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Research Article

Fecal carriage of extended spectrum beta-lactamase and fluoroquinolone resistant gene in non-typhoidal *Salmonella enterica* isolates from food-producing animals and humans

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Abstract

This study seeks to determine the feacal carriage of extended spectrum beta-lactamase and fluoroquinolone resistant non-typhoidal Salmonella enterica isolates from food-producing animals and humans. A total of three hundred (300) fecal samples were collected using sterile universal containers from food-producing animals namely (Chicken [100], Pig [100] and humans (100) from Onicha Local Government Area of Ebonyi State and analyzed for the presence of non-typhoidal *Salmonella enterica* using standard microbiological techniques. Phenotypic detection of extended-spectrum beta-lactamase (ESBL) were done by disc diffusion and Double Disk Synergy Test. Molecular characterization for ESBL and fluoroquinolone-resistant genes were done by PCR with specific primers. The result shows that non-typhoidal *Salmonella* species (NTS) accounted for 25 % and 17 % in poultry and pig fecal sample respectively while 60 % and 40% were phenotypic ESBL producers respectively. When compared statistically there is significant difference among isolates confirmed ESBL-positive ($P< 0.05$). Also, none of the 16 (58 %) NTS isolated from humans harbored ESBL phenotype. PCR analysis with β-lactam specific primer detected the presence of blaOXA 50 % and 50 %, blaSHV 36 %, and 64 %, blaTEM 43 % and 57 %, blaCTX-M 36 % and 64 % in poultry and pig respectively. Fluoroquinolone resistant gene *QnrA* was present in 0 and 100 % of poultry and pig respectively. *QnrB* was 40 % and 60 % in poultry and pig isolates respectively. *QnrS* was present in 64 % isolates of poultry and 13 % isolates in pig. The high prevalence of genes encoding beta-lactamases and fluoroquinolone resistance (*TEM*, *SHV*, *CTX-M* and *OXA*, (*qnrA*, *qnrB* and *qnrS*) were present more in poultry and pig than in humans and demonstrate a significant public health threat from consumption of food-producing animal harboring such pathogenic resistant genotype if not properly controlled.

Keywords: Extended spectrum beta-lactamase, Fluoroquinolone, non-typhoidal *Salmonella enterica,* feacal carriage

1. INTRODUCTION

Non-typhoidal salmonellosis refers to illnesses caused by all serotypes of *Salmonella* except for Typhi, Paratyphi A, Paratyphi B (tartrate negative), and Paratyphi C [1, 2]. (Gal-Mor *et al.,* 2014; Majowicz *et al.,* 2020). The disease Salmonellosis is a major public health problem worldwide. NTS usually causes self-limiting gastroenteritis associated with nausea, abdominal pain, vomiting and inflammatory diarrhoea. In some cases, specific strains among the serovars can cause bacteremia majorly in young children and immune compromised patients. Incubation of NTS after ingestion of the pathogen is between 6 and 12 h [1]. (Gal-Mor *et al.,* 2014). It is estimated that, each year in the United States, about 1.4 million persons are infected with Non-typhoidal *Salmonella,* which results in 15,000 hospitalizations and 580 deaths [2, 3]. (WHO, 2017; Majowicz *et al.,* 2020). In many regions such as South East Asia, there is an absence of official *Salmonella* surveillance data but it is estimated that up to 22.8 million cases occur annually with 37,600 deaths [2].(Majowicz *et al.,* 2020). There are more than 2,500 serovars of *Salmonella* enterica that have been identified. However, the majority of human cases of Non-typhoidal salmonellosis are caused by a limited number of serovars, which may vary from country to

country and over time [4]. (Hendriksen *et al.,* 2011). Invasive *Salmonella* spp. can spread beyond the gastrointestinal mucosa to infect other sites such as the bloodstream, the meninges, bone or joint spaces [5]. (Crump *et al.,* 2011). Serovars such as *S. choloraesuis* and *S. dublin* cause more invasive disease than other serovars [6]. (Jones *et al.,* 2008). *S. enteriditis* and *S. Typhimurium* have traditionally been the most frequently isolated serovars from humans worldwide [4, 7]. (Hendriksen *et al.,* 2011; Fashae *et al.,* 2010). Some other serovars have been reported to be more prevalent in specific regions or within countries, such as the prevalence of *S. weltevreden* and *S. stanley* in some South East Asian countries [4, 8]. (Hendriksen *et al.,* 2011; Lee *et al.,* 2009). However, Non-typhoidal *Salmonella* spp. are zoonotic agents and foods of animal origin are the main sources for Non-typhoidal *Salmonella* spp. transmission [7]. (Fashae *et al.,* 2010).

Human Non-typhoidal (NT) salmonellosis manifests clinically as self-limiting gastroenteritis in healthy individuals but may be severe in populations with compromised or low immunity (the young, the elderly and people with debilitating disease conditions) especially in developing nations [9]. (Hohmann, 2001). Transmission of NT *Salmonella* to humans is mostly linked to the consumption of contaminated poultry and poultry products [10, 11]. (Braden, 2006; Heredia and Garcia, 2018). While in the developed world, the incidence of *Salmonella* contamination along the food chain is treated seriously with proactive measures incorporated in the food chain to prevent outbreaks [12]. (Álvarez-Fernández *et al.,* 2012); the reverse is the case in the developing nations where food-borne infections are given less attention. The lack of focused surveillance systems and data collection on circulating *Salmonella* serovars in most developing countries make it difficult to define the severity of the problem. One major contributing factor may be due to the burden of other debilitating infections such as human immunodeficiency virus (HIV) that relegate foodborne infections like NT salmonellosis to the priority list in most developing countries.

Previous studies on NT salmonellosis in Nigeria have revealed diverse *Salmonella* serovars in both animals and man [7, 13, 14]. (Fashae *et al.,* 2010; Smith *et al.,* 2016; Agbaje *et al.,* 2019). However, the risk of infection with *Salmonella* has been worsened by the acquisition and spread of resistance traits to antimicrobials, a possible consequence of excessive and widespread use of antimicrobials in animal productions [15, 16]. (Ojo *et al.,* 2012; Omoshaba *et al.,* 2017). Studies have been undertaken largely on human clinical isolates exhibiting resistance to particular antimicrobials, such as fluoroquinolones and beta-lactams [17]. (Onyenwe *et al*., 2020). AMR to beta-lactams antibiotics mostly occurs due to the carriage of extended-spectrum beta-lactamase (ESBL) resistance plasmids in NTS. ESBLs are often plasmid-mediated enzymes and have various genotypes [17, 18] (Onyenwe *et al*., 2020; Joseph *et al.,* 2023). Through mutational events of amino acids surrounding the active site, ESBL genes evolved from the most predominant ESBL genes such as TEM-1, TEM-2, and SHV-1 genes [17, 18]. (Onyenwe *et al*., 2020; Joseph *et al.,* 2023). This led to the emergence of ESBLs with expanded substrate profiles that empowers them with the ability to hydrolyze all penicillins, cephalosporins, and monobactams. Interestingly, in the past decade, the massive spread of CTX-M-type ESBL gene which has become the main epidemic genotype worldwide has been described [17, 19, 20]. (Onyenwe *et al*., 2020; Yhiler *et al*., 2019; Sharma *et al*., 2010). Study has shown that the majority of ESBLs in *Salmonella* are derivatives of the TEM, SHV, and CTX-M (cefotaximase) *β*-lactamase families [21]. (Eguale *et al*., 2017) including the report of OXA in *Salmonella* species [19]. (Yhiler *et al*., 2019) commonly found in *Pseudomonas aeruginosa.* Additionally, fluoroquinolone resistance target sites in *E. coli* are the topoisomerases, such as DNA gyrase (topoisomerase II) - the primary site, and topoisomerase IV - the secondary target which are both vital enzymes in bacterial DNA replication [21, 22]. (Eguale *et al*., 2017; Dupouy *et al.,* 2019). Plasmid-mediated quinolone resistance (PMQR) gene, the first identified PMQR gene, also termed *qnrA1*, was discovered in 1998 [21, 23]. (Eguale *et al*., 2017; Martínez-Martínez *et al.,* 1998). Plasmids which harbor PMQR genes may also carry genes exhibiting resistance to beta-lactams [24]. (Jeong, 2011). Although AMR genes can spread clonally; the transmission of mobile genetic elements harboring AMR genes between bacteria, including from commensal to pathogenic Enterobacteriaceae is evident [25]. (Newire *et al.,* 2013). In Nigeria, beta-lactams and fluoroquinolones are commonly used in the treatment of Gram-negative bacterial infections in both human and veterinary medicine. However, the misuse of these antibiotics has ostensibly led to an increase in bacterial resistance; thus, resulting in "difficult-to-treat'' bacterial infections.

Despite the arrays of studies highlighting enteric bacteria harboring *bla*TEM, *bla*SHV, *bla*OXA-1, *bla*CTX-M, *qnr A, qnrB, qnrS* genes [19, 22, 26, 27]. (Yhiler *et al*., 2019; Dupouy *et al*., 2019; Aasm¨ae *et al*., 2019; Ugbo *et al.,* 2020), there is paucity of information regarding their prevalence in human and food-producing animal. Thus, this study was designed to ascertain the fecal carriage of extended spectrum beta lactamase and quinolone resistant non-typhoidal *Salmonella enterica* isolates from food-producing animals and humans.

2. METHODS

2.1 Sample collection

Three hundred (300) fecal samples were collected using sterile universal containers from food-producing animals namely (Chicken [100], Pig [100] and humans (100) in Onicha Local Government Area of Ebonyi State. Human stool samples were aseptically collected from different wards which includes A&E, Male surgical ward, Female surgical ward, theatre ward, Labour ward, Orthopaedic ward, Pediatric ward, Female medical ward, Male medical ward, GOPD ward. Ten samples were collected randomly from each ward with their age discrepancies from General Hospital Onicha Igboeze, Isu Health Centre, Enyibuchiri Health Center Abaomege, Oshiri Health Centre and Ukawu Health Centre. All human and animal fecal samples collected were labeled and analyzed within 2 hrs of sample collection for bacteriological identification.

2.2 Isolation and identification of *Salmonella* species

The collected samples were analyzed for the presence of *Salmonella* specie by inoculating a loopful of each sample into a separate tube of sterile nutrient broth and incubate at 37°C for 24 hrs. After overnight incubation, a loopful of the turbid broth culture was aseptically seeded by streaking on sterile solidified *Salmonella/Shigella* agar (SSA) and was incubated at 37° C for 24 h. *Salmonella* specie from positive cultures were identified by their characteristic appearance (color, consistency, shape) on the differential media, motility, and biochemical tests as previously described as reference in the microbiology practical handbook [28]. (Iroha *et al*., 2019).

2.3 Detection of Extended-spectrum beta-lactamase (ESBL)-producing Non typhoidal *Salmonella* species

The production of ESBL was phenotypically confirmed by the Double Disk Synergy Test (DDST) method using Non-typhoidal *Salmonella* isolates which exhibited reduced susceptibility to 2nd and 3rd generation cephalosporins as previously described (Joseph *et al.,* 2023). All non-typhoidal *Salmonella* inoculum were adjusted to 0.5 McFarland turbidity standards and aseptically swabbed on the Mueller-Hinton agar (MHA) plates.

Thereafter, a disc of amoxicillin/clavulanic acid (20/10 µg) was placed at the centre of the plate while ceftazidime (30 µg) and cefotaxime (30 µg) discs were each adjacently placed 15 mm away from the centre disc of amoxicillin- clavulanic acid. Plates were then incubated for 18–24 hrs at 37 ◦C. ESBL production was phenotypically confirmed by an expansion of the zone of inhibition for either ceftazidime or cefotaxime in the presence of amoxicillin-clavulanic acid than in its absence giving a dumb-bell shape [18]. (Joseph *et al.,* 2023).

2.4 Molecular typing of *ESBL* and fluoroquinolone encoding genes

Extraction of DNA was done with the ZR fungal/bacterial DNA kit (Cat number: D6005). PCR mix contained up of 12.5 µL of Taq 2X Master Mix (New England Biolabs, M0270); 1 µL each of 10 µM forward and reverse primers; 2 µL of DNA template and 8.5 µL Nuclease free water. Oligonucleotide nucleotide primers used are shown in Table 1. The following PCR condition was used: An initial denaturation for 5 mins at 94 ◦C, followed by 36 cycles of denaturation for 30 secs at 94 ◦C, annealing for 30 secs at 55 ◦C, and elongation for 45 s at 72 ◦C; followed by a final elongation step for 7 min at 72 ◦C, and hold temperature at 4 ◦C. Electrophoresis was run at 80–150 V for about 1–1.5 h. Amplified PCR products were then visualized under UV transilluminator. Positive controls used for PCR assays were previously sequenced isolates that harbored the tested genes.

Table 1: Primer Sequence

Gene	Sequence (5' to 3')	Target	Reference
Beta-Lactamases			
TEM-F	ATAAAATTCTTGAAGACGAAA	blaTEM	[29]. Egwu *et al.* (2023)
TEM-R	GACAGTTACCAATGCTTAATC		
SHV-F	TTATCTCCCTGTTAGCCACC	blaSHV	[29]. Egwu *et al.* (2023)
SHV-R	GATTTGCTGATTTCGCTCGG		
OXA-F	TCAACTTTCAAGATCGCA	blaOXA	[30]. Ahmed *et al.* (2007)
OXA-R	GTGTGTTTAGAATGGTGA		
CTX-M-F	CGCTTTGCGATGTGCAG	blaCTX-M	[29]. Egwu *et al.* (2023)
CTX-M-R	ACCGCGATATCGTTGGT		
Plasmid-mediated quinolone resistance			
qnrA-F	ATTTCTCACGCCAGGATTTG	qnrA	[31]. Robicsek *et al.* (2006)
qnrA-R	GATCGGCAAAGGTTAGGTCA		
qnrB-F	GATCGTGAAAGCCAGAAAGG	qnrB	[31]. Robicsek *et al.* (2006)
qnrB-R	ACGATGCCTGGTAGTTGTCC		
qnrS-F	ACGACATTCGTCAACTGCAA	qnrS	[31].Robicsek *et al.* (2006)
qnrS-R	TAAATTGGCACCCTGTAGGC		

Statistical analysis

The raw data obtained in the course of this study were presented as mean ± standard deviation in tables and bar charts while relevant data were interpreted using simple descriptive statistics such as minimum, maximum, and one-way analysis of variance (ANOVA) with the aid of IBM Statistical Package for Social Sciences (SPSS) version 22 and Microsoft Excel 2013 software. $P < 0.05$ was considered to be statistically significant.

3. RESULT

The distribution of NTS accounted for 25 % and 17 % in poultry and pig fecal sample respectively while 60 % and 40% were phenotypic ESBL producers respectively and were statistically significantly ($P< 0.05$). Also, none of the 16 (58 %) NTS isolated from humans were ESBL producers (Table 2). Samples gotten from the Laboratory and theatres had the highest frequencies of isolated Salmonella species with 17 (34 %) and 9 (16 %). They were also the only isolates that were significantly ($P< 0.05$) ESBL-producers with a frequency of 6 (86 %) and 1 (14 %) respectively (Table 3).

PCR analysis with β-lactam specific primer detected the presence of blaOXA 50 % and 50 %, blaSHV 36 %, and 64 %, blaTEM 43 % and 57 %, blaCTX-M 36 % and 64 % in poultry and pig respectively Combination of β-lactam gene *bla*OXA + *bla*SHV + *bla*CTM was observed to be 0.0% isolate of human, poultry and pig origin. Fluorquinolone resistant gene *QnrA* was Present in 0 and 100 % of poultry and pig, then *QnrB* was 40 % and 60 % present in poultry and pig isolate. *QnrS* was Present in 64 % isolate of poultry and 13 % of pig. There was no Co-expression of fluoroquinolone and β-lactam gene- *Qnr* +*bla*OXA +*bla*SHV+*bla*CTM seen in ESBL-producing *Salmonella* (Table 4).

Table 2: Frequency of ESBL-producing *Salmonella* and Non-typhoidal *Salmonella* species from fecal samples of food-producing animals and humans in Onicha Local Government Area

S/N	Sample Source	No. of *Salmonella* specie (%)	ESBL (%)	*p-value*	Non-ESBL (%)	*p-value*
1	Poultry	39 (63)	6 (40)	1.9921	33 (70)	-1.9328
2	Pig	23 (37)	9 (60)	0.0887	14 (30)	-0.7297
	∑V	**62**	**15 (24)**		**47 (76)**	
	Sample Source	**No. of NTS (%)**	**ESBL (%)**		**Non-ESBL (%)**	
1	Poultry	7 (25)	2 (40)	-0.6732	5 (71)	0.3872
2	Pig	5 (17)	3 (60)	0.5491	2 (29)	0.6937
3	Humans	16 (58)	-	-	-	-
	∑V	**28**	**5 (42)**		**7 (58)**	

(Mean ± SD = 2.1 ± 0.521, ∑fv = 5.00)

KEY: NTS = Non-typhoidal *Salmonella* species, SD = Standard Deviation**, ∑V =** Summation of variables**.** Values represent means of data ± Standard Deviation (SD). Data was statistically analyzed at 95% level of confidence (P < 0.05).

Table 3: Frequency of ESBL-producing *Salmonella* species from fecal samples of humans in a hospital in Onicha Local Government area with respect to wards of admission

	Ward of admission	Nos. sampled	*Salmonella* specie (%) S.D	ESBL positive (%) S.D
1	A & E	10	4 (7) ±0.2131	-
2	Male Surgical	10	3 (5) ±0.9828	-
3	Female Surgical	10	3 (5) ±0.2187	-
4	Paediatrics	10	7 (12) ±0.7276	-
5	Labour Ward	10	4 (7) ±0.5483	-
6	Orthopaedic	10	1 (2) ±1.9341	-
7	Laboratory	10	17 (34) ±0.3817	6 (86) ±0.1938
8	Theaters	10	9 (16) ±1.8864	1 (14) ±1.6281
9	GOPD	10	4 (7) ±0.9715	-
10	Male Medical	10	3 (5) ±0.6829	-
	∑V	**100**	**56 (56)**	**7 (13)**

Key: SD = Standard Deviation**, ∑V =** Summation of variables**.** Values represent means of data ± Standard Deviation (SD). Data was statistically analyzed at 95% level of confidence (P < 0.05), A & E = Accident and emergency ward, GOPD = General outpatient department.

Table 4: Molecular Detection of Extended Spectrum β-Lactamase and Fluoroquinolone Resistant Genes in non-typhoidal *Salmonella* Isolates

Antimicrobial Class	Genetic Marker Probe	No. (%) of positive isolates by origin		
		Human (n=0)	Poultry (n=7)	Pig (n=5)
β-lactam	*bla*OXA	0 (0)	2 (50)	2 (50)
	*bla*SHV	0 (0)	1(36)	2(64)
	*bla*TEM	0 (0)	3 (43)	4 (57)
	*Bla*CTX-M	0 (0)	1 (36)	2 (64)
	*bla*OXA + *bla*SHV	0 (0)	0 (0)	0(0)
	*bla*OXA+*bla*CTX-M	0 (0)	0(0)	0 (0)
	*bla*OXA +*bla*SHV+*bla*CTX-M	0 (0)	0(0)	0(0)
Fluoroquinolone	*Qnr*A	0 (0)	0(0)	2 (100)
	*Qnr*B	0 (0)	2(40)	3(60)
	*Qnr*S	0 (0)	2(64)	1(36)

Key: F-β: Fluoroquinolone and β-lactam

4. DISCUSSION

ESBL and fluoroquinolone gene were not detected from human sample. Also, resistance due to ESBLs was demonstrated by using the phenotypic confirmatory disc diffusion method, which is relatively cheaper and easy to carry out. However, the sensitivity of this method substantiate with molecular detection techniques, which are more sensitive and reproducible for confirmation of false positive results.

In contrast with our findings, molecular analysis of *Salmonella* strains demonstrated high dissemination of *blaTEM* (71.4%; 40) followed by *blaCTX-M*-1 (48.2%; 27), and *blaSHV* (19.6%; 11) [32]. (Al- Mayahi and Jaber, 2020). These findings are close to other results in Iraq [33]. (Aljanaby and Medhat, 2017), Pakistan [34]. (Saeed *et al*., 2020), and Bangladesh [35]. (Ahmed *et al*., 2014). TEM, SHV and CTX-M has been reported in most studies in Nigeria [17, 19, 36, 37, 38]. (Onyenwe *et al*., 2020; Yilher *et al*., 2019; Ugwu *et al*., 2020; Akinyemi *et al*., 2015; Iroha *et al* 2012) while in Abakaliki and Afikpo, *Qnr* gene was not reported [29].(Egwu *et al.,* 2023).

The absence of ESBL and florouquinolone gene can be used to explain the need for continuous use of this drug class for treatment of persistent salmonelloisis infection in patients.

*bla*OXA, *bla*TEM, *bla*SHV and *bla*CTX-M was common among food-producing animals. The emergence of ESBL-producing *Salmonella* reported, could be due to selective pressure imposed by the inappropriate use of broad-spectrum antibiotics such as the third generation cephalosporin as growth promoter and the treatment of bacterial infection in animal husbandry. Interestingly, in this study, some of the strains from food producing animal that produced ESBL gene can be used to explain why persistent salmonelloisis could occurs among the populace despite them receiving treatment with 3GCs. The implication of this is the potential for spread of emerging ESBL producing *S. typhi* in Onicha, which will add to the prevailing public health burdens in the state.

PCR analysis with β-lactam specific prime detected the presence of *bla*OXA 50 % and 50 %, *bla*SHV 36 %, and 64 %, *bla*TEM 43 % and 57 %, *bla*CTX-M 36 % and 64 % in poultry and pig respectively. Combination of β-lactam gene *bla*OXA + *bla*SHV + *bla*CTM was observed to be 0.0% isolate in human, poultry and pig samples. *Salmonella* spp. co-producing CTX-M- and TEM-type β -lactamases have been documented in a few case reports from Nigeria [17, 19, 36]. (Onyenwe *et al*., 2020; Yilher *et al.*,2019; Ugwu *et al*., 2020) Bangladesh [39]. (Ahmed *et al*., 2012) and India [40]. (Karthikeyan *et al*., 2011). Plasmid analysis of the resistant isolate revealed that the blaCTX-M and blaTEM genes were located in the same plasmid, which carried the ISEcp1 element upstream of the blaCTX-M gene to facilitate mobilization and expression [40]. (Karthikeyan *et al*., 2011). The present study is important in understanding the mechanism of resistance operating in these common pathogens, which are also endemic in most area in Nigeria.

Fluorquinolone resistant gene *QnrA* was present in 0 % and 100 % of poultry and pig respectively, while *QnrB* was 40 % and 60 % present in poultry and pig isolates respectively. *QnrS* was present in 64 % isolates from poultry and 13 % from pigs. Studies have shown quinolone resistance in salmonellae to be as a result of mutations in the DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV encoding (*parC* and *parE*) genes [17, 19, 41]. (Onyenwe *et al*., 2020; Yhiler *et al*., 2019; Ye *et al*., 2018). Other studies have also reported the presence of plasmid mediated quinolones resistant (PMQR) genes carried by the ESBL-producing plasmid, which facilitates the selection of higher-level resistance to quinolone drugs [17, 42, 43, 44]. (Onyenwe *et al*., 2020; Riyaaz *et al*., 2018; Carfora *et al*., 2018; Jacoby *et al*., 2014; Kongsoi *et al*., 2015).

There was no Co-expression of fluoroquinolone and β-lactam gene- *Qnr* +*bla*OXA +*bla*SHV+*bla*CTM seen in ESBL-producing non-Typhoidal *Salmonella.* This study has shown the presence of resistant genes encoding floroqunonlone and ESBL producing NTS predominantly in poultry and pigs. This could be associated with the extensive use of this antibiotic during chicken and pig rearing/production. There is an indication that the route of acquiring these genes are zoonotic and continually eating under cooked pork and chicken, poor hygienic handling of meat and careless playing with domestic animals can transfer these genes to humans and if not controlled can lead to outbreak of Salmonellosis among human population.

5. CONCLUSION

There is greater prevalence of genes encoding beta-lactamases and fluoroquinolone resistant (*blaTEM, blaSHV, blaCTX-M, blaOXA* and *blaCMY,* (*qnrA*, *qnrB* and *qnrS*) present in poultry and pig than in humans. This demonstrated a significant threat in spread of genes across human and can cause an outbreak if control approach is not put in place. ESBL producing NTS are more prevalent in animal than human and the danger associated to this is that continually eating under cooked chicken and pork plus other poor hygienic handling of this animal can actually be a route to human infection. The wide spread of fluoroquinolone and ESBL producing NTS could be associated with the extensive use of antibiotic during chicken and pig rearing/production and *Salmonella* isolates from human may be due to unhygienic handling and consumption of under cooked chicken and pork. It is of public health importance because consumers are exposed to the risk of infection by fluoroquinolone and ESBL-NTS producing strain from the chicken and pork. This further highlights the need for rational use of antibiotics in livestock, poultry and pig farming, proper meat handling/cooking practices and enforcement of standard food safety by governmental regulatory agencies so as to prevent the risk of ESBL and fluoroquinolone resistant bacteria mediated foodborne diseases.

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Ethical consideration: Ethical approval with reference No: SMOH/ERC/042/21 was obtained from the Research and Ethics Committee of Ebonyi State Ministry of Health, Abakaliki, Nigeria. All experiment in this study was executed following relevant national and international guidelines

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