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Analysis of Multi-Drug Resistance of Diarrhoeagenic *Escherichia coli* and *Shigella dysenteriae* Infections among Children (≤5 Years) at the Benue State Teaching Hospital, Makurdi, Nigeria

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ABSTRACT
Diarrhoea is a dreaded illness reported to cause high mortality globally, especially in children under five years of age. This study investigated the prevalence and multi-drug resistance profile of two bacterial species: *Escherichia coli* and *Shigella dysenteriae,* in 100 diarrhoeal children. Antimicrobial sensitivity tests were carried out on isolates using 12 antibiotics. *E. coli* infection was high (29%), and the degree of infection was not associated with the level of drug resistance (χ^2 =1.79, P>0.05). Children with age 0-12 months were the highest infected, constituting 34.5% of the infected children, whereas drug resistance was highest in the age bracket 13-24 months and lowest in age bracket 0-12 months (62.5%). Drug resistance to *E. coli* and *S. dysenteriae* was insignificantly higher in females (74.2%) than in males (67.5%) (χ^2 =0.32, P>0.05). None of the 12 drugs tested was 100% sensitive to *E. coli* and *S. dysenteriae*. Drug action was dependent on the level of resistance (χ^2 =15.68, P<0.05). Drug resistance was 93.1% in ampicillin and 86.2% in tetracycline. Two cases of *S. dysenteriae* (2% prevalence) were reported and not associated with the age and sex of the children. Drugs with 100% resistance were ampicillin, ceftriaxone, and augmentin. The hospital management should explore the potent antimicrobial agents against the bacterial species highlighted in this report. This study has given vital information needed in treating and controlling diarrhoea among children.

KEYWORDS: diarrhoea; *Escherichia coli*; *Shigella dysenteriae*; multi-drug resistance

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1. Introduction

Illnesses arising from diarrhoea have been reported to cause about four million deaths worldwide yearly among children, especially those under five years of age. Children within this age bracket have weak immunity; hence, they more susceptible to infections than older ages [1]. Diarrhoea accounts for 13.2% of all childhood deaths

worldwide [2, 3]. The prevalence of chronic diarrhoeal illnesses worldwide every year is 3% to 20%, depending on the location [2]. Chronic diarrhoea is a common devastating illness causing infant mortality in pediatric medicine [3]. The illness is challenging since it is caused by many pathogens ranging from viruses and bacteria to protozoans. Treatment is difficult because of drug resistance among pathogens possibly because of the resistance genes possessed by the variants and the combined effects or interactions of many unrelated organisms that manifest as diarrhoea [2, 3, 5].

Among the leading causes of diarrhoea worldwide are species of *Escherichia* and *Shigella* [6, 7]. Strains of *E. coli* such as *E. coli* O157:H7 that are considered diarrhoeagenic possess virulence factors and manifest clinically as bloody diarrhoea [6, 8]. *Shigella* infestation often manifests in children as fever, watery diarrhoea, and crampy abdominal pain [8].

Although treatment of diarrhoea can be achieved using potent antibiotics, multi-drug resistance to antimicrobial agents is a challenge [9]. Antimicrobial resistance is a huge threat in the treatment and management of diarhoea among children and in older ages. Microorganisms that develop resistance to drugs possess resistance genes in their genomes, thereby frustrating treatments by rendering medicines ineffective while the illness persists to the point of morbidity. Therefore, the selection of drugs is guided by the results of resistance profile and susceptibility pattern [9, 10].

Cases of diarrhoea among children are high in developing countries [9, 11]. In order to effectively control and treat this ailment, there is a need to analyze a wide spectrum of antimicrobial agents for susceptibility patterns against diarrhoeagenic *E. coli* and *Shigella* species. The Benue State University Teaching Hospital (BSUTH) in Makurdi, Nigeria was chosen as the study site because it is much patronized by Benue residents due to the presence of medical specialists and facilities needed in orthopedics. The two bacteria were selected because they were previously reported to dominate bacterial diarrhoeal cases in children [2, 7, 9]. This study intended to determine the prevalence of *E. coli* and *S. dysenteriae* among diarrhoeal children as patients in the BSUTH and analyze the multi-drug resistance pattern of the two tested bacteria.

2. Materials and Methods

2.1 Study Site

The study was carried out at the Benue State University Teaching Hospital (BSUTH) Makurdi in January-May 2021. All sampling collection and laboratory analyses were done at this location. Makurdi is the capital of Benue State (7° 47' and 10° 0' E; Latitude 6° 25' and 8° 8' N) with an estimated population of 4,253,641. There are primary, secondary, and tertiary health facilities within the Makurdi metropolis. The notable ones are Federal Medical Centre, Benue State University Teaching Hospital, and the Nigerian Air force Hospital. The BSUTH receives a high influx of orthopaedic patients, being well equipped with many medical experts. It is the only statutory hospital saddled with the responsibility of teaching and training medical students at Benue State University.

2.2 Sampling and Sample Collection

Ethical clearance was duly obtained for this study. One hundred diarrhoeal children (in-patients) were consulted through their parents and guardians, where each subject produced stool samples in sterilized containers. Parameters such as sample code, collection time, name, age, sex, and parental occupation were recorded in a laboratory record book. Samples were labelled with a corresponding sample code and well-handled before analysis.

2.3 Sample Size Determination

Purposive sampling method was used. Sample size was determined using a conventional sample size formula [5]:

$$n = \frac{Z^2 pq}{e^2}$$

where:
n= sample size,
Z= the value of the z-table at 95% with confidence interval of 1.96,
p=prevalence of diarrhoeal among children in Nigeria at 7.6 % (0.076) [9],
q= 1-p, and
d= sampling error at 5%.

Based on the above sample size formula, 100 stool samples were obtained from diarrhoeal children as patients in the hospital.

2.4 Microbial Isolation and Identification

E. coli and *S. dysenteriae* were cultured and isolated following standard microbiological practices [12]. Cultural characteristics were noted (for the purpose of identification only), including color, elevation, consistency, shape, and forms. Slides were prepared and viewed using x4 and x10 objectives for identification across ten fields of view. The degree of presence of the bacteria was recorded as heavy (+++) when bacteria were observed in all fields of view, moderate (++) when bacteria were observed in 50% of all fields of view, and low (+) when bacteria were seen in one to three field/s of view [12]. Biochemical tests (urease, indole, oxidase, and catalase) were carried out using standard protocol [13].

2.4.1 Urease Test

Urease test detected the ability of pathogens to produce urease enzyme. Colonies were inoculated on the agar slant of urea agar and incubated at 37^{o}C. Observations were made after four hours and after overnight incubation. The development of purple-pink color indicated the production of urease [13].

2.4.2 Indole Test

This was carried out using the Kovac's reagent and peptone water. Isolates were inoculated with peptone water broth and incubated at 37°C for 48 h. A 0.5 mL of Kovac's reagent was added as the mixture was shaken gently. A pink or red color in the alcohol layer (top of the culture) indicated a positive reaction [13].

2.4.3 Oxidase Test

This was carried out using an oxidase reagent placed on a piece of Whatman No. 2 filter paper. A test colony was picked with a sterile loop and smeared over a small area impregnated with filter paper. A deep purple color appearing in 20 s indicated a positive result. A deep purple color in 10-60 s indicated a positive result [13].

2.4.4 Catalase test

In the catalase test, the reagent degraded hydrogen peroxide (H_2O_2) and released oxygen that was detected as effervescence. A drop of 3% H_2O_2 was placed on test colonies on nutrient agar. Observation was made for effervescence. Prompt effervescence indicated catalase production [13].

2.5 Antibiotic Sensitivity Tests

A total of 12 commercially available antimicrobial discs (Abtek Biological Ltd, U.K.), commonly used as antimicrobial agents in the BSUTH, based on preliminary information, were tested on the isolates for sensitivity tests. They were Gentamycin (Gen) at 10 ug/disc, Erythromycin (Ery) at 15 ug/disc, Ampicillin (Amp) at 10 ug/disc, Ceftriaxone (Cef) at 30 ug/disc, Cotrimoxazole (Cot) at 25 ug/disc, Cefixime (Cf) at 30ug/disc, Tetracycline (Tet) at 30 ug/disc, Streptomycin (Str) at 10 ug/disc, Cloxacillin (Cxc) at 5 ug/disc, Amoxicillin (Amx) at 25ug/disc, Cefuroxime (Cxm) at 30ug/disc, and Ceftazidime (Caz) at 30ug/disc. Sensitivity test was carried out using the Kirby–Bauer disc diffusion method [10, 12]. Antibiotic impregnated discs of 8-mm diameter were used for the test. After incubation, the diameter of the zone of inhibition was measured and compared with the zone diameter interpretative chart (CLSI) [10] to determine the sensitivity of the isolates to antibiotics. Standard strain of *Shigella ATCC25922* was used as a control. Resistance to a drug was indicated as R, while the degree of sensitivity was rated as +4, +3, +2, and +1 in descending order of sensitivity [12].

2.6 Data Analysis

Data were analyzed on the SPSS (Statistical Package for the Social Science) software 20.0 version. Prevalence was calculated in simple proportion and percentages (%). The degree of association was determined using the Chi-Square test as a non-parametric statistical tool at a 95% confidence limit. The level of significance was determined at the $P \leq 0.05$ threshold.

3. Results and Discussion

Total prevalence of diarrhoeal cases in the study was estimated at 31% (29% *E. coli* and 2% *S. dysenteriae* infections) (Table 1). Table 2 gives the degree of *E. coli* infection in the studied population and the level of drug resistance. From a total of 29 cases out of 100 subjects (29% prevalence), 13 children had a heavy infection (13% prevalence), 14 children had a moderate infection (14% prevalence) and two children had a low infection (2% prevalence). This may be a primary opportunistic contaminant causing diarrhoea among the children. The degree of infection was associated with its prevalence (χ^2 =9.17, P<0.05). The 13 heavily infected children recorded 66.7% drug resistance out of 156 drug tests, while those with moderate infection recorded 73.2% drug resistance out of 168 drug tests. The same trend was observed among children with low *E. coli* cases, where 62.5% drug resistance was recorded. Hence, the degree of infection was not associated with drug resistance level (χ^2 =1.79, P>0.05) since drug resistance was not based on the degree of infection.

Table 3 gives the age distribution of 29 diarrhoeal children infected with *E. coli* in the study area. Those with age 0-12 months were the highest (10 children), constituting 34.5% of the infected children with a total prevalence of 10%, followed by five children each (5% prevalence) in other age brackets except in 25-36 months with only four children (4% prevalence). However, infection was not associated with the age of the children (χ^2 =3.93, P>0.05) as each group had equal chances of being infected with *E. coli*. Drug resistance was highest in the age bracket 13-24 months (81.7%) and lowest in the age bracket 0-12 months (62.5%). However, all age brackets had equal chances of drug resistance to *E. coli* since there was no significant association between age and drug resistance (χ^2 =2.96, P>0.05). Among the 29 *E. coli*-infected children, there were 19 males (65.5%) with a prevalence of 19% and 10 females (34.5%) with a 10% prevalence (Table 4). This difference was statistically insignificant (χ^2 =2.79, P>0.05) as the two sexes had equal chances of having *E. coli*. Drug resistance to the organism was more in females (74.2%) than in males (67.5%), but it was not significantly established (χ^2 =0.32, P>0.05).

Table 5 gives the resistance and sensitivity profile of the 12 different drugs tested on the 29 *E. coli* cases. Out of 348 drug tests, sensitivity was 105 (30.2%), while resistance was 243 (69.8%). None of the 12 drugs was 100% sensitive to the test organism. Chi-square result showed that drug action depends on the level of resistance (χ^2 =15.68, P<0.05). The most sensitive of all drugs were cefixime and augmentin, with 13 positive cases out of 29 (44.8%), followed by amoxicillin, cefuroxime, and cotrimoxazole (37.9% each). Drug resistance was 93.1% in ampicillin, where 27 negative cases were observed out of 29 samples, followed by tetracycline with 25 negative cases (86.2%). Among the sensitive 105 cases as shown in Figure 1, tests with +4 level of action were eight, and they included drugs such as ceftriaxone (three cases), cefixime (two cases), tetracycline (one case), cefuroxime (one case) and augmentin (one case). The +3 level of sensitivity was the highest (71 out of 105) with 20.4%. All 12 drugs were in the +3-action category, but the highest number of samples was found in cefixime (11 cases) and amoxicillin (10 cases). A total of 23 cases obtained a +2 level of action, which accounted for 6.6%.

Strains of *E. coli,* which are part of the normal microorganisms of the large intestine, are also responsible for diarrhoea at an elevated level in children as opportunistic pathogens [6]. Thus, diarrhoeagenic strains of *E. coli* have been reported

Table 1. Summary of the prevalence of diarrhoeal cases among children ≤5 years old.

Measures	*E. coli*	*S. dysenteriae*	Total cases
Number of positive subjects	29	2	31
Total number of samples	100	100	100
Prevalence	29%	2%	31%

Table 2. Degree of *E. coli* infection among children (≤5 years) with diarrhoeal cases and level of drug resistance.

Degree of infection	Number	Prevalence	% Drug sensitivity	% Drug resistance	Total drug test
Heavy	13	13%	52 (33.3%)	104 (66.7%)	156
Moderate	14	14%	45 (26.8%)	123 (73.2%)	168
Low	2	2%	8 (33.3%)	15 (62.5%)	24
Total	29	29%	105 (30.2%)	242 (69.5%)	348

Note: χ^2 (Degree and Prevalence Infections) =9.17, DF=2, P=0.01 (P<0.05); χ^2 (Degree of Infection and Drug Resistance) =1.79, DF=2, P=0.409 (P>0.05).

Table 3. Age distribution of *E. coli* infection among children (≤5 years) with diarrhoeal cases and level of drug resistance.

Age (months)	Number	Prevalence	% Drug sensitivity	% Drug resistance	Total drug test
0-12	10	10%	45 (37.5%)	75 (62.5%)	120
13-24	5	5%	11 (18.3%)	49 (81.7%)	60
25-36	4	4%	16 (33.3%)	32 (66.7%)	48
37-48	5	5%	17 (28.3%)	43 (71.7%)	60
49-60	5	5%	16 (26.7%)	44 (73.3%)	60
Total	29	29%	105 (30.2%)	243 (69.8%)	348

Note: χ^2 (Age and *Escherichia coli* Infection)=3.93, DF=4, P=0.415 (P>0.05); χ^2 (Age and Drug Resistance)=2.96, DF=4, P=0.564 (P>0.05).

Table 4. Sex distribution of *E. coli* Infection among children (≤5 years) with diarrhoeal cases and level of drug resistance.

Sex	Number	Prevalence	% Drug sensitivity	% Drug resistance	Total drug test
Male	19 (65.5%)	19%	74 (32.5%)	154 (67.5.0%)	228
Female	10 (34.5%)	10%	31 (25.8%)	89 (74.2%)	120
Total	29	29%	105 (30.2%)	243 (69.8%)	348

Note: χ^2 (Sex and *Escherichia coli* Infection)=2.79, DF=1, P=0.095 (P>0.05); χ^2 (Sex and Drug Resistance)=0.32, DF=1, P=0.574 (P>0.05).

Table 5. Multi-drug resistance and sensitivity profile of *E. coli* infection among children (≤5 years) with diarrhoeal cases.

Drug	Resistance	Sensitivity	1+	2+	3+	4+	Total S (%)
Gentamycin	23	6	0	2	4	0	6 (20.7%)
Ampicillin	27	2	0	0	2	0	2 (6.9%)
Ceftriaxone	19	10	0	1	6	3	10 (34.5)
Cotrimoxazole	18	11	0	4	7	0	11 (37.9%)
Cefixime	16	13	0	0	11	2	13 (44.8%)
Tetracycline	25	4	0	1	2	1	4 (13.8%)
Streptomycin	20	9	0	1	8	0	9 (31.0%)
Cloxacicin	22	7	0	3	4	0	7 (24.1%)
Amoxillin	18	11	0	1	10	0	11 (37.9%)
Cefuroxime	18	11	1	1	8	1	11 (37.9%)
Cefazidime	21	8	0	2	6	0	8 (27.6%)
Augumentin	16	13	2	7	3	1	13 (44.8%)
Total	243 (69.8%)	105 (30.2%)	3 (0.9%)	23 (6.6%)	71 (20.4%)	8 (2.3%)	105/348 (30.2%)

Note: χ^2 (Combined Drug and Action) =15.68, DF=1, P=0.000 (P<0.05).

to possess virulence factors that produce distinct clinical manifestation. This observation corroborates the present findings where *E. coli* was 29% prevalent and hence could be described as the diarrhoeagenic type. In many of the children affected by this pathogen, the degree of infection was heavy. This position aligns with other reports describing diarrhoeagenic strains of *E. coli* as a major cause of diarrhoea [6, 14]. Although *E. coli* infection bears no relationship with the age of the affected children, the young ages (<12 months) were the most affected. This agrees with previous findings reporting a higher presence of diarrhoeagenic *E. coli* in younger children, especially those within 3-12 months, than in older children [5], possibly due to low immunity to infections at younger ages. Regardless of age, male and female antibiotic resistance to *E. coli* was high in this study. With the exception of cefixime and augmentin, all other drugs were highly resistant to a large extent, notably ampicillin and tetracycline. When diarrhoea is persistent due to drug resistance, *E. coli* may cause hemolytic-uremic syndrome [6].

The two *S. dysenteriae* cases (2% prevalence) shared an equal level of heavy and moderate degrees of infection (Table 6). The moderate level had higher percentage of drug resistance (58.3% than the heavy level (41.7%) although differences were not significant (χ^2=2.76, P>0.05). *S. dysenteriae* infection was observed in age brackets 25-36 and 37-48 months old, but age was not associated with the infection or level of drug resistance (χ^2 =2.76, P>0.05) (Table 7). Both sexes were equally infected, but males had a higher level of drug resistance (58.3%) than females (41.7%) but insignificant (Table 8). Thus, *Shigella* infection in this study cannot be substantially attributed to demographic parameters. Among 24 drug tests carried out on the test organism (Table 9), resistance and sensitivity were equal (50% each). One hundred percent sensitivity was recorded in gentamycin, cloxacillin, streptomycin, and amoxicillin. Drugs with 100% resistance were ampicillin, ceftriaxone, and augmentin. None of the sensitive drugs had +4 level of action, but they occurred largely at +3 level (9 out of 24), most especially Gentamycin and Streptomycin, as shown in Figure 2. In the present work, *S. dysenteriae* infection, where present, was either heavy or moderate with an average

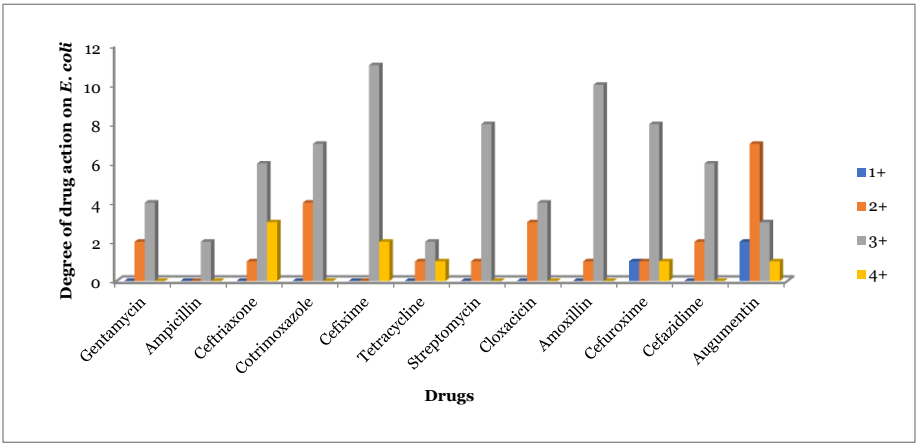

Figure 1. Effect of individual drug on *E. coli*.

level of drug resistance. The pathogen was sensitive to gentamycin, cloxacillin, streptomycin, and amoxicillin but resistant to antibiotics such as ampicillin, ceftriaxone, and augmentin. This agrees with previous reports describing *Shigella* infections as antimicrobial-resistant [9, 10, 15].

Some authors reported the genetic and evolutionary basis of multi-drug resistance in bacteria [16, 17]. Most microbes reproduce by dividing every few hours allowing them to evolve rapidly and adapt quickly to new environmental conditions. During replication, mutations (changes in the genetic material) arise, and some of these mutations may help an individual microbe survive exposure to an antimicrobial agent. Other causes of drug resistance are: gene transfer (resistance gene transfer among microbes), inappropriate use of an antimicrobial agent, and wrong diagnostics [17]; all these cannot be ruled out from the present outcome.

In this study, the combined effect of the two organisms (*E. coli* and *Shigella*) gave a total prevalence of 31%. This could be attributed to the fact that diarrhoea is caused by many species of bacteria and other organisms such as protozoa. Diarrhoeal infection is linked to many infectious microorganisms and opportunistic pathogens across diverse related and unrelated groups such as viruses, bacteria, and protozoans. Infection is aggravated when there is a synergy of mixed infectious pathogens within and between groups of organisms causing the illness. Secondly, resistance could be genetic in nature, such that microbial genetic resistance and mutation cannot be ruled out. Thirdly, the efficacy of each drug differs in addressing the specific and multiple causal pathogens [10, 16, 17].

It is also possible that the unaffected children in this work did not have diarrhoea but other forms of illnesses. Thus, the present work was limited to the two test organisms evaluated among in-patient children. Nevertheless, the study has successfully given an insight into the antimicrobial susceptibility pattern of common

Table 6. Degree of *S. dysenteriae* infection among children (≤5 years) with diarrhoeal cases and level of drug resistance

Degree of infection	Number	Prevalence	% Drug sensitivity	% Drug resistance	Total drug test
Heavy	1	1%	7 (58.3%)	5 (41.7%)	12
Moderate	1	1%	5 (41.7%)	7 (58.3%)	12
Total	2	2%	12 (50.0%)	12 (50.0%)	24

Note: χ^2 (Degree of Infection and Drug Resistance) =2.76, DF=1, P=0.097 (P>0.05).

Table 7. Age distribution of *S. dysenteriae* infection among children (≤5 years) with diarrhoeal cases and level of drug resistance.

Age (months)	Number	Prevalence	% Drug sensitivity	% Drug resistance	Total drug test
25-36	1	1%	5 (41.7%)	7 (58.3%)	12
37-48	1	1%	7 (58.3%)	5 (41.7%)	12
Total	2	2%	12 (50.0%)	12 (50.0%)	24

Note: χ^2 (Age and Drug Resistance) =2.76, DF=1, P=0.097 (P>0.05).

Table 8. Sex distribution of *S. dysenteriae* infection among children (≤5 years) with diarrhoeal cases and level of drug resistance.

Sex	Number	Prevalence	% Drug sensitivity	% Drug resistance	Total drug test
Male	1	1%	5 (41.7%)	7 (58.3%)	12
Female	1	1%	7 (58.3%)	5 (41.7%)	12
Total	2	2%	12 (50.0%)	12 (50.0%)	24

Note: χ^2 (Sex and Drug Resistance) =2.76, DF=1, P=0.097 (P>0.05).

Table 9. Multi-drug resistance and sensitivity profile of *S. dysenteriae* infection among children (≤5 years) with diarrhoeal cases.

Drug	Resistance	Sensitivity	1+	2+	3+	4+	Total S (%)
Gentamycin	0	2	0	0	2	0	2 (100%)
Ampicillin	2	0	0	0	0	0	0 (0.0%)
Ceftriaxone	2	0	0	0	0	0	0 (0.0%)
Cotrimoxazole	1	1	0	0	1	0	1 (50%)
Cefixime	1	1	0	0	1	0	1 (50%)
Tetracycline	2	0	0	0	0	0	0 (0.0%)
Streptomycin	0	2	0	0	2	0	2 (100%)
Cloxacicin	0	2	0	1	1	0	2 (100%)
Amoxillin	0	2	1	1	0	0	2 (100%)
Cefuroxime	1	1	0	0	1	0	1 (50%)
Cefazidime	1	1	0	0	1	0	1 (50%)
Augumentin	2	0	0	0	0	0	0 (0.0%)
Total	12 (50%)	12 (50%)	1 (4.2%)	2 (8.3%)	9 (37.5%)	0 (0.0%)	12/24 50.0%

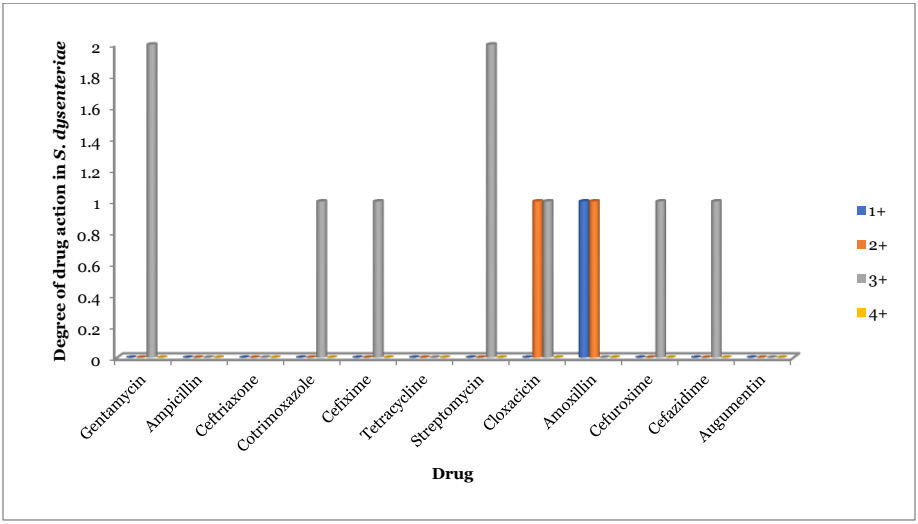

Figure 2. Effect of individual drug on *S. dysentriae*.

agents used in the treatment of diarrhoeal cases caused by *E. coli*. Potent drugs identified in the report could be explored for effective treatment and management of diarrhoea among children in the study area.

4. Conclusion

E. coli and *S. dysenteriae* infections occurred at 29% and 2%, respectively, among the children. Infections were not significantly attributed to the demographic status of the affected children. None of the 12 drugs tested was 100% sensitive to the two bacteria. *E. coli* resisted ampicillin and tetracycline, while *S. dysenteriae* resisted ampicillin, ceftriaxone, and augmentin. Potent antimicrobial agents against the microbes highlighted in this report should be explored by the hospital management. This study has given vital information needed in the treatment and control of diarrhoea among children.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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