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Prevalence of Antimicrobial Resistance Non-Typhoidal *Salmonella* Isolated from Livestock in Abia State, Nigeria

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ABSTRACT

This present study isolated and investigated the antibiotic profiles of Non-typhoidal *Salmonella* (NTS) from fecal samples of livestock in Abia state, Nigeria. 384 feces samples of each of four animals (cows, goats, pigs, poultry) collected from 9 local government areas in the state were analyzed between 2019 and 2022. Standard microbiological procedures were followed in isolation and identification of the *Salmonella* isolates and the identified NTS confirmed with PCR. Antibiotic susceptibility test on these organisms were done using the Kirby Bauer disk diffusion method and a panel of 15 commercially sold antibiotics: ciprofloxacin, aztreonam, cefpodoxime, colistin, cefoxitin, cefotaxime, cotrimoxazole, imipenem, tetracycline, amoxicillin– clavunalic acid, ceftazidime, ampicillin, cefepime, ceftriazone, and gentamicin. Presence of five resistance genes: SHV, CTX-M, IMP, TEM and mcr-1 were determined in the isolates. The identified NTS in the animals confirmed using PCR were *Salmonella enterica*, Var Typhimurium, *Salmonella enterica* var. Enteritidis and *Salmonella Dublin*. The results show that the highest prevalence of NTS in animals in Abia state was from Aba South LGA (7.14%), while the least was from Umunneochi LGA (2.98%). Others fell between 7.14% and 2.98%. In conclusion, data from this study indicates a varied prevalence of NTS across different local government areas, with notable antibiotic resistance observed among the isolates. The findings underscore the critical need for continuous surveillance and the implementation of effective antibiotic stewardship programs to mitigate the spread of resistance.

Keywords: antibiotic profile, Non-typhoidal Salmonella, Abia animal farms, public health

Introduction:

Non-typhoidal Salmonella (NTS) infections represent a significant public health concern globally, with a high burden in sub-Saharan Africa, including Abia, Nigeria. Overuse and indiscriminate use of antibiotics have been linked to the prevalence of antibiotic-resistant non-typhoidal *Salmonella* in farm animal feces in other parts of Nigeria and the occurrence of these resistant strains poses significant public health risks, one of which is foodborne infection (Jibril et al., 2021). Because *Salmonella* is mainly transmitted to humans through foods of animal origin, consumption of such foods without proper investigation places humans at high risk. Igbinosa et al., (2023) investigated their prevalence using 150 fecal, water, and feed samples from ten poultry farms in Delta State between January and August 2020 and identified *Salmonella* serovars such as: *Salmonella* Enteritidis (39.5%) and other *Salmonella* serovars (30.2%). They further reported that the serovars exhibited multiple antibiotic-resistance, elaborated resistant genes, and exhibited virulence determinants, which demonstrated their potential of transmission to humans resulting to difficult-to-treat infections. Similarly, Edward et al., (2021) reported multidrug resistant *Salmonella* species from abattoir wastewater in Abia state, Nigeria. Other studies in Nigeria have reported resistance to multiple antibiotics such as augmentin, amoxicillin, ampicillin, tetracycline, sulphonamides, ciprofloxacin, cefuroxime, ceftazidime, ofloxacin, ciprofloxacin and gentamicin as well as identified the presence of virulence factors and biofilm formation in *Salmonella* serovars (Ajayi et al., 2023; Igbinosa et al., 2023; Jibril et al., 2021; Orum, Ishola & Adebowale, 2022; Sánchez, 2023). Additionally, research across Nigeria has highlighted the prevalence of NTS in poultry farms, with identified risk factors including poor biosecurity practices, inadequate vaccination, and water source contamination, contributing to NTS persistence and infection rates (Igbinosa et al., 2023; Jibril et al., 2021). However, such researches have scarcely investigated and reported NTS prevalence in the fecal matters of goats, cows, and pigs. The presence of antibiotic-resistant strains in these farm animal feces as well as in poultry increases the likelihood of transmission to humans through contaminated products and lead to economic losses.

Methodology

Questionnaire investigation

Questionnaires were prepared and distributed to the farm managers and they supplied the information on it.

Isolation of non-typhoidal salmonella

Enrichment of organism

The method outlined by Kebede et al. (2016) was adopted by adding 25 g of aseptically weighed fecal sample into 225 ml of buffered peptone water in a conical flask. The mixture was gently shaken for 2 minutes before being incubated at 37°C for 18 hours. Following the incubation period, 1 ml of the peptone culture was transferred into a test tube containing 10 ml of sterile Mueller Kauffmann Tetrathionate broth. Simultaneously, 0.1 ml of the culture was transferred into another test tube with 10 ml of sterile Rappaport Vassiliadis broth. Both test tubes were shaken and then incubated, with the Tetrathionate broth at 37°C and the Rappaport Vassiliadis broth at 42°C, for 18 to 24 hours. Post-incubation, a loopful of each broth culture was subcultured on xylose lysine deoxycholate agar (XLD) and brilliant green agar (BGA). These agar plates were incubated at 37°C for 24 to 48 hours. The colonies were then observed for characteristic growth patterns: red colonies with black centers on XLD and pink colonies with a red zone on BGA, which are indicative of Salmonella isolates. *Salmonella* typing was done using polyvalent and monovalent antisera from ThermoFisher Scientific. Species of non-typhoidal *Salmonella* isolated were confirmed with PCR.

Preparation and standardization of Inoculum:

A sterile wire loop was used to pick about 3 well-isolated colonies of the test organisms that are similar in appearance and emulsified in 3ml sterile physiological saline.

The turbidity standard was shaken to mix well, and then the turbidity of the suspension was matched to it by comparing their turbidities against sheet of paper. (Cheesbrough, 2010). Inoculum was standardized with 0.5 McFarland corresponding to 1.5×1^{8} cfu/ml cells.

Molecular Characterisation of Isolates

Bacterial genomic DNA extraction

Extraction was done using a ZR fungal/bacterial DNA mini prep extraction kit supplied by Inqaba South Africa. A heavy growth of the pure culture of the suspected isolates was suspended in 200 microliters of isotonic buffer into a ZR Bashing Bead Lysis tubes, 750 microliter of lysis solution was added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tube were centrifuged at 10,000xg for 1 minute.

Four hundred (400) microliters of supernatant were transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000 xg for 1 minute. One thousand two hundred (1200) microliters of fungal/bacterial DNA binding buffer were added to the filtrate in the collection tubes bringing the final volume to 1600 microliter, 800 microliter was then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000xg for 1 minute, the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) microliter of the DNA Pre-wash buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000xg for 1 minute followed by the addition of 500 microliter of fungal/bacterial DNA Wash Buffer and centrifuged at 10,000xg for 1 minute.

The Zymo-spin IIC column was transferred to a clean 1.5 microliter centrifuge tube, 100 microliter of DNA elution buffer was added to the column matrix and centrifuged at 10,000xg microliter for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20 degree for other downstream reaction.

DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2 µl of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the “measure” button.

16S rRNA Amplification

The 16s RRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3’ and 1492R: 5'-CGGTTACCTTGTTACGACTT-3’ primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a UV transilluminator.

Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10µl, the components included 0.25 µl BigDye® terminator v1.1/v3.1, 2.25µl of 5 x BigDye sequencing buffer, 10µM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions was as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min.

Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou & Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes & Cantor 1969).

MOLECULAR IDENTIFICATION OF RESISTANCE GENES

Amplification of SHV, TEM, CTX-M, IMP, and mcr-1 genes

The 5 genes from the isolates were amplified using each gene primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microliters for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as in figure below. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator. TEM was resolved at 200V for 15mins, IMP was resolved on a 1% agarose gel at 130V for 30 minutes whilst Mcr-1 was resolved on a 1% agarose gel at 130V for 25 minutes.

Table 1. PCR Conditions for The Detection of Resistant Genes

Gene	Primer	PCR conditions	Amplicon	Reference
16srRNA	F: 5'-AGAGTTTGATCMTGGCTCAG-3' R: 5'-CGGTTACCTTGTTACGACTT-3'		1500bp	Lane, 1991.
Mcr-1	F: 5'-CGGTCAGTCCGTTTGTTC -3' R: 5'- CTTGGTCGGTCTGTAGGG- 3'	ID95ºC/5m, D95ºC/30s, A58ºC/30s, E72ºC/30s, F72ºC/5m	309bp	Liu et al., 2016
SHV	F: 5'- CGCCTGTGTATTATCTCCCT-3' R: 5'-CGAGTAGTCCACCAGATCCT-3'	I.D 95ºC/5min D 95ºC/30m A 56ºC/40s E 72ºC /50s F72ºC/5min	293bp	Gholipaur et al., 2014.
CTM-X	F: 5'-CGCTTTGCGATGTGCAG-3' R: 5'-ACCGCGATATCGTTGGT-3'	ID 95ºC/5m, D 95ºC/30s, A 52ºC/30s, E 72ºC/30s, F 72ºC/5m	550bp	Yazdansetad et al., 2019
IMP	F: 5'- GGAATAGAGTGGCTTAAYTC-3' R: 5'- GGTTTAAYAAAACAACAACC-3'	ID95ºC/5m, D95ºC /30s, A 48ºC/30s, E72ºC/30s, F72ºC/5m	256bp	Poirel, Walsh, Cuvillier & Nordmann, 2011.
TEM	F: 5'-TTTCGTGTCGCCCTTATTCC-3' R: 5'-ATCGTTGTCAGAAGTAAGTTGG-3'	I.D 95ºC/5min, D 95ºC/30s, A 58ºC/30s, E 72ºC/30s, F 72ºC/5min	403bp	Gholipaur et al., 2014.

Key: I.D (Initial denaturation); D (denaturation); A (annealing); E (extension) for 35 cycles F (final extension).

Antibiotic Susceptibility Test

Kirby- Bauer Disc diffusion technique:

Antimicrobial sensitivity testing was performed according to Kirby- Bauer disc diffusion method recommended by the NCCLS (2004) and CLSI (2012) and also described by Cheesbrough (2010) was used. A panel of fifteen (15) antibiotics which include: ampicillin, aztreonam, ceftazidime, ceftriazone, ciprofloxacin, colistin, cotrimoxazole, gentamicin, imipenem, tetracycline, amoxicillin-clavunalic acid, cefepime, cefoxitin, cefpodoxine and cefotaxime was used in this assay. About five (5) antibiotic discs each were placed on inoculated Mueller Hinton agar surface and the plates incubated at 35ºC for 24hrs. Zones of growth inhibition around the discs were measured to the nearest millimetre

Results

In the determination of prevalence of non-typhoidal *Salmonella* in animals in Abia state, Aba south local government area was found to have the highest prevalence (12, 7.14%) while Umunneochi local government area had the least (5, 2.98%). Others fall between them (table 2).

Table 2. Occurrence of non-typhoidal *Salmonella* in animals in Abia State

LGA	Number of samples	Number of Isolates	Percentage Occurrence
Aba N	168	9	5.36%
Bnd	168	9	5.36%
Ik	168	9	5.36%
Aba S	168	12	7.14%
Isk	168	7	4.17%
Oss	168	6	3.57%
UmN	168	7	4.17%
UmS	168	6	3.57%
Unc	168	5	2.98%

Key: LGA = Local government areas, AbaN = Aba North, Bnd = Bende, Ik = Ikwuano, AbaS = Aba South, Isk = Isuikwuato, Oss = Osisioma, UmN = Umuahia North, UmS = Umuahis South, Unc = Umunneochi.

The result of the sequenced isolates from animals: K1(Po) from poultry, K4(Pg) from pig, K5(Cw) from cow, K6(Gt) from goat, K8(Po) from poultry and K9(Po) from poultry indicated three species of non-typhoidal *Salmonella* including *Salmonella enterica* var Enteritidis, *Salmonella enterica var* Typhimurium and *Salmonella* Dublin (fig.1).

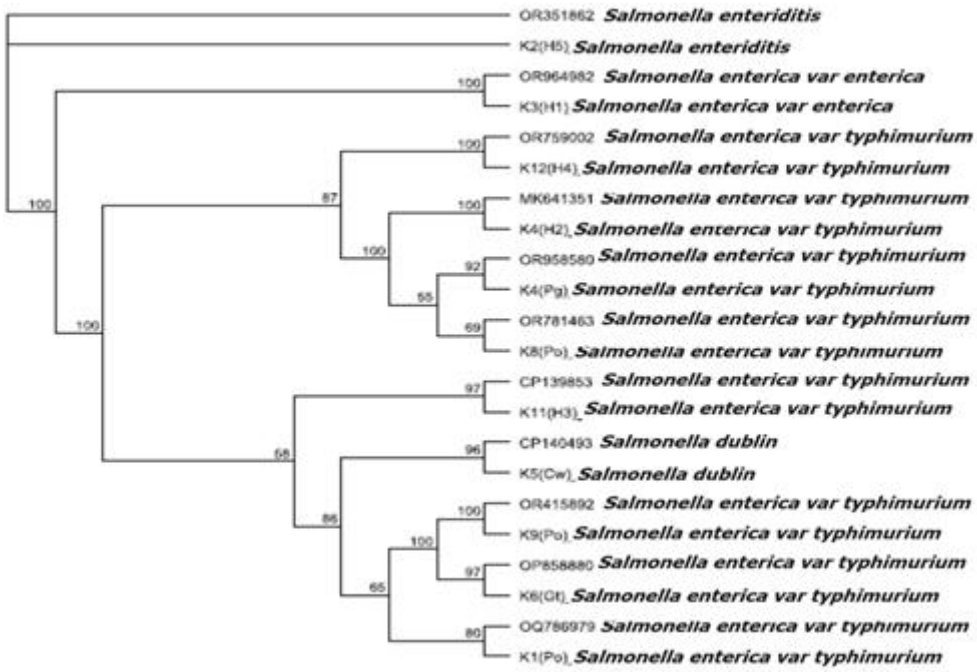

Fig 1 Phylogenetic tree of the sequenced isolates

The antimicrobial profile of the isolates showed that resistance by isolates from goat was highest to imipenem antimicrobial with six (6) LGAs showing total (100%) resistance to it followed by amoxicillin clavunalic acid which had total (100%) resistance in five (5) LGAs (Table 3).

Isolates from pig had the highest (100%) resistance to imipenem in seven (7) LGAs followed by amoxicillin- clavunalic acid with total (100%) resistance in five LGAs (Table 4).

The cow isolates showed highest resistance to two antibiotics namely; aztreonam (ATM) and imipenem (IPM) with isolates from seven LGAs being total resistant to the antibiotics (Table 5).

The highest resistance by poultry isolates was directed to amoxicillin-clavunalic acid which recorded total resistance (100%) in six (6) LGAs, followed by resistant to imipenem and aztreonam with each having total resistance (100%) in five Local Government Areas (Table 6).

Table 3. **Antimicrobial resistance profile (%) of NTS isolates from goat faecal waste in Abia State.**

	LGA / Number(%) resistant isolates								
Antimicrobial	**Aba N**	**Bnd**	**Ik**	**Aba S**	**Isk**	**Oss**	**UmN**	**UmS**	**Unc**
	n = 3	**n = 3**	**n =1**	**n = 3**	**n = 2**	**n = 3**	**n = 1**	**n = 1**	**n = 2**
CIP	2(66.67%)	1(33.33%)	1 (100%)	3 (100%)	1 (50%)	1(33.33%)	0 (0%)	0 (0%)	1 (50%)
ATM	3 (100%)	2(66.67%)	1 (100%)	30(0%)	1 (50%)	2(66.67%)	0 (0%)	1 (100%)	1 (50%)
CPD	2(66.67%)	2(66.67%)	0 (0%)	2(66.67%)	1 (50%)	3 (100%)	0 (0%)	1 (100%)	1 (50%)
CT	2(66.67%)	1(33.33%)	0 (0%)	2(66.67%)	0 (0%)	1(33.33%)	1 (100%)	0 (0%)	0 (0%)
FOX	0 (0%)	0 (0%)	0 (0%)	2(66.67%)	1 (50%)	0 (0%)	0 (0%)	1 (100%)	1 (50%)
CTX	1(33.33%)	1(33.33%)	1 (100%)	2(66.67%)	0 (0%)	1(33.33%)	0 (0%)	0 (0%)	1 (50%)
SXT	2(66.67%)	0 (0%)	0(0%)	1(33.33%)	1 (50%)	1(33.33%)	0 (0%)	0 (0%)	1 (50%)
IPM	3 (100%)	2(66.67%)	1 (100%)	2(66.67%)	1 (50%)	3 (100%)	1 (100%)	1 (100%)	2 (100%)
TE	1(33.33%)	0 (0%)	1 (100%)	2(66.67%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
AMC	2(66.67%)	2(66.67%)	1 (100%)	3 (100%)	0 (0%)	2(66.67%)	1 (100%)	1 (100%)	2 (100%)
CAZ	3 (100%)	1(33.33%)	0 (0%)	2(66.67%)	0 (0%)	3 (100%)	0 (0%)	0 (0%)	1 (50%)
AMP	3 (100%)	2(66.67%)	1 (100%)	2(66.67%)	2 (100%)	2(66.67%)	0 (0%)	1 (100%)	1 (50%)
FEP	1(33.33%)	2(66.67%)	1 (100%)	2(66.67%)	0 (0%)	2(66.67%)	0 (0%)	0 (0%)	1 (50%)
CRO	1(33.33%)	1(33.33%)	0 (0%)	2(66.67%)	2 (100%)	1(33.33%)	0 (0%)	1 (100%)	1 (50%)
CN	0 (0%)	3 (100%)	1 (0%)	1(33.33%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Key: LGA = Local government ares, AbaN = Aba North, Bnd = Bende, Ik = Ikwuano, AbaS = Aba South, Isk = Isuikwuato, Oss = Osisioma, UmN = Umuahia North, UmS = Umuahis South, Unc = Umunneochi. CIP = Ciprofloxacin, ATM = Azreonam, CPD = Cefpodoxime, CT = Colistin, FOX = Cefoxitin, CTX = Cefotaxime, SXT = Cotrimoxazole , IPM = Imipenem, TE = Tetracycline, AMC = Amoxicillin – Clavunalic acid, CAZ = Ceftazidime, AMP = Ampicillin, FEP = Cefepime , CRO = Ceftriazone, CN = Gentamicin.

Table 4. Antimicrobial resistance profile (%) of Non-typhoidal *Salmonella* isolates from pig faecal waste in Abia State.

	LGA / Number (%) resistant isolates							
Antimicrobial	**Aba N**	**Bnd**	**Ik**	**Aba S**	**Isk**	**Oss**	**UmN**	**UmS**
	n = 2	**n = 3**	**n = 3**	**n = 3**	**n = 2**	**n = 1**	**n = 3**	**n = 1**
CIP	2 (100%)	2(66.67%)	2(66.67%)	1(33.33%)	0 (0%)	1 (100%)	2(66.67%)	0 (0%)
ATM	2(100%)	2(66.67%)	2(66.67%)	2(66.67%)	0 (0%)	1 (100%)	1(33.33%)	1 (100%)
CPD	1 (50%)	3 (100%)	2(66.67%)	2(66.67%)	1 (50%)	0(0%)	2(66.67%)	1 (100%)
CT	1 (50%)	0 (0%)	1(33.33%)	2(66.67%)	1 (50%)	0 (0%)	1(33.33%)	0 (0%)
FOX	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1(33.33%)	1 (100%)

CTX	0 (0%)	0 (0%)	2(66.67%)	2(66.67%)	1 (50%)	1 (100%)	1(33.33%)	1 (100%)
SXT	0 (0%)	0 (0%)	1(33.33%)	1(33.33%)	0 (0%)	0 (0%)	2(66.67%)	1 (100%)
IPM	2 (100%)	3 (100%)	3 (100%)	3 (100%)	1 (50%)	1 (100%)	3 (100%)	1 (100%)
TE	1 (50%)	0 (0%)	2(66.67%)	0 (0%)	2 (100%)	0 (0%)	1(33.33%)	0 (0%)
AMC	1 (50%)	1(33.33%)	3 (100%)	3 (100%)	2 (100%)	0 (0%)	3 (100%)	1 (100%)
CAZ	2 (100%)	2(66.67%)	1(33.33%)	2(66.67%)	1 (50%)	1 (100%)	0 (0%)	0 (0%)
AMP	2 (100%)	0 (0%)	3 (100%)	1(33.33%)	1 (50%)	1 (100%)	2(66.67%)	1 (100%)
FEP	2 (100%)	1(33.33%)	2(66.67%)	1(33.33%)	0 (0%)	1 (100%)	2(66.67%)	1 (100%)
CRO	0 (0%)	1(33.33%)	1(33.33%)	1(33.33%)	0 (0%)	0 (0%)	1(33.33%)	0 (0%)
CN	0 (0%)	3 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Key: LGA = Local government ares, AbaN = Aba North, Bnd = Bende, Ik = Ikwuano, AbaS = Aba South, Isk = Isuikwuato, Oss = Osisioma, UmN = Umuahia North, UmS = Umuahis South, Unc = Umunneochi. CIP = Ciprofloxacin, ATM = Azreonam, CPD = Cefpodoxime, CT = Colistin, FOX = Cefoxitin, CTX = Cefotaxime, SXT = Cotrimoxazole, IPM = Imipenem, TE = Tetracycline, AMC = Amoxicillin – Clavunalic acid, CAZ = Ceftazidime, AMP = Ampicillin, FEP = Cefepime, CRO = Ceftriazone, CN = Gentamicin

Table 5. Antimicrobial resistance profile (%) of Non-typhoidal *Salmonella* isolates from cow faecal waste in Abia State.

Antimicrobial	**LGA / Number (%) resistant isolates**							
	Aba N	**Ik**	**Aba S**	**Isk**	**Oss**	**UmN**	**UmS**	**Unc**
	n=2	**n = 2**	**n = 3**	**n = 1**	**n = 1**	**n = 3**	**n = 1**	**n = 1**
CIP	2 (100%)	2 (100%)	3 (100%)	0 (0%)	0 (0%)	2(66.67%)	0 (0%)	0 (0%)
ATM	2 (100%)	2 (100%)	3 (100%)	1 (100%)	1 (100%)	1(33.33%)	1 (100%)	1 (100%)
CPD	2 (100%)	1 (50%)	3 (100%)	0 (0%)	0 (0%)	3 (100%)	1 (100%)	0 (0%)
CT	0 (0%)	2 (100%)	1(33.33%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)
FOX	0 (0%)	1 (50%)	1(33.33%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)
CTX	1 (50%)	1 (50%)	0 (0%)	1 (100%)	0 (0%)	1(33.33%)	1 (100%)	1 (100%)
SXT	1 (50%)	0 (0%)	1(33.33%)	0 (0%)	0 (0%)	3 (100%)	0 (0%)	0 (0%)
IPM	1 (50%)	2 (100%)	3 (100%)	1 (100%)	1 (100%)	3 (100%)	1 (100%)	1 (100%)
TE	2 (100%)	1 (50%)	2(66.67%)	0 (0%)	0 (0%)	1(33.33%)	1 (100%)	1 (100%)
AMC	2 (100%)	1 (50%)	3 (100%)	0 (0%)	1 (100%)	3 (100%)	1 (100%)	1 (0%)
CAZ	1 (50%)	2 (100%)	1(33.33%)	0 (0%)	1 (100%)	3 (100%)	0 (0%)	0 (0%)
AMP	1 (50%)	2 (100%)	2 (66.67%)	0 (0%)	1 (100%)	2(66.67%)	1 (100%)	1 (100%)
FEP	0 (0%)	2 (100%)	2 (66.67%)	1 (100%)	1 (100%)	1(33.33%)	1 (100%)	0 (0%)
CRO	0 (0%)	1 (50%)	3 (100%)	0 (0%)	1 (100%)	1(33.33%)	1 (100%)	0 (0%)
CN	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)	3(33.33%)	0 (0%)	0 (0%)

Key: LGA = Local government ares, AbaN = Aba North, Bnd = Bende, Ik = Ikwuano, AbaS = Aba South, Isk = Isuikwuato, Oss = Osisioma, UmN = Umuahia North, UmS = Umuahis South, Unc = Umunneochi.

Table 6. Antimicrobial resistance profile (%) of Non-typhoidal *Salmonella* isolates from poultry faecal waste in Abia State.

	LGA / Number (%) resistant isolates							
Antimicrobial	**Aba N**	**Bnd**	**Ik**	**Aba S**	**Isk**	**Oss**	**UmS**	**Unc**
	n = 2	**n = 3**	**n = 3**	**n = 3**	**n = 2**	**n = 1**	**n = 3**	
CIP	2 (100%)	1(33.33%)	1(33.33%)	2(66.67%)	0 (0%)	1 (100%)	1(33.33%)	0 (0%)
ATM	2 (100%)	3 (100%)	3 (100%)	2(66.67%)	1 (50%)	1 (100%)	1(33.33%)	2 (100%)
CPD	1 (50%)	1(33.33%)	3 (100%)	1(33.33%)	1 (50%)	0 (0%)	3 (100%)	0 (0%)
CT	1 (50%)	1(33.33%)	1(33.33%)	3 (100%)	0 (0%)	1 (100%)	1(33.33%)	1 (50%)
FOX	0 (0%)	0 (0%)	2(66.67%)	2(66.67%)	0 (0%)	0 (0%)	3 (100%)	1 (50%)
CTX	2 (100%)	3 (100%)	2(66.67%)	3 (100%)	1 (50%)	1 (100%)	1(33.33%)	1 (50%)
SXT	0 (0%)	1(33.33%)	3 (100%)	1(33.33%)	0 (0%)	1 (100%)	3 (100%)	0 (0%)
IPM	2 (100%)	2(66.67%)	2(66.67%)	3 (100%)	2 (100%)	1 (100%)	3 (100%)	0 (0%)
TE	2 (100%)	0 (0%)	2(66.67%)	1(33.33%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)
AMC	2 (100%)	3 (100%)	2(66.67%)	3 (100%)	0 (0%)	1 (100%)	3 (100%)	2 (100%)
AMP	2 (100%)	2(66.67%)	3 (100%)	2(66.67%)	0 (0%)	1 (100%)	3 (100%)	1 (50%)
FEP	1 (50%)	0 (0%)	1(33.33%)	1(33.33%)	1 (50%)	1 (100%)	2(66.67%)	0 (0%)
CRO	1 (50%)	0 (0%)	1(33.33%)	1(33.33%)	1 (50%)	1 (100%)	2(66.67%)	1 (50%)
CN	0 (0%)	0 (0%)	1(33.33%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Key: LGA = Local government ares, AbaN = Aba North, Bnd = Bende, Ik = Ikwuano, AbaS = Aba South, Isk = Isuikwuato, Oss = Osisioma, UmN = Umuahia North, UmS = Umuahis South, Unc = Umunneochi.

Detected resistance genes in the non-typhoidal *Salmonella* isolates

The screening of resistance genes in the non-typhoidal *Salmonella* isolates with PCR revealed five resistance genes including SHV, TEM, IMP, CTX-M and mcr-1 genes (figs. 2, 3, 4, 5 and 6).

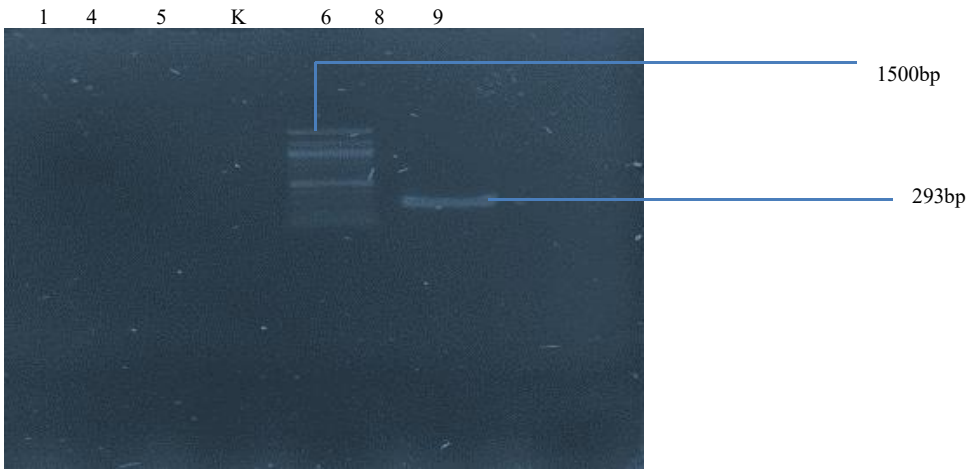

Fig. 2: Agarose gel electrophoresis of SHV gene of bacterial isolates.

Lane 6 represents the SHV gene bands (293bp) isolated from goat in Osisioma LGA. Lane K represents the 100bp Molecular ladder.

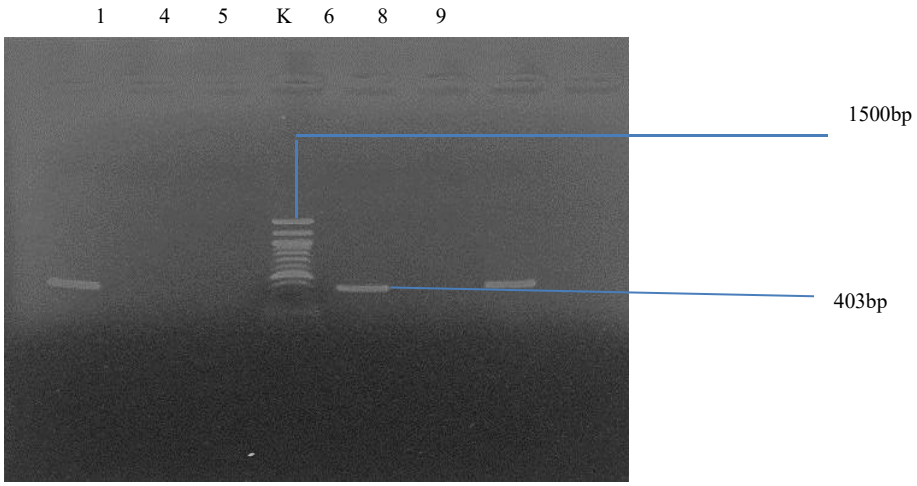

Fig. 3: Agarose gel electrophoresis showing identified TEM genes in the isolates.

Key: Lane 1,6 and 9= Positive TEM gene at 403bp from isolates. 1= isolate from poultry in Aba South LGA, 6= isolate from goat in Osisioma LGA, 9= isolate from poultry in Aba North LGA.

Lane K = 100bp molecular ladder.

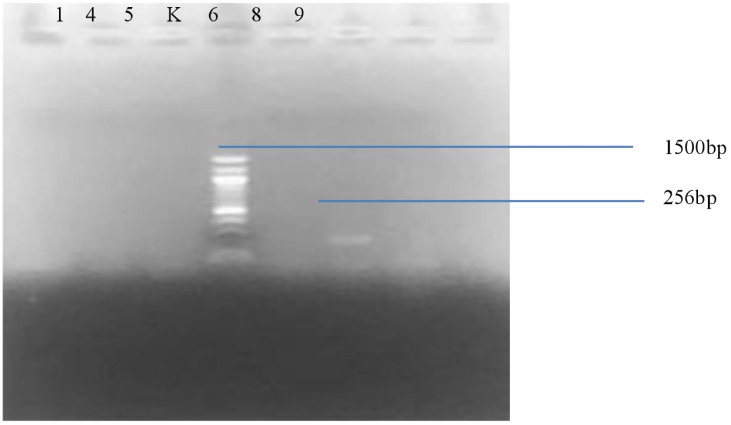

Fig. 4: Agarose gel electrophoresis of IMP gene identified in the isolates.

Key: Lane 8 = IMP gene band (256bp). Lane K = 100bp molecular ladder.

8= Non- typhoidal salmonella isolate from poultry in Umuahia South LGA.

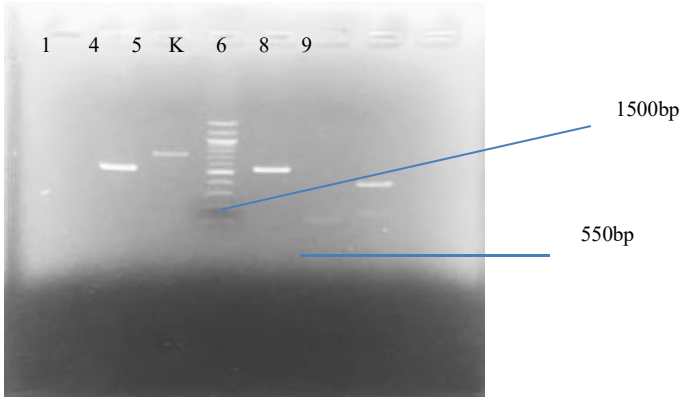

Fig.5: Agarose gel electrophoresis of CTX-M genes identified in the isolates.

Key: Lane 5 and lane 8 = CTM-X genes band (550bp). Lane K = 100bp molecular ladder.

5 = Non-typhoidal *Salmonella* isolate from cow in Ikwuano Local government area.

8 = Non-typhoidal *Salmonella* isolate from poultry in Umuahia South Local government area.

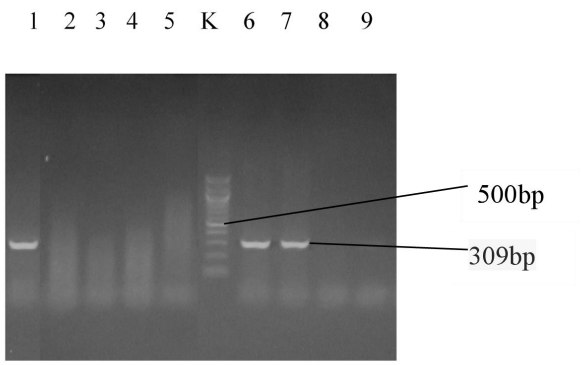

Fig. 6: Agarose gel electrophoresis showing the amplified mcr- 1 gene.

Key: Lanes 1, 6 and 7 = amplified mcr-1 gene bands at 309bp.

1 = cow isolates from Ikwuano. 6 = poultry isolate from Umuahia South LGA . 7 = poultry isolate from Aba North LGA.

Lane K =100bp molecular ladder.

Discussion

The variations of NTS occurrence in the animals surveyed across the 9 local governments in Abia (Tables 2 and 3) might be linked to differences in livestock population density, farming practices, veterinary services, or presence of specific abattoirs (Akpabio & Kalu, 2015).

Antimicrobial resistant profile (%) of NTS isolates from goat faecal waste in Abia state showed highest resistance to Imipenem antimicrobial with six (6) LGAs showing total (100%) resistance to it. The next was amoxicillin clavunalic acid which had total (100%) resistance in five (5) LGAs (Table 4). Antibiotics like azreonam (ATM), cefpodoxime (CPD), imipenem (IPM), amoxicillin – clavulanic acid (AMC), ceftazidime (CAZ), and ampicillin (AMP) exhibited resistance rates of 66.67% to 100% in most LGAs. Also, there is variability in resistance patterns across different LGAs. For instance, while some antibiotics show consistent resistance across LGAs (e.g., Ampicillin), others exhibit varying resistance rates. This variability may reflect differences in antibiotic usage practices, hygiene standards, or other environmental factors across the regions. Further, some antibiotics showed emerging resistance patterns, such as cefoxitin (FOX) and ceftriazone (CRO), which displayed resistance in certain LGAs despite being previously effective in others. This suggests the potential for the emergence and spread of antibiotic-resistant strains of *Salmonella* in the region. High levels of resistance to important antibiotics like ciprofloxacin (CIP) and imipenem (IPM) are particularly concerning as these are commonly used antibiotics for treating severe *Salmonella* infections in humans. The presence of multidrug-resistant strains, as indicated by resistance to multiple antibiotics, raises serious public health concerns and highlights the need for effective antibiotic stewardship programs requiring a multifaceted approach involving collaboration between veterinarians, healthcare professionals, policymakers, and the community to ensure responsible antibiotic use and prevent further spread of antibiotic-resistant bacteria. These findings underscore the importance of continuous surveillance of antimicrobial resistance in both animal and human populations. Implementing effective control measures, such as prudent antibiotic use in agriculture and healthcare settings, improved sanitation practices, and promoting alternative disease prevention strategies, is essential to mitigate the spread of antibiotic resistance.

Similar to the findings in the previous table, table 5 indicated that across the different local government areas (LGAs) in Abia State, antibiotics like ciprofloxacin (CIP), azreonam (ATM), cefpodoxime (CPD), and imipenem (IPM) showed consistent high resistance rates, ranging from 66.67% to 100%. Resistance patterns vary across different LGAs, reflecting differences in antibiotic usage, farming practices, and environmental factors. For instance, while some antibiotics like ceftazidime (CAZ) and ampicillin (AMP) showed high resistance rates in certain LGAs, they exhibit lower resistance rates in others. Certain antibiotics, such as colistin (CT), cefoxitin (FOX), and ceftriazone (CRO), showed emerging resistance patterns in specific LGAs. This suggests the potential for the spread of resistant strains within the pig population, which could pose challenges for both animal health and public health. Multidrug resistance, defined as resistance to multiple antibiotics, was evident among NTS isolates from pig fecal waste. Antibiotics like ampicillin (AMP), amoxicillin – clavulanic acid (AMC), and cefepime (FEP) showed resistance in combination with other antibiotics, indicating the presence of multidrug-resistant strains. Igbinosa et al., (2023) and Edward et al., (2021) have reported similar ampicillin resistance among the Salmonella serovars they investigated.

Others, such as gentamicin (CN), showed limited effectiveness against NTS isolates from pig fecal waste, with resistance observed across all LGAs. This highlights the importance of judicious antibiotic use and the need for alternative strategies for controlling *Salmonella* infections in pigs as also documented by Jibril et al., (2021).

Overall, the highest resistance was offered by Imipenem. The total (100%) resistance against Imipenem occurred in seven (7) LGAs. This was followed by amoxicillin- clavunalic acid with total (100%) resistance in five LGAs.

Human fecal waste NTS isolates as shown in table 6 demonstrated high levels of resistance (100%) to various antibiotics such as ciprofloxacin (CIP), azreonam (ATM), cefpodoxime (CPD), colistin (CT), imipenem (IPM), amoxicillin – clavulanic acid (AMC), ceftazidime (CAZ), ampicillin (AMP),

cefepime (FEP), and ceftriazone (CRO) in one or more local government areas (LGAs). This indicated a disturbing trend of widespread resistance among *Salmonella* strains affecting humans in the region. Similarly, Orum, Ishola and Adebowale (2021) reported multidrug resistance patterns of *Salmonella* species (n = 77; 100.0%) resistant to cefuroxime, n = 73; 94.8%), resistant to chloramphenicol, meropenem resistant (n = 72; 93.5%), gentamicin (n = 69; 89.6%), and tetracycline (n = 64; 83.1%) from poultry farms in Ibadan. In the present study, unlike the variability observed in resistance patterns among animal isolates, the resistance profiles of NTS isolates from human fecal waste appear more consistent across different LGAs. This uniformity in resistance patterns suggests that certain antibiotic-resistant strains may be prevalent among human populations throughout the region. Further, the effectiveness of several commonly used antibiotics, including tetracycline (TE) and gentamicin (CN), appeared to be limited against NTS isolates from human fecal waste, with no observed susceptibility in any of the LGAs. The complete resistance evident in commonly used antibiotics for the treatment of *Salmonella* infections in humans, such as ciprofloxacin and ceftriazone, indicate the potential for treatment challenges and increased morbidity and mortality associated with Salmonella infections, underscoring the need for enhanced surveillance and control measures.

The NTS cow isolates from Abia State offered highest resistance to two antibiotics namely; aztreonam (ATM) and imipenem (IPM) with isolates from seven LGAs being total resistant to the antibiotics. Antibiotics such as ciprofloxacin (CIP), azreonam (ATM), cefpodoxime (CPD), colistin (CT), imipenem (IPM), amoxicillin – clavulanic acid (AMC), ceftazidime (CAZ), ampicillin (AMP), and ceftriazone (CRO) showed resistance rates ranging from 50% to 100% in various LGAs (table 7). Variable resistance patterns were also evident across different LGAs. For instance, while some antibiotics like cefoxitin (FOX) and cotrimoxazole (SXT) showed resistance in certain LGAs, they exhibited lower resistance rates in others. This variability may be influenced by factors such as antibiotic usage practices, farming techniques, and environmental conditions in each LGA. Cefotaxime (CTX) and gentamicin (CN), showed emerging resistance patterns in specific LGAs. This suggests the potential for the spread of resistant strains within the cow population, which could have implications for both animal health and public health. Moreso, multidrug resistance was evident, with resistance observed against multiple antibiotics in some LGAs. This highlights the presence of multidrug-resistant strains, which pose challenges for both veterinary and human medicine. Antibiotics like tetracycline (TE) showed limited effectiveness against NTS isolates from cow fecal waste, with high resistance rates observed in most LGAs. This underscores the importance of judicious antibiotic use and the need for alternative strategies for controlling *Salmonella* infections in cows.

Among the poultry fecal waste NTS isolates (table 8), the highest resistance was noticed against amoxicillin-clavunalic acid (100%) total resistance in six (6) LGAs, followed by resistant to imipenem and aztreonam which recorded each total resistance (100%) in five Local Government Areas. Ciprofloxacin (CIP), azreonam (ATM), cefpodoxime (CPD), colistin (CT), imipenem (IPM), amoxicillin – clavulanic acid (AMC), ceftazidime (CAZ), and ampicillin (AMP) show resistance rates ranging from 50% to 100% in various LGAs. Similar variability in resistance patterns were evident: cefotaxime (CTX) and sulfamethoxazole - trimethoprim (SXT), showed higher resistance in certain LGAs but lower resistance rates in others. Certain antibiotics, such as cefoxitin (FOX) and gentamicin (CN), showed emerging resistance patterns in specific LGAs, a situation for the potential for the spread of resistant strains within the poultry population.

Multidrug resistance is evident among NTS isolates from poultry fecal waste, with resistance observed against multiple antibiotics in some LGAs. This highlights the presence of multidrug-resistant strains, which can complicate treatment options for both poultry and human infections. tetracycline (TE) showed limited effectiveness against NTS isolates from poultry fecal waste, with high resistance rates observed in most LGAs. This underscores the importance of judicious antibiotic use and the need for alternative strategies for controlling *Salmonella* infections in poultry.

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