Original Research Article

PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *E. coli* AND *Salmonella spp* ISOLATED FROM DIARROEIC CHILDREN IN SELECTED HEALTH CENTRES IN SOKOTO, NIGERIA

ABSTRACT

Aim: To determine the prevalence and antibiotic susceptibility patterns of *E. coli* and *Salmonella spp.* associated with childhood diarrhoea in our locality

Study design: Cross-sectional study

Place and Duration of Study: School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto between May and October 2017

Methodology: A total of 236 faecal samples were collected from children less than or equal to five years and were processed, isolates were identified following standard bacteriological procedures. Antibiotic susceptibility test was performed using disc diffusion method.

Result: About 96/236 (40.7%) of the sample yielded growth of *E. coli*, and 14/236 (5.9%) yielded growth of *Salmonella* species. *Salmonella* spp were 100% sensitive to ciprofloxacin, ofloxacin and ceftriaxone whereas they demonstrated low sensitivity of 35.7%, 14.3% and 7.1% to cefuroxime, ceftazidime and cotrimoxazole respectively and none of the isolates was sensitive to ampicillin and augumentin. *E.coli* on the other hand were 73.9% sensitive to ceftriaxone, 69.8% to ciprofloxacin, 62.5% to gentamycin and 61.5% sensitive to ofloxacin. Sensitivity of *E. coli* to cefuroxime and cotrimoxazole was very low and none of the isolates was sensitive to ampicillin and augumentin. **Conclusion:** The prevalence of *E. coli* causing **i**nfectious diarrhoea among children in Sokoto is significantly high. Both bacterial agents presented with marked resistance to most antibiotics. Ceftriaxone, ciprofloxacin and ofloxacin were found to be drugs of choice in the treatment of bacterial diarrhoea caused by both *E. coli* and *Salmonella*.

Keywords: Antibiotic susceptibility, *E. coli*, *Salmonella* spp, diarrhoea

1. Introduction

Diarrhoea is a significant public health problem with high morbidity and mortality among children below the age of five especially in developing countries (1). It ranks second after pneumonia among the causes of death in under- five (2). Globally, it is responsible for 526,000 childhood death, this means that 1400 children die of diarrhoea yearly, 60 children die hourly and a child dies every 60 seconds (3). The prevalence of diarrhoea is intense in sub-Saharan Africa where it accounts for 295,000 deaths in children below the age of five years in 2015. Nigeria ranked second after India with 77,000 diarrhoea death in children below five years of age (3). Pathogens associated with diarrhoea include bacteria, viruses, parasites and some fungi. In poor resource nations, rotavirus and *E. coli* are implicated as the major cause of diarrhoea among children in the study group (4). Most of these agents are transmitted through faecal oral route. The surveillance for the causative agents of infectious diarrhoea is important in developing countries in order to accurately document the burden of the disease (5). Usually indiscriminate use of antibiotics prompts resistance and increases infectious disease mortality not only in developing countries but also in developed countries. Progressive increase in antimicrobial resistance among enteric bacteria pathogens in developing countries is becoming a critical area of concern (6). Enteric bacteria play a major role in diarrhoea; it is however disturbing that many of these agents pose a serious problem of multiple drug resistance with severe consequence on public health. Many reports have described resistance of enteric bacteria to antimicrobial agents especially the commonly used amoxicillin and cotrimoxazole with rising treatment failures (7, 8, 9, and 10). This may be linked with the high frequency with which antimicrobials are used in empirical treatment of infections (8). Periodic antibiogram will assist clinicians to assess local susceptibility rates which will help in determining antibiotic empirical therapy and monitoring current resistance trend (11). The aim of this work is to determine the prevalence of some enteric pathogens and their antibiotic susceptibility patterns in our locality as this will help policy makers to formulate drug policy and make the best choice of antibiotics in the treatment of bacterial diarrhoea

2. MATERIALS AND METHOD

2.1 SAMPLE COLLECTION

A total of 236 stool samples were collected from diarrhoeic children below five years of age after completion of a semi structured questionnaire adopted from Mulatu *et al.,* (12). The samples were transported in an ice-tray box to the Medical Microbiology Laboratory of School of Medical Laboratory Science, Usmanu Danfodiyo University Sokoto, in not later than 60 minutes of collection for bacteriological analysis. Written informed consent was obtained from parent or guardian of each child while ethical approval (SKHREC/026/017) was obtained from the Ministry of Health, Sokoto State.

2.2 SAMPLE ANALYSIS

Samples were cultured on Selenite F broth and incubated at 37^{o}C for 16 hours after which it was sub-cultured onto Xylose lysine deoxycholate citrate agar (Titan, India) and Deoxycholate citrate agar (HiMedia, India) for the isolation of *Salmonella spp*. MacConkey agar (HiMedia, India) was used for the isolation of *Escherichia coli* and the isolates were identified using conventional biochemical tests such as Gram's staining, motility test, carbohydrate fermentation, Simmons citrate, tryptophan hydrolysis, oxidase test, urease test , Kligler iron agar, lysine decarboxylase following standard procedures.

2.3 ANTIBIOTIC SUSCEPTIBILITY TESTING

Antibiotic susceptibility of isolates was determined using modified Kirby-Bauer (13) disk sdiffusion method as recommended in CLSI (14). Standard bacteria suspension equivalent to 0.5 McFarland standards which yielded a uniform suspension containing 10^{5}-10^{6} cells/ml was employed in the susceptibility testing. The bacteria suspension were tested against standard antibiotics (Rapid Labs, Uk and Oxoid, UK) on Mueller Hinton agar (Accumix,Tulip Diagnostics(p) Ltd, India). These are commonly used and available antibiotics in Sokoto.. The antibiotics include Ofloxacin 5 µg,

Ciprofloxacin 5 µg, Gentamycin 10 µg, Cefuroxime 30 µg, Ceftazidime 30 µg, Ampicillin10 µg, Cotrimoxazole 5 µg, Amoxycillin clavulanate 10 µg, Chloramphenicol 30 µg and Ceftriaxone 30 µg). ATCC strain of *E. coli* 25922 was used as control. The percentage resistance was calculated by dividing the number of isolates resistant to a particular antibiotic by the total number of isolates multiplied by 100

3.0 Results and Discussion

As shown in figure 1, of the 236 stool samples examined, 110 (46.7%) enteric pathogens were identified. Of these enteric bacteria, 96 (40%) were *E. coli*, and 14 (5.9%) were *Salmonella* species. The prevalence of bacterial diarrhoea was found to be higher in children within the age group 6-24 months than older infants. Table 1 shows that children within the age range of 13-24 month had the highest positive culture of 43 (46%) for *E. coli* while those within the age range 49-60 month had a high positive culture of 2 (15%) for *Salmonella spp.* Females had a higher positive culture of 42 (43.2%) for *E. coli* while males had a high positive culture of 11 (7.9%) for *Salmonella spp.* Children residing in rural areas had a high positive culture of 62 (45%) and 11 (7.9%) for *E. coli* and *Salmonella spp* respectively. Chi square analysis showed that there was no significant association between culture positivity and age, gender or residence.

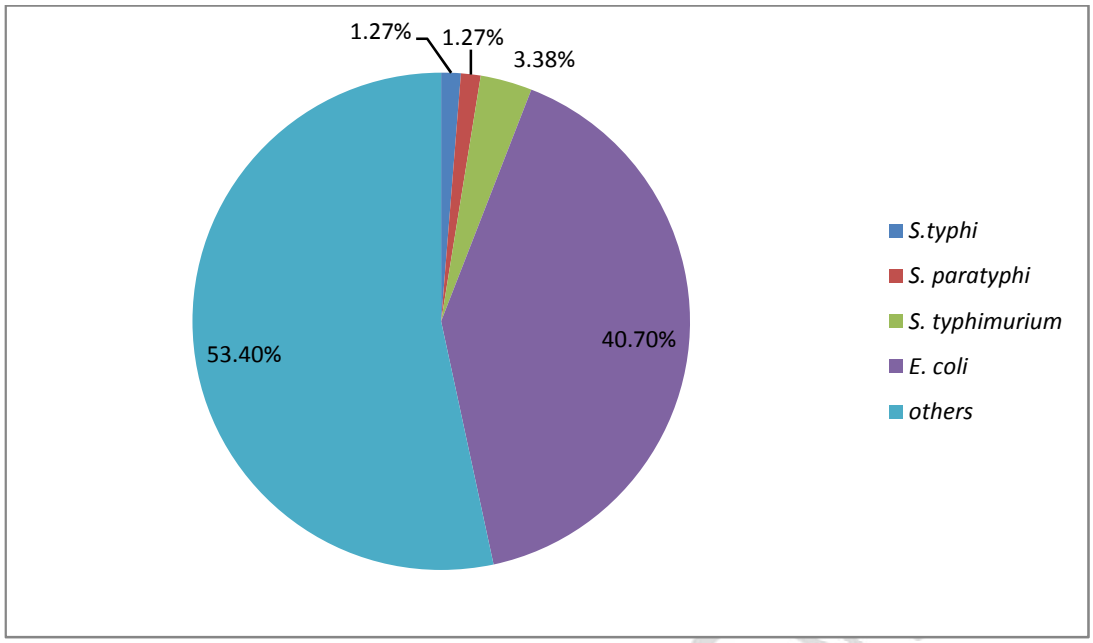

Figure 1: Prevalence of *E. coli and Salmonella* species isolated from diarrhoeic children

Table 1: Distribution of *E. coli* and *Salmonella* with Demographic Characteristics among Children with Diarrhoea in Selected Health Centres in Sokoto

Age(month)	*E.coli* (N)		*Salmonella*(N_1)		X^2 value	P-value
	Pos	Neg	Pos	Neg		
	N (%)	N (%)	N (%)	N (%)		

< 6	5 (21.7)	18 (78.3)	1 (4.3)	22(95.6)	10 .84	0.370
6-12	28 (40.0)	42 (60.0)	4 (5.7)	66 (94.3)		
13-24	48 (**51.**0)	46 (49.0)	3 (3.1)	91 (97)		
25-36	10 (34.5)	19 (65.5)	4 (13.7)	25(85.35)		
37-48	3 (43.0)	4 (57.0)	0 (0.0)	7 (100.0)		
49-60	2 (15.0)	11(85.0)	2(**15**.0)	11 (85.0)		
Gender						
Male	51(37.0)	87(63.0)	11(**7.9**)	127(92.0%)	3.495	0.479
Female	42(**43.2**%)	55(66.8%)	3 (3.1%)	94 (97.0%)		
Residence						
Urban	34(34.0%)	65(66.7%)	5 (5.2%)	94 (97.0%)	4.195	0.123
Rural	62 (**45**%)	75(55.0%)	9 (**7.0**%)	128(93.0%)		

P< 0.05 Pos=Positive Neg=Negative X^2 = chi square N is total number of *E.coli*=96 N_1 is the total number of *Salmonella*=14

Figure 2 shows that *E. coli* isolates were highly susceptible to ceftriaxone, moderately susceptible to ciprofloxacin, gentamycin and ofloxacin while they were resistant to cotrimoxazole and cefuroxime. *Salmonella* isolates were highly susceptible to ceftriaxone, ciprofloxacin, gentamycin, ofloxacin and chloramphenicol. All isolates of *E. coli* and *Salmonella* were resistant to both amoxicillin clavulanate and ampicillin.

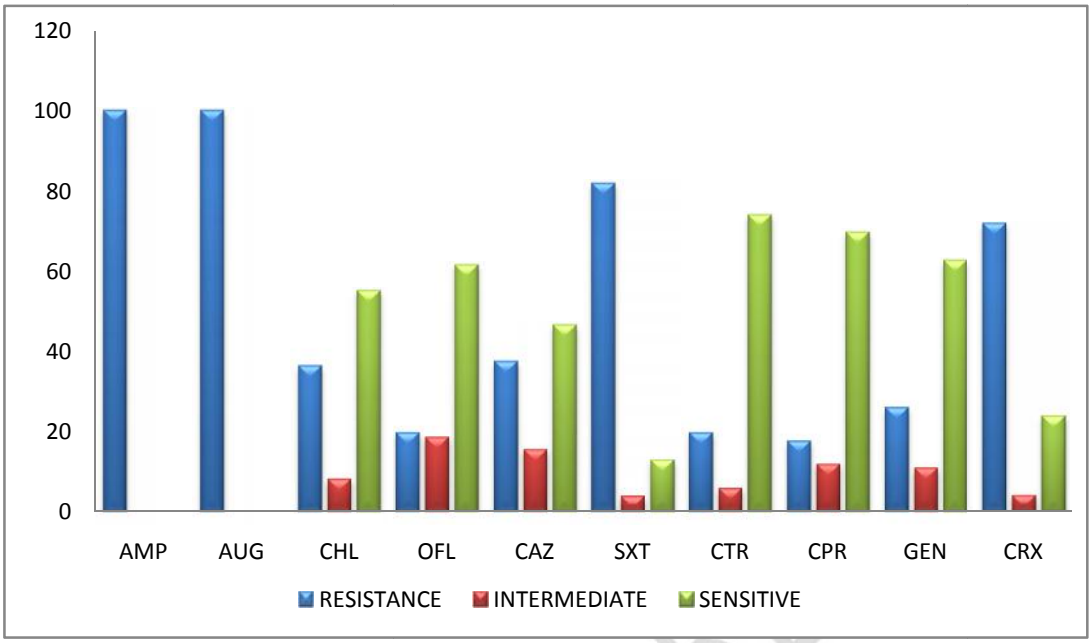

Figure 2: Antibiotic Susceptibility Pattern of *E. coli* Isolates

Abbr= Abbreviation, AMP= Ampicillin, AUG= Amoxycillin clavulunate, CRX= Cefuroxime ,CAZ= Ceftazidime, SXT= Cotrimoxazole ,CHL= Chloramphenicol CTR= Ceftriaxone, CPR= Ciprfloxacin ,OFL= Ofloxacin,, GEN= Gentamycin

The “sensitive” category means that the isolates are inhibited by the usually achievable concentrations of the antibiotics when the dosage recommended to treat the site of infection is used.
The “intermediate” category includes isolates with antibiotics minimum inhibitory concentrations that approach usually attainable blood and tissue levels, and for which response rates may be lower than for susceptible isolates.
The “resistant” category means that isolates are not inhibited by the usually achievable concentrations of the antibiotics with normal dosage schedules.

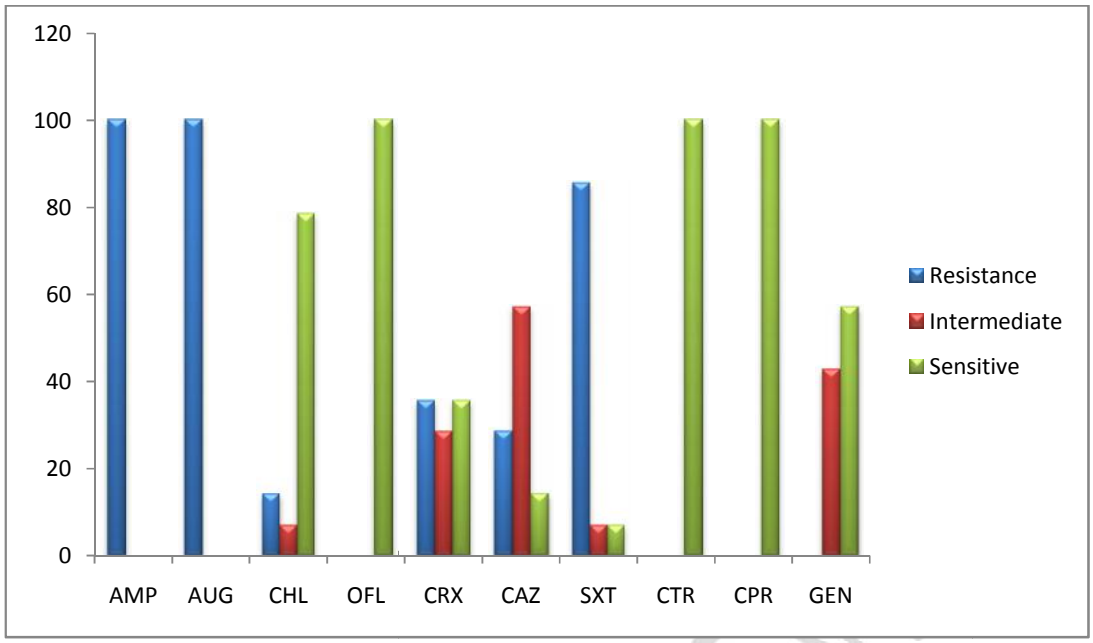

Figure 3: Antibiotic Susceptibility Pattern of *Salmonella* Isolates

Abbr= Abbreviation, AMP= Ampicillin, AUG= Amoxycillin clavulunate ,CRX= Cefuroxime ,CAZ= Ceftazidime, SXT= Cotrimoxazole ,CHL= Chloramphenicol CTR= Ceftriaxone, CPR= Ciprfloxacin ,OFL= Ofloxacin,, GEN= Gentamycin

The "sensitive" category means that the isolates are inhibited by the usually achievable concentrations of the antibiotics when the dosage recommended to treat the site of infection is used.
The "intermediate" category includes isolates with antibiotics MICs that approach usually attainable blood and tissue levels, and for which response rates may be lower than for susceptible isolates.
The "resistant" category means that isolates are not inhibited by the usually achievable concentrations of the antibiotics with normal dosage schedules.

In this study, *E. coli* was more implicated as a cause of diarrhoea with a prevalence of 40.7%. This is in agreement with the findings in Tamil Nadu, (15) and South East Nigeria, (16) that reported the prevalence of *E. coli* in diarrhoea to be 36% and 41% respectively. This shows that *E. coli* is a leading cause of diarrhoea not only in this region. Although, *E. coli* prevalence findings from this work is low when compared to the report of 61.7% by Uma *et al.,*(17) and it is high compared to the report of 4.6% in China, (18) and 22.9% in Tanzania, (19). The reason(s) for this is not properly understood by the scope of this work.

Salmonella specie prevalence in this study is 5.9%, this did not concord with the findings in previous studies where lower prevalence was obtained (12, 18, and 22). Indeed, 8.7% prevalence was reported in Nigeria (16) and 18.6% in India (15). The disparity in our findings could be as a result of different geographical location and different cultural practices that might have exposed the children to various types of hygienic practices.

Antimicrobial resistance in enteric pathogen is of major concern in developing countries, where the rate of diarrhoeal disease is high due to poor sanitary and socioeconomic condition. The rise in antibiotic resistance poses serious threat to the treatment of infectious diseases and this call for serious concern because of prevalence of infectious diseases.

In this study, *E. coli* demonstrated 100% resistance to ampicillin; this in no doubt is the outcome of the increased misuse and abuse of the drug in both symptomatic and asymptomatic illnesses. This finding is comparable to previous report of 90.8%, 93%, 100% and 86.8% (20, 15, 16 and 21) .The high level of resistance to ampicillin may be due to the action of penicillin binding proteins and also betalactamases that rapidly inactivate peniciliins.

The 100% resistance of *Salmonella* to ampicillin in this research is comparable to the work of Manikandan and Amsath (15) but is contrary to the report of Mei qu *et a.l* (18), the disparity here may be because ampicillin is no longer in use in the country with low resistance. It is worrisome that 100% of the *Salmonella spp.* was resistant to amoxycillin clavulunate which is known to be broad-spectrum antibiotics with proven clinical efficacy. The high rate of resistance to amoxycillin clavulunate may be due to hyper production of the chromosomal class C β-lactamase and the production of inhibitor-resistant TEM (IRT) enzymes. This is in tandem with the findings of Ugwu *et al.* (22) that reported 82.0% resistance to amoxycillin clavulanate but it contradicted the work of Clarence *et al.* (16) that reported 55.6%.The difference in resistance of the same isolate from different countries can be as a result of real localized resistance problems and also from methodological differences in susceptibility testing and breakpoint criteria.

E. coli demonstrated moderate resistance of 36.5% to chloramphenicol, 37.5% to ceftazidime, 26% to gentamycin, and low resistance rate of 17.8% to ciprofloxacin 19.8% to both ofloxacin and ceftriaxone. This may be because these antibiotics are rarely employed in the treatment of diarrhoea in children in this geographical location. This moderate resistance is comparable to previous report

(23). However this is contrary to the findings of Manikandan and Amsath, (15) that reported 3% resistance to ciprofloxacin, 2% to gentamycin, and 43% to chloramphenicol. *E .coli* resistance was low compared to the findings of Ugwu *et al.* (22) that reported 91% resistance to ceftriaxone, 78% to ofloxacin, 100% to cefuroxime and 78% to gentamycin. The disparity here may be due to methodological differences in susceptibility testing.

Salmonella species demonstrated 100% susceptibility to ceftriaxone, ciprofloxacin, ofloxacin and gentamycin with appreciably high sensitivity to chloramphenicol 85.7%, ceftazidime 71.5% and cefuroxime 64.3%. This is comparable to work of Adnan, (24) that found *Salmonella spp.* to be 100% susceptible to ciprofloxacin, 96% to gentamycin 90% to chloramphenicol and is contrary to the work of Ugwu *et al.* 22) that reported 100% resistance to gentamycin, 100% to ceftazidime, 100% to cefuroxime, 100% to ceftriaxone 69% to ofloxacin and 82% to amoxycillin clavulunate.

4.0 Conclusion

E. coli and *Salmonella* spp were significantly associated with diarrhoea among children in Sokoto and there was a marked resistance among the *E. coli* isolated. Amoxycillin and cotrimoxazole which are mostly administered to diarrhoeic children were found to show high resistance in this work. Selective use of antibiotics is paramount, this is important due to poor medical service, poor quality of drugs and non –compliance to drug therapy which all aid the emergence of antibiotic resistance. It is recommended that the pattern of resistance be monitored as the susceptibility of bacterial pathogens responsible for diarrhoea is reducing. Ceftriaxone, ciprofloxacin and ofloxacin were found to be potent agents against *E. coli* and *Salmonalla* causing childhood diarrhoea.

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Competing interest: Authors have declared no competing interest exist

Consent: Informed written consent was obtained from each parents or guardian of subjects prior to sample collection.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki

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