Plasmid-Mediated Quinolone Resistance Genes Associated With Ciprofloxacin Resistance in *Salmonella Typhimurium* from Cattle Faeces from Abattoir in Keffi, Nasarawa State, Nigeria

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***Abstract*-** The development of antimicrobial resistance by members of the genus *Salmonella* has a significant impact on the antibiotic treatment of *Salmonella* infections. This study investigated the plasmid-mediated quinolone resistance (PMQR) genes associated with Ciprofloxacin resistance in *Salmonella typhimurium (S. typhimurium)* from cattle faeces from abattoir in Keffi, Nasarawa State, Nigeria. A total of Four hundred (400) samples of cattle faeces were collected from abattoir and *S. typhimurium* were isolated and identified using standard microbiological methods. Antimicrobial susceptibility of the isolates was tested and interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) protocol. Plasmid-Mediated Quinolone Resistance (PMQR) genes in ciprofloxacin resistant isolates were screened for using polymerase chain reaction (PCR) method. Of the 400 samples, the occurrence of S. *typhimurium* was 46 (11.5%). The isolates were more susceptible to gentamicin (87.1%), chloramphenicol (78.3%), streptomycin (69.6%) and ciprofloxacin (67.4%). The occurrence of multi-drug resistance (MDR) isolates was 46 (100%). The order of occurrence of PMQR genes in the ciprofloxacin resistant isolates was: *aac (6')-Ib-cr* (66.7%) > *oqx*AB and *qnr*B (26.7%) > *qnr*S (20.0%). Gentamicin, chloramphenicol, streptomycin and ciprofloxacin, were the most effective antibiotics against the isolates. All the isolates were MDR and the most common PMQR gene detected in the ciprofloxacin resistant isolates was *aac (6')-Ib-cr*. Further studies on the molecular diversity of PMQR *S.typhimurium* isolates from the study location should be carried out.

I. INTRODUCTION

The genus *Salmonella* is a gram negative rod shaped bacteria belonging to the family of Enterobacteriaceae, and are facultative anaerobic, non-spore forming, non-lactose fermenting bacteria possessing motility with peritrichous flagella which causes salmonellosis (Kongsoi *et al.*, 2016).

Non-typhoidal *Salmonella* are zoonotic pathogens and are the important causes of foodborne infection because *Salmonella* have a broad host range and are strongly associated with animal and plant products (Gal-mor *et al.*, 2014). *Salmonella typhimurium* and *S. enteritidis* are natural pathogen of cattle and beef which are common reservoirs for human infection (Costa *et al*., 2012; Kongsoi *et al.*, 2016). The Infections due to NTS range from mild diarrheal disease to severe systemic infections (Neelem Taneja *et al*., 2014) and are of significant public health concern globally (Adesiji *et al*., 2014). Non-typhoidal *Salmonella* infections are transmitted to humans primarily through consumption of contaminated foods from animal origin (Ranjbar *et al*., 2011).

Antimicrobial resistance in NTS is a significant public health problem both in developed and developing countries (Adesiji *et al.*, 2014). Several literatures have reported an increasing number of cases of multi-drug resistant foodborne *Salmonella enterica* infections in both developed and developing countries, with few options left for antimicrobial treatment (Uche *et al.*, 2017; Shu-kee *et al.*, 2015). Examples of increases in resistance in NTS in developing countries, particularly in sub-Saharan Africa, the Indian subcontinent and Southeast Asia, are exemplified by numerous outbreaks caused by multi drug resistant non-typhoidal salmonella; these are frequently resistant to the newer quinolones both in hospitals and the community over wide geographical areas (Kagambega *et al.*, 2017).

Fluoroquinolones exhibit potent antibacterial activity against *Salmonella* and are usually the first drug of choice for treatment of life-threatening salmonellosis due to multidrug-resistant strains (Kongsoi *et al.*, 2016). In the early 1990s quinolone antibiotics have been approved for use in food producing animals; but the misuse and use of sub-growth inhibitory concentration of these antibiotics in animals has been reported as one of the factors that endanger quinolones resistance in NTS strains (Beneduce *et al*., 2008; Rankotonirina *et al*., 2013; Kongsoi *et al.*, 2016; Tajbakhsh *et al.*, 2016), which may be chromosomal or plasmid mediated. The most common PMQR genes reported in salmonellae and other enterobacteriacae are *oqx*AB, *qnr*B, *qnr*S and *aac(6')-Ib-cr*.

Non-typhoidal *Salmonella* is the primary foodborne zoonotic agent of salmonellosis in many countries (Hoelzer *et al.*, 2011). *Salmonella typhimurium* and *Salmonella enteritidis* are natural NTS pathogens of cattle and beef which are common reservoirs for human infection (Costa *et al*., 2012; Kongsoi *et al.*, 2016). Infection due to NTS ranges from mild diarrheal disease to severe systemic

infection both in human and other animals (Tanija *et al*., 2014). These zoonotic pathogens are spread by consumption of contaminated food sources (Ranjbar *et al*., 2011; Costa *et al.,* 2012). Fluoroquinolones are usually the first drug of choice for treatment of life-threatening salmonellosis due to multidrug-resistant strains (Kongsoi *et al.,* 2016). However, increased use of these agents in animal husbandry has led to concomitant emergence of quinolone resistance NTS (Rankotonirina *et al*., 2013; Tajbakhsh *et al.,* 2016).There are several reports of fluoroquinolone resistance *S. typhimurium* in different countries and continents of the world (Chattaway *et al.,* 2016). In Nigeria, few studies have reported the presence of *S. typhimurium* resistant to fluoroquinolones (Chattaway *et al.,* 2016). However, there is dearth of such information in the study location. This study thus, investigated the presence of plasmid mediated quinolone resistance genes in *S. typhimurium* isolated from cattle faeces from abattoir in Keffi, Nasarawa State, Nigeria.

Non-typhoidal *Salmonella* is a primary foodborne zoonotic pathogen that causes salmonellosis in different parts of the world (Kongsi, *et al*., 2016). Previous studies have shown that NTS are resistant to fluoroquinolones (Hernamdez, *et al.,* 2011; Raktonirina, *et al.,* 2013; Tajbakhsh *et al*., 2016). However, no such studies have been reported in the study area. Hence, this study investigated the plasmid mediated Quinolones resistance genes associated with ciprofloxacin resistance in *S.typhimurium*, a common NTS, isolated from cattle faeces from abattoir in Keffi, Nasarawa State, Nigeria. Antimicrobial resistance in NTS is a significant public health problem both in developed and developing countries.

II. Material and Methods

Study Location

The study Location was Keffi. Keffi is geographically situated on latitude 8º50ˈN and longitude 7º52ˈ E. Keffi town is about 850m above sea level and it is the North-West of Lafia, the state capital. It is 53 km away from Abuja (Capital of Nigeria) in the Guinea Savannah region of Nigeria (Akwa *et al*., 2007).

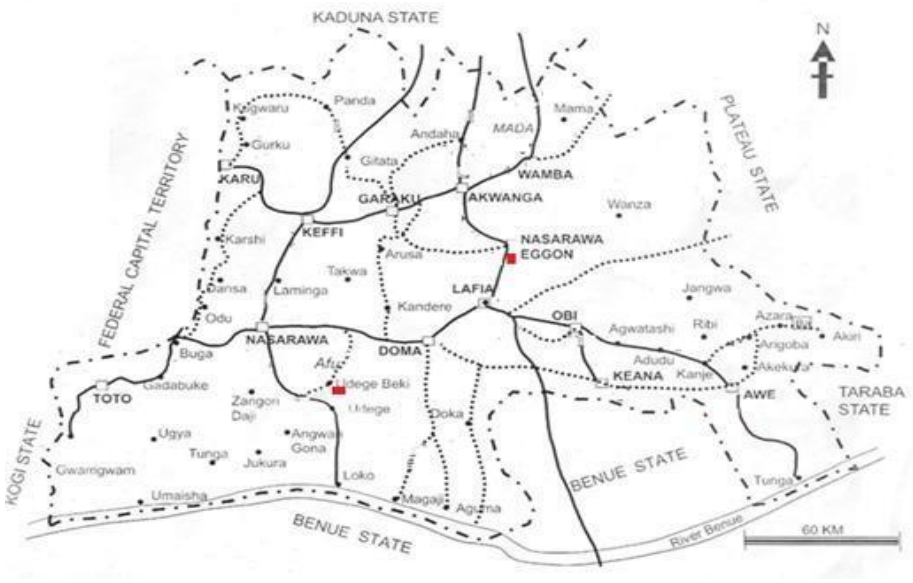
Sample Size

The sample size was calculated manually, using the formula below as earlier described by Fisher *et al.* (2002).

$$N=Z^2P\sum/d^2$$

Where: N= desired sample size (when the population >10,000); Z= standard normal deviate, usually set at 1.96, which usually correspond to 95% confidence level; P= proportion in the target population, set at 50% (0.5) d= tolerated margin of error.
The proportion was estimated as p< (0.5) and q< (0.5) for non infection confidence estimated used was 95% (≥1.5) confidence interval with degree of accuracy of d (0.05). The designed effect of 1 was used. The sample size was obtained

N= (1.96)2× 0.5× 0.5÷ (0.05)2
=0.9604÷0.0025
= 384



Map showing study location in Keffi, Nigeria (Source: Akwa *et al*., 2007).

Sample Collection

A total of 400 samples (fresh and non-freshly voided materials) were collected randomly from fecal matter at Keffi abattoir and transported to Microbiology Laboratory, Nasarawa State University for analysis using aseptic transport medium container.

Isolation of *Salmonella* species

The method described by Cheesbrough (2006) was used. The fecal matter samples ascetically collected from Keffi abattoir was first inoculated on Xylose Lysine Deoxycholate (XLD) agar and Selenite F enrichment broth (pH 7.4) and incubated aerobically at 37ºC for 24 h. After incubation, all the tubes showing turbidity were sub-cultured on xylose-lysine deoxycholate (XLD) agar and *Salmonella-Shigella* agar (SSA) and then incubated at 37ºC for 24 h for the selective isolation of *Salmonella* species. Red colonies with black centre were taken as presumptive *Salmonella* species. Colonies not resembling *Salmonella* spp were further re-incubated at 37º C for overnight. Presumptive *Salmonella* isolates were sub-cultured unto freshly prepared Xylose Lysine Deoxycholate (XLD) agar and Selenite F enrichment broth (pH 7.4) media and incubated to obtain pure isolates as described by Iroha *et al*. (2016).

Identification of the Isolates

Presumptive *Salmonella* colonies were further identified by Gram staining reaction and biochemical tests according to the method of Cheesbrough (2006), and outlined below.

Gram Staining

A small portion of cultural organism was transferred onto a clean grease-free glass slide, and emulsified in a drop of distilled water until a thin homogeneous film is obtained, then the wire loop was re-sterilized and the thin homogeneous film was allowed to air-dry, and heat-fixed by passing through flame. The slide was then flooded with crystal violet for 1 min, and then rinsed with distilled water. The stain was again flooded with Lugol's iodine for 1 min, and rinsed with distilled water and then decolorized, rapidly with acetone alcohol until no more colour appeared to flow from preparation and rinsed appropriately with distilled water. The stain was then counter-stained with neutral red for 1 min, and rinsed with distilled water and allowed to air dry and viewed microscopically using x100 oil immersion objective. Gram positive organism retains the dark blue colour inferred by the iodine/crystal violet complex, while Gram negative organisms appear red; maintaining the colour of the secondary dye. *Salmonella* species is a Gram negative rod.

Biochemical tests

The following biochemical tests were carried out on the suspected *Salmonella* species isolates: Catalase test, Indole, Nitrate reduction, Urease production, Citrate utilisation and glucose fermentation tests

Indole Test

A colony of the organism from culture plate was inoculated unto 5 ml tryptone broth and incubated at 37ºC for 24 h. After which a few drops of Kovac's reagent was added to the overnight tryptone broth culture, and shaken. A positive reaction is indicated by the development of red ring colour in the reagent layer above the broth within 10 min observed. *Salmonella* species is negative for this reaction.

Methyl Red/Voges-Proskauer Test

A pure culture of test organism was inoculated in to MR-VP medium and incubated at 37ºC for 72 h after which the culture was divided in to two portion. To the first portion three drops of methyl red was added and formation of red colour was indicative of methyl red positive. To the second portion 10 drops of 40% KOH (Potassium hydroxide) was added, followed by four drops of alpha-napthol was added and observed for 30 min. Formation of pink/red colour indicates Voges-Proskauer positive and formation of yellow colour indicates Voges-Proskauer negative. *Salmonella is* positive for methyl red and negative for Vorges-Proskauer.

Citrate Utilization Test

A pure culture of the organism was inoculated as a single streak on the slant surface of citrate agar and was incubated at 37ºC for 24 h. Blue color on the medium indicated the presence of alkaline products and it is therefore positive, while green color is negative. *Salmonella* species gave negative citrate utilization reaction.

Catalase test

A pure colony of the organism was streaked aseptically on Nutrient agar slant and incubated at 37ºC for 24 h. Three drops of Hydrogen peroxide (H_2O_2) was added to the slant and observed for bubbling gas. *Salmonella* species is positive for this test.

Urease test

This was be performed by inoculating the organism into Urea broth and incubated at 37ºC for 24 h. Intense pink color indicated positive; otherwise, it is negative. *Salmonella* species are negative for urease production.

Molecular detection of Plasmid-mediated Quinolones resistance genes

DNA Extraction

The DNA of ciprofloxacin resistant isolates was extracted using boiling method as described by Porteous *et al.* (1994). Briefly, following the purification, one pure colony of ciprofloxacin resistance isolate, was inoculated into 2 ml of LB broth and incubated at 37ºC for 18 h. Exactly 200 µl of the Lysogeny broth (LB) culture was then transferred into Eppendorf tube and centrifuged in a micro centrifuge at 3200 revolutions per minute (rpm) for 2 min at room temperature. The supernatant was discarded leaving the cells in the

tube. The cells were washed twice with washing buffer. Exactly 0.5 ml of sterile phosphate buffer was added to the pellet and votexed for 5 sec. It was then heated at 90ºC for 10 min, then cooled down rapidly by freezing for 10 min. It was then centrifuged at 3200 rpm for 1 min to separate the DNA and the cells debris. Exactly 300 µl of the supernatant containing the DNA was then transferred into Eppendorf tube and stored at -10ºC for further use.

Amplification of Target Genes

The DNA amplification of target plasmid-mediated quinolone resistant genes in ciprofloxacin resistant isolates was carried out using single plex method by modification of the method earlier described in a work by (Ranjbar 2017) Briefly, the reaction was carried out in 25 µl reaction volume in artificial tubes which is made up of 5 µl master mix, 2.4 µl primers (0.4 µl each of forward and reverse primers), 0.5 µl of $MgCL_2$, 1.5 µl of DNA template and 15.6 µl of nuclease free water. The reaction tubes were placed in the holes of the thermer cycler and its door was closed. Then *qnr*A, *qnr*B, and *qnr*S genes were amplified under the following conditions: Initial denaturation at 94ºC for 5 min followed by 32 cycles of amplification at 94ºC for 45 sec each, annealing at 53ºC for 45 sec, with final extension at 72ºC for 5 min. (Jiang *et al.*, 2008).

The amplification condition for detection of *aac (6')-Ib-cr* was carried out as follows: initial denaturation at 95ºC for 5 min, annealing at 59ºC for 40 sec and initial extension at 70ºC for 30 secs and with final extension at 72ºC for 5 min.

III. Data Presentation and Analysis

Isolation and Identification of *Salmonella typhimurium*

The cultural, morphological and biochemical characteristics of *S.typhimurium* isolated from fecal samples of cattle in Keffi abattoir, Nasarawa State, Nigeria as shown in Table 4.1 The colourless colonies with black colouration in SSA which was gram negative, non lactose fermenting organism with rod shape, motile peritrichous flagella and TSI positive were identified as *S.typhimurium* as shown in Table 4.1. Transparent colonies with black centre by *Salmonella* species on SSA and red translucent colonies by *Salmonella* species on XLD. Red to Pale red translucent colonies with black centre colonies by *Salmonella* species on XLD showed morphological characteristics of *S.typhimurium*. Brilliant green agar showed pale red with black centre colonies in line with characteristics as reported according to the methods of Cheesbrough (2006).

Occurrence of *Salmonella typhimurium*

Of the 400 samples of cattle fecal matter obtained, the occurrence of *S. typhimurium* was 46 (11.5%) as shown in Table 4.2

Cultural, Morphological and Biochemical characteristics of *Salmonella typhimurium* isolated from cattle faeces from abattoir in Keffi, Nasarawa State, Nigeria

Cultural Characteristics	**Morphological Characteristics**		**Biochemical Characteristics**									**Inference**
	Gram	**Morphology**	**MOT**	**UR**	**LYS**	**H2S**	**ONPG**	**NIT**	**MAL**	**IN**	**MR**	
Transparent colonies with black centre by *Salmonella* species on SSA	-	Rod shape	+	-	+	+	-	+	+	_	+	*Salmonella Species*
Red to Pale red translucent colonies with black centre colonies by *Salmonella* species on XLD and BGA agar												

MOT = Motility test; UR = Urease test; LYS = Lysine utilization; H_2S = H_2S production test; ONPG = Onpg-Galactosidase test; NIT = Nitrate test; MAL = Maltose fermentation test; IN = Indole fermentation test; MR = Methyl red; MCA = MacConkey agar; SSA = Salmonella Shigella agar; BGA = Brilliant green agar

Occurrence of *Salmonella typhimurium* from cattle faeces from abattoir in Keffi, Nasarawa State, Nigeria

Samples	No. of Samples	No (%) *Salmonella typhimurium*
Fecal matter	400	46 (11.5%)

Antimicrobial Resistance Profile

The antimicrobial resistance profile of the isolates is as shown in Table 4.3. The isolates were more resistant to nalidic acid and tetracycline (100.0%); sulphamethoxazole/trimethoprim (87.0%) and ceftriaxone (60.9%) but less resistant to gentamicin (12.9%) and chloramphenicol (21.7%) respectively as shown in Table 4.3.

Antibiotic Resistance Phenotype

The antibiotic resistance phenotypes of the *Salmonella typhimurium* isolates is shown Table 4.4 .The isolates were distributed into different antibiotic resistance phenotypes and the commonest was TE-SXT-CAZ-NA-CIP (10.9%)

Multiple Antibiotic Resistance (MAR) Index

The MAR indices of the *S. typhimurium* isolates are as shown in Table 4.5. The isolates with MAR ≥ 0.2 are defined as MAR isolates and the commonest MAR index was 0.5 (43.5%) as shown in Table 4.5.

Antibiotics Resistance Profile of *Salmonella typhimurium* from cattle faeces from abattoir in Keffi, Nasarawa State, Nigeria

Antibiotics	Disc Content (µg)	No. (%) Resistance (n=46)
Amoxicillin/ Clavunalic acid (AMC)	30	18(39.1)
Ceftazidime (CAZ)	30	20(43.5)
Ceftriaxone (CRO)	30	28(60.9)
Chloramphenicol (C)	30	10(21.7)
Gentamicin (CN)	10	6(12.9)
Sulphamethoxazole Trimetheprim (SXT)	25	40(87.0)
Streptomycin (S)	30	14(30.4)
Ciprofloxacin(CIP)	5	15(32.6)
Nalidixic acid (NA)	30	46(100.0)
Tetracycline(TE)	30	46(100.0)

Antibiotic Resistance Phenotypes of *Salmonella typhimurium* from cattle faeces from abattoir in Keffi, Nasarawa State, Nigeria

Antibiotic Resistance Phenotypes	Frequency (%) (n=46)
TE,SXT,NA	2(4.3)
CRO,TE,SXT,NA	4(8.7)
AMC,TE,SXT,NA	2(4.3)
CRO,TE,SXT,NA	2(4.3)
TE,SXT,CAZ,NA,CIP	5(10.9)
TE,SXT,S,NA,CIP	3(6.5)

CN,TE,SXT,NA,CIP	1(2.2)
CRO,TE,SXT,CAZ,NA	2(4.3)
CRO,TE,S,CAZ,NA	2(4.3)
CRO,TE,SXT,S,NA	3(6.5)
C,AMC,TE,SXT,NA	1(2.2)
AMC,CRO,TE,SXT,NA	3(6.5)
C,TE,SXT,CAZ,NA,CIP	2(4.3)
CRO,TE,SXT,S,CAZ,NA	2(4.3)
AMC,CRO,TE,SXT,CAZ,NA	3(6.5)
C,AMC,TE,SXT,S,NA,CIP	2(4.3)
C,AMC,CRO,TE,SXT,S,NA	3(6.5)
CN,AMC,CRO,TE,SXT,CAZ,NA	4(8.7)

AMC = Amoxicillin/Cluvanulic acid; C = Chloramphenicol; CAZ = Ceftazidime; CRO = Ceftriaxone; CIP = Ciprofloxacin; CN = Gentamicin; NA = Nalidixic acid; S = Streptomycin; SXT = Sulphamethoxazole/Trimetheprim; Te = Tetracycline

Multiple Antibiotics Resistance (MAR) index of *Salmonella typhimurium* from cattle faeces from abattoir in Keffi, Nasarawa State, Nigeria

No. of antibiotics to which isolate is resistant (a)	No. of antibiotics tested (b)	MAR Index (a/b)	Frequency (%) n=46
10	10	1.0	(0.0) 0
9	10	0.9	(0.0) 0
8	10	0.8	(0.0) 0
7	10	0.7	(19.6) 9
6	10	0.6	(15.2) 7
5	10	0.5	(43.5) 20
4	10	0.4	(17.4) 8
3	10	0.3	(4.3) 2
2	10	0.2	(0.0) 0

Classification of Antibiotic Resistance

The classes of antibiotic resistance of the *S. typhimurium* isolates are as shown in table above All the isolates were MDR with percentage occurrence of 100%.

Molecular Detection of Plamid Mediated Quinolones resistance genes

Molecular detection of PMQR genes in the ciprofloxacin resistant *S. typimurium* isolates is as shown in Table 4.7. The occurrence of *aac(6')-1b-cr* (66.7%) gene was high while *oqx*AB/*aac-(6')-1b-cr* (13.0%), *qnr*B/*aac-(6')-1b-cr* (13.0%), *qnr*S/ *aac(6')-1br-cr* (13.0%) and *oqx*AB/*qnr*B/ *qnr*S /*aac(6')-Ib-cr* (13.3%) showed low Quinolones resistance genes across the table.

Classes of Antibiotics Resistance of *Salmonella typhimurium* isolated from cattle faeces from abattoir in Keffi, Nasarawa State, Nigeria

Classes of Antibiotics Resistance	No. (%) *S. typhymurium* (n=46)
MDR	46(100)
XDR	0(0.0)
PDR	0(0.0)
NMDR	0(0.0)

NMDR = Non-multi-drug resistance; MDR = Multi-drug resistance (non-susceptible to ≥1 agent in ≥3 antimicrobial categories); XDR = Extensive drug resistance (non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); PDR=Pan drug resistance (non-susceptible to all antimicrobial listed) (Magiorakos *et al.*, 2012).

Molecular Detection of Plamid-mediated Quinolones Resisitance genes in ciprofloxacin Resisitance *Salmonella typhumurium* isolated from cattle faeces from abattoir in Keffi, Nasarawa State, Nigeria

Plasmid-mediated quinolone resistance genes	No. (%) *S. typhimurium* (n=15)
*Oqx*AB	4 (26.7%)
*Qnr*B	4 (26.7%)
*Qnr*S	3 (20.0%)
aac(6')-Ib-cr	10 (66.7%)
*oqx*AB/*qnr*B	4 (26.7%)
*oqx*AB/*qnr*S	3 (20.0%)
*qnr*B/*qnr*S	3 (20.0%)
*oqx*AB/*qnr*B/*aac(6')-Ib-cr*	3 (20.0%)
*oqx*AB/*aac(6')-Ib-cr*	2 (13.3%)
*qnr*S /*aac(6')-Ib-cr*	2 (13.3%)
*qnr*B/*aac(6')-Ib-cr*	2 (13.3%)
*oqx*AB/*qnr*B/ *qnr*S /*aac(6')-Ib-cr*	2 (13.3%)

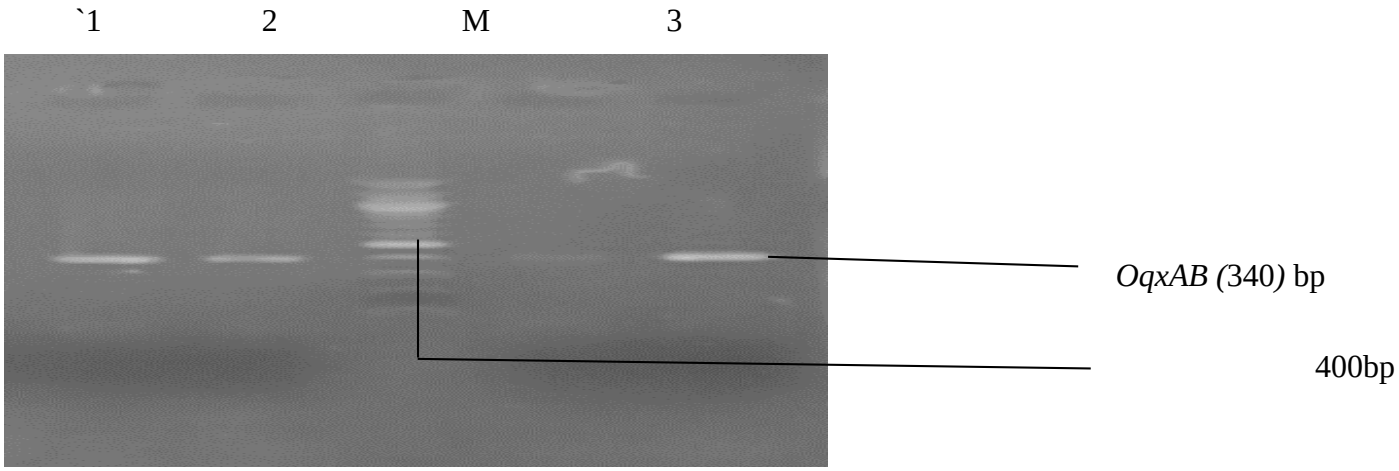


Plate 4.1: Agarose gel electrophoresis of the amplified *oqxAB* genes from the *Salmonella typhimurium* isolates. Lanes 1, 2, 3 and 4 represent the *oqxAB* bands, Lane M represents the 1500bp molecular ladder.

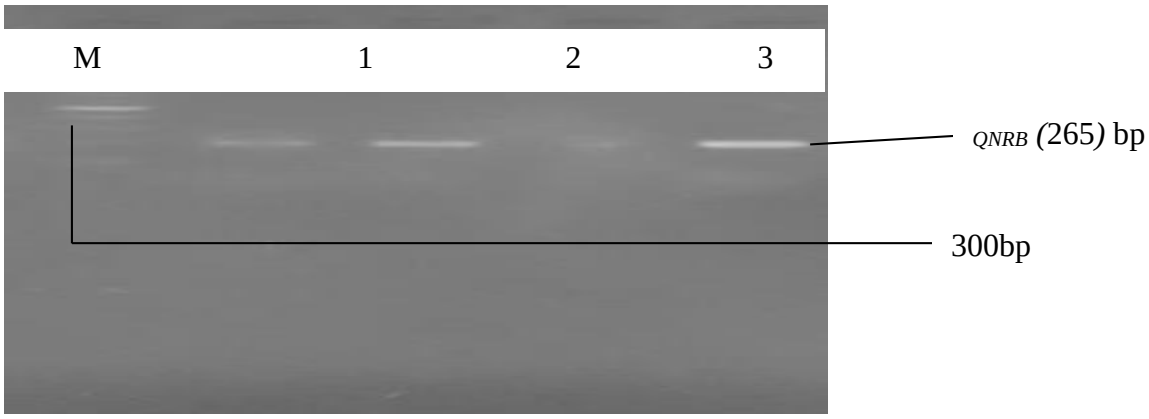


Plate 4.2: Agarose gel electrophoresis of the amplified *QNRB* genes from the *Salmonella typhimurium* isolates. Lanes 1, 2, 3, and 4 represent the *QNRB* bands, Lane M represents the 1500bp molecular ladder.

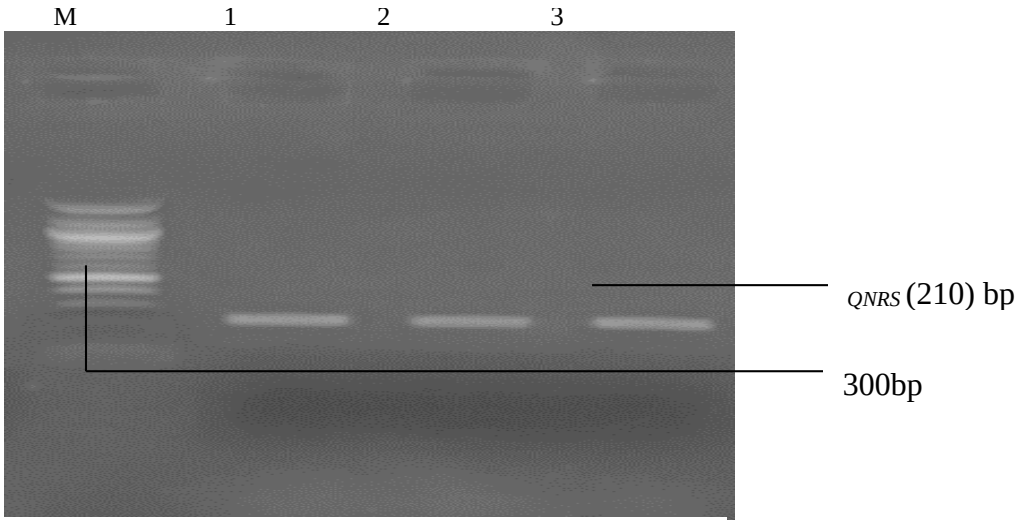


Plate 4.3: Agarose gel electrophoresis of the amplified *QNRS* genes from the *Salmonella typhimurium* isolates. Lanes 1, 2 and 3 represent the *QNRS* band, Lane M represents the 1500bp molecular ladder.

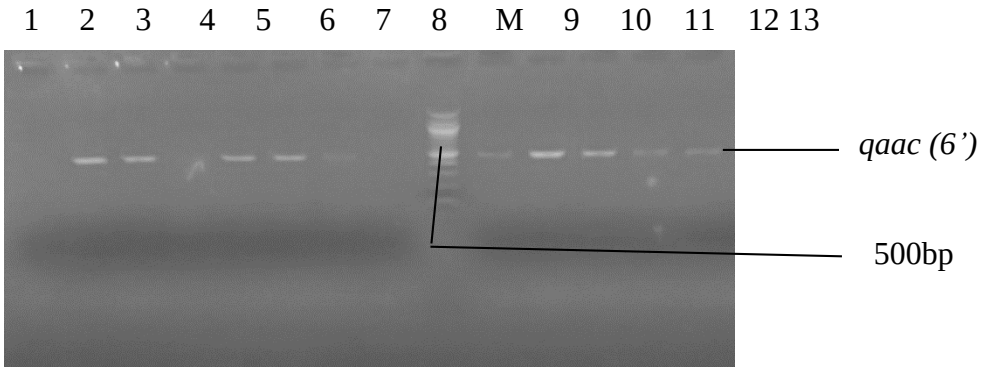


Plate 4.4: Agarose gel electrophoresis of the amplified *qaac (6')* genes from the *S.typhimurium* isolates. Lanes 2, 3, 5, 6, 7, 9, 10, 11, 12 and 13 represent the *qaac (6')* band, Lane M represents the 1500bp molecular ladder, while lane 4 shows no band.

IV. Discussion of Findings

Over the years, the proportion of *Salmonella typhimuruim* with reduced Fluoroquinolones susceptibility has increased in many countries of the United States, United Kingdom and south East Asia (Yoke-Kqueen, *et al*., 2008). This Study focused on molecular detection of plasmid mediated quinolones resistance (PMQR) genes in ciprofloxacin resistance *S.typhimurium* isolates following almost non availability of such report in the study location. The occurrence of S. *typhimurium* from fecal samples of cattle obtained in the study location was expected and is in agreement with the study earlier reported by Tenaja *et al.* (2014), Thai *et al.* (2018) and Ari (2017). The 11.5% significant occurrence of S. *typhimurium* in the study location was higher than 5% reported by Ari (2017) and 3.4% by Kagembega *et al.* (2017). The study however showed a lower occurrence compared to 12% (Shu-Kee *et al.* 2015) and 18.5% (Thai *et al.* 2018) previously reported in other studies. The occurrence of this bacteria in fecal sample may have a public health since the cow dung may contaminate meat or may also be useful as organic manure for cultivation of vegetables and likely serve as agent of food borne diseases that cause morbidity and mortality worldwide (Smith *et al*., 2016).

The S. *typhimuirum* isolates observed in this study were less susceptible to ceftriazone, sulphamethoxazole trimethoprin, nalidixic acid and tetracycline and this finding is in agreement with the study earlier reported by Hakanen *et al.,* (2005), Kotilainen *et al*., (2005) & Miriagou *et al*., (2004). The less susceptibility of the isolates to antibiotics mentioned may be due to inappropriate use of such antibiotics both in Hospital and agricultural settings Mir *et al.,* (2015), Marshal &Levy, (2011).The 0%, 0%, 13% and 18% susceptibility to nalidixic acid, tetracycline, sulphamethaxole trimethoprin and ceftriazone as reported in the study showed same pattern of no and low susceptibilities along the same line of antibiotics earlier described by Kagembega *et al*., (2017) in similar study.

The high susceptibility of the isolates to amoxicillin/clavulanic acid, ceftazidine, chlorophenicol, gentamycin, streptomycoin and ciprofloxacin was not surprising and this however justifies their use as common drug of choice for treatment of salmonella infections (Ari 2017).

The high susceptibility of the isolates to amoxycilin/clavulanic and chloramphenicol and streptomycin observed in this study is in agreement with the earlier studies described by Ari (2017) and Rajashekhara (2014). The percentage of the isolates to antibiotics mentioned above was 60.9% to 78.3% with gentamicin having the highest susceptibility pattern of 87.1%.

The occurrence of multidrug resistance S.*typhimurium* in the location as was observed in the study not surprising again and this is in agreement with the study earlier described by Migma *et al.* (2001). The multi drug resistance (MDR) isolates occurrence was 100%, this had a slight disagreement with 94.5%, 47.7%, and 78% reported by Taneja *et al.* (2014), Mir *et al.* (2015), and Yoke-Kqueen *et al*., (2008) respectively in previous studies. The occurrence of multidrug resistance isolates observed in this study may have public health implication since multi drug resistance S. *typhimurium* have been reported to cause Salmonellosis that is difficult to be treated using antibiotics.

The detection of Plamid mediated quinolone resistance genes namely *oqx*AB, *qnr*B, *qnr*S and *aac(6')-Ib-cr* in this study is in agreement with the study earlier reported by Maima *et al.* (2011); Taneja *et al.* (2014) and Hooper *et al.* (2016). Our finding in this study shows that *aac(6')-Ib-cr* genes was the main gene detected in ciprofloxacin resistance isolates and this finding is consistent with the study earlier reported by Stefana Sabtcheva *et al.* (2009) that reported 60.7% and Migma *et al.* (2011) which reported 66.7%.
In addition, the percentage occurrence of *oqx*AB (26.7%), *qnr*B (26.7%) and *qnr*S (20.0%) was less than 37.1 % reported by Shreya *et al.* (2018). The detection of the plamid mediated quinolone resistance gene observed in this study was indication that may be responsible for ciprofloxacin resistance.

V. Summary Conclusion

The findings from this study were as follows:

i. The occurrence of *S. typhimurium* in cattle faeces from abattoir in Keffi, Nasarawa State, Nigeria was 46(11.5%).
ii. *Salmonella typhimurium* isolates were more susceptible to Gentamicin (87.1%) Chloramphenicol (78.3%), Streptomycin (69.6%) and Ciprofloxacin (67.4%).
iii. The most common antibiotic resistance phenotype of the isolates was TE-SXT-CAZ-NA-CIP with percentage occurrence of 10.9%.
iv. All the isolates were MAR isolates and the commonest MAR index was 0.5 (43.5%)
v. All the isolates were multi drug resistant (MDR).
vi. (V) The detection of *aac(6')-Ib-cr* (66.7%) gene was higher than other Plasmid-mediated quinolones resistance genes in ciprofloxacin resistance isolates.

The occurrence of *S. typhimurium* was high and antibiotics namely gentamicin, chloramphenicol, streptomycin, ciprofloxacin and amoxycillin/clavulanic acid were very effective against the isolates. All the isolates were MDR isolates and harbors plasmid-mediated quinolones resistance genes and the most common was aac-(6')-1b-cr gene

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