

Antibacterial Spectrum and Susceptibility of Bacterial Pathogens Causing Diarrheal Illnesses: Cross Sectional Study of Patients Visiting Health Facility in Lake Victoria Region - Kenya

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ABSTRACT

Diarrheal illness has been studied extensively among children under five years, however little information is known among population over five years. This study determined the spectrum and antibiotic susceptibility of bacterial pathogens causing diarrhea in above five-year-old patients in a health facility along Lake Victoria region. In a cross-sectional study, 400 patients aged five years and above with diarrhea visiting Port Victoria health facility along Lake Victoria region were recruited in this study from 2013 to 2016. Stool samples were collected and cultured following standard microbiological procedures. Bacterial agents were isolated, identified and subjected to antibiotic susceptibility test. A total of 317 organisms (283 bacteria, 31 parasites and 3 others) were identified from 303 (76%) patients. Most frequently isolated pathogens being Non O1 *Vibrio cholerae* 98(30.9%), *Salmonella species* 97(30.6%), *Shigella species* 47(14.8.0%) and *Aeromonas species* 38(12.0%). Non O1 *Vibrio cholerae* isolates were less sensitive to tetracycline, ampicillin and nalidixic acid. *Salmonella species* were less sensitive to ampicillin but sensitive to nalidixic acid. *Shigella species* isolates were more sensitive to chloramphenicol, ciprofloxin and nalidixic acid. Similar trend was observed for *Aeromonas species* isolates. The susceptibilities expressed were fairly good. However, some of the resistances shown pose a threat to public health, especially in unexposed populations. With the observed trends in this study, bacterial species have responded differently to antibiotics. It may be beneficial for clinicians to consider antibiotic susceptibility testing prior to management of patients to slow down resistance development.

Key words: Diarrheal illness, Bacterial pathogens, Antibiotic susceptibility, *Vibrio cholerae*, *Salmonella*

INTRODUCTION

Diarrheal diseases are common world-wide and among the leading causes of morbidity and mortality in developing nations. World Health Organization (WHO) report showed that diarrheal disease was the fifth most common infection accounting to mortality of 3.7% (2.2 million) in 2004¹. In 2010, 1.4 million people, across all ages, died from diarrheal diseases, and the age-standardized mortality rate was 20.9 per 100,000, which has fallen to 49% between by 2010². Diarrhea is the global second leading cause of mortality, and nearly one in five children under the age of five die as a result of dehydration, weakened immunity or malnutrition associated with diarrhea³.

Mortality rates among children declined globally from 146 per 1,000 in 1970 to 79 per 1,000 in 2003⁴. However, the situation in Africa is strikingly different compared with other regions of the world. African region shows the smallest reductions in mortality rates and the most marked slowing down trend⁵. Diarrheal illness is the third leading cause of morbidity and mortality in children younger than 5 years of age in Africa and were responsible for 30 million cases of severe diarrhea and 330,000 deaths in 2015⁶. Diarrhea remains a major public health problem in East African, for instance, the prevalence of diarrhea among children less than five years ranged from 11-54% with an average of 27% in Kenya, Ethiopia, and Somalia from 2012 to 2017⁷. Between

2001 and 2003, diarrhea was responsible for 11.2 % hospitalized cases in Kenya. Fatality rate was 8.0% with more adults than children⁸. Diarrhea is the third most common pediatric diagnosis in rural western Kenya and particularly among adults with HIV infection^{9,10,11}. Although extensive diarrheal studies have been carried out in children below the age of five years, paucity exists in individuals older than this age. Therefore, this study sought to determine bacterial agents causing diarrhea in individuals older than five years and also determine the antibiotic susceptibility test using commonly available antibiotics.

Diarrhea is the passage of three or more loose stools per day by an individual¹². It is usually a symptom of gastrointestinal infection. Diarrhea diseases due to infection constitute a major burden of disease¹². Systematic analysis for the Global Burden of Disease (GBD) indicated that there are various aetiologies for diarrhea in the cause list for 2010². Diarrhea can be caused by bacteria, viruses or parasites^{13,14,15}. Among the major diarrheal bacteria include *Vibrio species*, *Salmonella paratyphi*, *Shigella*, *Enterotoxigenic E. coli*^{16,17,18}. In fact, some constitute acute infectious diarrhea¹⁹. Cholera remains an important cause of illness in many developing countries and has been estimated to result in more than 120,000 deaths each year²⁰. The most readily recognized bacterial agents of gastroenteritis in industrialized countries are *Salmonella* spp and *Campylobacter* Spp^{13,21,22}. Bacillary dysentery due to either *Shigella dysenteriae* type 1 or Entamoeba contributes to a large degree of bloody diarrhea illness based on reports from Kenya, Egypt, Ethiopia and Nicaragua^{23,24,25}. The various *Shigella species* can cause diarrhea or the syndrome known as bacillary dysentery. Severe acute diarrhea is more common in travelers and older people. Travelers are exposed to drinking contaminated water and eating contaminated food prepared by other people at their destinations whereas older people have lower immunity to fight against pathogens causing diarrhea²⁶. Globally, *Shigella* was the second leading cause of diarrheal mortality in 2016, accounting for 212 438 deaths among all ages and responsible for 63 713 deaths among children younger than 5 years²⁷.

World-wide, the etiology of bacteria causing diarrhea appears to differ depending on the geographical area. For example, a report from Spain indicated that *Campylobacter* spp. and *Salmonella* spp. were the primary bacterial pathogens, accounting for 22.2 % and 16.4% of cases of acute diarrhea in children respectively²⁸. In Ecuador, *Shigella* spp. and *Campylobacter jejuni* were reported to be the main etiological causes of diarrhea²⁹. Whereas in Turkey, *Salmonella* spp. (25.6%) and *C. jejuni* (18.3%) were the main causes of acute gastroenteritis in children³⁰. In developing countries, *Shigella*, non-

typhoidal *Salmonella* (NTS), *Campylobacter*, and *Yersinia* are the major enteropathogens causing diarrhea across all ages³¹. 22.2% diarrheal cases were caused by *Aeromonas* species in Pakistan and Bangladesh between 2009 and 2012³². A Case-Control Study in Ifakara, Tanzania reported 47.8% children with diarrhea caused by enteropathogen of which *Shigella* species was the most prevalent³³.

As much as in Kenya *Vibrio* species has been isolated from stool of diarrheal patients during outbreaks^{9,10,11} a prevalence of 18.8% and fatality rate of 2.5% was reported among diarrheal patients across all the ages⁹. *Salmonella* species have been isolated in studies carried out in Kenya^{34,35,36,37,38,39,40,41,42,43}. However, a prevalence of 14 % was reported among children of less than 12 years of age⁴⁴. In a study carried out in Kenya on patients with sporadic bloody diarrhea, *Shigella* species was the most prevalent pathogen affecting 44% of the studied population followed by *Salmonella* species with 17%⁴⁵. In two population-based studies, 22.3% and 23% incidences of shigellosis in Nairobi, Kenya were reported^{46,47}. *Aeromonas* associated diarrheal study in Kenya reported 0% isolation of this pathogen from less than 5 years old children with diarrhea³². However *Aeromonas Hydrophila* was isolated from River Njoro in Kenya in 2012⁴⁸. Another study in India reported 17.7% prevalence of *Aeromonas hydrophila* from patients with diarrhea⁴⁹. The assumption was, there could be other more bacterial causing diarrheal illness. Therefore, this study was designed with an aim of determining if there could be other more bacterial pathogens causing diarrhea in addition to the ones that are documented.

In Africa, including Kenya, the diagnosis of diarrheal illness is based on clinical and laboratory investigations of pathogens causing diarrhea. Clinically, the doctor investigates the patients based on the presenting syndromes i.e. acute watery stool, three or more episodes of diarrhea per day or bloody diarrhea by examining the rectum. Laboratory investigations involve two aspects i.e. the use of simple microscopy technique on a stool sample to be able to identify protozoa or helminthes causing diarrhea. The second laboratory diagnosis is microbiological culture of stool sample to be able to identify bacteria causing diarrhea.

In treatment of different bacterial diseases, antibiotics such as tetracycline, doxycycline, erythromycin and streptomycin are generally used⁵⁰. Antimicrobial resistance is one of the most important public health problems that directly relates to disease management and control⁵¹. Resistance to antibiotics is an increasingly common problem in human medicine. Its management is the subject of urgent debate. Efforts to reduce this resistance are based on the assumption

that it is maintained in bacterial populations as a result of exposure to antibiotics, and restricting the use of antibiotics⁵². With few exceptions, the major classes of antibiotics used to manage infections with enteric bacteria include the beta-lactams (penicillin derivatives), carbapenems, the fluoroquinolones, the aminoglycosides, and TMP-SMZ. Because most *Enterobacter* species are either very resistant to many agents or can develop resistance during antimicrobial therapy, the choice of appropriate antimicrobial agents is complicated^{53,54}. In Kenya there are diverse commonly prescribed antibiotics for treating infections caused by enteric bacteria. These include, tetracycline, gentamicin, doxycycline, norfloxacin, ciprofloxacin, streptomycin, chloramphenicol, cotrimoxazole, Nalidixic acid, Ampicillin, cefotaxime e.t.c⁴⁷. Antibiotics such as tetracycline, doxycycline, norfloxacin, ciprofloxacin and streptomycin are used with adjunct in rehydration therapy and are critical in the treatment of septicemia patient^{50,55,56}. Resistance to many of these drugs in general *Vibrio* pathogens have been reported in various African countries including Kenya^{51, 57,58,59,60,61,62}. Antibiotic resistance of bacterial pathogens isolated from childhood diarrhea in four provinces of Kenya reported resistance of 100% of *Vibrio* pathogens to Nalidixic acid and chloramphenicol; sensitivity of 100% to ciprofloxacin and tetracycline and resistance of 50% to ampicillin⁶⁰.

Studies carried out for more than 10 years ago have reported resistance of *Salmonella* strains to commonly prescribed antibiotics in some parts of Kenya^{34,35,36,37,38,39,40,41}. A study carried out in Kenya reported *Salmonella* level of resistance ranged from 44.4% to 75% for ampicillin; 22.2% to 50% for chloramphenicol; 33.3% to 66.7% for tetracycline; 0% to 25% for nalidixic acid and sensitivity of 100% to ciprofloxacin⁶⁰. Treatment of shigellosis by appropriate antimicrobial agents has proven efficacious in shortening the duration of fever, diarrhea and toxemia and apparently in reducing the risk of lethal complications as well⁶³. The increase in the number of *Shigella* isolates resistant to most of the antibiotics (sulfonamides, tetracyclines, ampicillin, and trimethoprim-sulfamethoxazole (TMP-SMX) available in countries where the choice of treatment is limited³⁴, represents an important health problem. The World Health Organization (WHO) recommends that all suspected cases of shigellosis based on clinical features be treated with effective antimicrobials (antibiotics)⁶⁴. If *Shigella* dysenterea has been isolated, Beta-lactams (Ampicillin, amoxicillin, third-generation cephalosporins (cefixime, ceftriaxone), and pivmecillinam); Quinolones: (Nalidixic acid, ciprofloxacin, norfloxacin, and ofloxacin); Macrolides (Azithromycin) and others (sulfonamides, tetracycline,

cotrimoxazole, and furazolidone) can be used for treatment. Resistance of this organism against antibiotics is has been reported in Kenya¹². A study carried out among children in four provinces of Kenya reported sensitivity of 75% to 100% of *Shigella* pathogen to ciprofloxacin and nalidixic acid; 50% to 100% to chloramphenicol; 20% to 100% to tetracycline and resistance of 33.3% to 80% to ampicillin⁶⁰.

Rationale

From the literature, diarrheal illness has been studied extensively among children under five years, however, little information is known among the population over five years. Furthermore, different levels of resistances and sensitivities of these organisms against commonly prescribed antibiotics have been reported in some parts of Kenya^{12,60,61,62}. However, little information has been reported in the current study site. Therefore, this study was designed to determine etiological agents causing diarrheal illnesses among individuals of five years and above and determine antibiotic susceptibility profile of these enteric pathogen and advice the clinicians on the appropriate antibiotics to use for managing diarrheal patients.

METHODS

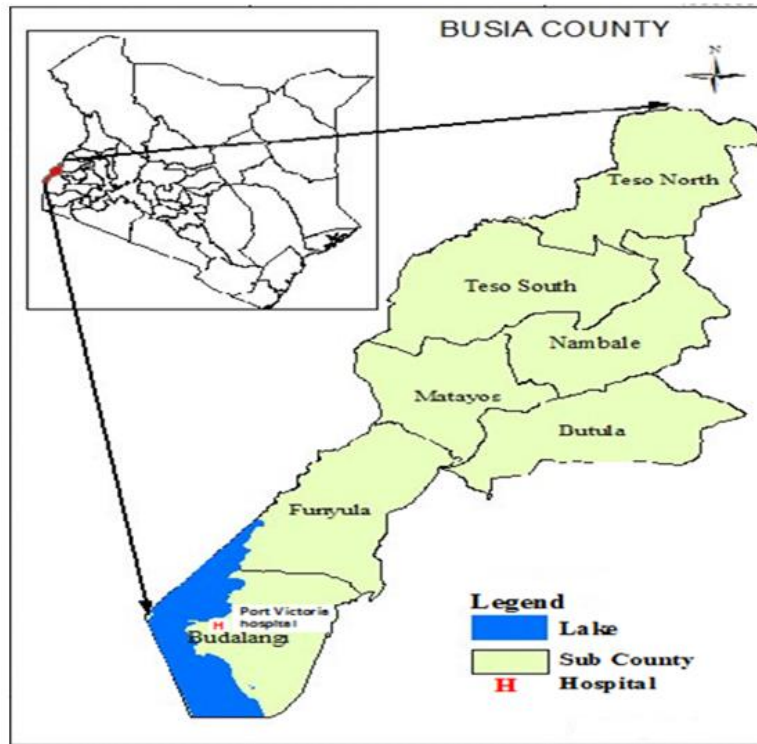
Study design

This was a cross sectional study involving patients presenting at Port Victoria hospital with three or more episodes of diarrhea per day during rainy season (April, May, June) and (August and September). A total of 100 samples were collected every year within these five months, 20 samples per months and an average of 1 sample per-day.

Study site

The study took place at Port Victoria Sub-county hospital (Figure 1) in Busia County, Kenya. This health facility borders Lake Victoria and located about 2 km away. Majority of residents are fisher folk from Luo and Luhya communities. The hospital offers the following services: Antenatal, Antiretroviral Therapy, Basic comprehensive Emergency Obstetric Care, Caesarean, Curative In-patient Services, Curative Outpatient Services, Family Planning, Growth Monitoring and Promotion, HIV Counseling and Testing, Immunization, Integrated Management of Childhood Illnesses, Prevention of Mother to Child transmission of HIV, Radiology (e.g. X-ray, UltraScan, MRI, etc.), Tuberculosis Diagnosis, and Treatment, Laboratory and pharmaceutical services. Among the most diagnosed diseases are Malaria, HIV, diarrhea, pneumonia, tuberculosis etc. The climate of the area is warm and hot with two distinct seasons, dry and rainy.

FIGURE 1: A map of Kenya showing Port Victoria Sub-county hospital



Source: <https://www.google.com/search?g=kenya+busia+county+map>

Inclusion criteria

Patients with diarrhea of three or more episodes per day, who are five years and above
Patients willing to consent to participate in the study.

Exclusion Criteria

Patients with diarrhea of less than three episodes per day and patients below five years of age
Patients unwilling to consent to participate in the study.

Sample determination

The formula by fisher *et al.* 1998 was used to determine the minimum number of diarrheal patients to be sampled in this study. We suspected diarrheal illness is common perhaps 50% suspected cases during rainy season. The formula was

$$n = \frac{Z_{1-\alpha/2}^2 P(1-P)}{d^2}$$

where;

n=Sample size $Z_{1-\alpha/2}^2 = 1.96$ P= Expected proportion in population (50%)

d=Absolute precision.

Diarrheal causing pathogen isolation rate was assumed to be 50% and level of significance was 5%. Therefore, P=0.5, d=0.05 (5% absolute precision).

Therefore, $n = 1.96^2 \times 0.5 \times (1-0.5) / 0.05^2 = 384.16$ patients. This was rounded to 400 stool samples from the same number of patients.

Procedure for Study Participants

All patients attending Port Victoria Sub-County Hospital and who met the inclusion criteria were sampled during rainy seasons (April to June) and August to September. A total of 100 samples were collected every year within these five months, 20 samples per months and an average of 1 sample per-day. The study purpose was explained to the patients. Those who accepted to participate in the study were requested to sign consent for recruitment.

A questionnaire was used to collect socio-demographic information such as age, gender, area of residence and occupation. Guardians/parents of minors/children were interviewed on their behalf. Patients were recruited from the outpatient clinic. We defined any diarrhea as the passage of three or more liquid stools within 24 hours, or any number (more than three times) of loose stools within 24 hours as per

WHO definition. Stool samples were collected on the same day the patient arrived at the hospital with episodes of diarrhea. Identification of bacterial pathogens and antimicrobial susceptibility was conducted following standard microbiological processes.

Specimen Collection

Stool / rectal swabs were collected from patients presenting to the hospital with diarrhea during rainy season using sterile spatula and placed in sterile polybags. These were transferred into Carry Blair transport medium (Thermo Fisher Scientific Inc, USA). The specimen tubes were transported in cooler boxes from the health facility to KEMRI, CIPDCR– Busia laboratories within 48 hours. After stool processing, the culture plates were decontaminated before disposal. The study subjects benefited from free diagnosis.

Stool Processing

Microscopy Procedures

Microscopy is a technique that involves the evaluation of a stool sample under Columbus microscope (Olympus BH-2, Optical Co. Ltd, Japan) to determine the presence of ova and parasite that infect the lower digestive tract, causing symptoms such as diarrhea. Most parasites have more than one form through which they develop. Many have a mature form and a cyst and/or egg (ova) form. A small amount of the stool specimen was placed onto a microscope slide. Two drops of saline were added and mixed well and placed under a microscope. This was examined at X40 for presence or absence of ova or cyst.

Microbiological Procedures

A loopful of stool sample was inoculated into alkaline peptone water; pH 8.2 and incubated for 8 hours at 37°C. Subculture was done on TCBS (Central drug house Ltd, India), XLD (Hardy diagnostics, USA) and Mackonkey (Oxoid, UK) and further incubated at 37°C for 24 hours. Direct culture was carried out on TCBS, XLD, Campylobacter blood free agar (Oxoid, UK), and enrichment in Selenite F medium (Himedia laboratories PVT Ltd India) to isolate the bacterial pathogens. Rectal swabs were processed in the same manner.

Identification of Bacterial Pathogens

Standard methods for the identification of both Enterobacteriaceae and non-enterobacteriaceae was followed (Edward and Ewing). These included colony morphology on TCBS, XLD and Mackonkey; gram stain reaction and other biochemical tests like motility in saline, oxidase production and susceptibility to vibriostatic compound O/129 at 10 and 150 micrograms (Oxoid, USA). Further

identification was done on API20NE and API20E. (API bioMerieux, Sa, France).

Isolation and Characterization of Isolates

Within a maximum of 2 hours upon arrival at the KEMRI laboratories, the stool samples/rectal swabs were plated on TCBS using cotton swab and left to incubate overnight at 37°C. In the second day, TCBS plates were examined for growth of typical yellow or blue-green colonies. Further incubation was done for an additional 24 hours if no growth was identified.

Sensitivity to vibrio-static agent O129

Typical colonies were inoculated on blood agar with a sterile wire loop. Filter discs containing the vibrio-static agent O129, 10 µg and 150 µg, respectively were added to the agar surface. The resultant was incubated at room temperature for 30 minutes and then overnight at 37°C.

Oxidase test

On day 3, an oxidase strip was placed in a clean Petri dish. Using a sterile applicator stick, colonies from the TCBS agar plate were taken and rubbed in the strip. The immediate development of deep blue or purple colour was a positive test result. If there was no colour change or weak colour change after 10 seconds, this was considered a negative test result. *V. cholerae* O1 NCTC 11218 as positive control and *Escherichia coli* NCTC 10418 as negative control were used.

Motility Test Procedure

Motility refers to ability of bacteria to change direction. This is important when bacteria require moving away or towards repellents or attractants respectively. Motile bacteria are effective root colonizers and can swim towards root exudates or other nutrient gradients earlier than non-motile bacteria. A colony of a young (18 to 24 hour) culture growing on agar medium was touched with a sterile needle. This was stabbed down the center of the tube to about half the depth of the medium. This was then incubated at 35°C-37°C and examined daily for up to 7 days. Positive motility test was determined if organisms spread out into the medium from the site of inoculation.

A negative motility test was determined if the organisms remained at the site of inoculation. These Organisms were again placed on a microscope glass slide with a drop of distilled water; a similar slide was set as a control by adding a drop of saline. A small loop of culture was emulsified in saline using separate wires and this was covered by a glass cover slip. This was examined under x40 and oil immersion

lenses and the presence/absence of motility were confirmed.

Agglutination Test Procedure

Agglutination is the clumping of cells such as bacteria or red blood cells in the presence of an antibody or complement. An agglutination test is carried out on slides and is called slide agglutination tests. Serotyping of bacteria is a good example of slide agglutination tests. For bacterial serotyping, a saline emulsion of the bacterial colony is made on a glass slide and a drop of specific antiserum is added and mixed. Antibodies bind to the antigens on the surface of bacteria and aggregate them resulting in visible clumps. One drop of saline and one drop of anti-sera was placed on microscope glass slide. The test organism was then emulsified (flame between each drop). The slide was rotated for 30 seconds and the presence of visible agglutination recorded. The presence of agglutination in the anti-sera denoted a positive reaction. Non- agglutination was observed in the saline portion.

Drug Susceptibility Testing Procedure

A susceptibility analysis is a test that determines the “sensitivity” of bacteria to an antibiotic. It also helps the doctor determine which drugs are likely to be most effective in treating the infection. A sterile wire loop was used to inoculate pure colonies of bacterial isolates onto Muller Hinton agar (Himedia Laboratories PVT Ltd, India) plate. About three to four different antibiotic disks was spread on each agar plate containing bacterial inoculums. The plate was sealed using a parafilm and incubated for 12-24 hours at 37°C. Antibiotic inhibition zones were taken using a ruler.

The results noted down was interpreted either as sensitive or resistant depending on the standard measurement of a specific antibiotic disks.

Five antibiotics were used for susceptibility testing, Chloramphenicol, Ciprofloxacin, Ampicillin, Nalidixic acid and tetracycline.

Data Analysis

Collected data was cleaned and entered into a Microsoft excel for Windows database developed for this purpose. Analysis was done by SPSS version 20. Univariate analysis was carried out to summarize socio-demographic factors and other frequencies. Descriptive statistics was done to determine the frequency of bacterial pathogens from stool samples.

Ethical considerations

Ethical approval for this study (KEMRI/SSC 1503) was sought from the national ethical review committee at KEMRI. The study set-up and purpose were explained to the study participants/caregivers after which written informed consent was sought. For confidentiality purposes, patients’ names were not used on labels, on forms, or elsewhere. Instead unique patient identification numbers were used for identification of samples.

RESULTS

Out of 400 participants (male, 187 and female, 213) had the median age of 20 years with a range of 5–60 years. A total of 171(42.8%) patients had no formal education, 123 (30.8%) had attained primary education, 86 (21.8%) had secondary education and 20 (5.0%) had gone to college and university (tertiary education). 200 (50%) patients were involved in fishing, 50 (12.5%) were small scale traders, 20 (5%) were small scale farmers and 30 (7.5%) were fishing at the same time students and 50 (12.5%) students and 50 (12.5%) pupils (*Table 1*). The study sought to determine the spectrum of bacterial pathogen causing diarrhea among patients visiting Port Victoria hospital facility along Lake Victoria. From the findings, Non O1 *Vibrio Cholerae* and *Salmonella* pathogens seem to cause majority of diarrhea than the rest (*Table 2*).

TABLE 1. Socio-demographic Information of Participants

Age (years)	Gender		Total (%)
	Male	Female	
5 – 12	78 (19.5)	72 (18.0)	150 (37.5)
13 – 17	54 (13.5)	60 (15.0)	114 (28.5)
≥ 18	55 (13.8)	81 (20.3)	136 (34.0)
Total	187	213	400(100.0)

Education			
No Education	71 (17.8)	100 (25.0)	171 (42.8)
Primary	59 (14.8)	64 (16.0)	123 (30.8)
Secondary	50 (12.5)	36 (9.0)	86 (21.5)
Tertiary	7 (1.8)	13 (3.2)	20 (5.0)
Total	187	213	400

Occupation			
Fishing	111 (27.7)	89 (22.3)	200 (50.0)
Small scale traders	35 (8.7)	15 (3.8)	50 (12.5)
Small scale farmers	6 (1.5)	14 (3.5)	20 (5.0)
Fishing and students	21 (5.3)	9 (2.2)	30 (7.5)
Students	22 (5.5)	28 (7.0)	50 (12.5)
Pupils	27 (6.8)	23 (5.7)	50 (12.5)
Total	222	178	400

TABLE 2. Number (%) of Bacteria Pathogens Isolated and Parasites Identified n=317

Bacterial Pathogens	Frequencies n (%)
<i>Non O1 Vibrio Cholerae</i>	98 (30.9)
<i>Salmonella species</i>	97 (30.6)
<i>Shigella species</i>	47 (14.8)
<i>Aeromonas species</i>	38 (12.0)
<i>Aeromonas and Giardia lamblia</i>	3 (0.9)
<i>Giardia lamblia and Schistosoma mansoni</i>	11 (3.5)
<i>Giardia lambla</i>	6 (1.9)
Others	3 (4.4)

Sensitivity patterns of *bacterial species* to commonly prescribed antibiotics were determined. out of 98 non O1 *Vibrio cholerae* isolates, 86.7% were sensitive to chloramphenicol; 76.5% to ciprofloxacin; 65.3% to Tetracycline; 63.3% to Ampicilin and 53.1% to Nalidixic acid. Sensitivity patterns of *Salmonella* species to commonly prescribed antibiotics were also determined. out of 97 *Salmonella* species isolates, 80.4% each were sensitive to chloramphenicol and ciprofloxacin; 79.4% to Tetracycline; 50.5% to Ampicilin and 70.1% to Nalidixic acid in. Out of 47 *Shigella* species isolates, 76.6% were sensitive to chloramphenicol; 74.5 to ciprofloxacin; 61.7% to Ampicilin; 70.7 to Nalidixic acid and 70.2% to Tetracycline. Sensitivity

patterns of *Aeromonas species* to commonly prescribed antibiotics were determined. Majority of the bacteria responded well to Chloramphenicol, Ciprofloxacin and Nalidixic acid and poorly to ampicillin. Out of 41 *Aeromonas* species isolates, 73.2% were sensitive to chloramphenicol; 73.2% to ciprofloxacin; 53.7% to Ampicilin; 70.7 to Nalidixic acid and 68.3% to Tetracycline (**Table 3**). A total of 317 organisms (283 bacteria, 31 parasites and 3 others) were identified from 303 patients. These were: 98(30.9%), Non O1 *Vibrio cholera*; 97(30.6%), *Salmonella species*; 47(14.8.0%), *Shigella species*; 38(12.0%), *Aeromonas species*; 6(1.9%), *Giardia lambla*. 3(0.9%), others. About 3(0.9%) had a mixed infection of

Aeromonas species and *Giardia lamblia* and 11(3.5%), *Giardia lamblia* and *Schistosoma mansoni* (**Table 2**). Sensitivity patterns of *bacterial species* to commonly prescribed antibiotics were determined. out of 98 non O1 *Vibrio cholerae* isolates, 86.7% were sensitive to chloramphenicol; 76.5% to ciprofloxacin; 65.3% to tetracycline; 63.3% to ampicillin and 53.1% to nalidixic acid. Sensitivity patterns of *Salmonella* species to antibiotics were as follows: out of 97 *Salmonella* species isolates, 80.4% each were sensitive to chloramphenicol and ciprofloxacin; 79.4% to Tetracycline; 50.5% to ampicillin and 70.1% to nalidixic

acid. Out of 47 *Shigella* species isolates, 76.6% were sensitive to chloramphenicol; 74.5 to ciprofloxacin; 61.7% to ampicillin; 70.7 to nalidixic acid and 70.2% to Tetracycline. Sensitivity patterns of *Aeromonas species* to commonly prescribed antibiotics were determined. Majority of the bacteria responded well to chloramphenicol, ciprofloxacin and nalidixic acid and poorly to ampicillin. Out of 41 *Aeromonas* species isolates, 73.2% were sensitive to chloramphenicol; 73.2% to ciprofloxacin; 53.7% to ampicillin; 70.7 to nalidixic acid and 68.3% to tetracycline (**Table 3**).

TABLE 3. Susceptibility Pattern for Bacterial Isolates

Non O1 <i>Vibrio cholerae</i> species (%). n=98	
Antibiotics	Sensitive
Chloramphenicol	85 (86.7)
Ciprofloxacin	75 (76.5)
Ampicillin	62 (63.3)
Nalidixic acid	52 (53.1)
Tetracycline	64 (65.3)
<i>Salmonella</i> species (%). n=97	
Chloramphenicol	78 (80.4)
Ciprofloxacin	78 (80.4)
Ampicillin	49 (50.5)
Nalidixic acid	68 (70.1)
Tetracycline	77 (79.4)
<i>Shigella</i> species (%). n=47	
Chloramphenicol	36 (76.6)
Ciprofloxacin	35 (74.5)
Ampicillin	29 (61.7)
Nalidixic acid	39 (83.0)
Tetracycline	33 (70.2)
<i>Aeromonas</i> species (%). n=41	
Chloramphenicol	30 (73.2)
Ciprofloxacin	30 (73.2)
Ampicillin	22 (53.7)
Nalidixic acid	29 (70.7)
Tetracycline	28 (68.3)

DISCUSSION

The study was designed to determine pathogens isolated from patients with diarrhea who were five and more years living along Lake Victoria region of Kenya. In this study, the most affected were young individuals aged between 5 and 12 years. This is because this age bracket have poor personal hygiene including not washing hands before and after eating⁶⁵. In this study, individuals with no or lower education were more likely to have diarrhea when compared with individuals with higher education. This finding was similar with other studies, where the prevalence of diarrhea varies according to education. Children whose mothers didn't know how to read and write⁶⁶ had relatively high diarrhea cases than those who knew how to read and write. Thus education provides the knowledge on the rules of hygiene and clean feeding^{67,68}.

Majority of the studied group regardless of the gender were fishermen and women. Exposure to drinking of contaminated untreated water directly from Lake Victoria⁴⁵ during fishing might have contributed to transmission of known enteric pathogens which are passed through contaminated food and water. In this study, four types of bacterial pathogens were isolated and two parasitic pathogens were identified. These bacterial pathogens were Non O1 *Vibrio cholerae*, *Salmonella species*, *Shigella species* and *Aeromonas species*. As much as the diarrheal infection is a common problem worldwide⁶⁹, the isolation of the first three bacteria from patients with diarrhea have been reported in previous studies including Kenya^{24,43,60,70,71,72}.

In this study Non O1 *Vibrio cholera* 98(30.9%) were the leading isolates causing diarrhea in the studied population. The ecosystem of Non O1 *Vibrio cholerae* being Salty and brackish environment⁷³ contradicts the existence of the bacteria in fresh waters of Lake Victoria. However, human activities may alter water ecosystems⁷⁴. Lake Victoria is well characterized with extensive fishing⁷⁵ and this is evident from this study that majority of diarrheal patients were fishermen and women. Pollution of the Lake Victoria with discharge of raw sewage dumping of domestic and industrial waste, fertilizer and chemicals from farms which in turn increase the invasive water hyacinth⁷⁶ may alter the salinity of waters thus favoring the existence of these bacteria. Evaporation throughout the year due to high temperatures along Lake Victoria may lead to relatively increased salinity in freshwater bodies. In addition non O1 *Vibrio cholera* possess additional virulence factors that enable it to survive in various ecosystems⁷⁷. Although, studies carried out in other places of Kenya have reported the isolation of serotypes of *Vibrio cholera* pathogen from stool of both children and adults^{45,60,24,62}.

The prevalence of these *Vibrio* species was lower especially among the children compared to the current study which constituted both children and adults. The prevalence difference could be due to groups of study participants where the previous obtained samples from children and the current from both the children and adults. Other studies in Kenya have reported isolation of this pathogen from environmental samples^{61,62}. Another study carried out in Nigeria reported lower isolation of non O1 *Vibrio cholera* from only adult patients with acute diarrhea compared to the current¹⁴ which studied all patients with three or more episodes of diarrhea per day excluding patients with two or one episodes of diarrhea per day.

Salmonella typhi causes typhoid fever which is spread by eating or drinking food or water contaminated with the feces of an infected person. *Salmonella species* is associated with poor sanitation and poor hygiene⁷⁸. Port Victoria being among the areas in Kenya with poor sanitary condition may have contributed to existence of these bacteria. The finding of this investigation also confirms earlier observations of the isolation of *Salmonella* from patient with diarrhea elsewhere^{79,80,81}. In this study, *Salmonella species* was the second prevalent organism. In comparison with other studies carried out elsewhere, the prevalence of this organism in other studies carried out in Kenya^{45,39,40,43,60}, Korea¹⁸ and Ethiopia⁸¹ were lower compared to the current study. This difference could exist because previous studies considered characterization of *Salmonella species* into specific serotypes compared to the current which did not characterize *Salmonella species* into various serotypes types.

Shigella species causes human dysentery in man^{82,83}. Report from the World Health Organization is that, the bacteria leads in the cause of diarrhea in human⁸⁴ worldwide. In this study, *Shigella species* was third affecting 47(14.8%) patients presenting in the health facility with diarrhea. *Shigella species* has been isolated from stool of patients with diarrhea in Masaai communities in Kenya⁷⁰. Consumption of meat by Masaai communities and fish by the participants in the current study might have served as associated factors for *Shigella* infection since this pathogen is known to be zoonotic in nature. Other previous studies have also reported *Shigella species* to cause travelers' diarrhea^{85,26,86}, however the current study reports *Shigella species* to cause diarrhea in the domestic population who did not have a history of travel for the last one year. Isolation of *Aeromonas species* from clinical samples is of great concern. This bacterium was first reported from a river sample in Kenya⁴⁸. However, in this study was least isolated from patients with diarrhea. In other previous studies carried out elsewhere, this bacterium was isolated from travelers with diarrhea, healthy carriers⁷² and immune-compromised individuals due to HIV^{87,88}.

However, in the current study the patient had no history of travel for the last one year and their HIV status was not determined. Its isolation in the current study is linked to the fact that majority of patients were fishermen and women who come into contact with and drink water directly from the lake during fishing. This might have served as a risk factor for its transmission as evidenced by the fact that, *Aeromonas species* had been isolated from a fresh water river samples in Kenya⁴⁸ and elsewhere⁸⁹. In the current study, all isolated pathogens were subjected to five commonly prescribed antibiotics. Sensitivity pattern showed varied sensitivities to all tested antimicrobials. Hence, there was variation in drug sensitivity patterns among all isolates compared to other studies carried out elsewhere. As much as over 50% of all the isolates were sensitive to all tested antimicrobials. The resistance shown by these pathogens still poses a threat in their management. In the current study, the predominance of Non O1 *Vibrio cholera* from diarrheal patients probably reflects a high prevalence in the community.

Tetracycline and ciprofloxacin among others have been generally considered as the drug of choice for the treatment of cholera. However, the resistance profiles indicate that these antibiotics will be less effective for treating non-O1 *V. cholera* infections. In deed comparing with another previous study in Kenya⁶¹, the resistance of this pathogen against tetracycline in the current study is slightly higher than the previous ⁶¹. However the resistance of ciprofloxacin against *Vibrio* species was lower in the current study than the previous because this drug is expensive for the population in the studied site and it is not purchased over the counter⁶¹. In another study conducted along Lake Victoria, Kenya more than 10 years ago showed that, all *Vibrio* species⁴⁵ isolated were susceptible to all antibiotics that were tested in this study. This difference in antibiotic susceptibility is that the previous study considered patients who had only bloody diarrhea while the current considered all the patients with diarrhea. To add on this, the previous study was carried out more than 10 years ago of which resistance might have developed along the way. In another study carried out elsewhere⁹⁰, *Vibrio* species reported lower resistance to tetracycline and ciprofloxacin, perhaps because it was not the drug of choice to treat infection caused by this pathogen. In the current study, resistances of *Vibrio Cholerae* Non O1 was slightly more against Nalidixic acid and slightly lower against Ampicillin compared to the previous study, 30% and 42.5% respectively in China⁹⁰. This difference in susceptibility could be due to geographical location of the current and previous studies as it is ascertained by other studies that resistances of bacteria are different basing of geographical distribution⁹¹.

The current study identified and determined susceptibilities of *Salmonella* species against various antibiotic. Generally, susceptibilities to chloramphenicol, ciprofloxacin and tetracycline were excellent, while susceptibility of Nalidixic acid and Ampicillin were fairly good. However, resistances of some isolates to these antibiotics in the current study are of great concern especially in the studied individuals, because these may rise with time. The current study noted some differences in all the five antibiotics tested with previous study carried out in Kenya³⁷ where by all isolates of *Salmonella* species from sixteen adults were susceptible to ciprofloxacin, chloramphenicol and nalidixic acid while 82% of the isolates from 22 children were resistant to ampicillin, tetracycline and chloramphenicol. This difference could be explained by the fact that the previous study considered fewer blood culture samples compared to the sample size of stool cultures in the current study. There was also variation in antibiotic resistance and susceptibilities of all these tested antibiotics against *Salmonella* isolates between the current study and the previous study in Kenya³⁸. The difference could be due to the fact that the previous study obtained its samples from an outbreak which probably had different species of *Salmonella* from the current. In another study carried out in Kenya⁴¹, all *Salmonella* isolates were sensitive to Ciprofloxacin while in the current study, some isolates were resistant to this drug. This is because previous study was carried out more than 10 years ago of which resistance might have developed along the way. The current study shows that 77(79.4%) and 49(50.5%) *Salmonella* isolates are sensitive to Tetracycline and Ampicillin while the previous study shows all to be resistant⁹². The previous study obtained samples from poultry while the current from human. About 93.1% of *Salmonella* species were resistant to both Tetracycline and Ampicillin in a previous study⁹³. The previous study considered both samples from both human and animals while the current samples were from only human. On the other hand, study conducted in India reported that 78.4% *S. typhi* showed resistance to chloramphenicol which is conventionally used to treat typhoid fever⁹⁴, the current study shows opposite results in that 80.4% are sensitive to chloramphenicol which is again frequently prescribed in the treatment of typhoid cases.

In this study, testing of *Shigella* species against five antibiotics has revealed various susceptibility and resistance patterns. Both patterns have revealed differences in the current and previous studies in Kenya. In the current study susceptibility of this pathogen is lower to ciprofloxacin and higher to ampicillin and tetracycline than the previous study²⁴.

In the current study susceptibility of this pathogen is lower to ciprofloxacin and nalidixic acid and higher to chloramphenicol, ampicillin and tetracycline than the previous study^{45,60}. The differences between the current and the previous studies is that, the previous studies obtained their samples from children while the current considered both children and adults. As the antimicrobial sensitivity test results imply, 74.5% and 83.0% of the *Shigella* species isolates to be sensitive to two antimicrobials: ciprofloxacin and nalidixic acid respectively. Differently, results have been reported from Ethiopia where 100% *Shigella* species isolates were found sensitive⁸¹ and 96.4% *Shigella* species isolates in China were resistant to nalidixic acid⁹⁵. Sensitivity to ciprofloxacin in the present study is slightly lower than the result found from Gondar University where 91.1% were found to be sensitive⁹⁶. In comparison to a previous study in Tehran, the percentage of resistance of *Shigella* species against ampicillin is slightly lower with the current study which was 10%⁹⁷. In the present study only 10(21.3%) of the isolates are resistant to tetracycline and this does not agree with related studies in Iran, India, Chile, and Nepal whose resistance was more than 50.0%^{47,98,99,100,101}. Study done in India found all *Shigella* species isolates sensitive to Chloramphenicol¹⁰², while in the current study, 36(76.6%) were sensitive and 6(12.8%) resistant. The findings of the current study do not agree with the previous Uzbekistan where some strains of *Shigella flexneri*, 28(90.4%) and *Shigella sonnei* 1(4.8%) were resistant¹⁰³. The resistance differences between the current and the previous studies could be due to heritable factors that determines intestinal environment⁸⁷ thus heritable factors of the studied individuals in the previous studies are different from the individuals in this study.

More than a half of the isolated *Aeromonas species* were susceptible to all antibiotics except Ampicillin whose susceptibility was almost half. Unlike in the previous study whose all the isolates were susceptible to Ciprofloxacin⁸⁹, only 30(73.2%) in the current study were susceptible. This difference could be attributed by the fact that the previous study considered specimen from environment and clinical sources while the current concentrated on the clinical samples only. Susceptibility of *Aeromonas species* to Tetracycline in the current study was lower than in the previous study 94.4%⁸⁹. The data from the present study indicated 53.7% of *Aeromonas species* being susceptible to Ampicillin a situation that does not concur with results from sensitivity pattern of the previous study that showed varied sensitivities to all tested antimicrobials¹⁰⁴. The previous study had 88% some biotype resisting ampicillin and 100% other biotype susceptible. The reason for varied susceptibilities in the previous study was that resistant biotype are frequently

isolated than the susceptible ones a situation that could be related to our case. As much as in the current study indicate *Aeromonas species* as the least isolated pathogen from patients with diarrhea still some 17.1% and 14.6% were resistant to chloramphenicol and Nalidixic acid respectively the reason of which could be these antibiotics are still commonly prescribed in typhoid cases.

Limitation of the study

This study did not consider HIV status of the participants because the study was interested to know the type of organisms that cause diarrhea regardless of whether patients had HIV or not. It did not also analyze antibiotic testing in relation to sex and age rather it generalized the analysis. The study also did not determine whether diarrhea was caused by the virus to check the 97 samples without parasite or bacteria whether they had viruses or not.

CONCLUSION

Bacterial isolates in this study responded differently to antibiotics as compared to other previously done studies. The susceptibilities expressed were fairly good; however, some of the resistances shown pose a threat to public health, especially in unexposed populations. With the observed trends, bacterial species in different geographical settings have responded differently to antibiotics. It may be beneficial for clinicians to consider antibiotic susceptibility testing prior to management of patients to slow down resistance development.

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