Original Research Article

Detection of Plasmid-borne*qnr* Genes in Quinolone-Resistant *Salmonella typhi* from Patients Attending National Hospital, Abuja, Nigeria.

ABSTRACT

Aims: The aim of this study was to investigate and report the presence of the *qnr* genes among *S. typhi* isolated from stool of patients with suspected typhoid fever, atNational Hospital, Abuja, Nigeria

Study design: Cross sectional study

Place and Duration of Study: Department of Microbiology, Nasarawa State University, Keffi, between October 2020 and April 2022.

Methodology: Stool samples of patients with suspected typhoid fever were collected by standard methods in sterile disposable containers. After analysis of stool, microscopic observations and culture analysis, *Salmonella typhi* was isolated, antibiotic susceptibility testing was carried out on the isolate, and the bacterial genome was extracted by boiling method. PCR for detection of qnr genes including *qnrA, qnrB* and *qnrS* was done by specific primers, then PCR products were run using gel electrophoresis and visualized by gel documentation system.
Results:6.6% of samples isolated (10/150) were positive for *S. typhi*. Antibiotic resistance among the isolates in decreasing order were as follows: imipenems (100.0%), cefuroxime (100.0%), cefotaxime (100.0%), nalidixic acid (90.0%), amoxicillin/ clavulanic acid (90.0%), levofloxacin (80.0%),ceftriaxone/sulbactam (70.0%), ciprofloxacin (70.0%), gentamicin (70.0%), and ofloxacin (60.0%).The most common antibiotic resistant phenotype was AUG- CTX-IMP-OFX-CN-NA-CXM-CRO-CIP-LBC at 20.0%. Multiple antibiotic resistance (MAR) was observed in 100% (10/10) of the isolates with the common MAR indices being 0.8 and 0,9 (30.0%). The only positive *PMQR* genes were *qnrA* and *aac(6')-Ib-cr* .
Conclusion: The *S. typhi*isolates showed high resistance to all the isolates, and all isolates were MAR, with resistance to 9 and 8 antibiotics being the most predominant. In addition, *qnr*A resistance gene was the most common gene expressed.

*Keywords: Salmonella typhi;Stool; antibiotics; Resistance; qnr*A.

1. INTRODUCTION

Salmonella enterica serovar Typhi (S. Typhi), the causative agent of typhoid fever, which is a common human-specific pathogen, has been widely acknowledged as the primary cause of mortality resulting from enteric pathogen infections (1,2). The Organism is a Gram-negative, rod shape, flagellated and aerobic bacteria; it is posing a great danger to human health, most notably in low- and middle-income countries (3).Every year, there are 21 million reported cases of typhoid fever, resulting in 200,000 fatalities globally (3). For a long time, the medication for Salmonellosis infections were Penicillin, Chloramphenicol and trimethoprim. An increase in resistance to such antibiotics and many more drugs led to the emergence of newer antibiotics, known as the Fluoroquinolone groups (4). The un-responsiveness of Fluoroquinolones are due two mechanisms: chromosomally mediated mutations in topoisomerase's quinolone resistance determining regions (QRDR) and quinolone resistance determining region mutations; resistance genes belong to qnr groups plasmids mediated play a role in Fluoroquinolone resistant (5). Fluoroquinolone resistance is conferred by both chromosomal and plasmid- encoded fluoroquinolone resistance (6).
Determinants belonging to *qnr* have been found in a numeral of enterobacterial species from different parts of the world, including Asia, America, Europe and Africa (7, 8). So far, six variants (*qnrA*1 to *qnrA*6) have been discovered. Quinolones produced by other plasmids qnrB (*qnrB*1 to *qnrB*5) and *qnrS* (*qnrS*1 and *qnrS*2) resistance determinants outlined in enterobacterial species (9).
The current study aimed to investigate the presence of *qnrA*, *qnrB* and *qnrS* genes in quinolone-resistant Salmonella typhi (*S. typhi*) isolated from stool samples of patients with suspected typhoid fever in National Hospital, Abuja, Nigeria.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Media

Bacteriological media that were used in this study include: Salmonella-Shigella (SSA) Agar; Nutrient agar (NA); Mueller-Hinton agar (MHA); Mueller-Hinton broth (MHB); Bismuth sulfite agar (BSA); Selenite F- Broth (SFB); Xylose Lysine Deoxychocolate agar (XLD); Simmons Citrate agar (SCA); Triple Sugar Iron agar (TSI); and Peptone water (PW).

2.1.2 Equipment

The equipment used in this study include: Autoclave, Oven, Incubator, Refrigerator/Freezer, Thermocycler, Gel electrophoresis machine, Laminar air flow cabinet, Microscope, Spectrophotometer, UV illuminator, Centrifuge, Touch plate Super Mixer, Microwave oven, Electronic weighing balance, Vortex machine, and Gel Doc system.

2.1.3 Chemicals and reagents

The chemicals and reagents that were used in this study include: Acridine orange, Carbol fuschin, Crystal violet, Ethanol, Xylene solution, Creatinine, Pottasium hydroxide, Kovac's reagents, Ethydium bromide, Iodine solution, EDTA and Glycerol

2.1.4 Bacteria Isolates

Confirmed *S. typhi*isolates from the stool of patients were obtained and used for this study.

2.1.5 Study Location

The study was carried out at the National Hospital, Central Business District, Abuja (NHA). NHA is a 400 - bed hospital (Secondary health facility) that renders specialist services in all areas of medicine, located inGarki, within the Federal Capital Territory of Nigeria.

2.2 Methods

2.2.1 Sample Collection

A total of 150 stool samples of patients with suspected cases of typhoid fever were collectedusing sterile container and transported using icepack to the Microbiology Laboratory, NasarawaState University, Keffi for analysis.

2.2.2Isolation and Identification of *Salmonella typhi*

Salmonella typhi was isolated and identified using Gram staining, indole test, methyl red test, Voges-Proskauer test, citrate test and oxidase test as described by [10] and further identified using KB003HI25 TM identification kits following manufacturer's instruction.

2.2.3 Antibiotic Susceptibility Testing

The antibiotic susceptibility test of theisolates was carried outusing the Kirby-Bauer disc diffusion method as modified by the Clinical and LaboratoryStandards Institute - CLSI [10]. Briefly, (3) pure colonies of the isolate from stool samples of patients in the selected hospital was inoculated into 5 ml sterile 0.85% (w/v) NaCl (normal saline) and the turbidity of the bacteria suspension was adjusted to the turbidity equivalent to 0.5 McFarland standard. The McFarland's standard was prepared as follows; 0.5 ml of 1.172% (w/v) $BaCl_2.2H_2O$ was added into 99.5 ml of 1% (w/v) H_2SO_4[11]. A sterile swab stick was soaked in the standardized bacteria suspension and streaked on the Mueller-Hinton agar plates and the antibiotics disc were aseptically placed at the centre of the plates and allowed to incubate at room temperature for 1 hour before re-incubating at 37ºC for 17 hours. The discs used include:Gentamicin (CN: 10 µg), Amoxicillin/Clavulanate (AMC: 30µg), Cefotaxime (CTX: 25 µg), Imipenem/Cilastatin (IMP: 10 µg), Ofloxacin (OFX: 5 µg), Nalidixic acid (NA: 30 µg), Cefuroxime (CXM: 30 µg), Ceftriaxone/Sulbactam (CRO: 30 µg), Ciprofloxacin (CIP: 5 µg), and Levofloxacin (LBC: 5 µg).After incubation, the diameters of the zones of inhibition were measured to the nearest millimeter (mm) using a ruler and the result of the susceptibility test was interpreted using susceptibility breakpoint earlier described by CLSI [10].

2.2.4Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index of the isolates was determined as described previously [12] using the formula:

$$\text{MAR Index} = \frac{\text{No antibiotics isolate is resistant to}}{\text{No. of antibiotics tested.}}$$

2.3 Molecular detection of Quinolone resistance genes

2.3.1 DNA extraction

The bacterial DNA was extracted by a method described by Abimiku *et al* [13] with minor modification. Ten (10) milliliters of an overnight broth culture of the bacterial isolate in 1 ml Luria-Bertani (LB) were spun at 14000 rpm for 3 minutes. The supernatant was discarded, and the harvested cell pellet was re-suspended in 1 ml sterile distilled water and transferred into 1.5 ml centrifuge tube and centrifuged at 14000 rpm for 10 minutes. The supernatant was discarded carefully. The pellet was re-suspended in 100 µl of sterile distilled water by vortexing. The tube was centrifuged again at 14000 g for 10 minutes, and the supernatant was discarded carefully. The cells were re-suspended in 500 µl of normal saline and heated at 95°C for 20 minutes. The heated bacterial suspension was cooled on ice for 10minutes and spun for 3 minutes at 14000 rpm. The supernatant containing the DNA was transferred to a 1.5-ml microcentrifuge tube and stored at -20^{o}C for other reactions.

2.3.2Amplification of Target Genes

The DNA amplification of target plasmid–mediated Quinolone resistant genes in ciprofloxacin resistant *S. typhi* isolates was carried out using single plex method by modification of the method earlier described by [14]. 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 thermal cycler was closed and the door was closed. Then *qnrA, qnrB*, and *qnrS* 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 [14].

The amplification condition for detection of *aac (6) -1b-cr* was carried out as follows; initial denaturation at 95ºC for 20 min, annealing at 59ºC for 40 sec and initial extension at 70ºC for 30 sec and with final extension at 72ºC for 5 min [14]. The primers used are listed in Table 1.

2.3.3 Agarose gel electrophoresis

The PCR products (10 µl) were evaluated on a 1.5% (w/v) Agarose gel (Gibco Life Technologies, Paisley, United Kingdom) at 100 mV for 60 min using BIO-RAD Power Pac 3000; and a molecular weight marker (1-kb DNA Ladder) was used as a standard. The DNA bands were then visualized and photographed under UV light using UVitec and Video copy processor after staining the gel with ethidium bromide as described by [15].

Table 1. Primers and their sequences

Target gene	Primer Sequence	Annealing Temperature (°C)	Amplicon size (bp)	Reference
qnrA	5'-CCGCTTTTATCAGTGTGACT-5' 3'-ACTCTATGCCAAAGCAGTTG-3'	55	188	[16]
qnrB	5'-GATCGTGAAAGCCAGAAAG G -5'3'-ACGATGCCTGGTAGTTGTCC-3'	54	469	[16]
qnrC	5'-GGGTTGTACATTTATTGAATC G -5'3'-CACCTACCCATTTATTTTCA -3'	54	308	[16]
qnrD	5'-CGAGATCAATTTACGGGGAAT A-5' 3'-AACAAGCTGAAGCGCCTG -3'	57	582	[16]
QnrS	5'-ACGACATTCGTCAACTGCA A- 5' 3'-TAAATTGGCACCCTGTAGG C- 3'	55	417	[16]
aac(6')-Ib	5'-TTGCGATGCTCTATGAGTGGCT A-5' 3'-	57	482	[16]

	CTCGAATGCCTGGCGTGTTT-3'			
Class1 Integron	5'-TCCACGCATCGTCAGGC -5' 3'-CCTCCCGCACGATGATC-3'	55	280	[16]

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of *Salmonella typhi*

Theorganism which grew with colorless colonies onSalmonella-Shigella (SSA) Agar, black metallicsheen on Bismuth Sulphite Agar, Gram negative, rod shape, nitrate-positive,Hydrogen sulphide-positive, andMethyl red-positive was identified as *S. typhi*.

3.2 Occurrence of *Salmonella typhi*

The occurrence of*S. typhi* was 6.7 % (10/150). In relation to age ofpatients, the occurrence of *S. typhi* was highest at age >50yrs (17.6%), but lowest in age ≤ 10 (0.0%) as shown in Table 2.

Table 2. **Age Distribution of the Study Population**

Age	No of Samples	Number (%) *S. typhi*
≤ 10	15	0(0.0)
11- 20	18	1(5.6)
21- 30	35	3(8.6)
31- 40	35	2(5.7)
41- 50	30	1(3.3)
> 50	17	3(17.6)
Total	150	10(6.7)

3.3 Antimicrobial Resistance Profile

The Antibiotic Resistance of S.*typhi* isolates from of patients with suspected typhoid fever in University of National Hospital, Abuja, is as given in Table 3.The isolates from NHA were totally resistant to Cefotaxime (100.0%), imipenem (100.0%), and cefuroxime (100.0%), with all other antibiotics having a resistance percentage not less than 60%.

Table 3. Antimicrobial resistance profile of S.*typhi*from stool of patients with suspected typhoid fever in National Hospital, Abuja, Nigeria

Antibiotics	Disc Content (µg)	No. (%) resistance in S.*typhi*(n=10)
Amoxicillin/Clavulanate (AMC)	30	9(90.0)
Cefotaxime (CTX)	25	10(100.0)
Imipenem/Cilastatin (IMP)	10	10(100.0
Ofloxacin (OFX)	5	6(60.0)

Gentamicin (CN)	10	7(70.0)
Nalidixic acid (NA)	30	9(90.0)
Cefuroxime (CXM)	30	10(100.0)
Ceftriaxone/Sulbactam (CRO)	30	7(70.0)
Ciprofloxacin (CIP)	5	7(70.0)
Levofloxacin (LBC)	5	8(80.0)

3.4 Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance (MAR), which is the resistance of microorganisms to at least two (2) antibiotics was observed in all the isolates. The commonest indices were 0.9 (30.0%), 0.8 (30.0%), and 1.0 (20.0%) as shown in Table 4.

3.5 Molecular Detection of Plasmid Mediated Quinolone resistant genes

The plasmid mediated quinolone resistant genes detected in quinolone resistant isolates is as shown in table 5. The only positive *PMQR* genes were *qnrA* and *aac(6')-Ib-cr.*

Table 4. . Multiple Antibiotics Resistance (MAR) Index of *Salmonella typhi* isolates from stool of patientsfrom National Hospital, Abuja, Nigeria

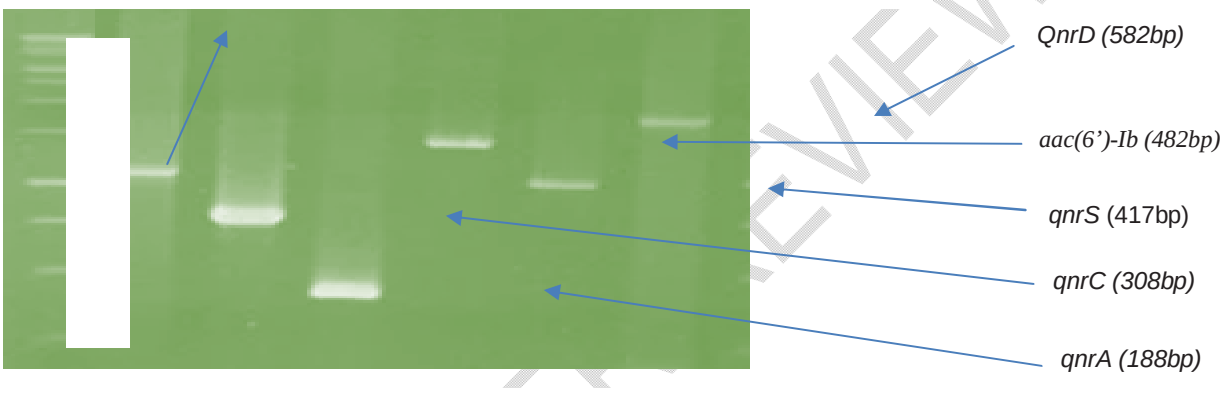
No. of Antibiotic Resistance (a)	No of Antibiotics Tested (b)	MAR Index (a/b)	No (%) of MAR Isolates (n =17)
10	10	1.0	2(20.0)
9	10	0.9	3(30.0)
8	10	0.8	3(30.0)
7	10	0.7	1(10.0)
6	10	0.6	0(0.0)
5	10	0.5	1(10.0)
4	10	0.4	0(0.0)

Table 5. Molecular Detection of plasmid mediated Quinolone Resistance genes in Quinolone resistant *Salmonella typhi*National Hospital, Abuja, Nigeria.

Quinolone resistance Genes	No. (%) of *S. typhi*) (n = 1)
*Qnr*A	1(100.0)
*Qnr*C	0 (0.0)
QnrD	0 (0.0)
*Qnr*S	0 (0.0)

aac(6')-Ib-cr	0 (0.0)
qnrA + qnrC	1 (100.0)
qnrC + qnrD	0 (0.0)
qnrD + qnrS	0 (0.0))
qnrS + aac(6')-Ib-cr	0 (0.0)
qnrA + qnrC + qnrD +qnrS + aac(6')-Ib-cr	1 (100.0)

**MAR isolates are those with resistance to at least two antibiotics [15]*



3.6 Discussion

Salmonella typhi, which is known for being resistant to several antibiotics is the causative agent for Typhoid fever. It is a major health worry as it causes of death and infections in many developing nations [17], as well as illnesses in Africa, particularly Nigeria. In our study, 10(6.7%) identified as *S. typhi.* This percentage is lower than 13% reported in a study by Jubair *et al* in Iraq [18], 47% reported by Jabeen *et al* in Pakistan [19], and 80% in Middle Eastern countries reported in a study by Rahman *et al* [20].

The occurrence of *S. typhi* from stool of patients in relationship to their age was higher at age > 50 years (17.6%), closely followed by age 21-30 years (8.6%). These figures differ from reports in a studies by Jubair *et al* in Iraq [18], Aljanaby *et al* in Iraq [21] and Safdar *et al* in Pakistan [22], where age 21-30 years had a higher occurrence 15.8%, 16.2% and 16.7% respectively. The higher rates of occurrence among adults in these studies might be due to exposure and lack of good hygiene especially with food.

The high rate of resistance of the isolates to antibiotics such as cefuroxime, cefotaxime, imipenem, amoxicillin/clavulanate, and nalidixic acid (all above 90%) as observed in this study is probably due to the abuse and misuse of antibiotics. The isolates were highly resistant to

cefuroxime, cefotaxime and imipenem (all 100%). This was in contrast with findings from a study in Iraq by Njum *et al* [23], where isolates were sensitive (100%) for ceftriaxone, cefepime, cefazolin and chloramphenicol. Furthermore, similar resistance to antibiotics including Ofloxacin(60.0%), gentamicin(70.0%), and ciprofloxacin(70.0%) was observed in a study by Njum *et al* and Namratha *et al.,*2015 [24]. In Iraq and India respectively. including The resistance of these isolates to antibiotics means they could be presented as the best option for treatment of infections caused by *S. typhi* in this region.

The Multiple antibiotic resistance (MAR), which is the resistance of microorganisms to at least two (2) antibiotics was observed in all 15 isolates (100.0%). The three most common PMQR gene. (qnrA, qnrB, and qnrs) are known to lead and cause the spread of quinolone-resistant *S.typhi*, and they were chosen for use in this study. However, the only positive *PMQR* genes were *qnrA* and *aac(6')-Ib-cr* with percentage occurrence of 100.0% respectively**.** In South Korea, the *qnrB* gene was amplified in 70.0% of *S.typhi* strains isolated [25]. In Iran, *qnrA* was found in 30.4% of the *S.typhi* isolates, *qnrS* was detected in over 50.0%, while qnrB was detected in 1.1% [24]. A study on *qnr* genes in *S.typhi* isolates from India found that *qnrB* was the most common *qnr* gene [26].

4. CONCLUSION

This study demonstrated that cephalosporins (ceftriaxone, cefuroxime and cefotaxime) and carbapenems (imipenem), are fully effective against *S.typhi* clinical isolates from typhoid fever patients. The findings of this study report the presence of plasmid-mediated quinolones-resistant genes in *S.typhi* clinical isolates from patients with suspected typhoid fever in Abuja, Nigeria. The *qnrA* gene was the visible gene. Resistance genes may contribute to the quinolone resistance of *S. typhi*. With the appearance of plasmid-mediated quinolones-resistant genes in*S.typhi*, it's important to limit the use of quinolones and have good infection control to keep resistant strains from spreading.

CONSENT

All authors declare that 'written informed consent was obtained from the patient approved parties for publication of this study. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

Appropriate ethical committee approval was obtained prior to start of the research and is available for review (Attached to this publication draft)

REFERENCES

1. Marchello CS, Birkhold M, Crump JA. 2020. Complications and mortality of typhoid fever: a global systematic review and meta-analysis. *J Infect* 81:902–910.
2. Hancuh M, Walldorf J, Minta AA, Tevi-Benissan C, Christian KA, Nedelec Y, Heitzinger K, Mikoleit M, Tiffany A, Bentsi-Enchill AD, Breakwell L. 2023. Typhoid fever surveillance, incidence estimates, and progress toward typhoid conjugate vaccine introduction - worldwide, 2018-2022. *MMWR Morb Mortal Wkly Rep* 72:171–176.
3. Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive salmonella infections. *Clin Microbiol Rev*. 2015 Oct;28(4):901-37.

4. Adel WA, Ahmed AM, Hegazy Y, Torky HA, Shimamoto T. High prevalence of ESBL and plasmid-mediated quinolone resistance genes in *Salmonella enterica* Isolated from Retail Meats and Slaughterhouses in Egypt. *Antibiotics*. 2021 Jul;10(7):881.
5. Zhao X, Yang J, Zhang B, Sun S, Chang W. Characterization of integrons and resistance genes in *Salmonella* isolates from farm animals in Shandong province, China. *Frontiers in microbiology*. 2017 Jul 12; 8:1300.
6. Monte DF, Lincopan N, Fedorka-Cray PJ, Landgraf M. Current insights on high priority antibiotic-resistant *Salmonella enterica* in food and foodstuffs: A review. *Current opinion in food science.* 2019 Apr 1; 26:35-46.
7. Calayag AM, Paclibare PA, Santos PD, Bautista CA, Rivera WL. Molecular characterization and antimicrobial resistance of *Salmonella enterica* from swine slaughtered in two different types of Philippine abattoir. *Food microbiology*. 2017 Aug 1; 65:51-6.
8. Okorie, C. N., Emencheta, S. C., Ezeugwu, D. N., Iganga, J. C., Nkereuwem, R. N., Ozioko, C. C., Onuigbo, E.B. & Attama, A. A. (2024). Molecular Characterization and Resistance Profiling of Multidrug-Resistance *Salmonella* Species Isolated from Southeast Nigerian River. *Tropical Journal of Natural Product Research*, *8*(4).
9. Concha C, Miranda CD, Rojas R, Godoy FA, Romero J. Characterization of a novel variant of the quinolone-resistance gene qnrB (qnrB89) carried by a multi-drug resistant *Citrobacter gillenii* strain isolated from farmed salmon in Chile. *Antibiotics*. 2021 Mar 10(3):236.
10. Cheesbrough M. District laboratory practice in tropical countries: Cambridge university press; 2006.
11. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI Informational Supplement M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
12. Krumperman PH. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Applied and environmental microbiology*. 1983 Jul 46(1):165-70.
13. Abimiku RH, Ngwai YB, Nkene IH, Bassey BE, Tsaku PA, Ibrahim T, Tama SC, Ishaleku D, Pennap GR. Molecular Diversity and extended spectrum beta-lactamase resistance of diarrheagenic *Escherichia coli* from patients attending selected health care facilities in Nasarawa state, Nigeria. *International Journal of Pathogen Research*. 2019 Sep 2;3(1):1-8.
14. Jang S, Kelley KW, Johnson RW. Luteolin reduces IL-6 production in microglia by inhibiting JNK phosphorylation and activation of AP-1. Proceedings of the National Academy of Sciences. 2008 May 27;105(21):7534-9.
15. Aljanaby AA, Medhat AR. Research article prevalence of some antimicrobials resistance associated-genes in *Salmonella typhi* isolated from patients infected with typhoid fever. *J. Biol. Sci*. 2017;17(4):171-84.
16. Herrera-Sánchez MP, Castro-Vargas RE, Fandiño-de-Rubio LC, Rodríguez-Hernández R, Rondón-Barragán IS. Molecular identification of fluoroquinolone resistance in *Salmonella* spp. isolated from broiler farms and human samples obtained from two regions in Colombia. *Veterinary World*. 2021 Jul;14(7):1767
17. Jubair HH, Al-Buhamrah NA, Al-Fatlawi SG. Molecular characterization of quinolones resistant *Salmonella typhi* isolates from patients infected with Typhoid fever in Al-Najaf province, Iraq. *Journal of Population Therapeutics and Clinical Pharmacology*. 2023 Mar 13;30(3):11-9.
18. Jubair HH, Al-Buhamrah NA, Al-Fatlawi SG. Molecular characterization of quinolones resistant *Salmonella typhi* isolates from patients infected with Typhoid fever in Al-Najaf province, Iraq. *Journal of Population Therapeutics and Clinical Pharmacology*. 2023 Mar 13;30(3):11-9.
19. Jabeen K, Saleem S, Jahan S, Nizamudin S, Arshad F, Huma ZE,& Haq FU. Molecular characterization of extensively drug resistant *Salmonella enterica Serovar Typhi* clinical isolates from Lahore, Pakistan. 2023. *Infection and Drug Resistance*, 2987-3001.

20. Rahman BA, Wasfy MO, Maksoud MA, Hanna N, Dueger E, House B. Multi-drug resistance and reduced susceptibility to ciprofloxacin among *Salmonella enterica* serovar *typh*i isolates from the Middle East and Central Asia. *New microbes new Infect*. 2014;2(4):88–92.
21. Aljanaby AA, Medhat AR. Research article prevalence of some antimicrobials resistance associated-genes in *Salmonella typhi* isolated from patients infected with typhoid fever. *J. Biol. Sci*. 2017;17(4):171-84.
22. Safdar A, Muhammad R, Maria R, Rafia A, Maimona S, Irfan A, Areej S, & Laiba Q. A Molecular analysis: detection of plasmid-borne qnr genes in quinolone-resistant S*almonella typhi*isolates from patients at a tertiary care hospital in Bahawalpur, Pakistan. *Journal of Population Therapeutics and Clinical Pharmacology*, 2024.*31*(3), 1049–1057. https://doi.org/10.53555/jptcp.v31i3.5078
23. Njum AA, Hassan RN, Alwan JA. Identification of Antibiotic-Resistant Genes in *Salmonella Typhi* Isolated From Typhoid Patient in Samawa City. *Iraqi J Sci*. 2019;60(5):980–4.
24. Namratha KG, Sreeshma P, Subbannayya K, Dinesh PV, & Champa H. Characterization and antibiogram of *Klebsiella spp*. isolated from clinical specimen in a rural teaching hospital. *Sch. J. App. Med. Sci*, 2015: *3*(2E), 878-883.
25. Geetha VK, Yugendran T, Srinivasan R, Harish BN. Plasmid-mediated quinolone resistance in typhoidal *Salmonellae:* a preliminary report from South India. *Indian J Med Microbiol*. 2014;32(1):31–4.
26. Kim H Bin, Park CH, Kim CJ, Kim E-C, Jacoby GA, Hooper DC. Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. *Antimicrob Agents Chemother*. 2009;53(2):639–45.