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Prevalence of Non-Typhoidal *Salmonella* among HIV/AIDS Patients and Poultry Chicken in Ekiti State

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Authors' contributions

This work was carried out in collaboration between both authors. Authors AOO and OOB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Both authors AOO and OOB managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Salmonella is an important cause of infection in both humans and animals. This study was therefore carried out to identify the non-typhoidal *Salmonella* isolates from poultry chicken which is one of the main animal reservoirs of Salmonellosis and from HIV/AIDS patients in Ekiti State, Nigeria. A total of 200 samples were collected altogether (100 human feacal samples, 50 human blood and 50 feacal samples from broiler chicken) and analysed using standard methods. The antimicrobial susceptibilities were also carried out using disc diffusion technique. A total of 18 Non-typhoidal *Salmonella* isolates were obtained from human samples with prevalent rate of 12% and 4 Non typhoidal *Salmonella* isolates were from poultry broiler-chicken with prevalent rate of 8%. There was no significant difference in number of male and female as there were 10 males and 8 females infected with Non-typhoidal *Salmonella*, with a median age of 30 years. Although, almost all the human and Broiler-Chicken non-typhoidal *Salmonella* isolates showed resistance to more than one antibiotic but Ampicilin, Tetracycline and Gentamycin showed 100% resistance rate to Broiler-chicken isolates while Ceftriaxone and Ofloxacin showed least resistance among human isolates. The average resistances to seven commonly prescribed antibiotics were more in Broiler-

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Chicken (78.6%) than in Humans (53.9%). However, the high resistance showed in poultry suggested that there might likely be spread of these resistance strains to human in this study environment.

Keywords: Salmonellosis; prevalence; NTS; HIV/AIDS; Nigeria.

1. INTRODUCTION

Salmonella are found worldwide in cold and warm blooded animals (including humans), and in the environment. They cause illnesses like typhoid fever, paratyphoid fever, food borne illness [1] and bacterimia illness [2]. It has also been reported to cause a huge global burden of morbidity and mortality among both the health and immunocompromised individual [3]. *Salmonella* infections are zoonotic and can be transferred between humans and non-human animals. Many infections are due to ingestion of contaminated food. However, S*almonella* serotypes are generally distinguished into two broad classes: Typhoidal *Salmonella* and non tyhoidal *Salmonella*. A distinction is made between enteritis *Salmonella* and *Salmonella* typhoid/paratyphoid *Salmonella*, because of a special virulence factor and capsule protein (virulence antigen) posses by S. typhi and *paratyphi* which can cause serious illness. *Salmonella typhi* is adapted to humans and does not occur in other animals. The typhoidal *Salmonella* causes typhoid fever and enteric fever with the disease endemicity observed in tropic and sub-tropic and has become a major public health problem in developing countries of the world [4] including Nigeria where they constitute serious sources of morbidities and mortalities [5]. However, on the other hand, Non-typhoidal *Salmonellae* (NTS) are an important cause of infectious diarrhoea world-wide. It also remains an important cause of invasive disease, particularly in developing countries, likely secondary to the high prevalence of coexisting malnutrition, malaria and HIV infection [6]. It is also a leading etiology of community- acquired bacteremia in patients with HIV infection in developed or developing countries [2,7,8].

Non typhoidal *Salmonella* is mainly spread to humans through foods most especially meat, poultry and their products contaminated or infected with NTS. Spread by cross-contamination occurs when *Salmonella* contaminates ready-to-eat food: for example, when food that will not be cooked further is cut with a contaminated knife or via the hands of an infected food handler. *Salmonella* can spread from person-to-person via the hands of an infected person [9]. NTS have a worldwide distribution; there were 93.8 million episodes estimated and 155,000 deaths each year attributable to NTS [10]. About 142,000 Americans are infected each year with *Salmonella enteritidis* from chicken eggs, and resulted to about 30 deaths [11]. The U.S. Government reported as many as 20% of all chickens were contaminated with *Salmonella* in the late 1990s and 16.3% were contaminated in 2005 [2]. A 2010 study of worldwide burden of *Salmonella* gastroenteritis estimated 2·5 million cases of the disease and 4100 deaths per year in Africa [10]. In Nigeria, morbidity associated with illnesses due to *Salmonella* continues to be on the increase and, in some cases, resulting in death. Thus, the purpose of this study was to identify the non-typhoidal *Salmonella* isolates from poultry chicken which is the main animal reservoir of Salmonellosis and from HIV/AIDS patients attending Federal Medical Centre, Ido Ekiti. The antimicrobial resistance pattern was examined to determine the resistance to some commonly prescribed antibiotics.

2. MATERIALS AND METHODS

2.1 Ethical Consideration, Questionnaire and Informed Consent

The ethical clearance (ERC/2013/08/20/50B) for this research was given by Federal Medical Centre (FMC) Ethical Committee after due processes had been followed. Administration of questionnaires was done to the consented subjects to obtain the demographic data and other relevant information.

2.2 Human Samples

A total of one hundred and fifty HIV/AIDS patients attending Federal Medical Centre, Ido Ekiti participated in this study. One hundred stool samples and fifty blood samples were collected. Stool samples were pre-enriched in Rappaport vassiliadis soy broth overnight while the blood sample were cultured on Brain heart infusion broth for 7 days and then sub cultured on

Bismuth Sulphide Agar and MacConkey Agar. Colonies were then identified [12,13].

2.3 Livestock Samples

From September, 2013 to February, 2014, a total of 50 feacal samples from broiler-chicken were collected with sterile swab sticks and transported within 1 hour of collection to the laboratory. The swab was pre enriched in Rappaport vassiliadis soy broth overnight and sub cultured on BSA and MacConkey Agar [12,13].

2.4 Identification of Isolates

The isolates were identified as non-typhoidal *Salmonella* with the following biochemical tests: Substrate Utilization test with Kligler Iron Agar, Urease, Citrate, Indole, Motility and Oxidase test using standard methods described by Ochei and Kolkahtar [12]; Cheesbrough [13].

2.5 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed using Mueller-Hinton agar by Disc diffusion method as described by CLSI [14]. The following antibiotics were tested: ciprofloxacin (5 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ofloxacin (5 ug), ampicillin (10 µg), tetracycline (30 µg), and gentamycin (10 µg) and purchased from OXOID company.

2.6 Statistical Analysis

The data generated from this study were analysis using SPSS version 16.0 (SPSS Inc. Chigago IL).

3. RESULTS

Eighteen human non-typhoidal *Salmonella* isolates were isolated from September, 2013 to February, 2014 at Federal Medical Centre, Ido Ekiti. These isolates were gotten from HIV/AIDS patients with sporadic cases seeking medical attention. It was very difficult to incriminate any particular food associated with the sporadic cases as the entire participant identified with the meat and various foods.

Among the eighteen human isolates, sixteen *Salmonella* isolates were from feacal samples. The remaining two isolates were from the blood samples (Table 1). There were 10 males and 8 females, with a median age of 30 years (Table 2). All the patients were immunocompromised with the CD 4^+ count of < 200 cells/ ul.

Table 1. Prevalence of non typhoidal *Salmonella* (NTS) from human samples (HIV/AIDS patients)

	Faecal	Blood	Total
Positive	16 (16.0)	2(4.0)	18 (12)
Negative	84 (84.0)	48(96.0)	132 (88)
Total	100(100.0)	50 (100.0)	150 (100)

Table 2. Prevalence of NTS in relation to age and sex among HIV/AIDS patients

Age group	No	Sex	
	Positive	Male	Female
1-10	1	0	1
11-20	4	2	2
21-30	11	6	5
31-40	2	2	0
41-50	0	0	0
51-60	0	0	0
61-70	0	0	0
>70	0	0	0
Total	18	10	8

However, the prevalence of *Salmonella* among the Broiler-chicken in poultry farm in Ekiti State University, Ado-Ekiti showed that out of the 50 feacal samples collected from broiler-chicken, 4 (8.0%) non-typhoidal *Salmonella* were isolated between September, 2013 to February, 2014 (Table 3).

Table 3. Prevalence of NTS from feacal sample of chicken-broiler

	Faecal samples
Positive	4 (8.0)
Negative	46 (92.0)
Total	50 (100.0)

The antimicrobial susceptibilities of the non-typhoidal *Salmonella* were revealed in (Fig. 1). Of the 18 human NTS isolates, 12 (66.7%) isolates showed resistance to Ciprofloxacin and Ampicillin each, 11 (61.1%) isolates showed resistance to Tetracycline, 10 (55.6%) NTS were resistance to Gentamycin while 8 (44.4%) isolates showed resistance to Ceftriaxone and Ofloxacin and 7 (38.9%) NTS isolates were resistance to Ceftazidime with an average resistance to seven tested antibiotics of 53.9% (Table 4). However, out of the 4 NTS isolated from poultry and tested with seven antibiotics commonly prescribed, all the NTS isolates (100%) showed resistance to Ampicillin,

Tetracycline and Gentamycin, 3 (75.0%) isolates were resistance to Ciprofloxacin and Ofloxacin while 2 (50%) *Salmonella* isolates showed resistance to Ceftriaxone and ceftazidime (Fig. 1.) with an average resistance to seven antibiotics of 78.6% (Table 4).

4. DISCUSSION

Non-typhoidal *Salmonella* isolation from poultry and clinical sources has not received much attention in Ekiti State, Nigeria. However, the objective of this work was to isolate NTS from poultry and HIV/AIDS patients, since poultry remains one of the major routes of transmission of this organism. The result obtained showed varying frequencies in the isolation of non-typhoidal *Salmonella* in this study. Four (8.0%) out of the fifty broiler-chicken tested had NTS. This may be due to the fact that most poultry birds were usually given antibiotics which might have lead to few numbers of isolates in this study. This agreed with CDC [15] that reported the prevalence of NTS among chickens in Michigan as 9.2%.

However, the prevalent rate of isolates from human (HIV/AIDS patients) varies according to the type of samples; feacal sample had 16% while blood had 4% isolation rate. This may be due to the fact that faeces being a non-sterile sample while blood being a sterile sample; which may account for the higher prevalence of non-typhoidal *Salmonella* in the faecal sample [16].

In the demographic data of patients whose stool and blood samples were examined, it was observed that there was no much difference between infections in male as compared to female. This may be due to the fact that Humans and warm blooded animals are considered to be the primary reservoir of non-typhoidal *Salmonella* and subsequent shedding can result in the spread to humans and their environs [17].

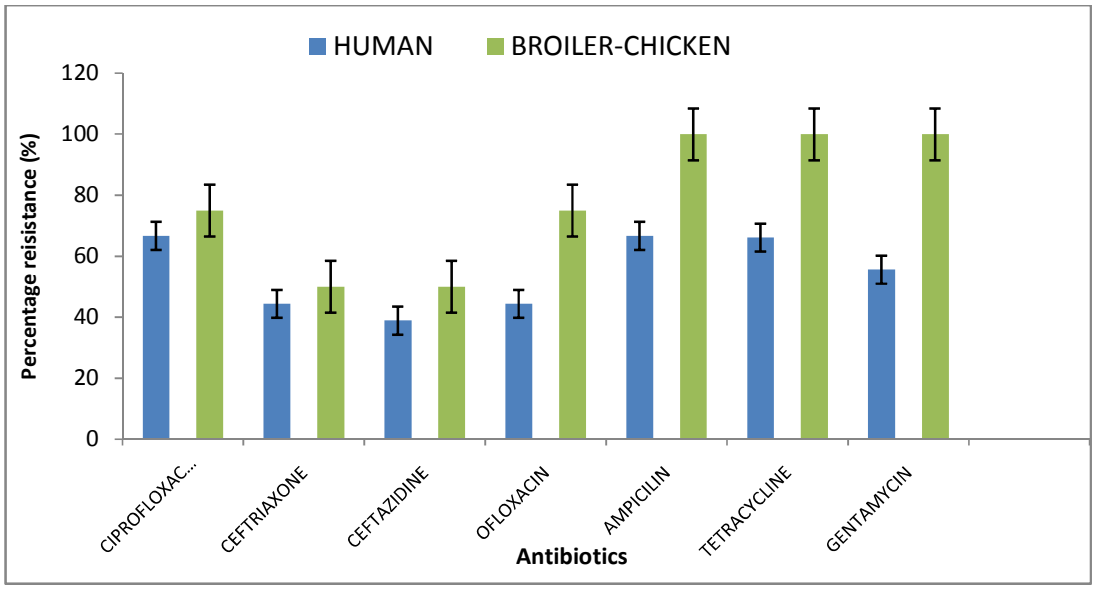

Fig. 1. Antibotics resistance of non-typhoidal *Salmonella* from both human (HIV/AIDS patients) and animal

Γ= Standard bar error

Table 4. Average resistance of NTS to seven selected antibiotics

Organisms	Average resistance to seven antibiotics tested
Non-typhoidal *Salmonella* (Human) n =18%	9.7 53.9
Non-typhoidal *Salmonella* (Poultry) n=4%	3.1 78.6

The age distribution of non-typhoidal *Salmonella* isolates showed a high prevalent in age group 21-30 years with an average median age of 30 years.

Almost all the human and broiler-chicken *Salmonella* isolates showed resistance to more than one antibiotic, suggesting that antimicrobial resistance is wide spread in both human and livestock [2]. High resistance rate was seen among the poultry bird especially to antibiotics like Ampicillin, Tetracycline and Gentamycin. This may be due to the fact that the poultry birds were usually given antibiotics and this has created a pressure in antibiotics resistance in human. Reports have indicated that the use of antimicrobials for growth-promotion prophylaxis and treatment of food-animals increases the prevalence of resistance in human pathogens, particularly non-typhoidal Salmonellosis. This practice is common in Asia and the United Kingdom and in some other European countries where fluoroquinolones have been approved for animal use [18,19]. In this study, the cephalosporin class of antibiotics (Ceftazidine and Ceftriaxone) showed lesser resistance to isolates from poultry. Although, the study conducted by Chenng et al. [2] showed no resistance of ciprofloxacin and ceftriaxone to either human or poultry isolates. However, the increasing smuggling of poultry products into our country, as a result of the ban in importation of these products in recent times, might be, among other factors, a likely reason for the emergence of reduced susceptibility to fluroquinolones and cephalosporins in our environment. There has been a suggestion that the use of antimicrobials in animal growth should be phased out gradually, since similar benefits could be obtained by improving other aspects of animal care, such as hygiene [18]. However, the molecular study of these isolates was not done; therefore, it was very difficult to ascertain their epidemiological relatedness. Although many research had shown relatedness in human isolates and that of their environment [1,2].

In conclusion, poultry could be a potential source of non-typhoidal *Salmonella* infection in human especially in HIV/AIDS patients and as such, there is a need to study the molecular relatedness of the isolates from both human and poultry so as to ascertain the source of infection in HIV/AIDS patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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