Enteric *Salmonella*species and IntestinalHelminthesCo-infectionamong Typhoid Fever Patients attending PHC Clinics inthe NorthernZone of NasarawaState, Nigeria

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ABSTRACT---*Diarrheal disease continues to be an important cause of morbidity and mortality in developing countries and globally, intestinal parasites, and Salmonella species remain major contributors to acute enteric infections. The study was aimed at determining the co-infection of Salmonella species and helminthic parasites among typhoid fever patients attending Primary Health Care (PHC) clinics in the Northern zone of Nasarawa state, Nigeria. A total of 400 stool samples were aseptically collected of which 390(97.5%) samples were positive for Salmonella species and intestinal parasites. Of these, 70(17.5%), 50(12.5%), 29(7.3%), 03(0.8%) and 197(49.3%) samples were positive for Ascarislumbricoides, Schistosomamansoni, Hook worm, S. stercoralis and Salmonella species respectively. The overall Salmonella species-helminthes co-infection was 15%. Multiple parasitic infections were also observed in 19 (7.3%) patients. The prevalence of Ascarislumbricoides, Hook worm, S. mansoni, and S. stercoralis were highest 34.3%, 12.8%, 13.5% and 2.4% among ages 11-20, 21-30, 31-40 and 51-60 respectively. Salmonella species were highly susceptible to Amikacin, Gentamycin, Ciprofloxacin, Ceftriaxone,and Chloramphenicol and resistant to Tetracycline, Amoxicillin, Amoxycillin/clauvulanic acid. It is therefore, imperative that measures such as health education, improvement of safe water supply, sanitation facilities and continuous monitoring of microbiological, parasitological and antimicrobial surveillance are crucial.*

Keywords---Co-infection, *Salmonella* species, helminthes, typhoid fever, Nasarawa state

1. INTRODUCTION

Gastrointestinal parasitic infections are amongst the most common infections worldwide. These cases are attributed to three common intestinal parasites: *Ascarislumbricoides*, hookworm, and *Trichuristrichiura*. Important determinant of helminthes transmission are climate, with adequate moisture, warm temperature, poverty, inadequate water supply and sanitation [1]. The global prevalence of helminthic diseases is estimated to be 478 million children for *A. lumbricoides*; 280 million for hookworms and 347 million children for *T. trichiura*[1]. Reports of prevalence studies have indicated that the triad of *Ascarislumbricoides,Trichuristrichiura* and the hookworm species are common infections in Nigeria. Very important determinants of transmission of these infections are climate with adequate moisture and warm temperature, poverty and inadequate water supply and sanitation [1]. The most common effect of helminthic infection is a subtle and insidious constraint on normal physical development, resulting in children failing to achieve their genetic potential for growth and suffering from the clinical consequences of iron deficiency anaemia and other nutritional consequences.

Enteric *Salmonella* species are bacteria that are responsible for typhoid fever.The bacteria can survive in water for one week, two weeks in sewage and one month in ice cream.However, boiling of water or milk destroys the organisms[2].

The disease-Typhoid fever like the gastrointestinal parasitic infections is one of the most important life threatening but under estimated enteric infectious disease.The incidence of typhoid fever is significantly high. Typhoid fever incidence was estimated by [3] and [4] to be approximately 21.7 million cases worldwide with more than 700,000 deaths, of which infants, children and adolescents in South-central and South-eastern Asia experience the greatest burden of illness.

It is a major health problem in all parts of the world where safe drinking water and sanitation is inadequate.The incidence of infection and death caused by typhoid fever has been greatly increased by many factors. Among these are;

crowded and impoverished populations with a combination of poor sanitation and hygiene, exposure to unsafe water and food and unavailability of vaccines and high cost of effective antimicrobial chemotherapy [5]. Besides, the effectiveness of antimicrobial chemotherapy is also being challenged by the emergence of antibiotic resistance [6] and [7].

In Nigeria the infection is not only endemic but constitutes a great socio-medical problem.The distribution pattern of typhoid fever in Nigeria seems uncertain.Some studies found that enteric fevers are more prevalent in males than in females [8], while [9] found no influence of age, sex, and social class on the distribution pattern of *S.typhi/paratyphi.*

Thus, thiscross sectional study was conducted to determine the Co-infection and prevalence of *Salmonella* speciesand intestinal helminthesamong clinically diagnosed typhoid fever patients attending Primary Health Care Clinics (PHC) in the Northern zoneand antimicrobial susceptibility of *Salmonella* species to commonly used antibiotics.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out among typhoid fever patients attending Primary Health Care Clinics (PHC) in the Northern zone of Nasarawa state, Nigeria to determine the prevalence of *Salmonella* species and helminthic parasites co-infection

2.2 Ethical Clearance

Ethical clearance was obtained in accordance with the code of ethics for biomedical research involving human subjects from the Nasarawa state Ministry of Health committee on infectious diseases, official consent was also obtained from the patients and parents or guardians of children.

2.3 Sample Collection

A total of 400 stool samples were collected fromclinically diagnosed typhoid fever patients that presented themselves for typhoid fever treatment into sterile plastic universal containers from May, 2012- April, 2013. All samples were aseptically labelled appropriately and packed well in an ice pack and taken to the Microbiology laboratory of Nasarawa State University Keffi, Nasarawa State for isolation and other microbiological analyses.Relevant demographic, clinical and laboratory data were also recorded and transferred to the questionnaire prepared for this study.

2.4 Isolation

A loop full of each stool sample was homogenized in normal saline and then inoculated into Tetrathionate broth (Oxoid Ltd USA) at room temperature and incubated at 37^0C for 24 hours.The resultant enriched culture was then sub-cultured onto Bismuth sulphite agar (Oxoid Ltd USA) and incubated at 37^0C for 24 hours.The isolated non-lactose fermenter colonies were observed microscopically and thereafter, screened through biochemical tests and serology [10] for *Salmonella spp*.

2.5 Parasitological Examination of Stool

Stool specimens were examined for the presence of cysts, eggs, trophozoites and larvae of intestinal parasites. Slides were prepared directly for wet mount in saline as well as in iodine and were microscopically examined under low power (X10 magnifications) and then under high-power (X40 magnification).Finally the sample was concentrated using formalin ethyl acetate technique. Iodine stained slides were then prepared and examined microscopically.

2.6 Antibiotic Susceptibility Testing

Kirby-Bauer disc diffusion method was used to determine the susceptibility of all the pure strains of *Salmonella*species isolates [10].The antibiotics impregnated discs used with their concentration were: Amikacin (30µg), Amoxicillin (25µg), Amoxicillin/Clauvalinic acid (30µg), Chloramphenicol (30µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Gentamicin (10µg) and Tetracycline (30µg) (Oxoid Ltd USA).Zone of inhibition diameters were measured to the nearest millimetre using a ruler. Results were interpreted using the criteria of the National Committee for Clinical Laboratory Standards.

2.7 Statistical Tools Used

To achieve the objectives of this research work, Chi-square test and correlation analysis were utilised to assess significant differences. A difference was taken as significant at a p-value less than 0.05.

3. RESULTS

Out of the 400 stool samples collected aseptically from clinically diagnosed typhoid fever patients, 390(97.5%) samples were positive for intestinal helminthes and *Salmonella* species. Of these, 70(17.5%), and 50(12.5%) samples were positive for *Ascarislumbricoides* and *Schistosomamansoni*, while 29(7.3%), 03(0.8%) and 197(49.3%) samples were positive for Hook worm, *S. stercoralis* and *Salmonella* species respectively. With respect to the nature of stool, watery stool had the highest prevalence (55.8%) of *Salmonella* species,while stools mixed with blood had the highest

prevalence of *Ascarislumbricoides*(78.1%). Hook worm, had highest prevalence (27.4%) in mucoid stools. *Schistosomamansoni* and *Strongyloidesstercoralis* on the other hand had highest prevalence in mucoid stools (29.0%) and stools mixed with blood (3.1%) respectively (Table 1). Theoverall *Salmonella* species and helminthesco-infection was 15% with 9.1% and 10.6% co-infection among males and females respectively. The overall prevalence of helminthes in the study was 38% while that of *Salmonella* species was 49.3%. Multiple parasitic infections were also observed in 12(3%) patients.

The prevalence of intestinal helminthes among clinically diagnosed typhoid fever patients with respect to sex showed that female patients had the highest burden (38.5%) of infection than the males (37.4%). The prevalence of *Ascarislumbricoides*, Hook worm, *S. mansoni*, and *S. stercoralis*with respect to different age groups were highest 34.3%, 12.8%, 13.5% and 2.4% among ages 11-20, 21-30, 31-40 and 51-60 respectively (Table 3). *Salmonella* species infection however, was highest (55.4%) among the females, with ages 31-40 having the highest burden of *Salmonella* infection.

Salmonella species were highly susceptible to Amikacin (90.8%), Gentamycin (87.4%), Ciprofloxacin (85.1%) and Ceftriaxone (78.2%). *Salmonella*species isolates were highly susceptible to Chloramphenicol. The bacterial isolates were however resistant to Amoxicillin, Amoxicillin/clauvulanic acid and Tetracycline (Table 4).

4. DISCUSSION

Typhoid fever, a worldwide bacterial disease and intestinal helminthesinfectionsare both endemic in Nigeria [11] and in areas where there is high endemicity, co-infection is an inevitable reality. This is because both enteropathogensshare social and pathological circumstances that are imperative for their transmission and pathogenesis. They are both water-borne and food-borne and when in the blood, *Salmonella*species attach to the tegument of helmintheslike Schistosomesand could serve as a source and vehicle of *Salmonella* infections [12]. This study revealed 15% concurrent*Salmonella* species and intestinal helminthes infections among typhoid fever patients attending primary Health Care clinics in the Northern zone of Nasarawa state, with 14% and 9.1% in female and male respectively.This incidence though higher than what [13]and [11]worked in Jos, Plateau state and Kaduna state respectively, it is probably because this was on the different types of intestinal helminthes while theirs on *Schistosoma* species.The correlation analysis also showed a strong relation of these groups of enteropathogens. From the study, watery and loosed stool had higher prevalence of the enteropathogens (Table 1) and with respect to *Salmonella* species infection, watery and mucoid stools had highest prevalence of 55.8% and 54.8% respectively. This is in agreement to what [14] observed in their studies among acutegastroenteritis children in Federal Capital Territory Abuja, Nigeria.Among the helminthes, *Ascarislumbricoid*es (17.5%) was the most prevalent parasite followed by *Schistosomamansoni* (12.5%). The dominancy of *A. lumbricoides* among thesehelminthes in this study is in agreement with the findings of [14] and also higher among ages 11-20 years.

Salmonella species showed low resistance level,36.8% and 26.4% for *S. typhi* and *S. paratyphi* respectively to Chloramphenicol in this study, this could be due abandoning of prescribing the drug by the responsible health personnel before a long time ago.

5. CONCLUSION

The study showed a relationship between *Salmonella*species and intestinal helminthesinfections.The co-infection of *Salmonella* species with helminthescould complicate treatment of enteric fever caused by *Salmonella* bacteria. This is because the worms could provide a focus for multiplication of this bacterium, which are then released into the blood stream, causing septicemia. This explains why chemotherapy for *Salmonella* infection must be administered with anti-helminthic treatment to be efficient. This association can also result in early relapses of typhoid fever as opined by [16].

Since both diseases share social circumstances such as poverty and hygiene, governments' involvements in the improvement of the standard of living of individuals in areas of high endemicity is hereby recommended. Also, cases of the disease should be detected and promptly treated to avoid further transmissions. The populace should be educated on the importance of personal hygiene and appropriate drugs should be made available at reduced and affordable costs. A continuous monitoring of microbiological, parasitological and antimicrobial surveillance is crucial.

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Table 1: Distribution of Enteropathogenswith respect to nature of stool examined among typhoid patients

Nature of Stool	No.Examined	No.Enteropathogens (%)					Total
		A	BC	D		E	
Watery	165	92(55.8)	04(2.4)	01(0.6)	05(3.0)	02(1.2)	124
Mucoid	62	34(54.8)	09(14.5)	17(27.4)	18(29.0)	00(---)	88
Loose Stool	141	60(42.6)	32(22.7)	06(4.3)	21(14.9)	00(---)	125
Mixed with blood And mucus	32	11(34.4)	25(78.1)	05(15.6)	06(18.8)	01(3.1)	53
Total	400	197(49.3)	70(17.5)	29(7.3)	50(12.5)	03(0.8)	390

Key: A= *Salmonella* spp; B= *Ascarilumbricoides*; C= Hook worm; D= *Schistosomamansoni*;
E= *Strongyloidesstercoralis*

Table 2: Distribution of *Salmonella*-helminthic parasites among typhoid patients with respect to Sex

Sex	No. examine	NO. Enteropathogens (%)				Total	Co-infection(%)
		ABC	DE				
Male	187	79(42.2)	33(17.6)	11(5.9)	23(12.3)	3(1.6)	70(37.4) 17(9.1)
Female	213	118(55.4)	37(17.4)	18(8.5)	27(12.7)	-	-82(38.5) 31(14.6)
Total	400	197(49.3)	70(17.5)	29(7.3)	50(12.5)	3(0.8)	152(38.0) 60(15.0)

Key: A= *Salmonella* spp; B= *Ascarislumbricoides*; C= Hookworm; D=*Schistosomamansoni*;
E= *Strongyloidesstercoralis*

Table 3: Distribution of *Salmonella spp*-Helminthic parasitesamong typhoid patients with respect to Age

Age group	No. examined	No. Enteropathogens (%)				
		A	BC	D	E	
< - 10	68	31(45.6)	18(10.1)	03(1.7)	02(1.1)	00(0.0)
11-20	67	37(55.2)	72(34.3)	18(8.6)	22(10.5)	00(0.0)
21-30	80	54(67.5)	49(19.6)	32(12.8)	43(12.2)	00(0.0)
31-40	50	36(72.0)	23(13.5)	12(7.0)	23(13.5)	04(2.3)
41-50	59	21(35.6)	13(7.3)	07(3.9)	12(6.7)	04(2.2)
51-60	41	15(36.6)	07(5.6)	02(1.6)	08(6.3)	03(2.4)
61 >	35	03(8.6)	01(1.2)	01(1.2)	02(2.3)	02(2.3)
TOTAL	400	197(49.3)	70(17.5)	29(7.3)	50(12.5)	03(0.8)

Key: A= *Salmonella* spp; B= *Ascarislumbricoides*; C= Hookworm; D= *Schistosomamansoni*;
E= *Strongyloidesstercoralis*

Table 4: Antibiotic susceptibility of *Salmonella* species isolated from clinically diagnosed typhoid fever patients.

Antibiotics Used	*Salmonella typhi* (n=87)	*Salmonella paratyphi* (n=110)
Amikacin	79(90.8)	98(89.1)
Amoxicillin	17(19.5)	26(23.6)
Amoxicillin/clavulinic acid	22(25.3)	40(36.4)
Ceftriaxone	68(78.2)	89(80.9)
Chloramphenicol	55(63.2)	81(73.6)
Ciprofloxacin	74(85.1)	92(83.6)
Gentamycin	76(87.4)	87(79.1)
Tetracycline	6(6.9)	23(20.9)

n= Number of isolates

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