Middle East Journal of Applied Science & Technology (MEJAST)

(Peer Reviewed & Quarterly International Journal) Volume 2, Issue 2, Pages 36-45, April-June 2019

The Prevalence and Fluoroquinolone Susceptibility Pattern of *Salmonella typhi* among Patients Attending Tertiary Care Hospitals in Kaduna Metropolis, Nigeria

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Article Received: 22 December 2018 Article Accepted: 27 March 2019 Article Published: 30 June 2019

ABSTRACT

Typhoid fever has continued to pose a serious threat to public health in low income countries with poor hygiene standards and sanitary conditions. In Nigeria, enteric fever constitutes a socio-medical problem responsible for many cases of pyrexia of unknown origin. The study was designed at determining the incidence of reduced susceptibility to routinely prescribed fluoroquinolones to patients diagnosed with typhoid fever in Kaduna metropolis. Stool samples were collected from patients attending hospitals and cultured using selective isolation media with prior enrichment using the spread plate method. Presumptive isolates were identified and characterized using conventional biochemical methods and identified by specific antisera. Kirby-Bauer disk diffusion method was employed for the antimicrobial susceptibility testing using fluoroquinolones. 17.48% of the samples were found positive for the growth of Salmonella enterica serovar Typhi, confirming the typhoid fever. The prevalence between males and females differed with 11.17% and 5.34% respectively. Result of the sensitivity test showed resistant to fluoroquinolones. Fluoroquinolone-resistant strain of Salmonella Typhi in the study area highlights the need for a strong collaboration between the physicians and the laboratory to discourage the development of the fluoroquinolones-resistant strain of bacterial pathogens.

Keywords: Fluoroquinolone, Prevalence, Quinolone, Resistance, Samples, Significance, Susceptible.

1. INTRODUCTION

Typhoid fever is a life-threatening systemic illness caused by serovar of human-adapted pathogen, *Salmonella enterica* (Crump *et al.*, 2010). The disease is caused by *Salmonella enterica* subspecies *enterica* serovar Typhi, a pathogen specific only to humans and up-to-date remains an important public health problem (Crump *et al.*, 2015). Morphologically it is non-spore-forming, facultative anaerobic, gram-negative rods and predominantly motile by means of peritrichous flagella with diameters of around 0.7-1.5µm and lengths of 2-5µm with a few exceptions. Colonies are generally lactose non-fermenters. Recent global incidence of enteric fever has been reported to range from 11.9 million to 26.9 million per year with approximate case fatality rate of 1% (Crump *et al.*, 2015; Steele *et al.*, 2016). It is prevalent in developing countries of Africa as a result of poor sanitary conditions related to rapid population growth, increased urbanization, inadequate human waste treatment, limited water supply and overburdened health care systems (Eibach *et al.*, 2016). In Nigeria, the prevalence is between 12%-16% in patients presenting with febrile illness (Akinyemi *et al.*, 2005 and Ogunleye *et al*., 2005). Typhoid fever caused by multi drug resistant *Salmonella enterica* serovar Typhi has become one of the most challenging problems in medical therapeutics and a significant cause of morbidity and mortality over recent years. These strains have also caused out breaks throughout the world, especially in South America, Indian subcontinent, Africa and South-East Asia (Idowu *et al.*, 2017). The fluoroquinolones are potent, broad-spectrum antibiotics that have been used in medical practice for the treatment of severe or resistant infections since the late 1980s. As their name suggests, Quinolones are a group of antimicrobials with a 4-quinolone nucleus (Guan *et al.*, 2013). Fluoroquinolones differ from quinolones by the replacement of the eighth carbon atom of the backbone with a nitrogen atom and the addition of a fluorine atom at the sixth position, giving them more potent antibiotic action and a broader spectrum of activity (Klemm *et al.*, 2018). Their spectrum of efficacy against a wide range of Gram-positive and Gram-negative pathogenic

bacteria has led to widespread use worldwide. Fluoroquinolones target DNA gyrase and topoisomerase IV with varying efficiency in different bacteria and inhibit their control of supercoiling within the cell, resulting in impaired DNA replication (at lower concentrations) and cell death (at lethal concentrations) (Correia *et al.,* 2017). Antibiotic resistance is one of the most pressing global concerns in medicine, with highly resistant pathogens of many species proving difficult to treat. Against the backdrop there are few new drugs in development, so maintaining the utility of the currently available agents is of crucial importance. To make this possible, a thorough knowledge of the mechanisms of resistance and the fate of resistant strains are needed to understand the conditions where resistance is selected and persist. The evolution of antimicrobial resistance in bacteria is driven by the pressure of sustained exposure to antimicrobials. Such strong selective pressure has stark short and long-term consequences, as evidenced by more than half a century of antimicrobial usage and resistance evolution (Andersson *et al.,* 2010). The development of specific antimicrobial resistance patterns within sentinel organisms has spawned the popular term 'super-bug' (Adegoke *et al.,* 2017; Anzaldi *et al.,* 2011). In Nigeria, as elsewhere, rapid escalation of fluoroquinolones use has been paralleled by a similarly rapid increase in the prevalence of resistant bacteria, including enterics (Omigie *et al.,* 2009; Lamikanra *et al.,* 2011; Namboodiri *et al.,* 2011). In part because quinolone resistance evolved later but also perhaps because mechanisms conferring resistance to a wide range of drugs may have predominated before quinolone-specific mechanisms evolved. Fluoroquinolone-resistant strains are typically resistant to multiple antimicrobials (Lamikanra *et al.,* 2011; Namboodiri *et al.,* 2011); other factors may account for association of fluoroquinolone resistance and multiple resistances. Quinolones were widely used for decades elsewhere in the world so that resistance genes and resistant clones had already evolved by the time fluoroquinolones were significantly employed in Africa. Five years after fluoroquinolones were introduced in a community in Western Nigeria, *Escherichia coli* strains showing quinolone-specific resistance mechanisms were isolated (Lamikanra *et al.,* 2011). Although the majority of these isolates carried point-mutations in the quinolone-resistance determining regions of gyrA and parC, six strains bore the plasmid-encoded resistance gene qnrS1. It has been hypothesized that chloroquine contributed to selective pressure for fluoroquinolone-resistant bacteria in malaria endemic settings but available data do not support this hypothesis for Nigeria (Davidson *et al.,* 2008; Lamikanra *et al.,* 2011). Risk factors for the development of resistance in *Salmonella typhi* include overuse, misuse, and inappropriate antibiotic prescribing practices (Klemm *et al.,* 2018). Factors such as patient and time pressures and diagnostic uncertainties are some of the main forces behind irrational prescription of antimicrobial combinations (Chattaway *et al.,* 2016). Easy availability of drugs at the pharmacy without a prescription, use of allopathic drugs by traditional medicine practitioners such as homeopaths, unani and ayurvedic practitioners, and uncontrolled use of antibiotics in agriculture, animal husbandry and fisheries has further aggravated the problem. Due to an absence of active surveillance, data on fluoroquinolones consumption are lacking for many countries, making comparison of consumption and rates of resistance between different parts of the world difficult (Chattaway *et al.,* 2016), more so that due to lack of availability and cost, other higher generation quinolones are not used in resource-limited countries **(**Abd-Elfarag, 2015). These aims and objectives of the study are, to isolate and characterize *Salmonella enterica* serovar Typhi among the patients attending the tertiary health care centers in

the community, to determine the level of antibiotics resistance of the serotype isolated and the minimum inhibitory concentration and to determine the prevalence.

2. MATERIALS AND METHODS

2.1 Study design area. The study was carried out in six selected tertiary care hospitals in Kaduna Metropolis namely: General Hospital Kawo (N10^0 34.978′ E007^0 28.676′), Yusuf Dantsoho Memorial Hospital (N10^{0}31.259′ E007^{0}25.007′), Gwamna Awan General Hospital (N10^0 27.940′ E007^0 24.152′), Federal Neuropsychiatric Hospital (N10^0 27.919′ E007^0 25.895′), General Hospital Sabon-tasha (N10^0 26.933′ E007^0 28.676′) and Barau Dikko Teaching Hospital (N10^0 31.547′ E007^0 26.505′).

2.2 Study population. Two hundred and six (206) stool samples of were collected from patients (both male and female) with clinical symptoms of enteric fever attending six tertiary care hospitals in Kaduna metropolis based on ethical clearance and permission obtained from ministry of health, Kaduna State.

2.3 Samples collection. Sterile wide-necked leak proof screw cap bottles were used to collect the samples from the patients with the assistance of the hospital Laboratory Staff. The samples collected were then transported in thermo flasks packed with ice to Microbiology departmental laboratory, Kaduna State University, for immediate analysis within two hours of collection. When delay was inevitable (more than two hours), stool samples were placed in Stuart's transport medium and refrigerated immediately.

2.4 Data collection. Direct face-to-face questionnaires were filled for every person presenting stool sample and matched control. This questionnaire gathered information about the person's identity, typhoid fever history, typhoid fever predisposing factors, and drug-resistance predisposing factors.

2.5 Samples Analysis

All presumptive colonies obtained from the culture plates were screened by biochemical tests. Presumptive colonies of *Salmonella* species were further tested for somatic (O), virulence antigen (Vi) and flagella (H) antigens with Salmonella specific antiserum (R30957401/ZC18, Oxoid, UK).

2.6 Antibiotic Susceptibility Test of Salmonella typhi Isolates

Antibiotic susceptibility test of *Salmonella typhi* isolates were analyzed using Kirby-Bauer disc diffusion techniques described by Arora (2011). Given turbidity equivalent to the 0.5 McFarland standards (a density of 1x10^8 cells/ml), the isolates were inoculated on Muller-Hinton agar; Using disc dispenser, single disc fluoroquinolones (Oxoid, Basingstoke United Kingdom): PEF: Pefloxacin 5µg, OFX: Ofloxacin 5µg, CIP: Ciprofloxacin 5µg, LEV: Levofloxacin 5µg, NOR: Norfloxacin 10µg and NA: Nalidixic Acid 30µg were dispensed onto the surface of the agar plates. The plates were incubated aerobically at 37^{0}C for 18 hours after 30 minutes of applying the discs.

2.7 Statistical Analysis of Data

The data analysis was processed using SPSS version 23.0 (IBM SPSS, Chicago, USA). The p value were calculated by the bivariate correlation and Student's t test was applied to determine the association between the parameters

nalidixic acid and fluoroquinolone resistance based on disc zone diameter. A p value of ≤ 0.05 was considered as significant (Micheal, 2011; Sehra, 2013).

3. RESULTS AND DISCUSSION

The pathogens isolated were *Citrobacter* species with 28 (13.59%) occurrence, *Edwardsiella* species with 20 (9.71%), *Enterobacter* species with 19 (9.22%), *Escherichia coli* with 15 (7.28%), *Klebsiella* species with 21 (10.19%), *Morganella* species with 7 (3.39%), *Proteus* species with 25 (12.13%), *Salmonella* serotypes with 51 (24.76%), *Shigella* species with 13 (6.31%), while *Yersinia* species had 3 (1.46%) occurrence of isolates and 4 (1.9%) isolates were unidentified after the biochemical test as shown figure 1.0. Serological classification of these isolates by the rapid slide agglutination test with Salmonella 'O/Vi' specific antisera revealed thirty-six (70.59%) *Salmonella enterica* serovar Typhi and eleven (21.57%) *Salmonella enterica* serovar Paratyphi while four (7.84%) of the isolates were untypeable with the specific antisera as shown in Table 1.0.

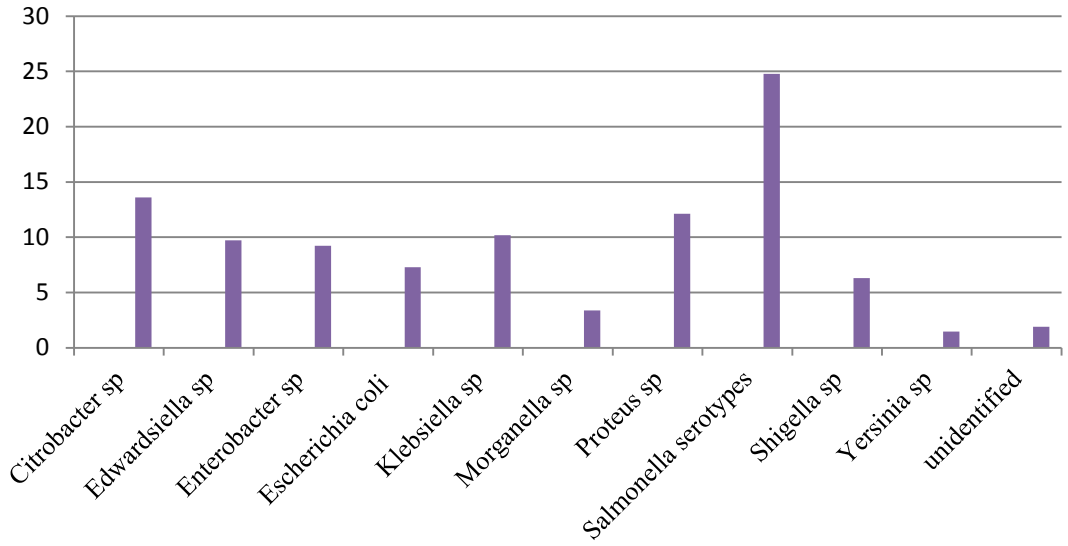

Fig 1.0: Results of Cultural, Morphological, and Biochemical Characteristics of Isolates from Stool Samples

Seventeen point four - eight percent (17.48%) of the isolates were confirmed and identified as *Salmonella enterica* serovar Typhi based on cultural, morphological, biochemical tests and Serotyping which was supported by Abdullahi *et al.* (2014) and Leopold *et al.* (2014). In the study, the prevalence rate of *Salmonella* was 17.5% Therefore, the result of this study was more in agreement with the findings of Akinyemi *et al.* (2005) and Ogunleye *et al*. (2005) who also conducted research investigation on *Salmonella* from humans in Nigeria. However, this was not in agreement with the observations of Adeleke *et al.* (2006) who reported prevalence rate of 3.5% of *Salmonella enterica* serovar Typhi in Zaria, Nigeria in a study on prevalence of typhoid fever and antibiotic susceptibility pattern of *Salmonella enterica* serovar Typhi. Of the 206 stool samples collected, 184 and 151 stool samples originated from males and females respectively. Thirty-six samples yielded *Salmonella enterica* serovar Typhi isolates, which gave an overall prevalence among humans submitting stool sample at 65%. It was observed that 12 (5.83%) was the recorded highest *Salmonella enterica* serovar Typhi isolates in hospital B while 2 (0.97%) was the lowest number of *Salmonella enterica* serovar Typhi isolates in hospital F. The number of *Salmonella enterica*

serovar Typhi isolates observed in hospitals A, D, and C and E were 11 (5.34%), 5 (2.43%), and 3 (1.46%) respectively. Out of the thirty-six samples that were positive for *Salmonella enterica* serovar Typhi, the highest frequency of occurrence was found in twelve (21%) hospital B while two (4%) hospital F had the least frequency of occurrence as summarized in Fig 2.0. Male patients (26, 23%) constitute the major subgroup affected with typhoid fever, and *Salmonella typhi*. These findings were similar to those observed in a study conducted in northern Nigeria by Ameh *et al.* (2004) where more males were implicated but the sex-related difference in infection rates did not vary significantly ($P < 0.05$). The skewed distribution ratio of 2:1 between males and females is difficult to explain, but may be caused by the fact that more males were examined or that the male population has a higher susceptibility to infections.

Table 1.0: *Salmonella* serotypes Isolated from Stool Samples

N	***Salmonella* serotypes number (%)**		
	Salmonella typhi	*Salmonella Paratyphi*	ND
51	36(70.59)	11(21.57)	4(7.84)

Key: n = Number of Isolates; ND = untypeable

The slight differences among the prevalence percentages might be due to the species differentiation, hygienic, environmental and geographic variation and technical limitations of the laboratory of the study.

Antibiogram of these isolates showed that, highest resistance was observed against ofloxacin and norfloxacin in hospitals F and D (80%). The percentage resistance was significantly high against norfloxacin in hospital A (72.73%). Two resistance patterns were observed among *Salmonella typhi*; most (n =2; 100 %,) were resistant to ofloxacin and norfloxacin, while 66.67% (n = 12; n = 3) showed resistance to the fluoroquinolones and 72.73% (n =11) exhibited resistance to norfloxacin. Nalidixic acid susceptibility was considered as a surrogate marker to predict decreased susceptibility to ciprofloxacin (Bhagra *et al.*, 2017; Makanjuola *et al.*, 2012). Generally, the *Salmonella typhi* isolates displayed more resistance (52.31%) and less susceptible (47.69%) to the fluoroquinolones while the isolates showed moderate to high resistance to nalidixic acid (Figure 3.0). **Levofloxacin**: The disc diffusion testing of four *Salmonella enterica* serovar Typhi isolates showing 80% resistance yielded zone of inhibition diameters of 0-20mm in hospital D ($p = 0.312$ not statistically significant, $r = 0.500$ marginal relationship). **Pefloxacin**: The zone diameters of twelve *Salmonella enterica* serovar Typhi isolates showing resistance ranged from 0-22mm and two *Salmonella enterica* serovar Typhi isolates revealed resistance of 20mm ($p = 0.234$ not statistically significant, $r = 0.573$ marginal relationship). **Ofloxacin**: *Salmonella typhi* isolates showing 100% resistance yielded zone diameter of 17.5mm-22.5mm in hospital F ($p = 0.020$ statistically significant, $r = 0.882$ strong relationship). **Norfloxacin**: *Salmonella typhi* isolates exhibiting 100% resistance showed zone diameter of 0-11.5mm in hospital F ($p = 0.006$ not statistically significant, $r = 0.936$ strong relationship). **Ciprofloxacin**: *Salmonella typhi* isolates showing 80% resistance showed zone of inhibition diameter of 0-25.5mm in hospital D ($p = 0.099$ marginally significant, r = strong relationship). Table 2.0 showed

the characteristics of the two hundred and six people with typhoid fever in the study area while Table 3.0 revealed the predisposing factors associated with exposure to *Salmonella enterica* serovar Typhi within Kaduna metropolis.

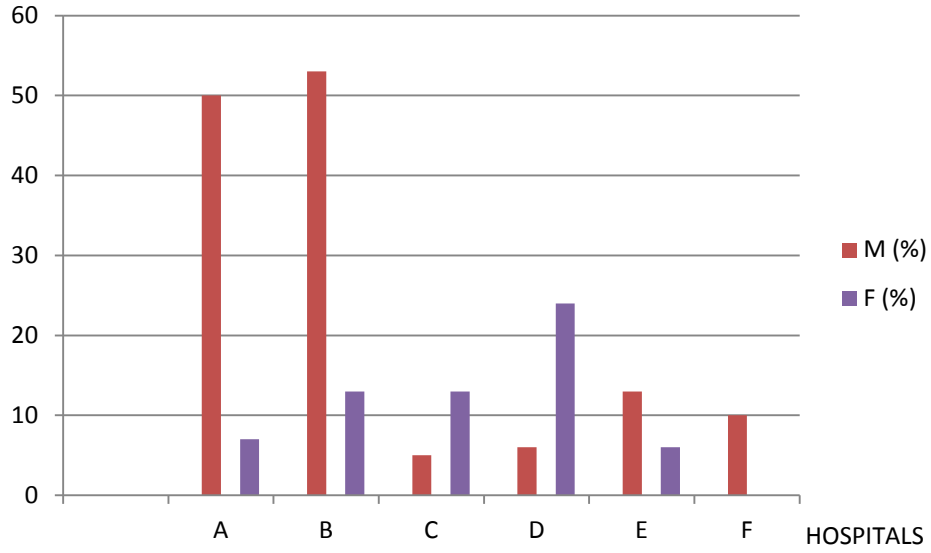

Fig 2.0: Prevalence of *Salmonella enterica* serovar Typhi in Patients

Key: M = Male; F = Female

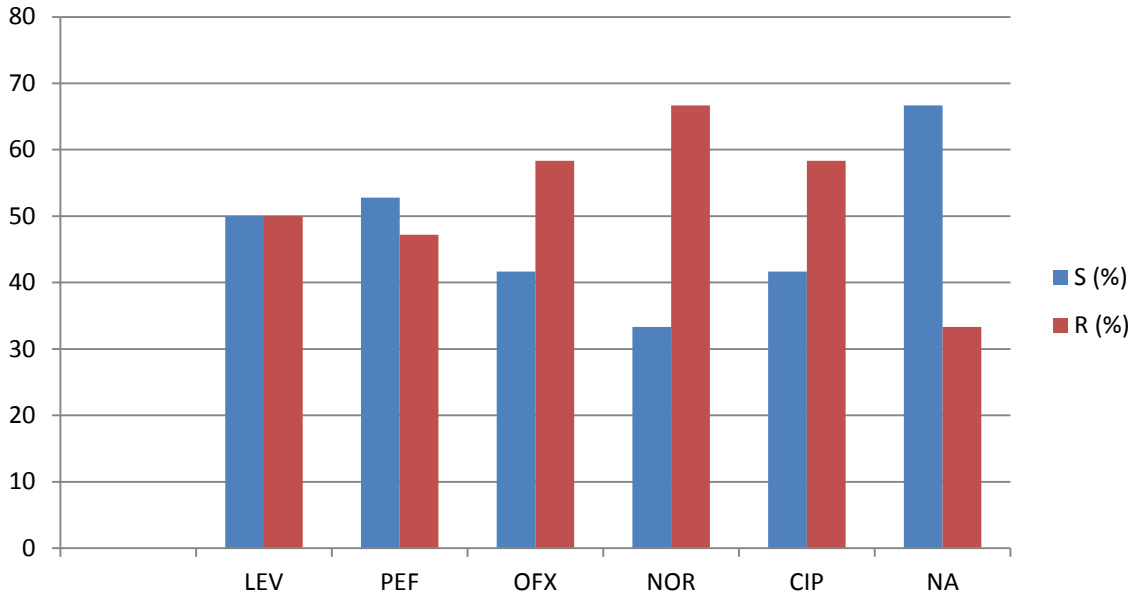

Fig 3.0: Percentage Occurrence and Distribution of Antibiogram of *Salmonella typhi* in the Study Area

Key: S = Susceptible; R = Resistance; LEV= levofloxacin; PEF= pefloxacin; OFX= ofloxacin; NOR= norfloxacin; CIP= ciprofloxacin; NA= nalidixic

Only sixty-six of the study cases were having domestic animals. On sanitary issues, all the respondents with typhoid fever except one, used pit latrines or flush toilets. One volunteer used the bush for defecation. One hundred and forty-one persons reported to wash their hands with soap and water after toilet, and two hundred and three also did the same before eating, but most cases eat with spoons. The sources of drinking water were pipe borne water,

tube-wells, and water hawkers, and none used surface water for drinking. Thirteen persons reported staying near to the dumping sites and thirty-eight respondents agreed being in close contact with family members who recently suffered from typhoid fever. Respondents in most cases are living in densely populated houses with poor sanitary conditions. This coupled with poor hygienic practices constitute pre-disposing factors. The scarcity of these basic amenities weighs heavily on public health, and the development of fluoroquinolone – resistant typhoid fever in the study area. The occurrence of fluoroquinolone – resistant *Salmonella enterica* serovar Typhi incidence increased the risk of typhoid fever. From Table 2.0, the frequency of typhoid fever was higher in 21-40 years age groups with one hundred and eleven (54%) followed by ≤ 20 (66), 41-60 (25), and ≥ 60(3) with (32%), (12%) and (2%), respectively. However, there is no infection among children with age group 0-4 months. The distribution of typhoid fever cases by age group was based on the risk factors that presented age specific typhoid fever incidence. This study revealed the average age of typhoid infection is lower in the study area with high incidence. In the two sexes, males were more infected than their female counter parts in the study cases with one hundred and eleven (54.4%) and ninety-four (45.6%) respectively. Similarly, the matched-control showed males were more infected fifty-six (46.7%), than their females, forty-seven (39.2%), and counterparts. Also, there is a significant difference between the rate of infection among the two sexes at $P < 0.05$ and 5 degree of freedom, because, the calculated chi-square (x2) value was less than the tabulated value (11.070) in the hospitals studied.

Table: 2.0: Percentage Characteristics of Patients with Typhoid Fever and Matched Controls

Characteristics	**Typhoid fever cases**	**Matched control**
Sampling sites	6	6
Sampling size	206	120
Sex:		
Female	94(45.6%)	47(39.2%)
Male	112(54.4%)	56(46.7%)
Age Category (years):		
≤20	66(32%)	37(38%)
21-40	112(54%)	50(64%)
41-60	25(12%)	13(16%)
≥60	3(2%)	2(2%)
Diagnostic Test	206	116
Medical Consultation	201	120

Table: 3.0: Percentage Predisposing Factors Associated with Exposure to *Salmonella typhi*

Predisposing factors	Typhoid fever Cases	Matched Control
On transit	**135 (65.53%)**	**51 (42.5%)**
Personal hygiene	141 (68.45%)	109 (90.83%)
Use of Cutleries	203 (98.54%)	85 (70.83%)
Proximity to waste dump site	13 (6.31%)	3 (2.5%)
Toilet facility	205 (99.52%)	120 (100%)
Contact with patients	38 (18.45%)	9 (7.5%)
Antibiotic use	201 (97.57%)	0 (0)
Antibiotic regimen	193 (93.69%)	0 (0)
Self medication	25 (12.14%)	13 (10.83%)
Cost of medication	116 (56.31%)	0 (0)
Drinking water source	206 (100%)	120 (100%)
Laboratory investigation	206 (100%)	37 (30.83%)
History of typhoid fever immunisation	59 (28.64%)	21 (17.50%)
Domestic animals	66 (32.04%)	10 (8.33%)

The high susceptibility of males to typhoid infection is attributed to exposure to high – risk factors associated with the development of fluoroquinolone – resistant typhoid fever in the study area. There is no significant difference in the results of the patients in the study case and matched-controls associated with development of resistant typhoid fever. The association of exposure to *Salmonella typhi* and development of resistance to Salmonella enterica serovar Typhi in this study has an odds ratio of 0.75 (95% CI: 0.13-3.15).

4. CONCLUSION

Antibiotics are the mainstay of therapy in typhoid fever to prevent the complications associated with severe illness and death of the patients. However, the reduced susceptibility of *Salmonella typhi* isolates to commonly used antibiotics continues to be a major problem for effective therapy of typhoid fever, particularly in Nigeria. The antibiogram of *Salmonella typhi* isolates showed resistance to all the commonly prescribed fluoroquinolones, this is the leading cause of treatment failure. The resistance could be as a result of rapid escalation of fluoroquinolones use as summarized by respondent to the administered questionnaire. The patients suffering from typhoid fever attending the tertiary health care centers are on referral after treatment failure. Nonetheless, a high infectious

disease burden warranting justifiable antimicrobial use, poor diagnostic test infrastructure and uptake and unregulated use of antibiotics combine to exacerbate the adverse effects that antimicrobial use has on resistance. The emergence of resistant *Salmonella typhi* isolates to fluoroquinolones calls for strict policy of routine screening of antibiotics effectiveness, before prescription to reduce the spread of resistant strains. Interventions are required to prevent the over-prescription of antibiotics and preventing unsanctioned use in humans and livestock. Emphasis must therefore be placed on disease prevention, which can include both short- and long-term measures that must be followed strictly. Short-term measures include vaccination of the high-risk population in endemic areas and rational and judicious antibiotic prescribing practices by clinicians. It is necessary to control the sale of antibiotics without prescriptions. In addition, antibiotics should be used more judiciously in outpatient and inpatient departments in hospitals and by private practitioners.

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