Original Research Article

Antibiotics susceptibility profile of Non-Typhoidal *Salmonella* Species using Selected 3rd Generation Cephalosporins antibiotics against Humans and Poultry Faeces in Ido-Ekiti, Ekiti State.

ABSTRACT

INTRODUCTION: Non-typhoidal *Salmonella* (NTS) isolates are among the most common bacterial pathogens associated with antimicrobial resistance (AMR) where animals are known to be the major reservoir of NTS despite improvements in hygiene and sanitation.
AIM: This study was conducted to investigate the prevalence of non-typhoidal *salmonella* species from humans and poultry faeces in Ido-Ekiti using selected 3rd generation cephalosporin antibiotics.STUDY LOCATION AND DURATION: The study was carried out at Federal Teaching Hospital Ido Ekiti for 3months between July 2023 and September 2023.
METHODOLOGY:A total of 300 faecal samples were collected (100 from humans and 200 from poultry) by random sampling and analyzedby enriching on Selenite F broth and cultured on XLD agar using standard method. Faecal samples were identified using morphology, microscopic and biochemical characteristics. Antibiotic susceptibility testwasdetermined using the disc diffusion method.
RESULTS: The prevalence of NTS in human and poultry sources were 9% and 11.5% respectively. Antibiotic susceptibility test revealed high resistance rates to 3rd generation Cephalosporins antibiotics among the poultry isolates, especially to Cefuroxime, Cefotaxime and ceftriaxone at 86.9%, 65.2% and 82.6% respectively while Ceftazidime at 88.9% only hada high resistance from human samples as compared to the poultry samples.
CONCLUSION: The study confirmed that poultries in Ido Ekiti are likely major sources of resistant NTS, and this poses a potential hazard to the public, especially those living close to the poultry and those rearing them.

Keywords: Non Typhoidal Salmonella (NTS), 3rd Generation Cephalosporins, Humans, Poultry birds.

1.INTRODUCTION

Nontyphoidal *Salmonella* (NTS) infections in Africa have been reported to have a mortality rate of 20-25% by 2020 [10]. Most non-typhoidal *Salmonella* (NTS) infections are caused by *Salmonella enterica Typhimurium or Salmonella enterica Enteritidis*. In 2019, there were 87,923 cases of NTS, the second most common bacterial gastrointestinal infection after campylobacteriosis [8]. Non-Typhoidal*Salmonella* (NTS) is a symptomatic infection caused by bacteria of the genus Salmonella [20]. It is defined as an infectious or toxic disease caused by an agent that enters the body through food. Infection with nontyphoidal serotypes of *Salmonella* usually results in food poisoning. The elderly, the young, and others with weakened immune systems are more likely to develop NTS. It is usually spread by eating contaminated meat, eggs, water, or milk [19]. Other foods can spread disease if they come in contact with people. Several domestic animals such as cats, dogs and reptiles can carry and spread the infection. Diagnosis of NTS is made by stool examination or blood test [22]. The main source of this pathogen is poultry and cattle. Although most cases of salmonellosis are resolved on their own, antibiotic treatment may be necessary in severe cases, they may progress rapidly to systemic infection, particularly in infants, older adults, and individuals with weakened immune systems, requiring prompt clinical treatment [2]. The high prevalence of NTS infections in Nigeria is linked

to various factors, including poor sanitation, inadequate access to clean water, and inadequate food handling and storage practices.

The problem of NTS infections is further complicated by the emergence of antimicrobial-resistant strains. Antibiotic resistant infections lead to longer, more severe illnesses and reduced treatment effectiveness [12]. NTS strains have been shown to develop resistance to antibiotics commonly used to treat infections. The spread of antimicrobial-resistant *Salmonella* is a major public health problem, as drug-resistant infections can cause high morbidity and mortality due to reduced disease duration and severity and reduced treatment efficacy [15,12]. Following Fajilade*et al.* [7], antimicrobial drug resistance is prevalent among NTS species where Isolates showed resistance to amoxicillin (100%), cotrimoxazole (100%), ampicillin (86%), chloramphenicol (100%) and ofloxacin (57%).

Cephalosporin C was discovered in the sea near a sewage outfall in Susiccu by the port of Cagliari in Sardinia by the Italian pharmacologist Giuseppe Brotzu in July 1945 [21]. Cephalosporin compounds were first isolated from a culture of *Acremonium strictum* from sewage in Sardinia in 1948 by the Italian scientist Giuseppe Brotzu [17]. Cephalosporins act similarly to beta-lactam antibiotics. They inhibit the synthesis of peptidoglycan in the cell wall of bacteria. Peptidoglycan is the bacterial exoskeleton that provides structural integrity and shape to cells and protects them from rupture [16]. These antibiotics are divided into several generations based on the period of drug development and their antimicrobial properties [16]. The difference lies in the molecular structure and has important therapeutic implications. The group of cephalosporins considered third generation is the most prescribed [18].

Third generation cephalosporins are drugs used to control and treat gram-negative and gram-positive bacteria. This drug belongs to the beta-lactam drug family, the most prescribed drug group. These cephalosporins are semi-synthetic analogues with different chemical substitutions in the C7 acylamide chain. This category includes ceftriaxone, cefdinir, cefixime, cefixime, cefpodoxime, ceftazidime, cefoprazone, ceftizoxime, and ceftibuten. It is a broad-spectrum antibacterial agent with activity against gram-negative and gram-positive bacteria and is more active against primary and secondary cephalosporin-resistant gram-negative bacteria. Interestingly, third generation cephalosporins are more resistant to beta-lactamase than first or second generation cephalosporins, especially *Salmonella, Klebsiella and Escherichia coli*. Several studies have reported 3rd generation cephalosporin resistance genes in non-typhoidal *Salmonella* isolated from humans and animals in Nigeria. A study conducted in Lagos, Nigeria by Iroha *et al*. [11] found that 38.4% of *Salmonella* typhoidal isolates were resistant to 3rd generation cephalosporins, some third generation cephalosporins include cefotaxime, ceftriaxone, and ceftazidime, as well as the oximino-monobactamaztreonam. The aim of the study??

2. MATERIALS AND METHODS

2.1 ETHICAL CONSIDERATION, QUESTIONNAIRE AND INFORMED CONSENT

The ethical clearance (ERC/2022/06/06/794A) for this research was given by Federal Medical Centre (FMC) Ethical Committee after due processes had been followed. Administration of questionnaires was given to the subjects who consented to obtain their demographic data and other relevant information. The study was conducted between July 2022 and November 2022.

2.2 HUMAN SAMPLES

A total of one hundred patients attending Federal Medical Centre, Ido Ekiti presenting with the study infection participated in this study and 200 poultry birds. One hundred faecal samples were collected and enriched in selenite F broth overnight and cultured on Xylose Lysine Deoxycholate (XLD) agar (Oxiod,uk) for 24hrs ,NTS Colonies were then identified [4].

2.3 POULTRY SAMPLES

A totalof 200 feacal samples from poultry birds werecollected with sterile swab sticks and transportedwithin 1 hour of collection to the laboratory. Theswab was pre enriched in enriched in ?selenite F broth overnight and cultured on Xylose Lysine Deoxycholate (XLD) agar (Oxiod,uk) for 24hrs ,NTS Colonies were then identified [4].

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 testusing standard methods described by Cheesbrough [5].

2.5 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility tests were performedusing Mueller-Hinton agar by Disc diffusionmethod as described by CLSI [6]. The followingantibiotics (Oxoid, Basingstoke, UK) were tested: Ofloxacin (OFX) (5µg), Pefloxacin (PEF) (5µg), Ciprofloxacin (CIP) (5µg), Levofloxacin (LEV) (5µg).were used in the study.

2.6 STATISTICAL ANALYSIS

The data generated from this study wasanalyzedusing SPSS version 16.0 (SPSS Inc.Chigago IL).

3. RESULTS

The overall prevalence of non-typhoidal *Salmonella* isolated in human and poultry faecal samples was 32(10.7%) isolated from July 2022 to November 2022 (Table 1). Nine (9.0%) non-typhoidal *Salmonella* isolates from human faecal were identified at Federal Medical Centre, IdoEkiti (Table 1). Based on their age, 61-70 were most prevalent at 55.5%.There were 3 males and 6females, and based on their occupation, farmers had a high prevalence at 44.4% as compared to traders(Table 2). All the patients were showing presenting symptoms of NTS. However, the prevalence of NTS among the poultry birds selected at Ido Ekiti poultry locations showed that out of 200 feacal samples collected, 23(11.5%) non-typhoidal *Salmonella* were isolated (Table 1).

Table 1: Overall prevalence of Non-typhoidal *Salmonella* (NTS) isolated from humans and poultryfaecal.

Nontyphoidal *Salmonella*	No of Patients (n= 100)	No of Poultry (n=200)	Total (n=300)
Positive	9 (9.0%)	23 (11.5%)	32 (10.7%)
Negative	91 (91.0%)	177 (88.5%)	268 (89.3%)
Total	**100 (100.0%)**	**200 (100.0%)**	**300 (100.0%)**

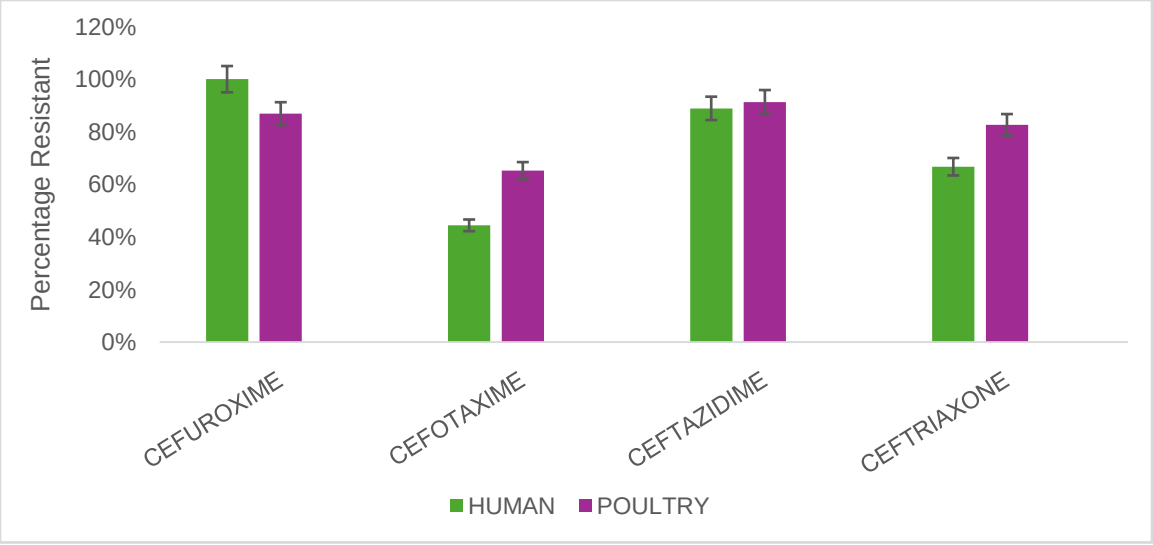
Table 2: Prevalence of NTS in relation to age, sex and occupation

	Positive(%)
AGE	
1-10	3 (33.3%)
11-20	1(11.1%)

61-70		5 (55.5%)
Total		**9 (100%)**
SEX		
Male	3 (33.3%)	
Female	6 (66.6%)	
Total		**9 (100%)**
OCCUPATION		
Trader	2 (22.2%)	
Farmer	4 (44.4%)	
None		3 (33.3%)
Total	**9 (100%)**	

The antimicrobial susceptibilities of the non-typhoidal*Salmonella* using four prescribed antibiotics were revealed in (Fig. 1).Of the 9 human NTS isolates, all 9 (100%)isolates showed resistance to Cefuroxime ,4 (44.4%) isolates showedresistance to Cefotaxime, 8(88.9%) NTS wereresistant to ceftazidime while6 (66.6%)isolates showed resistance to Ceftriaxone. However, out of the 23 NTS isolatedfrom poultry,20 (86.9%) and 15 (65.2%) isolateswere resistant to Cefuroxime and Cefotaxime respectively while 21(91.3%) and 19(82.6%) were resistant to ceftazidime and ceftriaxone respectively.

Fig 1: Prevalence of antibiotic resistance of NTSisolates from human and poultry faecal samples



4. DISCUSSION

Non-typhoidal *Salmonella* (NTS) is a very important pathogenic organism, ~~that~~ its prevalenceposing a serious threat to man [3] and its isolation from poultry and clinical sources has not received much attention in Ekiti State, Nigeria. ~~However~~, The aim of this study was to

isolate NTS from poultry and human' faecal samples in Ido Ekiti, and poultry is confirmed to remain one of the major routes of transmission of this organism. From the results obtained, Nine out of the one hundred gastroenteritis patients had NTS. Many studies have shown that Non-typhoidal *Salmonella* (NTS) is among the very common pathogens causing bacterial bloodstream and gastroenteritis in adults and children in sub-Saharan Africa as reported by Akyala*etal*. [1].Twenty-three out of two hundred poultry samples had NTS and the rate of isolation is higher compared to the isolation from humans. The prevalence of NTS from human faeces was 9.0% and poultry fecal sample had an 11.5% isolation rate, its prevalence among poultry faecal might be assumed as the main source of spread to humans. This agrees with Krawiec *et al*. [14] who reported a 64 (6.4%) prevalence rate of NTS among 100 samples collected from poultry and also agrees with Haeusler *et al.* [9] who reported that non-typhoidal *salmonellae* are isolated in 2–27% of stool culture-positive diarrhoeal illness. This is because Non-Typhoidal*Salmonella* species are prevalent and they occur as pathogenic bacteria in the intestines of domestic, poultry, and wild animals.

In the demographic data of humans with NTS, based on their sex, there was no difference statistically between infections in males as compared to females suggesting that anybody can be infected either male or female, most especially when coming in contact with the bacteria. This agrees with an earlier work of Akyala*etal*. [1] who reported that sex is not a predisposing factor to NTS bacteremia in immunocompromised hosts.The age distribution revealed a high prevalence in the age group61-70 years at 55.5% which showed it was statistically significant to the study. This is the most immunocompromised age of the patients which could be as a result of weak immunity to various illness, and the prevalence of NTS tends to increase among these age groups and this could have predisposed them to high prevalence among the age group. Also, the study conducted by Kaushik and LeClercq [13]~~also~~ showed a high prevalence of NTS among the age group 60 years and above. Based on their occupational status, the infection was more prevalent among farmers at 44.4%. This could be as a result of the farmers constant exposure to the poultry environment leading to a horizontal transmission from poultry to human and also the consumption of farmed food products that was grown with poultry faecal manure. This confirms that the spread of NTS is from poultry and livestock's which agrees with the study reported by WHO [23]?? that most of NTS are from poultry.
Antibiotic resistance of NTSisolates from sick patients and poultry faeces are shown in fig 1 andsuggests that antimicrobial resistance is widespread in both humans and poultry.High resistance rates to 3rd generation Cephalosporins antibiotics were seen among the poultry, especially to Cefuroxime, Cefotaxime and ceftriaxone while Ceftazidime only had a high resistance from human samples. This may be because poultry birds are usually given antibiotics which have created antibiotic resistance in humans. There have been reports indicating that the use of antimicrobials for growth promotion, prophylaxis, and treatment of food animals can increase the prevalence of resistance in human pathogens, particularly non-typhoidal *Salmonellosis*. Krawiec *et al.*[14] reported on the recent ban on the importation of poultry products into Nigeria that could have led to an increase in smuggling, which may be a likely reason for the emergence of resistance to 3rd generation cephalosporins.

CONCLUSION

The results of the study helped to explain the antibiotic susceptibility prevalence of NTS in human and poultry faeces in Ado Ekiti. The study confirmed that poultries in Ido Ekiti are likely major sources of resistant NTS, and this poses a potential hazard to the public, especially those living close to the poultry and those rearing them.There is a need to study the molecular relatedness of the isolates from both humans and poultry to confirm the source of infection in sick patients.

AVAILABILITY OF DATE AND MATERIALS

The authors declare that all the data supporting the findings are provided within the manuscript.

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