Antimicrobial Resistance Profile of *Salmonella* Typhimurium isolated from commercial poultry and poultry farm handlers in Nasarawa State, Nigeria

Abstract

Aim: This study was designed to investigate the antimicrobial resistance profile of *Salmonella* Typhimurium isolated from commercial poultry and handlers in Nasarawa State, Nigeria.

Study design: Investigative.

Place and Duration of Study: Microbiology Laboratory, Nasarawa State University, Keffi, Nigeria, from 1st November 2017 to 31th April 2018.

Methodology: A total of 1500 samples (poultry droppings, poultry flesh, feeds, handlers' faeces and hand swabs) were screened for the presence of *Salmonella* Typhimurium using pre-enrichment and selective enrichment culture media. Subculture of inoculated samples was done on Salmonella-Shigella agar and Xylose Lysine Deoxycholate agar. Presumptive *Salmonella* colonies were confirmedas serovar Typhimurium using both the conventional biochemical screening tests and Microgen Bio product GN identification system and slide agglutination test using polyvalent antisera. Antimicrobial susceptibility testing and interpretation were carried out as described by the Clinical Laboratory and Standards Institute guidelines.

Results: Resistance was highest to Augmentin (98.1%) and lowest to Imipenem (1.0%).No resistance was observed in all the isolates from poultry handlers to Ceftriaxone, Ceftazidime, Gentamicin and Streptomycin; but all were resistant to Ampicillin and Augmentin. The resistance of isolates from poultry and handlers to all the antibiotics is significant ($\chi^2 = 13.037$; $P = 0.01$). Most (86.7%, 92/106) resistant isolates belong to the multiple drug resistance class.The distribution of classes of resistance of isolates from poultry and handlers is significant ($\chi^2 = 318$;$P = 0.00$). MARI is greater than 0.2.

Conclusion: *Salmonella*Typhimurium with increasing multidrugresistance to antibiotics especially the β-lactam antibiotics has emerged in poultry.

KEYWORDS: *Salmonella*Typhimurium; Poultry; Nigeria; Multidrug resistance; Nasarawa.

1. INTRODUCTION

The Food and Agricultural Organization (FAO) of the United Nations has observed a high consumption of animal protein especially of poultry origin in developing countries [1]. The Nigerian Poultry is one of the most commercialized agricultural sub-sectors comprising of approximately180 million birds with the bulk of this sector being run as backyard poultry farmingas an additional source of income generation(National Veterinary Research Institute [NVRI] 2015).Poultry is one of the common carriers of non-typhoidal *Salmonella* [2].

Salmonella Enteriditis and *Salmonella* Typhimurium are known to be the serovars most commonly associated with human disease for which poultry are a major source of infection[3].*Salmonella* Typhimurium, being a zoonotic pathogen, can readily pass from animal to man, through consumption of contaminated food[4]. It is thesecond most widely studied pathogen in relation to antimicrobial resistance studies in Nigeria with a significant public health concern [5, 6].Notably, one of the major global causes of diarrhoeal diseases which can be mild or life threatening with severity depending upon host factors and serotype of *Salmonella* involved [3] and antibiotic therapy is required in severe infections among the young, elderly and the immune compromised [4].

However, persistent exposure of bacterial strains to a multitude of antibiotics has triggered an upward surge in antibiotic resistance which has become a global public health concern [7]. *Salmonella* is one of such microorganisms to which antibiotic resistant serotypes have emerged, having a direct effect on the food chain [3]. Acquisition of antibiotic resistance arises as a result of many factors, one of which is selective pressure from overuse or misuse of antibiotics in human and veterinary medicine as well as in disinfectants [4].

The expansion of poultry rearing and farming has made salmonellosis to become an important public health problem in Nigeria and other parts of the world causing heavy economic loss [9].In Nasarawa State, Nigeria, no documented evidence is known to the authors on antimicrobial

resistance profile of *S*. Typhimurium from poultry and handlers. This study thus investigatedthe antimicrobial resistance profile of *S.* Typhimurium from commercial poultry and handlers in Nasarawa State, Nigeria. The outcome of this study can have an overwhelming impact on public health, and the economy considering the booming poultry sector in Nigeria.In addition it will provide useful information to regulatory agencies, feed industries and poultry farm ownerson the effects of antibiotic misuse and overuse.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Nasarawa State, north central Nigeria. The State lies between latitude 7º 45' and 9º 25'N of the equator and between longitude 7º and 9º 37'E of the Greenwich meridian. It occupies an area of 27,117km^2 and a population of 1,869,377 as at 2006 census. Agriculture is the mainstay of the economy.The poultry population is unknown in Nasarawa State but as at 2017, Nigeria's production of poultry meat was estimated at 201,493 tonnes [10]. The State has three Senatorial Districts namely:Nasarawa North (NN), Nasarawa South (NS), and Nasarawa West (NW) [11]. The selected study areas were: Akwanga and Nasarawa Eggon (for Nasarawa North), Lafia and Keana (for Nasarawa South) and Keffi and Karu (for Nasarawa West). The average rain fall in March to October is 104.75 cm [12].

2.2. Sample Size Determination and Collection

The sample size was determined using the formular of [13]:

$$n = \frac{Z^2 P(1-q)}{d^2}$$

Where n = sample size

Z = Z statistic for a level of significance, 1.96 at 95% confidence interval

P = expected prevalence or proportion which was found to be 26%. [55] Hence, P = 0.26 from previous prevalence.

q = (1-p) = 1-0.26 = 0.74.

d = precision which is taken at 5% = 0.05

$$n = \frac{(1.96)^2 0.26(1-0.74)}{(0.05)^2} = 295$$

The total minimum number of each sample required was 295. However, 300 of each sample type, making a total of 1500, were collected across the senatorial districts from 1st November, 2017 to 30th April, 2018.

The stratified random sampling technique was employed in selecting the samples. Three hundred (300) samples each of faeces and hand swabs of poultry farm handlers; droppings, flesh and feed samples of poultry birds (Chicken) were obtained from six farms visited, following informed consent and voluntary participation of farm owners and workers. Ethical approval was obtained from the Ministry of Agriculture in Nasarawa State.Stool samples were collected in sterile stool containers whereas hand swabs were obtained using sterile cotton swabs immersed in sterile 0.85%buffered peptone water (BPW:Oxoid Ltd (Hampshire, UK). The poultry products (flesh, feeds and droppings) were also aseptically collected in sterile zip lock bags.

2.3. Isolation of Presumptive *Salmonella*

Isolation of *Salmonella* was carried out according to ISO 6579-1 (2017) as follows: pre-enrichment of samples in diluted (1:10) BPW with subsequent aerobic incubation at 37 ºC for 18 h.This was followed by selective enrichment in Rappaport-Vassiliadis Broth (RVB) with subsequent incubation at 42 ºC for 24 h.A loop full from the RVB was then sub-cultured by streaking onto SSA and XLD media respectively with incubation at 37 ºC for 24 h.The cultured plates were examined for the presence of typical colonies of *Salmonella* based on cultural and morphological characteristics on the media.

2.4. Identificationand Confirmation of*Salmonella* Typhimurium

Presumptive *S.* Typhimurium isolates were confirmed by Gram staining and both conventional and commercial biochemical tests. Gram staining and conventionalbiochemical tests (Triple sugar iron agar, urease test, indole test, methyl red test, ornithine decarboxylase test, lysine decarboxylase test, motility test, citrate utilization test)were conducted as described by [14]. The Microgen™ GnA+B-ID System Bioproducts Limited (Camberley, UK) for

biochemical identification was used for further characterization of the isolates strictly as specified by the manufacturer.All the isolates were further characterised by serotyping using polyvalent *Salmonella* antisera (Oxoid, UK) by slide agglutination test according to [15].

2.5 AntimicrobialSusceptibility Testing

Antimicrobial Susceptibility Testing on the isolates was done using the Kirby-Bauer disc diffusion technique as described by the Clinical and Laboratory Standards Institute [16]. Antimicrobial discs used were purchased from Oxoid (Basingstoke, England); and *Escherichia coli* (ATCC 35218 Microbiologics Inc MN, USA) was used as control.

2.6Determination of Multiple Antibiotic Resistance Indices in the resistant isolates

Multiple AntibioticResistance (MAR) is defined here as resistance to 2 or more of the antibiotics tested. The MAR Index was determined according to the method of [17]as described by [18]. From the result of the antimicrobial susceptibility testing, MARI was calculated using the following formula:$\text{MARI} = \frac{a}{b}$

(Where a = number of antibiotics to which an isolate is resistant to b = number of antibiotics against which the isolate was tested).

2.7Classification of Antimicrobial Resistance in the isolates

Antimicrobial resistance in the isolates were classified into: multidrug resistance (MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories); extensive drug resistance (XDR: non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); pan drug resistance (PDR: non-susceptible to all antimicrobial listed) [19].

2.8Statistical and Data Analysis

Statistical analysis was performed with SPSS version 20.0. Descriptive statistics was used to describe the result.

3. RESULTS

3.1.Antimicrobial Resistance Profile

Antibiotic Resistance Profile for the isolates is as shown in Table 2. Resistance was very low to Imipenem, low to Gentamicin, Streptomycin and Ceftazidime; moderate to Ciprofloxacin and Ceftriaxone; high to Sulfamethoxazole/Trimethoprim; and very high to Chloramphenicol, Ampicillin, Tetracycline, and Augmentin. No resistance was observed in all the isolates from poultry handlers to Ceftriaxone, Ceftazidime, Gentamicin and Streptomycin; but all isolates were resistant to Ampicillin and Augmentin. The resistances of the isolates from poultry and handlers to all the antibiotics is significant ($\chi^2 = 13.037$ $P = 0.01$).

Table 1: Antimicrobial Resistance Profile of *Salmonella* Typhimurium Isolates from poultry and handlers in Nasarawa State, Nigeria

Antibiotic	Disc Content (µg)	Poultry (n = 91)	Handlers (n = 15)	Number (%) resistant(n =106)
Ampicillin	10	84(92.3)	15(100.0)	99(93.4)
Augmentin	30	89(97.8)	15(100.0)	104(98.1)
Ceftriaxone	30	74(81.3)	0(0.0)	74(69.8)
Ceftazidime	30	50(54.9)	0(0.0)	50(47.2)
Imipenem	10	01(1.1)	0(0.0)	01(1.0)
Septrin	25	72(79.1)	11(73.3)	83(78.3)
Ciprofloxacin	5	58(63.7)	08(53.3)	66(62.3)
Gentamicin	10	40(44.0)	0(0.0)	40(37.7)
Streptomycin	10	45(49.5)	0(0.0)	45(42.5)
Chloramphenicol	30	74(81.3)	12(80.0)	86(81.1)
Tetracycline	30	88(96.7)	15(100.0)	103(97.2)

KEY: AMP = Ampicillin, CRO = Ceftriaxone, CAZ= Ceftazidime, AUG = Augmentin, IMP = Imipenem, SXT= Septrin, CIP = Ciprofloxacin, CN = Gentamicin, S= Streptomycin, C = Chloramphenicol, TET = Tetracycline

3.2.Antimicrobial Resistance Phenotypes of the isolates

Antimicrobial Resistance Phenotypes of the isolates are as shown in Table 2. It summarises the multiple antibiotic resistance pattern exhibited by the 106*S.* Typhimurium isolates. There are a total of 32 different resistance phenotypes with AMP, AUG, CRO, CAZ, C, CIP, S, CN, SXT, TEThaving the highest frequency of 25.0 % (26/106).

Table 2: Antibiotic Resistance Phenotypes of the*Salmonella* Typhimurium isolates frompoultry and handlers in Nasarawa State

S/N	Antimicrobial Resistance Phenotypes	Total No. (%) Isolates (n = 106)
1	AUG, TET	1(0.9)
2	AMP, AUG, CRO, TET	4(3.8)
3	AUG, C, SXT, TET	1(0.9)
4	AMP, AUG, C, TET	12(11.0)
5	AMP, AUG, CRO, CAZ, TET	1(0.9)
6	AMP, AUG, CAZ, SXT, TET	3(2.8)
7	AMP, AUG, CIP, SXT, TET,	3(2.8)
8	AMP, AUG, CRO, C, TET	3(2.8)
9	AMP, AUG, CRO, CIP, SXT, TET	2(2.0)
10	AMP, AUG, CRO, C, SXT, TET	11(10.0)
11	AMP, AUG, C, CIP, SXT, TET	5(4.7)
12	AMP, CRO, CAZ, CIP, S, SXT	1(0.9)
13	AMP, AUG, C, CN, SXT, TET	1(0.9)
14	AMP, AUG, CRO, C, CIP, S, CN,SXT, TET	1(0.9)
15	AMP, AUG, C, CIP, CN, SXT, TET	1(0.9)
16	AUG, CRO, CAZ, C, CIP, SXT, TET	2(2.0)

17	AMP, AUG, CRO, CAZ, C, SXT, TET	1(0.9)
18	AMP, AUG, CRO, CAZ, CIP, SXT, TET	1(0.9)
19	AMP, C, CIP, S, CN, SXT, TET	1(0.9)
20	AMP, AUG, CRO, CAZ, CIP, S, CN, SXT	1(0.9)
21	AMP, AUG, CRO, CAZ, C, CIP, SXT, TET	6(5.7)
22	AMP, AUG, CRO, C, CIP, S, SXT, TET	1(0.9)
23	AUG, CRO, CAZ, C, CIP, S, SXT, TET	1(0.9)
24	AMP, AUG, CRO, CAZ, C, S, SXT, TET	1(0.9)
25	AUG, CRO, CAZ, C, CIP, S, CN, SXT, TET	1(0.9)
26	AMP, AUG, CRO, CAZ, C, CIP, S, SXT, TET	4(3.8)
27	AMP, AUG, CRO, C, CIP, S, CN, SXT, TET	4(3.8)
28	AMP, AUG, CRO, CAZ, C, CIP, SXT, TET	1(0.9)
29	AMP, AUG, CRO, CAZ, C, CIP, S, SXT, TET	2(2.0)
30	AMP, AUG, CRO, CAZ, C, CIP, CN, SXT, TET	2(2.0)
31	AMP, AUG, CRO, CAZ, C, CIP, S, CN, SXT, TET	26(25.0)
32	AMP, AUG, CRO, CAZ, IMP, C, CIP, S, CN, SXT, TET	1(0.9)

AMP= Ampicillin, CRO= Ceftriaxone, CAZ= Ceftazidime, AUG= Augmentin, IMP=Imipenem, SXT= Septrin, CIP= Ciprofloxacin, CN = Gentamicin, S= Streptomycin, C= Chloramphenicol, TET= Tetracycline

3.3.Multiple Antibiotic Resistance Indices of the isolates

The Multiple Antibiotic Resistance Indices (MARI) of *Salmonella* Typhimurium isolates from poultry and poultry handlers is as shown in Table 3. The MARI of isolates greater than 0.2.MARI of 0.6 and 0.9 had the highest number of isolates 26(25.0%) in each. The MARI of isolates from poultry and handlers is significant (P = 0.00).

Table 3: Multiple Antibiotic Resistance Index (MARI) of *Salmonella* Typhimurium isolatesfrom poultry and their handlers

MARI	Poultry (n = 91)	Handlers (n = 15)	Total (%) MAR Isolates (n = 106)
0.20	1(1.1)	0(0.0)	1(1.0)
0.30	0(0.0)	0(0.0)	0(0.0)
0.40	13(14.3)	4(26.7)	17(16.0)
0.50	4(4.4)	6(40.0)	10(9.0)
0.60	21(23.1)	5(33.3)	26(25.0)
0.70	11(12.1)	0(0.0)	11(10.0)
0.80	13(14.3)	0(0.0)	13(13.0)
0.90	26(28.6)	0(0.0)	26(25.0)
1.00	2(2.2)	0(0.0)	2(2.0)

3.4 Classes of Antimicrobial Resistance in the resistantisolates

The various classes of antibiotic resistance in the isolates are as presented in Fig. 1. Out of the 106 isolates, a total of 92 were found to be multidrug resistant (MDR), 07 extensively drug resistant (XDR), 06 non-multidrug resistant (NMDR) and 01 Pandrug resistant (PDR). Their overall percentage prevalence is 86.7% MDR, 6.6% XDR, 5.7% NMDR and 1.0% PDR. The distribution of classes of resistance is significant ($\chi^2 = 318; P = 0.00$).

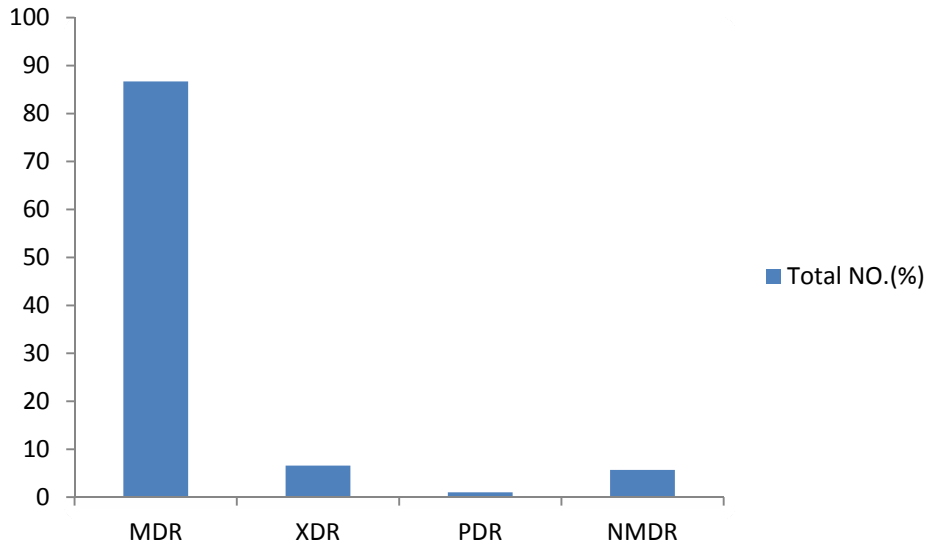

Fig. 1: Classes of Antibiotic Resistance in the *Salmonella* Typhimurium isolates from poultry and handlers in Nasarawa State

KEY: MDR = Multidrug resistance; XDR = Extensive drug resistance; PDR = Pan drug resistance; NMDR=Non-Multidrug resistance

3.5. Distribution of Antibiotic Resistance in relation to the Senatorial Districts

The representation of the classes of antibiotic resistance in this study in relation to the senatorial districts is as shown in table 4. The prevalence in each of the districts are; NW (95.0%)>NS (83.0%)>NN (70.0%). XDR and PDR only observed in NN and NS; 30.0% and 4.0% respectively. The NMDR recorded prevalence of 13.0% and 5.0% in NS and NW respectively. The distribution of classes of resistance of the isolates from poultry and handlerswithin the districts is significant. (χ2-value =36.57; *P*-value = 0.00).

Table 4: Distribution of the Classes of Antibiotic Resistance in the *S.*Typhimurium isolates in the Senatorial Districts of Nasarawa State

Classes of Resistance	No. (%) Isolates			Total (%)
	NN (n= 23)	NS (n= 24)	NW (n= 59)	(n = 106)

MDR	16(70.0)	20(83.0)	56(95.0)	92(86.7)
XDR	7(30.0)	0(0.0)	0(0.0)	7(6.6)
PDR	0(0.0)	1(4.0)	0(0.0)	1(1.0)
NMDR	0(0.0)	3(13.0)	3(5.0)	6 (5.7)
T-value	- 0. 32	- 0.84	-1.10	
P-value	0.7	0.45	0.34	
LOS	NS	NS	NS	

NN= Nasarawa North; NS= Nasarawa South; NW= Nasarawa West; MDR= Multidrug resistance; XDR= Extensive drug resistance; PDR= Pan drug resistance, **χ2-value = 36.57; *P* = 0.00**

DISCUSSION

Salmonella Typhimurium from poultry origin was found to exhibit high resistance to Tetracycline, Augmentin, Ampicillin, Chloramphenicol, Sulfamethoxazole-Trimethoprim and Ceftriaxone with moderate resistance to Ciprofloxacin. It however demonstrated low resistance to Ceftazidime, Gentamicin and Streptomycin. A similar trend in resistance to the current study for Ampicillin, Trimethoprim-Sulfamethoxazole and Tetracycline was also reported by [20] in Calabar, South eastern Nigeria and [21] in Thailand. A 100% resistance to Chloramphenicol was encountered in the studies of [22] in Egypt, which is higher than the 79.7% observed for this study but contrary to 27.3% reported by [23] in Tehran.

The resistance trend of the isolates from both poultry and handlers to Ampicillin, Tetracycline, Chloramphenicol and Trimethoprim-Sulfamethoxazole observed in this study was not surprising because they were several decades ago used as first-line drugs for therapy of severe salmonellosis[24].Unfortunately, an upsurge in reports on resistance to these drugs over time, has limited their usefulness in treatment and paved way for quinolone and third-generation cephalosporin antibiotics as preferred antibiotics for the treatment against salmonellosis[23].Nevertheless resistance to Sulfamethoxazole-Trimethoprim is indeed very alarming. For instance, Sulfomethoxazole-Trimethoprim is used in the prophylaxis management of opportunistic infections in HIV patients [25]. Tetracycline resistance obtained for the present study finding is not different from what is being reported in different parts of the world. This is

equally not surprising because it belongs to a class of antimicrobials most widely utilized for therapeutic purpose in livestock [26]. Thus the high resistance rates generally observed is an indication of its regular use within the study area [27].

Ciprofloxin has successfully been used in the treatment of septicaemic salmonellosis in humans and are also incorporated in poultry feeds [28].Ciprofloxacin, in this study recorded an overall resistance of 62.3% to *Salmonella* Typhimurium isolates from poultry and handlers. This is in agreement with the 68.4% reported in the findings of [29] in China. It is however, higher than the 18.4%, 26.32% and 48.7% observed in Nigeria, Morocco and China [9, 30, 31]. Evidently, fluoroquinolones administered in food producing animals belong to the same antibiotic class as those therapeutically used in human medicine [32]. Therefore non-typhoidal *Salmonella*, expressing resistance towards frequently used drugs in humans and animals poses a daunting socio-economic challenge in developing countries.

Cephalosporin resistance for poultry isolates in this study showed a resistance of 69.8% to Ceftriaxone and 47.2% to Ceftazidime. This is in agreementwith the findings of [33] in Ekiti south western Nigeria. Resistance of NTS to third generation Cephalosporins has been reported in developed and developing countries[34,35].The present study finding was found to be contrary to other reports on the dearth of *Salmonella* resistance to Cephalosporin in parts of Nigeria, Malaysia and Vietnam [36,37,38].B-lactam antibiotics especially the third generation extended spectrum Cephalosporins are mainly prescribed for treatment of infectious diseases in both human and veterinary medicine, and they are also used as feed additives to enhance the growth of food animals [27, 39]. Unfortunately, their widespread and unrestricted use has generated resistance [40] further evidenced by the findings of the current study.

Imipenem resistance, although rare in NTS had been detected in isolates from humans, livestock, wild animals and food [40] interestingly in this study, resistance to imipenem antibiotic was not encountered in handlers but very low in poultry.

In Nigeria, there are no strict laws on use of antibiotic both for humans and veterinary care [41, 42]. High resistance to third generation Cephalosporins as well as to the Penicilins, Tetracyclines and Chloramphenicol has been reported in Nigeria [43]. S. Typhimurium exhibiting antibiotic resistance has also been reported in many parts of the world [44,45,46,47].The observed high

levels of resistance is therefore an indication that these antibiotics are non-susceptible to the isolates particularly those from poultry originwithin the study area. Generally, *S*. Typhimurium from poultry origin where observed to exhibit higher resistancewhen compared to those from handlers; they were susceptible to the Aminoglycosides and beta-lactam Cephalosporin antibiotics. This clearly suggests that antibiotic mismanagement especially in poultry played a role in the observed trend. Inevitably, over reliance on antibiotics has become a threat to global animal and human health through the phenomenon of antimicrobial resistance [48].

Multidrug resistant (MDR) non-typhoidal *Salmonella* (NTS) has surfaced in Africa and is comparable to the increasing burden of NTS infections [49] which was ascertained by the high levels of MDR *Salmonella* Typhimurium encountered in the current study. The antibiotic resistance profile in this study suggests that selective pressure on antibiotics in the study area must have triggered the appearance of MDR strains of *S.*Typhimurium in the poultry reservoir. This clearly poses a threat to humans through the food chain [50]. The high rate of MDR *Salmonella* (86.7%) illustrated in the present study is in accordance with the findings of [51, 52, 53, 22, 54] respectively in Egypt. Similarly, [55] in India, reported a significant MDR *Salmonella enterica* isolates from poultry, exhibiting resistance to β-lactam antibiotics. The antibiotic resistance pattern observed in the present study covered a range of four to eleven antibiotics, which comprised of resistance to the β-lactams, Fluoroquinolones, Sulphonamides, Phenicol, Tetracycline and Aminoglycosides. *Salmonella* can therefore be said to have a predilection towards multiple and extensive drug resistance. This observation is in line with that of [56]. There have been reports from all over the world concerning the emergence of new MDR NTS strains in Poultry and is said to be responsible for around 12-33 million cases of gastrointestinal episodes on a yearly basis in Africa [51].Meanwhile, [36] concluded that the indiscriminate use of antibiotics at recommended or sub-therapeutic doses as feed additives in poultry farms can be linked to be attributable to the emergence of MDR *Salmonella* generally. The MDR pattern observed in the present study covered four to eleven antibiotics, which comprised of resistance to the β-lactams, third generation extended spectrum Cephalosporins and the Carbapenem. This study was able to discover distinct phenotypes exhibiting resistance to Penicillins, Cephalosporins, Phenicol, Quinolones, Tetracycline and Aminoglycoside. The most frequently isolated phenotypes in poultry, exhibited resistance to Ampicillin, Augmentin, Ceftriaxone, Ceftazidime, Chloramphenicol, Ciprofloxacin, Streptomycin, Gentamicin, Septrin

and Tetracycline (AMP, AUG, CRO, CAZ, C, CIP, S, CN, SXT, TET). A unique phenotype from poultry was observed to be resistant to all the eleven antibiotics used in this study, whereas in handlers, the most commonly isolated phenotype featured resistance to Ampicillin, Augmentin, Chloramphenicol, Ciprofloxacin, Septrin and Tetracycline (AMP, AUG, C, CIP, SXT, TET). There is therefore an indication based on the observed MDR pattern of *S*. Typhimurium in the current study that infection with this MDR strain can pose serious challenge in therapy management in the study area. It is on the basis of this trend of increasing MDR that [57] warned that *Salmonella* could transform into a super bacteria. This study thus, provides evidence to support their view.

The MAR index for this study was greater than 0.2 which implies antibiotic pressure and risk of *Salmonella* contamination within the study area.

CONCLUSION

*Salmonella*Typhimurium among poultry birds with increasing multidrug resistance to important antibiotics especially the β-lactam Cephalosporin and Fluoroquinolone antibiotics is indeed disturbing and calls for a proper antibiotic stewardship especially in veterinary medicine in order to curb the spread of antibiotic resistance in zoonotic pathogens, which can have a daunting effect on therapy.

REFERENCES

1. Nigerian poultry population.Sahel-Newsletter-Volume-11.2015.Retrieved January, 7 2019, Retrieved from www.sahelcp.com.
2. Tadesses Eguale. Non-typhoidal *Salmonella* serovars in poultry farms in Central Ethiopia: Prevalence and antimicrobial resistance. *BMC Vet Res*.2018; *14*(1):217.doi:10.1186/s12917-018-1539-4.
3. World health organization. Non-typhoidal*Salmonella*.2019. Retrieved January 8, 2019, from https://www.who.int/news-room/fact-sheets/detail/*Salmonella*- (non-typhoidal).
4. Cosby DE, Cox, NA, Harrison MA, WilsonJL, Buhr RJ, Fedorka-Cray, PJ. *Salmonella*and antimicrobial resistance in broilers: A review. *J Appl Poult Res*, 2015; *24*(3): 408-426.

5. Card R, Vaughan K, Bagnall M, Spiropoulos J, Cooley W, Strickland T, Davies R, & Anjum MF. Virulence characterisation of *Salmonellaenterica* isolates of differing antimicrobial resistance recovered from UK livestock and imported meat samples. *Front Microbiol. 2016;* 7:1–11.
6. Oloso NO, Fagbo S, Garbati M, Olonitola SO, Emmanuel JA, Mabel K A, *et al*. Antimicrobial resistance in food animals and the environment in Nigeria: A review.*Int. J. Environ. Res Public health*, 2018; *15*(6):1284.doi:10.3390/ijerph15061284.
7. Djeffal S, BakourS, MamacheB, Elgroud R, Agabou A, Chabou S. *et al*. Prevalence and clonal relationship of ESBL producing *Salmonella* strains from humans and poultry in North Eastern Algeria. *BMC Veterinary Research* 2017; *13*:132.https://doi.org/10.1186/s12917-017-1050-3.
8. Cosby DE, Cox NA, Harrison MA, Wilson JL, Buhr RJ, Fedorka-Cray PJ.*Salmonella*and antimicrobial resistance in broilers: A review. *J Appl Poult Res*, 2015; *24*(3): 408-426.
9. Agada GO, Abdullahi IO, Aminu M, Odugbo M. & Chollom SC. Prevalence and antibiotic susceptibility profile of *Salmonella* Isolates from commercial poultry and poultry farm-handlers in Jos, Plateau State. *British Microbiology Research Journal*, 2014; *4(*4):462479.
10. Knoema. (2019).Retrieved June 5, 2019, from httpd://Knoema.com/atlas/Nigeria/topics/Agriculture/Live-Stock-Production-Production-Quantity/Production-of-poultry-meat.
11. Geographical location of Nasarawa State. Retrieved January 12, 2019, fromhttp://en.m.wikipedia.org/wiki/Nasarawa_State.
12. Saliu EM, Vahjen W & Zentek J.Types and prevalence of extended–spectrum beta–lactamase producing Enterobacteriaceae in poultry. *Animal Health Research Reviews*, 2017; *18*(1), 46–57. Retrieved from http://doi.org/10.1017/S1466252317000020.
13. Daniel, WW. Biostatistics: A foundation for analysis in the health sciences. 7th edition. 1999, New York: John Wiley & Sons.
14. Cheesbrough M. *District Laboratory Practice in Tropical Countries,* Part II.Cambridge University press, Cambridge, U.K. 2006; pp442.

15. Kauffmann, F. Serological diagnosis of *Salmonella* species, Kauffmann White Scheme Minkagarord, Copenhagen, Denmark; 1974.
16. Clinical and Laboratory Standards Institute (CLSI).Performance standards for antimicrobial susceptibility testing.22nd informational supplement M100S. 2018 Wayne, PA, USA.
17. Krumperman, PH. Multiple antibiotic indexing *Escherichiacoli* to identifying risk sources of faecal contamination of foods. *Applied Environmental Microbiology*, 1983; 46: 165-170.
18. Ngwai YB, Gyar SD, Pennap G R I, Makut M D, Ishaleku D. Corosi S M.*et al*. Antibiogram of Non-Sorbitol Fermenting *Escherichia coli* Isolated from Environmental Sources in Keffi, Nigeria. *NSUK Journal of Science and Technology,* 4 (1&2), 2014; 152-163.
19. Magiorokos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al*.Multidrug- resistant, extensively drug -resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol and infect*, 2012; *18*(3):268-281.
20. Yhiler, N.Y.& Bassey, B.E. Primary sources of *Salmonella*speciesin poultry production settings in Calabar, Cross River State, Nigeria. *Donnish journal of Medicine and Medical Sciences,*2015; *2*:3 pp.047-051.
21. Lertworapreecha M, Noomee S, Sutthimusik S, Utarapichat, B, Tontikapong K. Multidrug resistant and extended spectrum β-lactamase producing *SalmonellaEnterica* isolated from food animals Phatthalung, Thailand. *SouthEast Asian J Trop Med Public Health.*2016; *47*:6.
22. El-Sharkawy H., Tahoun A, El-Gohary A, El-Abasy M, El-Khayat F, Gillespie T,*et al*. Epidemiological, molecular characterization and antibiotic resistance of *SalmonellaEnterica* serovars isolated from chicken farms in Egypt. *Gut Pathog*, 2017; *9*: 8.
23. Rizi KS, Najar-Peerayeh S, Bakhshi B, Rahbar M.Prevalence of ESBLs and Integrons in Clinical Isolates of *Salmonella spp*. From Four Hospitals of Tehran. *Int J Enteric Pathog*, 2015;3(1):e21820
24. Crump JA, Sjölund-Karlsson M, Gordon MA, Christopher MP. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management

of invasive *Salmonella* Infection. *Clin Microbiol Rev*, 2015; *28*(4): 901–937. 10.1128/CMR.00002-15.

25. Ibrahim T, & Olonitola OS. Antibiotic susceptibility of some *Salmonella* species isolated from diarrhoeal stools of HIV patients in Kaduna, Nigeria**.***NSUK Journal of Science & Technology*, 2016; *6*(2): 77 – 81.
26. International Standards Organization.Microbiology of the food chain-Horizontal method for the detection, enumeration and serotyping of *Salmonella*. ISO 6579-1:2017(E) Retrieved from https://www.iso.org/standard/56712.html
27. VanBoeckel TP, Gandra S, Ashok A, Coudron Q, Grenfell BT, Levin SA, *et al*.Global antibiotic consumption 2000 to 2010: an analysis of Cross Mark 742 national pharmaceutical sales data. *Lancet Infect Dis,* 2014*; 14*(8):742-50.
28. Griselda H, Gustavo HM, &Jorge E. Quantification of residual enroflox and ciprofloxacin in feather of broiler chicken by high performance liquid chromatography fluorescence after oral administration of the drug Jan-March. *J Adv Pharm Technolo Res*. 2016; *7*(1):2-5.
29. Li S, Zhao Y& Miao Z.Prevalence and Antibiotic Resistance of Non-typhoidal *Salmonella* Isolated from Raw Chicken Carcasses of Commercial Broilers and Spent Hen in Tai'an, China.*Frontiers in Microbiol*, 2017; *8*:2106.doi:10.3389/fcmicb.2017.02106.
30. KhallafM, Ameur N, Terta M, Lakranbi M, Senouci S& Ennaji M. Prevalence and antibiotic resistance of *Salmonella* isolated from chicken meat market in Rabat Morocco. *International Journal of Innovation and Applied Studies.* 2014; *6*(4):1123-1128.
31. Zhu Y, Lai H, Zou L, Yin S, Wang C, Han X *et al*. . Antimicrobial resistance and resistance genes in *Salmonella* strains isolated from broiler chickens along the slaughtering process in China. *Int J Food Microbiol*. 2017; *16*;259:43-51.doi:10.11016/j.ijfoodmicro.2017.07.023.Epub 2017.
32. AarestrupFM, Wegener HC& Collignon P. Resistance in bacteria of the food chain. Epidemiology and control strategies. *Expert. Rev Anti-infective Ther*. 2008; *6*(5):733-750.doi:10.1586/14787210.6.5.733
33. Oluyege AO, Ojo-Bola O. Prevalence of non-typhoidal *Salmonella* among HIV/AIDS patients and poultry chicken in Ekiti State. *BMRJ*2015; *6*(2):113-118.
34. Burke L, Hopkins KL, Meunier D, de Pinna E, Fitzgerald-Hughes D, Humphreys H *et al*. Resistance to third generation cephalosporins in human non-typhoidal *SalmonellaEnterica*

isolates from England and Wales 2010-2012.*Journal Antimicrobial Chemotherapy*. 2014; *69*(4):977-81.

35. Andoh LA, Ahmad S, Olson JE, DonsoKO, Newman MJ, OpintanJ.A.*et al*. Prevalence and characterization of *Salmonella* among humans in Ghana. *J. Tropical medicine and health*. 2017; *10*; 45:3.doi:10.1186/s41182-017-0043-z
36. Fashae K, Ogunsola F, Frank MA, Hendriksen RS. Antimicrobial susceptibility and serovars of *Salmonella* from Chicken and humans in Ibadan, Nigeria. *J infect Dev Ctries* 2010; *4*(8):484-494.
37. Woh PY, Thong KL, Behnke JM, Lewis JW& Zain SNM. Characterization of non-typhoidal *Salmonella* isolates from asymptomatic migrant food handlers in peninsular Malaysia. *Journal of food prot.*2017; *80*(8):1378-1383
38. Trung NV, Carrique-Mas, JJ, Nghia NH, Tu LTP, Mai HH, Tuyen, HT.*et al*. Non-typhoidal *Salmonella* colonization in chickens and humans in the Mekong Delta of Vietnam. *Zoonoses Public Health,* 2017; *64*(2):94-99. doi:10.1111/zph.12270.
39. European Medicines Agency. European surveillance of veterinary antimicrobial consumption. Sales of veterinary antimicrobial agents in 26 EU/EEA countries in 2013. Fifth ESVAC report, London, 2015. Retrieved from https://www.ema.europa.eu/en/veterinary-regulatory/overview/antimicrobial-resistance/european-surveillance-veterinary-antimicrobial-consumption-esvac
40. Fernandez J, Guerra B, Rodico MR. Resistance to Carbapenems in non-typhoidal *Salmonella enterica* serovars from humans, animals and food. *Vet Sci*,2018; *8;* 5(2). Pii:E40.doi.10.3390/vetsci5020040.
41. The European Committee on Antimicrobial Susceptibility Testing.Breakpoint tables for interpretation of MICs and zone diameters. 2015 Version 5.0. Retrieved fromhttp://www.eucast.org.
42. Ugwu IC, Anyanwu MU, UgwuCC, Ugwuanyi OW. Prevalence and antibiogram of generic extended –spectrum beta-lactam-resistant Enteriobacteriacea in Healthy Pigs. *Not Sci Biol*, 2015; *7*(3):272-280.
43. Federal Ministry of Agriculture and rural development. Antimicrobial use and resistance in Nigeria: Situation analysis and recommendations,2017. Retrieved November 09, 2018, from http://www.ncdc.gov.ng/themes/common/docs/protocols/56 1510840387.

44. Liljebjelke KA, Hofacre CL, White DG, Ayers S, Lee MD& Maurer JJ. Diversity of Antimicrobial Resistance Phenotypes in *Salmonella* Isolated from Commercial Poultry Farms. *Front. Vet. Sci,* 2017; 4:96.doi:10.3389/fvets.2017.00096.eCollection 2017
45. Nhung NT, Cuong NV, Thwaites G, Carrique-Mas (2016). Antimicrobial usage and antimicrobial resistance in animal production in South East Asia; A review. *Antibiotics* (Basel) 2016; 2:5(4), 37. Pii: E37.
46. Mion L, Parizotto L, Calasans M, Dickel EL, Pilotto F, Rodriguez LB*et al*.Effects of antimicrobials on *Salmonella* spp strains isolated from poultry processing plants.*Brazillian Journal of Poultry Science*, 2016; *18*(2) 337-342.
47. Akinyemi KO, Ajoseh SO, Iwalokun BA, Oyefulu CO, Fakorede CO, Abegunrin RO et al. Antimicrobial resistance and plasmid profiles of *Salmonella enterica* serovars from different sources in Lagos, Nigeria. *Health,*2015; *10*(06):758-772.
48. O'NeilJ.Antimicrobials in Agriculture and the environment: Reducing unnecessary use and waste.WellcomeTrust: London, U.K, 2015. Retrieved November 17, 2017, from https://amr-review.org/sites/default/files/Antimicrobials.
49. Feasey NA, Masesa C, Jassi C, Faragher EB, Mallewa J, Mallewa M. *et al*. Three epidemic of invasive multidrug–resistant *Salmonella* blood stream infection in Blantyre, Malawi, 1998-2014.*Clin Infect Dis*: 2015; 1; 61 Suppl 4:S363-71.doi.10.1093/cid/civ691.
50. Lai J, Wu C, WuC, Qi J, Wang Y, Wang H *et al*. Serotype distribution and antibiotic resistance of Salmonella in food- producing animals in Shandong province of China, 2009 and 2012. *Into J Food Microbiol*. 2014; *16*(180)30-8.doi 10.1016/j.ijfoodmicro.2014.03.030.Epub 2014 Apr 12
51. Al-Mustapha PA, Adetunji V, Ibrahim R& Adesiji, Y.Prevalence and antibiogram of Non-Typhoidal *Salmonella* isolates from poultry in Ilorin Kwara State. *International Journal of Infectious diseases*, 2018; *73*:163 Accessed, September 2018. Retrieved fromhttps://doi.org/10.1016/j.ijid.2018.04.3784.
52. Li S, Zhao Y, & Miao Z. Prevalence and Antibiotic Resistance of Non-typhoidal *Salmonella* Isolated from Raw Chicken Carcasses of Commercial Broilers and Spent Hen in Tai'an, China. *Frontiers in Microbiol*. 2017;*8*,2106.doi:10.3389/fcmicb.2017.02106.

53. Aziz SA, Latef GK, Shany SAS& Rouby SR.Molecular detection of integron and antimicrobial resistance genes in multidrug resistant *Salmonella* isolated from poultry, calves and human in Beni-suef governorate, Egypt. *Beni-suef University Journal of Basic and Applied Sciences,*2018; *7*(4)535-542.Retrieved from https://doi.org/10.1016/j.bjbas.2018.06.005.
54. Gharieb R, TartorYH, Khedr MHE.Non-typhoidal *Salmonella* in poultry meat and diarrhoeic patients: Prevalence, antibiogram, virulotyping, molecular detection and sequencing of class I integrons in multidrug resistant strains. *Gut Pathog*.15; *7*:34.doi:10.1186/s13099-015-0081-1.
55. Bhuvasnewari M, Shanmughapriya S, & Natarajaseenivasan K. Prevalence of multidrug resistant(MDR) *Salmonella* Enteriditis in poultry and backyard Chicken from Tiruchirappavi, India. *Microbiology Journal*,2015; *5*(2) 28-35.
56. Zhang L, Fu X, Xiong Z, Ma Y, Wei, Y, Qu, X (2018).Highly prevalent multidrug resistant *Salmonella* from chicken and Pork meat at retail markets in Guangdong China. *Front.Microbiol*. 2018; *10*(9)2104.doi:10.3389/fcmicb.2018.02104
57. Campioni F, Zoldan, MM, & Falcao JP. Characterization of Salmonella Enteriditis strains isolated from poultry and farm environments in Brazil. *Epidemiol. Infect*.2014; 142(7);1403-1410.doi10.1017/S0950268814000491.Epub 2014 Mar 14