Prevalence and Antibiotic Susceptibility Pattern of *Escherichia coli* and *Salmonella spp* Isolated from Diarrhoeic Children in Selected Health Centres in Sokoto, Nigeria

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Authors’ contributions

This work was carried out in collaboration between all authors. Author ZN designed the study, carried out the experiment, wrote the protocol and wrote the first draft of the manuscript. Authors SLK and JO managed the analyses of the data and the literature searches. All authors read and approved the final manuscript.

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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.

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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 [7,8,9,10]. 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 METHODS

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, Usman 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°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 diffusion 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, Cefazidime 30 µg, Amoxicillin10 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. RESULTS AND DISCUSSION

As shown in Fig. 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.

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.

![Fig. 1. Prevalence of *E. coli* and *Salmonella* species isolated from diarrhoeic children](image-url)
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) | Neg N (%) | *Salmonella*(N₁) | Neg N (%) | χ² value | P-value |
|------------|---------------|-----------|------------------|-----------|----------|---------|
| < 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)   |          |         |
| 49-60      | 2 (15.0)      | 11 (85.0) | 2 (15.0)         | 11 (85)   |          |         |

Gender

|  | Male            | Female        |
|----------------|----------------|
| Pos N (%)      | Neg N (%)      | Pos N (%)      | Neg N (%)      |
| Male           | 51 (37.0)      | 87 (63)       | 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 | Rural |
|----------------|-------|
| Pos N (%)      | Neg N (%) | Pos N (%)      | Neg N (%)      |
| 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  χ² = chi square  N is total number of *E.coli*=96  N₁ is the total number of *Salmonella*=14

Fig. 2. Antibiotic susceptibility pattern of *E. coli* isolates

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

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.

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 [12,18,20]. 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% [22,15,16,21]. The high level of resistance to ampicillin may be due to the action of penicillin binding proteins and also betalactamases that rapidly inactivate penicillins.

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 al. [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 clavulanate which is known to be broad-spectrum antibiotics with proven clinical efficacy. The high rate of resistance to amoxycillin clavulanate 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. [20] 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. [20] 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. [20] that reported 100% resistance to gentamycin, 100% to ceftazidime, 100% to cefuroxime, 100% to ceftriaxone 69% to ofloxacin and 82% to amoxycillin clavulanate.

4. 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 Salmonella causing childhood diarrhoea.

CONSENT

Informed written consent was obtained from each parent 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bryce J, Boschi-Pinto C, Shibuya K, Black R. WHO estimates of the causes of death in children. Lancet 2005;365:1147-52.
2. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black R. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities; an updated systematic analysis. The Lancet. 2015;385:430–40.
3. United Nations Children's Fund. One is too many: Ending child deaths from pneumonia and diarrhea. UNICEF, New York; 2016.
4. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black R. Global, regional, and national causes of under-5 mortality in 2000–15: An updated systematic analysis with implications for the sustainable development goals. The Lancet. 2016;6736(16):31593-8.
5. Gómez-Duarte OG, Arzuza O, Urbina D, Bai J, Guerra J, Montes O, Puello M, Mendoza K, Castro GY. Detection of *Escherichia coli* enteropathogens by multiplex polymerase chain reaction from children’s diarrheal stools in two Caribbean–Colombian cities. Foodborne Pathogens and Diseases. 2010;7(2):199–206.

6. Founou L, Founou R, Essack S. Antibiotic resistance in the food chain: A developing country-perspective. Front Microbiol. 2016;7:1881.

7. Duru EE, Agbagwa OE, Umoren OE. Bacterial agents associated with infantile diarrhea and their antibiotics susceptibility pattern in Port Harcourt, South-South, Nigeria. Journal of Medical Sciences and Public Health. 2014;3(1):01-12.

8. Ifeanyi Casmir, Ifeanyichukwu Cajetan, Isu Rosemary Nnennaya, Akpa Alexander Casmir, Ikeneche Nkiruka Florence. Enteric bacteria pathogens associated with diarrhoea of children in the Federal Capital Territory Abuja, Nigeria. New York Science Journal. 2009;2(7):62-69. ISSN: 1554-0200.

9. Langendorf C, Le Hello S, Moumouni A, Gouali M, Mamaty A-A, Grais RF, Weill FX, Page AL. Enteric bacterial pathogens in children with diarrhoea in Niger: Diversity and antimicrobial resistance. PLoS ONE. 2015;10(3):e0120275.

10. Alikhani MY, Hasheni SH, Aslani MM, Farajinia S. Prevalence and antibiotic resistance patterns of diarrheagenic *Escherichia coli* isolated from adolescents and adults in Hamedan, Western Iran. Iran J Microbiol. 2013;5(1):42–47.

11. Nolonwabo Nontongana, Timothy Sibanda, Elvis Ngwenya, Anthony I. Okoh. Prevalence and antibiogram profiling of *Escherichia coli* pathotypes isolated from the Kat River and the Fort Beaufort abstraction water. Int. J. Environ. Res. Public Health. 2014;11:8213–8227.

12. Mulatu Getamesay, Beyeke Getenet, Zeynudin Ahmed. Prevalence of *Shigella*, *Salmonella* and *Campylobacter Species* and their susceptibility patterns among under five children with diarrhea in Hawassa Town, South Ethiopia. Ethiopian Journal of Health Science. 2014;24(2):101-107.

13. Kirby-Bauer. Modified Kirby-Bauer disc diffusion method for antimicrobial susceptibility testing. Amer. J. Clin. Path. 1966;45:493-496.

14. Clinical Laboratory Standard Institute. Performance standards for antimicrobial susceptibility testing. Twenty-fifth informational supplements. CLSI Document M100-S25. Wayne, PA: Clinical and Laboratory Standard Institute. 2015;35:3.

15. Manikandan C, Amsath A. Antimicrobial resistance of enteric pathogens isolated from children with acute diarrhea in Pattukkottai, Tamil Nadu, India. Int. J. Pure Appl. Zool. 2013;1(2):139-145.

16. Clarence Suh Yah, Helen U. Chineye, Nosakhare Odeh Eghafona. Multi-antibiotics-resistance plasmid profile of enteric pathogens in pediatric patients from Nigeria. Biokemistri. 2007;19(1):35-42.

17. Uma B, Prabhakar K, Rajendran S, Kavitha K, Sarayn YL. Antibiotics sensitivity and plasmid profiles of *Escherichia coli* isolated from pediatric diarrhea. J. Glob. Infect. Dis. 2009;1(2):107-110.

18. Mei Qu, Bing Lv, Xin Zhang, Hanqiu Yan, Ying Huang, Haikun Qian, Bo Pang, Lei Jia, Biao Kan, Quanyi Wang. Prevalence and antibiotic resistance of bacterial pathogens isolated from childhood diarrhea in Beijing, China. Gut Pathogens. 2016;8:31.

19. Moyo Sabrina J, Gro Njolstad, Mateel Mecky I, Kitundu Jesse, Myrmeel Helge, Mylvaganam Haima, Maselle Samuel Y, Langeland Nina. Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dar es Salaam, Tanzania. Biomedical Central Pediatrics. 2011;11:19.

20. Ugwu M, Edeani G, Ejikeugwu C, Okezie U, Ejiofor S. Antibiotic susceptibility profile of *Escherichia coli* and *Salmonella* causing childhood diarrhea in Awka Municipality, South-Eastern Nigeria. Clinical Microbiology. 2017;8:277. ISSN :2327-5073.

21. Ayrikim A, Mulugeta K, Bayeh A, Endalkachew N, Melaku A. Antibiogram of *E. coli* serotypes isolated from children aged under five with acute diarrhea in Bahir Dar town. African Health Science. 2015;15(2):656–664.

22. Aggarwal P, Uppal B, Ghosh R, Prakash SK, Rajeshwari K. Highly-resistant *E. coli* as a common cause of paediatric
diarrhoea in India resistant *E. coli* as a cause of diarrhoea. Health Population and Nutrition. 2013;31(3):409-412. ISSN: 1606-0997.

23. Abdel-Nasser A. El-Moghazy, Mahmoud M. Tawfick, Mahmoud M. El-Habibi of enteric bacterial pathogens isolated from patients with infectious diarrhoea in Cairo, Egypt. Int. J. Curr. Microbiol. App. Sci. 2016;5(4):553-564.

24. Adnan S. Jaran. Antimicrobial resistance pattern and plasmid profile of some salmonella spp. isolated from clinical samples in Riyadh area. European Scientific Journal. 2015;11:6. ISSN: 1857-7881 (Print) e - ISSN 1857-7431.

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