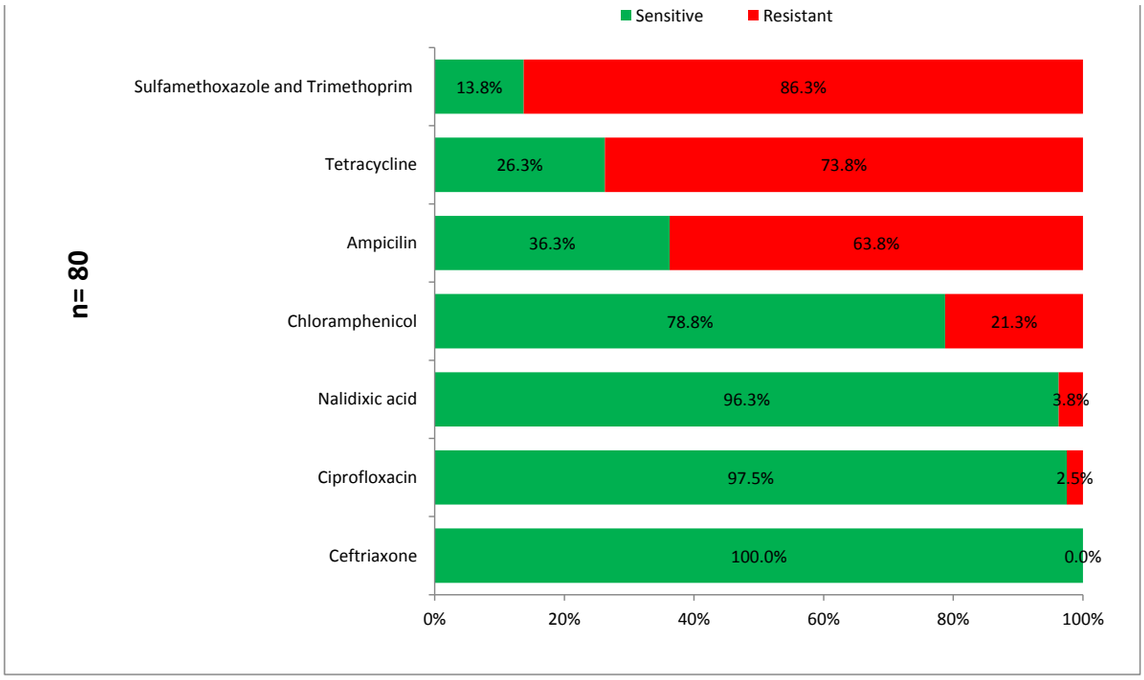

Figure 2
Antimicrobial resistance among the isolates of the Escherichia Coli genera

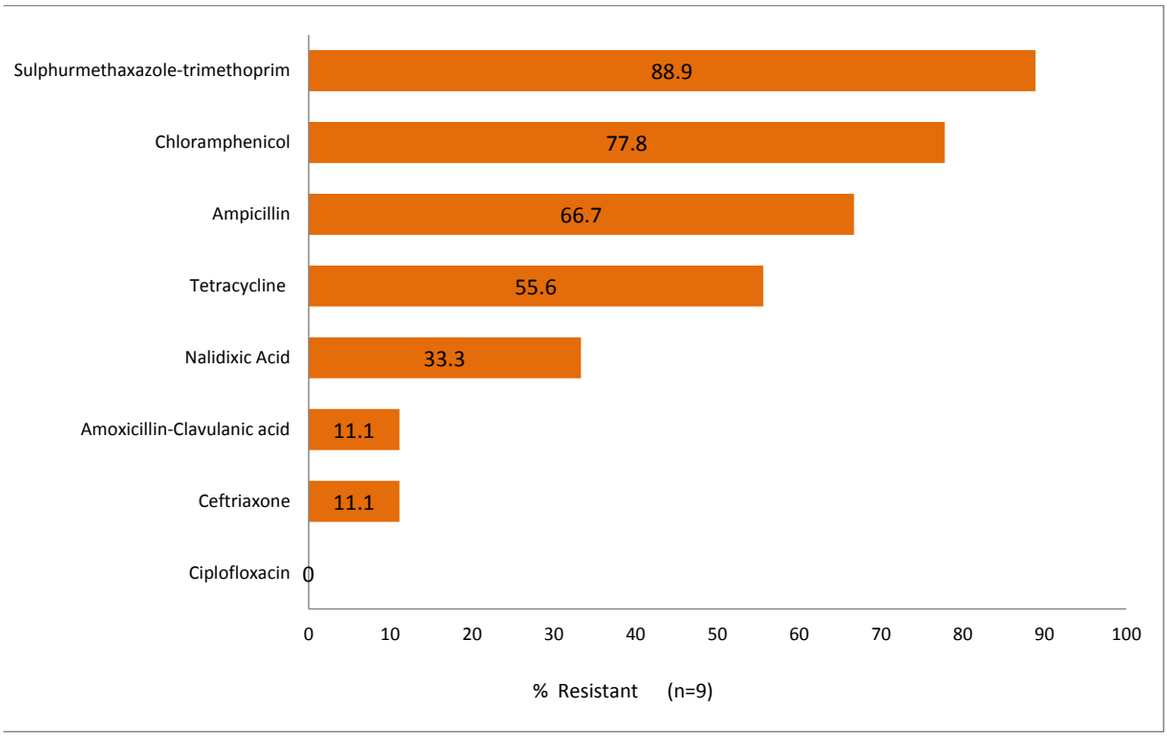

Figure 3
Distribution of multidrug resistance on the bacterial isolates

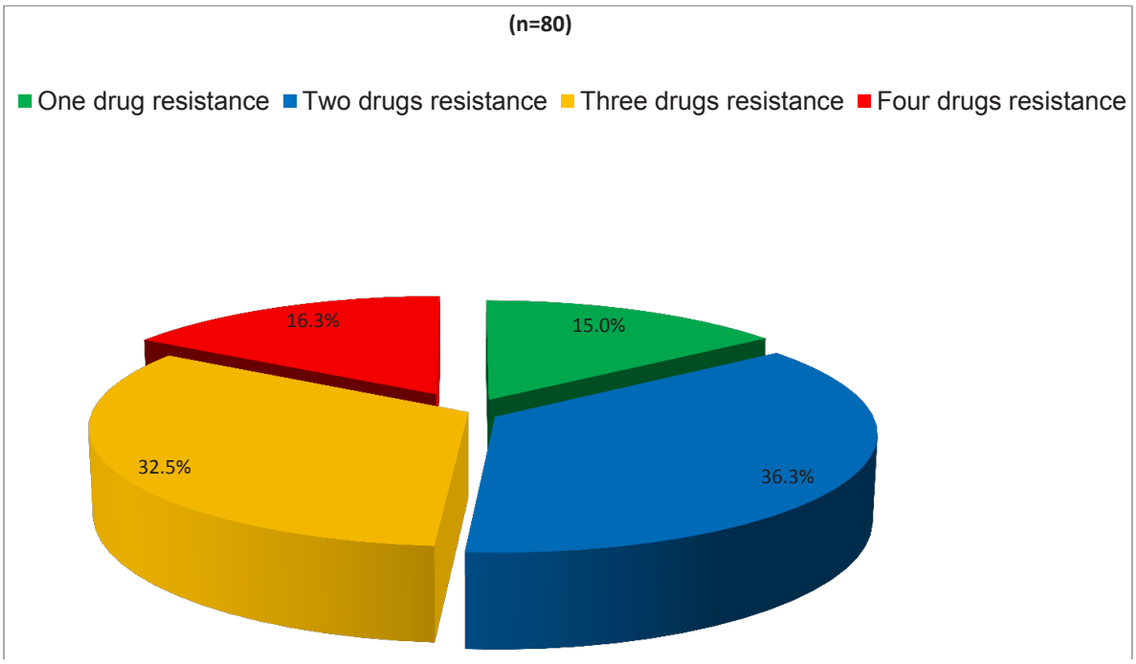

Figure 4
Comparison between prescribed antibiotics and antimicrobial resistance level

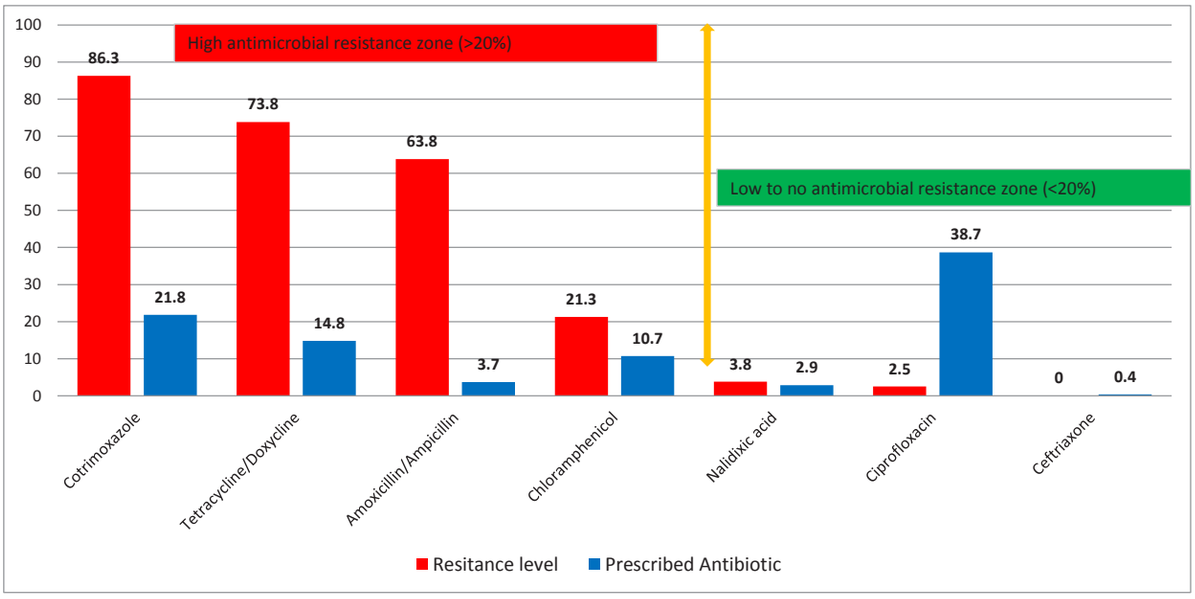

Table 1
Antimicrobial resistance among the isolates of the Shigella genera

	Sulfamethoxazole - Trimethoprim Resistant	Tetracycline Resistant	Ampicillin Resistant	Chloramphenicol Resistant	Nalidixic Acid Resistant	Ciprofloxacin Resistant	Ceftriaxone Resistant
Shigella Species (n=67)	97.0%	83.6%	58.2%	20.9%	4.5%	3.0%	0.0%
S. flexneri (n=40)	95.0%	85.0%	52.5%	20.0%	5.0%	5.0%	0.0%
S. dysenteriae (n=11)	100.0%	81.8%	54.5%	18.2%	9.1%	0.0%	0.0%
S. boydii (n=8)	100.0%	75.0%	87.5%	12.5%	0.0%	0.0%	0.0%
S. sonnei (n=8)	100.0%	87.5%	62.5%	37.5%	0.0%	0.0%	0.0%

DISCUSSION

Antibiotics misuse by both general public and health professionals is rampant in low income countries where laboratory facilities are limited. High antimicrobial resistance to the commonly prescribed antibiotics like sulphamethoxazole-trimethoprim (86.3%), tetracycline (73.8%), ampicillin (63.8) and chloramphenicol (21.3%) observed among enteric bacterial pathogens in our study is similar to that found in other studies done in Kenya (5, 12). In our study, *Shigella* species exhibited high resistance to these antibiotics; sulphamethoxazole-trimethoprim (97%), tetracycline (83.6%), ampicillin (58.2%) and chloramphenicol (20.9%). These findings agree with studies done in other parts of the world which indicate that resistance to low cost antibiotics has reached unprecedented proportions. Much higher resistance levels to ampicillin (97%), tetracycline (95%) and chloramphenicol (94%) have also been reported (13).In other studies, a high degree of resistance among the *Shigella* species to tetracycline 74%-97%, ampicillin 85%-100%, trimethoprim-sulfamethoxazole 69%-97% and chloramphenicol 77% have been demonstrated(14-16). Originally, these antibiotics were effective in many developing and developed countries but *Shigella* strains rapidly developed resistance to these agents. These are low-cost and widely available antibiotics that may be bought over the counter without prescription in many drug outlets in Kenya. The increase in resistance of *Shigella* species tosulphamethoxazole- trimethoprim and ampicillin has been reported worldwide (17-19).

The World Health Organisation has developed and applied criteria to rank antimicrobials according to their relative importance in human medicine, the ranking has the critically important, very important and important antimicrobials (20). There is, therefore, an urgent need to address the emerging resistance to the antimicrobial agents classified as critically important like the quinolone (nalidixic acid and ciprofloxacin) and third and fourth generation cephalosporin. The widespread use of nalidixic acid as a first-line drug for dysentery in many countries has resulted in the emergence of resistant strains. To date, this antimicrobial agent is no longer recommended as a first-line drug in the international guidelines, and the use of ciprofloxacin is currently encouraged instead.

High resistant S*higella*i solates to nalidixic acid have been reported in China (75%-94%) and countries in South Asia region like India, Nepal and Bangladesh which exceeded 60% (14,16,21). In a study done for 18 years (1990-2007), *Shigella sonnei* resistance to nalidixic acid increased from 1998 to reach a peak of 12.8% in 2004(22). Our findings suggest limited or minimal(3.8%) resistance to nalidixic acid, the findings collaborates with studies done in Madagascar, Senegal and United States where very limited nalidixic acid resistance in *Shigella* isolates was observed, 0.7%, 1% and 1% respectively (23-25). In the neighbouring country Tanzania and a published report on antibiotic use and resistance in Mozambique showed no resistance to nalidixic acid among the S*higella* spp (26, 27). Our findings further eluded, variation of nalidixic acid sensitivity among the *Shigella* strainsexist, all *S. boydii* and *S. sonnie* were sensitive while *S. flexneri* and *S. dysentriae* showed some emerging levels of resistance, 5% and 9% respectively. Emergence of ciprofloxacin resistance *S.flexneri* (2.5%) observed in our study was not reported from some studiesdone in Kenya, Tanzania, Senegal, Madagascar and Mozambique where all *Shigella* strains were sensitive to ciprofloxacin (5, 23, 24, 26, 27). High fluoroquinolones (ciprofloxacin) resistant *Shigella* species have been report in India, the level of resistance range between 30% and 55% (13, 21). In the United States, very limited (<1%) ciprofloxacin resistant *Shigella* species have been reported (25). Findings from the 18 years study in Belgium reported no ciprofloxacin resistance to *Shigella sonnei* isolates (22).

Resistance to three or more antimicrobial agents was used to define multidrug resistance (MDR) profiles (11). In our study, over half of the isolated *Shigella*species were multi drug resistant. The level of multidrug resistance varied among the four *Shigella* strains. *Shigella boydii* had the highest Multidrug resistance at 62.5% (5/8); *S. dysentriae* had 54.6% (6/11), *S. flexneri* had 52.5% (21/40) and *S. sonnei* had the least at 50.0% (4/8). The most common resistance pattern detected in *Shigella* strains combined sulphamethoxazole- trimethoprim, tetracycline and ampicillin. Our findings on multidrug

resistant *Shigella* collaborate with other studies done in some parts of Asia and Europe where 78.3% of the *S.flexneri* and 74.3% of the *S.boydii* were resistant to at least three antibiotics (14).Multidrug resistance in the predominant *S. sonnei* emerged in 2007, with 82% of total isolates being MDR (22). All (100%) *Shigella* species isolated in Iran were found to be multidrug resistance to at least three classes of antimicrobial agent (28). Currently, no vaccines against *Shigella* infection exist, both live and subunit parenteral vaccine candidates are under development (29).

Our study demonstrated high levels of antibiotic resistance to *Escherichia coli* isolates to commonly used antimicrobials; sulfamethoxazole-trimethoprim, chloramphenicol, ampicillin, tetracycline, and nalidixic acid. In the neighbouring country Tanzania, Diarrheagenic *E. coli* as a group exhibited high levels of antimicrobial drug resistance in diarrhoea cases to sulfamethaxazole-trimethiprine(86.9%),ampicillin (84.6%), tetracycline (80%), and chloramphenicol (42.3%) but were highly susceptible to quinolones like nalidixic acid (1.5%) and ciprofloxacin (0%)(30). Similar findings were reported where diarrheagenic *E.* coli pathotypes exhibited high levels of antimicrobial drug resistance to ampicillin (85%), sulfamethaxazole-trimethiprine (79%), tetracycline (65%), and nalidixic acid (28%) (31). Our study showed emerging resistance to third-generation cephalosporins (ceftriaxone) among the Enteroinvassive*Escherichia coli* pathotype, this finding collaborate with other findings where *E.coli* pathotypes showed low levels of resistance (32).

Multidrug resistant *Salmonella Typhi* to four drugs (sulfamethoxazole -trimethoprim, tetracycline, chloramphenicol and ampicillin) was reported in all (100%) our three isolates. All the isolates were 100% resistant to sulfamethoxazole-trimethoprim while the other three drugs had 66.7% resistance. These findings collaborates with other findings where the *Salmonella Typhi* was reported to be 70% multidrug resistant; sulfamethoxazole-trimethoprim (73%),tetracycline (62%), chloramphenicol (74%) and ampicillin (75%) (33). Similar findings on multidrug resistance *Salmonella Typhi* have been reported in the neighbouring country Uganda, where all isolates were found to be 76% resistant to five antibiotics including, ampicillin, tetracycline, sulfamethoxazole-trimethoprim. Chloramphenicol was resistant to 5% of the isolates while no resistance to nalidixic acid and ciprofloxacin was reported(34). No resistance to nalidixic acid, ciprofloxacin and ceftriaxone was reported in our study.Multidrug resistant S*almonella Typhi* has also been reported in Malawi and Mozambique (2009) where 100% (42) isolates tested were resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole while 4 (9.5%) were also resistant to nalidixic acid (35). The first-line antimicrobials should no longer be used as empirical treatment of suspected salmonellosis, instead parenteral third-generation cephalosporin such as ceftriaxone remain highly effective, representing possible treatment options for severe infections, especially among hospitalised patients.

Despite these findings highlighting the high resistance of *Shigella* to commonly prescribed antibiotics as well as recommendations by World Health Organisation encouraging the use of ciprofloxacin for the management of acute bloody diarrhoea, these high resistance antibiotics are still unnecessarily prescribed by health care providers or unnecessarily purchased directly by consumers. In our study, about 51% of the acute bloody diarrhoea patients were given prescriptions of antibiotics which had high antimicrobial resistance. Empirical treatment of bloody diarrhoea cases with highly resistant antibiotics by clinicians exacerbates the misuse of antibiotics. In spite of the seriousness of the issue, high antibiotic resistance is still not widely recognized as a problem, even among the health professionals. Raising awareness about resistance and educating health professionals, policy makers and the public can help to improve the rational antibiotic use.

In conclusion, the high levels of antibiotic resistance coupled with multidrug resistance among the commonly prescribed antibiotics like sulphamethoxazole-trimethoprim, tetracycline, ampicillin and chloramphenicol as well as high prescriptions of the antibiotics with high resistance calls for urgent reinforcement of the regulatory mechanism on the rational use of antibiotics and an all-round behaviour change. Although the drugs are obsolete, they are still preferred because of low cost and are readily available in most health facilities and drug outlets in Kenya. The emerging resistance to ciprofloxacin and ceftriaxone poses a serious public health threat in the management of enteric diseases in Kenya. Levels of resistance to ciprofloxacin and ceftriaxone should be constantly monitored to detect any shift in the quinolones and third generation cephalosporin resistance in order to measure the level of antibiotic pressure. These antimicrobials represent the critically important antibiotics used in the treatment of enteric diseases.To improve the quality of patient care, there is urgent need for a rational use of antimicrobials.

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