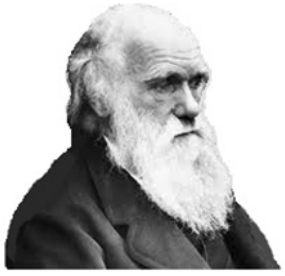
Available online at www.worldnewsnaturalsciences.com



World News of Natural Sciences

WNOFNS 11 (2017) 28-36 EISSN 2543-5426

Prevalence and Antibiotic Susceptibility Patterns of *Shigella* and *Salmonella* Causing *Diarrhoea* in Children Below 5 Years at Thika Level Five District Hospital

S. C. Kibet[1,a], **V. N. Matiru**[1], **P. W. Kibaba**[1,3,b], **A. Mucheke**[2] and **H. Louis**[2,3]

[1]Department of Medical Microbiology, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

[2]Department of Pure and Applied Chemistry, University of Calabar, P.M.B 1115 Calabar, CRS, Nigeria

[3]University of Chinese Academy of Sciences, UCAS, China

[a,b]E-mail address: kckibets2@gmail.com , kibaba88@gmail.com

ABSTRACT

The aim of the study was to isolate and identify the extent of *Salmonella* and *Shigella* inducing *diarrhoea* in children under five, and to determine their antibacterial susceptibility patterns. The method of investigation was a cross-sectional study. Samples were collected from children less than five years old afflicted with *diarrhoea* coming for treatment at Thika Level Five Hospital, Kiambu County. A total of 80 stool samples were collected. These were specifically examined for *Shigella* and *Salmonella*. In doing so, the samples were cultured in MacConkey and Xylose lactose deoxycholated (XLD) agar. The plates were subsequently incubated aerobically at 37 °C overnight. After incubation, suspected *Salmonella* and *Shigella* colonies were identified morphologically, marked, and the colonies were inoculated to biochemical tests for species identification as described in the Medical Laboratory Manual. Following this, colonies derived from purity plating through biochemical testing were sub-cultured onto nutrient agar to obtain pure colonies. The obtained pure colonies were then used to perform susceptibility tests to commonly prescribed antibacterial drugs, including Gentamicin, Ciproflaxicin and Erythromycin. Results showed 10 (12.5%) confirmed positive isolates, where 6 (60%) were *Salmonella* and 4 (40%) *Shigella*. With respect to the age, both isolates were found to be concentrated more at ages of 1-4 years. Herein, *Salmonella* – 1-2 years (33.33%), - 2-3years (50%); *Shigella* – 1-2 years (50%), – 2-3 years (25%). From the study, sex distribution (male or female) of the host isolates was equal (50-50%). Moreover, Ciprofloxacin and Gentamycin were the most potent antibiotics, whereas Ampicillin, Erythromycin, Cotrimoxazole and Sulfamethoxazole were highly resisted. According to study results,

Quinolones and Aminogylcosides are the antibiotics of choice for severe *diarrhoea* illnesses caused by *Salmonella* and *Shigella.*

Keyword**:** *Salmonella, Shigella,* Diarhoea, Children, Antibacterial

1. INTRODUCTION

Diarrhoea is defined as having loose or watery stools at least three times per day, or more frequently than normal for an individual who result to morbidity and mortality in children under fever in developing country and worldwide in general. The pathogenic microbes which are known to cause diarhoea are: *Salmonella, Shigella*, diarrheogenic *E. coli* and *Compyliobacter* and *Vibro cholera.* These pathogens can cause potentially serious diseases, which may be fatal, especially in children. The common route of infection by these pathogens is the ingestion of contaminated foods and drinks.

Shigella is a bacterium found exclusively in intestinal tract of man. It is a non-motile and glucose fermenting bacteria, gram-negative rod belonging to the family *Enterobacteriacea*e. All species of *Shigella* cause acute bloody *diarrhea* as a result patchy destruction of the colonic epithelium, which leads to formation of micro-ulcers and inflammatory exudates, and causes inflammatory cells. *Shigella* species, the causative agent of bacillary dysentery (*shigellosis*), are highly adapted human pathogens that are capable of invading and colonizing the intestinal epithelium resulting in a severe inflammatory colitis. *Shigellosis* symptoms include: fever, headache, malaise, anorexia, and occasionally vomiting, followed by excretion of profuse watery *diarrhoea* proceeding to bloody and/or mucoid *diarrhoea*. *Shigella* is an on-going global public health problem. Due to the fecal-oral transmission route of the organisms, the overwhelming burden of *shigellosis* is found in resource-poor settings with inadequate sanitation. With an estimated number of episodes exceeding 90 million per annum in Asia alone, *shigellosis* represents a significant proportion of the total number of bacterial gastrointestinal infections worldwide. Prompt treatment of *shigellosis* with appropriate antimicrobial agents not only shortens the duration and severity of the illness but also reduces microbial carriage and thus a spread of infection in the community, but unfortunately emergence of antimicrobial resistance has complicated the empirical therapy for treatment of *shigellosis*, due to the prevalence of antimicrobial resistance to some antibiotics used to treat *Shigella* sero groups.

Salmonella is a Gram-negative rod, facultative anaerobe, flagellated bacterium having a replication rate of 40 minutes at 37 °C but has the ability to grow at a wide range of temperatures, from 6 °C to 46 °C. This provides *Salmonella* with many opportunities to grow. Optimum growth occurs at a pH of 6.5–7.5. It is the pathogenic agent of *salmonellosis*, a major cause of enteric illnesses and typhoid fever, leading to many hospitalizations and deaths if no antibiotics are administered. *Salmonella* outbreaks are linked to unhygienic food preparation, cooking, reheating and storage practices that are contaminated with the microbe. The bacterium can be isolated from raw meat and poultry products as well as from milk and milk-based products. It is a ubiquitous human and animal pathogen. Antibiotic misuse by human leads to antimicrobial resistance to the *Salmonella* strain.

Most studies revealed that high numbers of patients had these signs and symptoms, of acute watery *diarrhoea*, loose stool. In a study, 196 children accessed between March 1994 and June 1996 at the paediatric emergency room of the teaching hospital, Universidad de Sao Paulo,

Brazil, the results showed the presence of bloody stools, vomiting, of which 28.6% had three or more episodes in the previous 24 hours, fever was measured or presumed by guardians in 59%. In the study done in Gaza strip, Palestine, mucous *diarrhoea* was predominant followed by vomiting and loss of weight, fever, chills and bloody *diarrhoea*. This study supports the conclusion from other studies that bacterial enteropathogens induce clinical illness characterized by fever, mucous *diarrhoea*, chills, vomiting, bloody *diarrhoea*, loss of weight or various combinations of these symptoms.

The antibiotics resistance of enteric bacteria has profound clinical implications because it threatens life and causes many of serious diseases such as acute gastroenteritis. Also, inappropriate prescription of antibiotics has prompted resistance, increased infections and mortality not only in developing countries but also in the developed countries. In a study conducted on Multidrug resistance(MDR) in *E.coli*, *Salmonella* and *Shigella* causing *diarrhoea* in children less than 5 years collected from Kenyatta National Hospital (KNH), showed that the highest resistance on *Salmonella* was shown by Trimetheprim sulphamethaxole (90%) followed by Ampicillin (81%), Streptomycin (75%) and Tetracycline and Chloramphenicol (56%). In the USA, two one-year old infants infected with *Salmonella enteridis* showed that they were resistant to Cefotaxime.

In the same study conducted on MDR (Multidrug resistant) at KNH, results showed that in *Shigella* only 7 out of the 12 antibiotics were effective to the isolates tested. Ciprofloxacin, Nalidix acid, Ceflazidine, Gentamicin, and Cefuroxime sodium were found to be effective on all the isolates, therefore showing 0% resistance. *Shigella* was shown to be 100% resistant towards 3 drugs, which were Trimethoprim - sulphamethaxazole, Tetracycline and Streptomycin. For this reason, microbial antibiotics resistance is receiving an increasing attention in light of the increasing incidence of bacterial infections resistant to antibiotic treatment. This has been attributed to Uncontrolled use of antibiotics due to the lack of antibiotics prescribing policy in *diarrhoeal* infections and for any kind of suspected infections, which is now becoming a major concern to the public health, putting a heavy burden on the health care system.

2. MATERIAL AND METHOD

The study area was in Thika Level 5 Hospital (TL5H) located in Kiambu County, Central province in Kenya. With respect to Fisher's formulae, 80 stool sample specimen collections were from children below 5 years, presenting with *diarrhoea* coming for treatment at TL5H, Kiambu County, in sterile disposable plastic containers (cary-blairs) within six hours after the collection.

Processing of the specimen was done through culture and microscopy. The stool sample or rectal swabs were first observed macroscopically for colour, texture and the presence of any extraneous material, such as blood, mucus, and pus. They were then cultured in MacConkey agar (oxoid), Xylose-Lactose-Desoxycholate (XLD) agar (oxoid). The plates were then incubated aerobically at 370 °C overnight for duration of 18-24 hours, suspected *Salmonella* and *Shigella* colonies were identified morphologically then biochemically as described in the Medical Laboratory Manual.

Gram staining was done by a smear of stool sample that was made on a glass slide, air dried/heat fixed and Gram stain procedure was undertaken where a series of stains were added

to the sample. The smear was first flooded with crystal violet for 60 seconds, after which the stain was gently rinsed off. It was then flooded with iodine for 30 seconds and decolorized by adding alcohol or acetone while holding the slide at an angle to allow a de-colouriser to drain, and stopped when runoff became clear. Finally a counterstain, safranin, was flooded on the smear for 60 seconds. The slide was then drained and allowed to air dry and examined under a light microscope using oil immersion to distinguish gram positive from gram negative bacteria. Pink-red short rods were observed for both isolated bacteria pathogen.

Each stool sample was cultured directly onto Xylose Lysine Desoxycholate agar (XLD), MacConkey agar. Approximately 1 gram of each sample was inoculated into 10 ml of Selenite F broth overnight and sub-cultured onto XLD agar, MacConkey agar and the plates incubated at 37 °C for 18-24 hours. The suspected colonies were identified by colony morphology and biochemical characteristics, *i.e*. *Salmonella* species appearing on XLD and MacConkey agar as colorless colonies with black centres owing to H_2S production. *Shigella* species colonies were identified on XLD and MacConkey agar as colonies appearing as transparent red and white colonies without black centers.

The colonies of suspected isolates for *Salmonella* and *Shigella* were picked and inoculated on different biochemical media. The organisms were incubated at 37 °C for 18-48 hours on Triple Sugar Iron (TSI) agar, Sulfide Indole motility, hydrogen sulfide test, Motility (SIM), Simmons' Citrate Medium, and Urea reaction.

The antimicrobial susceptibility of the confirmed positive isolate for *Salmonella* and *Shigella* was tested by Kirby-Bauer disc diffusion method for the commonly prescribed antibiotic for treatment of Gram negative bacterial infections, which are: Chloramphenicol (30 µg), Gentamycin (10 µg), Erythromycin (15 µg), Trimethoprim-Sulfamethoxazole (75 µg), Ampicillin (10 µg), Cotrimoxazole (75 µg), Ciprofloxacin (5 µg), and Amoxycillin/ Clavulanate (30 µg). Pure colonies were picked using a sterile wire loop to prepare turbidity by emulsifying in 1 ml of sterile peptone water, vortexed, and turbidity was then matched with 0.5 McFarland turbidity. A sterile disposable swab was then used to transfer the suspension onto Nutrient agar and commercial disks placed using a sterile loop. The plates were incubated for 18 hours after which the diameter of the inhibition zones were measured to nearest millimeter by use of a ruler. The diameters of the zones of inhibition for individual antimicrobial were translated into susceptible and resistant categories by referring to an interpretative table standard (sensitive >15 mm, intermediate 10-14 mm and resistant <9) according to National Committee for Clinical Laboratory Standards, NCCLS, 2010.

The data was analyzed using Microsoft Excel to generate descriptive statistics and then presented in tables, percentages and pie charts where necessary. The study was ethically approved by Ethical Review Board of Thika Level Five Hospital

3. RESULTS

A total of 80 stool samples were collected from patients below five years old, presenting with *diarrhea* attending Thika Level Five Hospital (TL5H). Among the 80 samples, 10 target bacteria were isolated: *Salmonella* 6, *Shigella* 4, as shown in **Figure 1**.

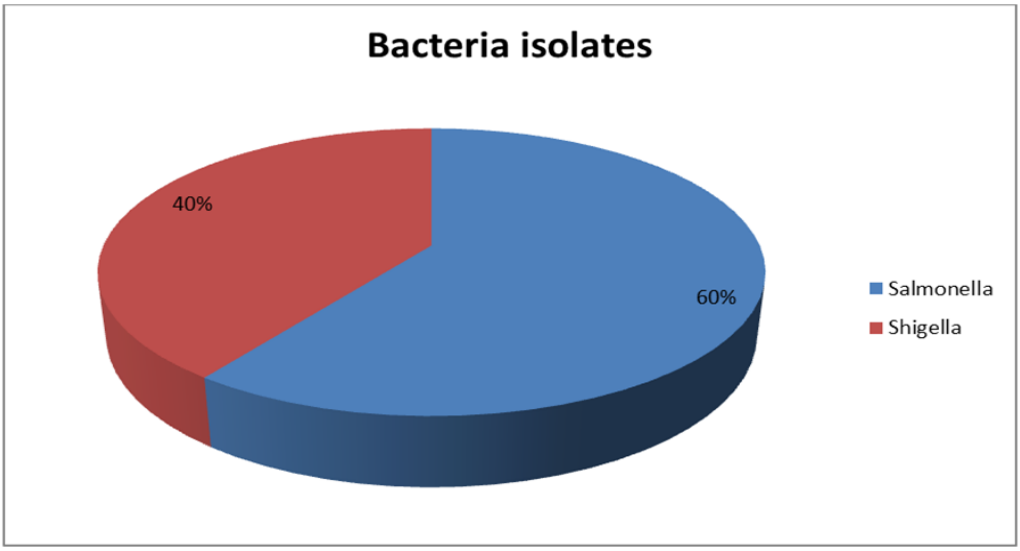


Figure 1. Bacteria isolates showing percentage of the more common pathogen

Primary culture plates werevshowing different appearance of the colony morphology of the isolates. *Salmonella* produced red colonies with black centres (1-2 mm). *Shigella* showed red/colorless colonies (1-2 mm) on xylose lactose deoxycholate (XLD) agar. *Salmonella* produced a transparent or colorless opaque with black centers (2-3 mm). *Shigella* produced convex colorless colonies, no black center on MacConkey agar. Gram staining reaction of the isolates viewed at ×100 appeared as short, red rods gram negatives.

Biochemical screening tests showed a positive confirmed *Salmonella* TSI (alkaline slant red, acid butt yellow, no gas production and H_2S production blackening), SIM (black coloration along stab line, no formation of red ring and non-motile no turbidity), Citrate utilization (positive reaction showing a deep prussian blue color), Urea (negative reaction showed by a pale yellowish-pink color. Tripple Sugar Iron test confirmed positive isolates for *Salmone*lla produced an alkaline slant (red), acid butt (yellow), H_2S blackening with no gas production. *Shigella* isolates characteristically produced an alkaline (red) slant and an acidic butt with little or no gas and no H_2S produced.

Children below five years old are susceptible to *diarrheal* infections caused by the two bacteria isolates. *Salmonella* was found more frequently at ages 1-4 years, whereas *Shigella* was more frequent occurring in ages 0-4 years, as shown in **Figure 2** and **Figure 3**.

The antibiotic susceptibility patterns of *Shigella* and *Salmonella* were tested using a commercial disk. The antibiotic with large inhibition zones were ciprofloxacin (32 mm) and Gentamycin (27 mm), thus were the most effective antibiotics against *Shigella* and *Salmonella*. While Ampicillin (6 mm) and Cotrimoxazole (6 mm) showed the smallest inhibition zone, thus were the highly resisted antibiotics, as are shown in **Figure 4**.

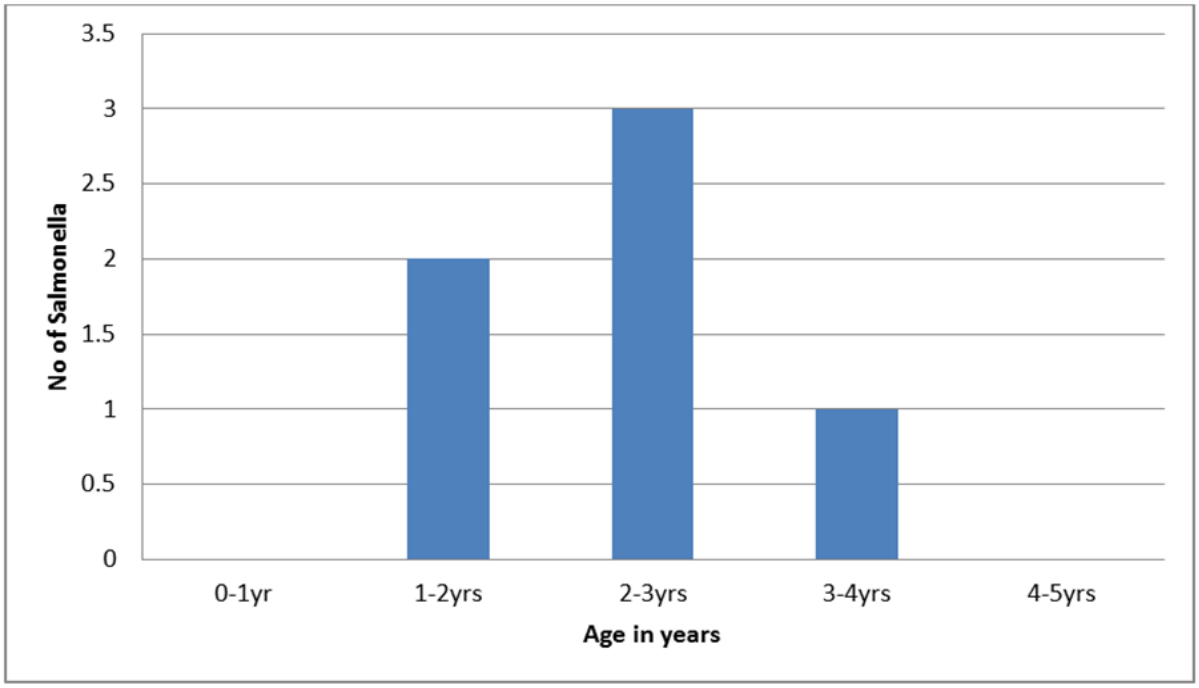


Figure 2. Number of *Salmonella* isolated with age of the children.

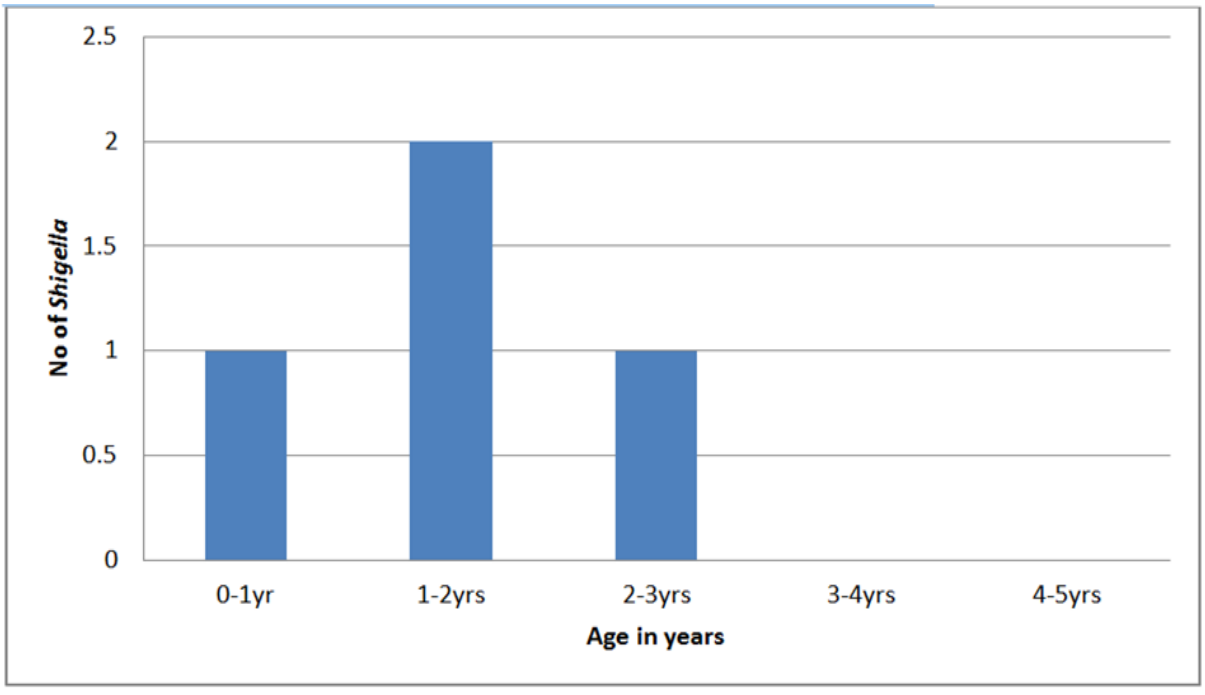


Figure 3. Number of *Shigella* isolated with age of the children.

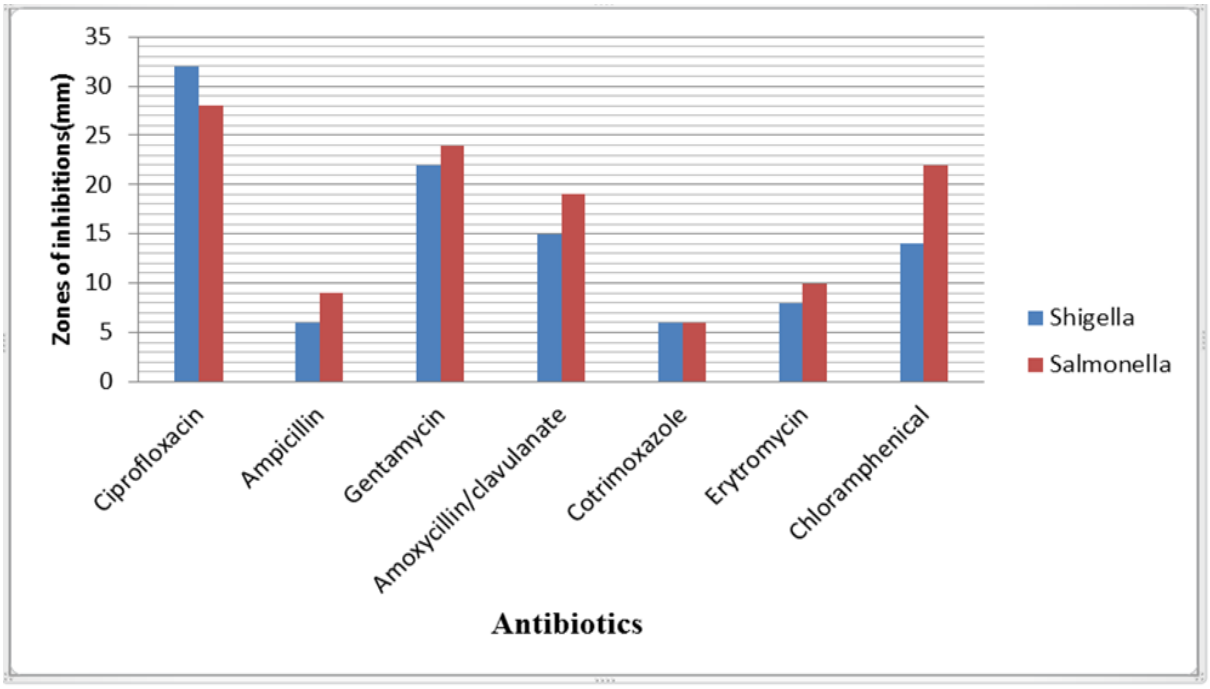


Figure 4. Antibiotic susceptibility patterns of the isolates.

4. DISCUSSION

Both isolates, *Salmonella* 60% and *Shigella* 40%, were found more frequently on the age group 1-4 years. Salmonella showed 1-2 years (33.33%), 2-3 years (50%) whereas *Shigella* 1-2 years (50%), 2-3 years (25%). These findings are consistent with other studies. The severity of the illness is mediated by different factors related to both, the patient nutritional status, presence of concomitant illness, and immunodeficiency status. This pattern reflects the combined effects of declining levels of maternally acquired antibodies and the lack of active immunity in infants. The introduction of food that may be contaminated with fecal bacteria and direct contact with human or animal feces when infants begin to crawl. Most enteric pathogens stimulate at least partial immunity against repeated infections or illnesses which helps to explain the declining incidences of disease in older children. Also from the results, the study found that there was no significant difference of the *diarrheal* infections caused by the isolated pathogens between boys and girls indicating that the virulence of infections was not gender related.

The isolates showed resistance to different classes of antibiotics used: for *Salmonella*, Ampicillin (50%), Sulfamethoxazole (66.67%), Erythromycin (50%) and Cotrimoxazole (50%), for *Shigella*, Ampicillin (75%) Erythromycin (50%), Chloramphenicol (50%) and Sulfamethoxazole (50%). Ciprofloxacin and Gentamycin were the most potent showing the highest effectiveness on all isolates: for *Salmonella*, Ciprofloxacin (100%), Gentamycin (83.33%), Amoxicillin/Clavulanic acid (83.33%) and Chloramphenicol (66.67%), for *Shigella*, Ciprofloxacin (75%), and Gentamycin (100%). The increase in a number of resistant strains to most antibiotics available represents an important health problem. This is probably due to inappropriate prescription of antibiotics (*i.e.* over the counter drugs). Also due to the shown resistance, it is important that *diarrheal* diseases are properly diagnosed, and susceptibility tests

properly carried out in order to reduce levels of resistance as emphasized so as to limit extensive and inappropriate use of antibiotics. Thus, continuous monitoring of antibiotic resistance in the county is imperative to ensure that severe *diarrhea* infections remain treatable.

The mechanisms of antimicrobial resistance are associated with intrinsic resistance, point mutations and acquired or extra chromosomal resistance. A wide range of molecular mechanisms, such as presence of beta-lactamases, dihydrofolate, acetyltransferase (CAT) enzymes have also been described. This has profound clinical implications because it threatens life and causes many of the serious diseases, such as acute gastroenteritis, especially in children whose immunity is developing. Increased infectious disease and mortality not only in developed countries has also prompted resistance.

5. CONCLUSIONS

In conclusion, *Salmonella* was the bacteria more isolated 6 (60%) and *Shigella* the least isolated with 4 (40%) from the study. With the presence of the two isolates in children under five years, there is a need that the age group 1-4 years should be monitored more closely in order to reduce infections and consequently be resistant to commonly prescribed drugs. There was no significant difference in the distribution of the isolated pathogen by sex.

The highest prevalence of antimicrobial resistance was to Aminopenicillins (Ampicillin) and sulfonamides (sulfamethoxazole). The classes of Quinolones (Ciprofloxacin) and Aminoglycosides (Gentamycin) were the most potent antibiotics against both bacteria isolates.

The study recommended for the following measures to be taken are: (1) Judicious use of antimicrobial therapy which requires education of health workers and patients, (2) Adequate lab diagnostic capability through conducting sensitive tests which is imperative to ensure that severe *diarrhea* infections remain treatable, (3) Government regulations to ensure that antimicrobial susceptibilities are monitored to effectively treat pathogens and also to curb indiscriminate use of antibiotics which has led to upraise of new resistant strains, (4) Emphasis should be placed on primary preventive measures, such as ensuring sewerage management and safe drinking water in the county. This would help to reduce exposure, or complete eradication to exposure to disease causing agents by these children.

References

[1] Guarner F., Schaafsma G.J. Probiotics. *Int J Food Microbiol* 1998; 39: 237–8.

[2] Van Niel C., Feudtner C., Garrison M.M., Christakis D.A. Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics* 2002; 109: 678–84.

[3] Huang J.S., Bousvaros A., Lee J.W., *et al*. Efficacy of probiotic use in acute diarrhea in children: a meta-analysis. *Dig Dis Sci* 2002; 47: 2625–34.

[4] Szajewska H., Mrukowicz J. Probiotics in the treatment and prevention of acute infectious diarrhea in infants and children: a systematic review of published randomized, double-blind, placebo controlled trials. *J Pediatr Gastroenterol Nutr* 2001; 33: S17–25.

[5] Rodrigues A.C., Nardi R.M., Bambirra E.A., Vieira E.C., Nicoli J.R. Effect of Saccharomyces boulardii against experimental oral infection with Salmonella typhimurium and Shigella flexneri in conventional and gnotobiotic mice. *J Appl Bacteriol* 1996; 81: 251–6.

[6] Czerucka D., Rampal P. Effect of Saccharomyces boulardii on cAMP- and Ca2+-dependent Cl^- secretion in T84 cells. *Dig Dis Sci* 1999; 44: 2359–68.

[7] Czerucka D., Dahan S., Mograbi B., Rossi B., Rampal P. Implication of mitogen-activated protein kinases in T84 cell responses to enteropathogenic Escherichia coli infection. *Infect Immun* 2001; 69: 1298–305.

[8] Fredenucci I., Chomarat M., Boucaud C., Flandrois J.P. S. boulardii fungemia in a patient receiving ultra-levure therapy. *Clin Infect Dis* 1998; 27: 222–3.

[9] Lherm T., Monet C., Nougiere B., *et al*. Seven cases of fungemia with S. boulardii in critically ill patients. *Intensive Care Med* 2002; 28: 797–801.

[10] Niault M., Thomas F., Prost J., Ansari H.F., Kalfon P. Fungemia due to Saccharomyces species in a patient treated with enteral S. boulardii. *Clin Infect Dis* 1999; 28: 930.

[11] Perapoch J., Planes A.M., Querol A., *et al*. Fungemia with Saccharomyces cerevisiae in two newborns, only one of whom had been treated with ultra-levure. *Eur J Clin Microbiol Infect Dis* 2000; 19: 468–70.

[12] Pletincx M., Legein J., Vandenplas Y. Fungemia with S. boulardii in a 1-year-old girl with protracted diarrhoea. *J Pediatr Gastroenterol Nutr* 1995; 21: 113–5.

[13] Carlstedt G., Dahl P., Niklasson P.M., Gullberg K., Banck G. Norfloxacin treatment of salmonellosis does not shorten the carrier stage. *Scand J Infect Dis* 1990; 22: 553-556.

[14] Neil M.A., Opal S.M., Heelan J., Giusti R., Ellen J., White R., Mayer K.H. Failure of ciprofloxacin to eradicate convalescent fecal excretion after acute salmonellosis: Experience during an outbreak in health care workers. *Ann Intern Med* 1991; 114: 195-9.

[15] Kazemi M., Gumpert T.G., Marks M.I. A controlled trial comparing sulfamethoxazole-trimethoprim, ampicillin and no therapy in the treatment of salmonella gastroenteritis in children. *J Pediatr* 1973; 83(4): 646-50.

(Received 02 June 2017; accepted 18 June 2017)