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Prevalent And Multiple Drug Resistance Indexes Of Typhoidal And Non-Typhoidal Salmonella Isolated From Stool Samples Of Hospitalized Subjects In Kano, North-West, Nigeria

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***Abstract*—Aim: The aims of the study were to determine the prevalent and multiple drug resistance indexes of typhoidal and non-typhoidal Salmonella isolates recovered from the stool samples of hospitalized patients in Kano metropolis.**
Study design: The study is a descriptive cross-sectional study.
Place and duration of study: Stool specimen was collected from each patient with some or all clinical features of salmonellosis that sign a consent form and transfer into wide-mouth screw crapped container. If daily is unavoidable stool samples were stored at 4^0C. Samples were analyzed at the laboratory of the author. This work was carried out between May, 2012 and March, 2014.
Methodology: The stool specimens were cultured onto deoxycholate citrate agar (DCA), *Salmonella-Shigella* agar (SSA) and brilliant Green agar (BGA) followed by confirmation of presumptive colonies using different biochemical tests and analytical profile index 20E. Serologic identification of *Salmonella* was performed by slide agglutination test using polyvalent O and H *Salmonella* antisera. Antibiotic susceptibility studies were performed by the disc diffusion method using ampicillin, chloramphenicol, ciprofloxacin, nalidixic acid and Trimethoprim-sulfamethoxazole.
Results: Although, the relationship between different age groups was not significantly associated ($P < 0.05$), patients under age bracket of 21-30 years were found to be more susceptible to *Salmonella* infections with 45 representing 9.0% followed in that order by 11-20 years (33), 31-40 years (27), ≤10 years (18) and >40 years (9) age groups, representing 6.6%, 5.4%, 3.6% and 1.8% respectively. The prevalent rate of *Salmonella* infections was significantly higher ($P > 0.05$) in males than the females patients with 78 (16.6%) and 49 (9.8%) respectively. The serovars of *Salmonella* Typhi was the most predominant with 34(25.8%) followed by *Salmonella* Typhimurium 31(23.5%), *Salmonella* Enteritidis 25(19.9%), *Salmonella* Paratyphi C 17(12.9%), *Salmonella* Paratyphi A 15 (11.4%) and *Salmonella* Paratyphi B was the least prevalent serovar with 12 (9.1%). Of the 132 isolates 118 (89.4%) were resistant to Ampicillin; 102(77.3%) resisted to Nalidixic acid; 53 (40.2%) resisted to Chloramphenicol, 35(26.5%) resisted to Cotrimazole while none (0.0%) resisted to Ciprofloxacine. 50 isolates were found resistant to 1 of 3 first-line anti-*Salmonella* antibiotics (ampicillin, cotrimoxazole and chloramphenicol). Highest MARI was recorded from 45 (39.5%) isolates with MARI of 9.0 with two phenotypic patterns which differed in two different antibiotics (ampicillin and nalidixic acid).
Conclusion: The frequency of *Salmonella* infections was highest among 21-30 year age group lowest in ≥40 year age group. However the rates of infection among all the six (6) age groups were not significantly associated. The prevalent rate of *Salmonella* infections was significantly higher ($P > 0.05$) in males than the females' patients. However, *Salmonella* Typhi was predominant followed by *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Paratyphi C, A and B. Most *Salmonella* serovars isolated from patients in Kano, Nigeria resisted to Ampicillin. They also resisted to Nalidixic acid, Chloramphenicol and Cotrimazole, in decreasing order. Ciprofloxacine remained effective against all the *Salmonella* isolates tested. 50 isolates were found resistant to 1 of 3 first-line anti-*Salmonella* antibiotics (ampicillin, cotrimoxazole and chloramphenicol).

***Keywords*—Salmonella serovars; Stool samples; Multiple antibiotic resistance index; Kano-Nigeria.**

1. INTRODUCTION

Salmonella infection also known as salmonellosis is an infection caused by ingesting *Salmonella* in food that is contaminated by faeces of animals or humans directly or indirectly [1]. On the basis of their pathogenicity and clinical importance, the salmonellae

may be divided into two groups: includes members of the genus that are involved as aetiologic agents of enteric fever (typhoidal salmonellosis) i.e. *Salmonella* Typhi and *Salmonella* Paratyphi Bacilli. These species are found only in the intestinal tract of man for whom they have a highest degree of pathogenicity and whom they frequently cause invasive disease [2]. *Group II:* Includes members of the genus that are involved as aetiologic agent of food poisoning (non typhoidal salmonellosis). The food poisoning groups are essentially parasites of animals from which man is occasionally infected. Their pathogenicity for man is relatively lows, the usual result of infection being the production of gastroenteritis. Nontyphoidal salmonellosis refers to illnesses caused by all serotypes of *Salmonella* except for Typhi, Paratyphi A, Paratyphi B (tartrate negative), and Paratyphi C [3].

Nontyphoidal salmonellae are a leading cause of bacterial diarrhoea worldwide; they are estimated to cause 94 million cases of gastroenteritis and 115,000 deaths globally each year. The risk of *Salmonella* infection among travelers returning to the United States varies by region of the world visited [4]. In one analysis, the incidence of laboratory-confirmed infections from 2004 through 2009 was 7.1 cases per 100,000 among travelers to Latin American and Caribbean, 5.8 cases per 100,000 among travelers to Asia, and 25.8 cases per 100,000 among travelers to Africa [5]. The true number of illnesses is much higher, because most ill people do not have a stool specimen tested. Travelers with salmonellosis were most likely to report visiting the following countries: Mexico (38% of travel-associated salmonellosis), India (9%), Jamaica (7%), the Dominican Republic (4%), China (3%), and the Bahamas (2%). Salmonellae have a wide range of hosts and are strongly associated with agricultural products [6]. The increasing centralization and industrialization of our food supply have enhanced the distribution of these hardy organisms [6].

The incubation period of nontyphoidal salmonellosis is 6–72 hours, and illness usually occurs within 12–36 hours after exposure. Illness is commonly manifested by acute diarrhea, with sudden onset of headache, abdominal pain, fever, and sometimes vomiting. The illness usually lasts 4–7 days, and most people recover without treatment. Salmonellosis outcomes differ by serotype [7]. Approximately 5% of people develop bacteremia or focal infection (such as meningitis or osteomyelitis). Infections with some serotypes, including Dublin and Choleraesuis, are more likely to result in invasive infections. Rates of invasive infections and death are generally higher among infants, older adults, and people with immunosuppressive conditions (including HIV), hemoglobinopathies, and malignant neoplasms. Individual serovars can be further characterized (typed) by a number of methods, including phage typing and antibiotic resistance profiles [8]. The most severe form of *Salmonella* infection is typhoid fever caused by serovars adapted to a human host, such as *Salmonella* Typhi and *Salmonella* Paratyphi. Infective dose can be quite low (10-100 cells) in vulnerable individuals or when contaminated food with a high fat content, like chocolate or cheese, is consumed [9]. Increasing antimicrobial resistance in typhoid and nontyphoid *Salmonella* species has been a serious problem for public health worldwide. The high rate of resistance is hampering the use of conventional antibiotics, and growing resistance to newer antimicrobial agents is aggravating the situation [10].

Salmonellae can be isolated from blood, stool, urine, bone marrow, duodenal aspirates and rose spots [10]. The organisms can usually be detected in 75-90% of patients during the first ten days of infection and in about 30% of patients during the third week in the blood [11, 12]. Blood culture is the only method that is routinely used for determination of antibiotics resistance in *Salmonella* infection from blood samples of patients. The results are obtained after nearly one week; therefore the early detection of disease is not possible [11]. Another major disadvantage is that its detection rate is only about 30%. Therefore, these necessitate the need for Stool culture technique that more effective method that cannot only diagnose typhoid fever and other *Salmonella* infection at an early stage but also provides information to enable the physician to start focused treatment from the onset of disease [13].

2. MATERIALS AND METHODS

2.1 Hospitals

The six most patronized hospitals were randomly selected including one Teaching Hospital (Aminu Kano Teaching Hospital), three specialist hospitals (Murtala Mohammed Specialist, Mohammed Abdullahi Wase Specialist and Sir Sunusi Specialist Hospital), one General Hospital (Sheik Waziru Gidado General Hospital) and one Private Hospital (Khadijat Memorial Private Hospital). All are situated within Kano metropolis. The selected hospitals are reference hospitals in the state where people from various parts of the state and neighboring states of various occupations attend. They gave more than 70% of health care delivery in the state at large.

2.2 Patients and Specimens

Patients (in and out) who patronized the six selected hospitals with some or all clinical symptoms of *Salmonella* infections (i.e. vomiting, diarrhoea, headache, abdominal pain, body ache, breathlessness, weight lost, constipation and anaemia) recruited to sign the consent form were used for the study.

Any patient (in and out) who brought his stool specimen to the laboratory reception of one of the six selected hospitals for stool analysis recruited to sign the consent form was used for the study.

Stool collected from each patient diagnosed positive for salmonellosis was used as sample for the study.

2.3 Collection and Handling of Specimens

Stool specimen was collected in wide-mouth screw crapped container. If daily is unavoidable stool samples were stored at 4 ^{0}C. Container was then labeled with specimen number, type of sample [12].

2.4 Isolation and identification of salmonellae

2.4.1; Presumptive isolation of *Salmonella*

Stool specimens collected were cultured onto SSA, BGA and DCA agar and incubated aerobically at 37^{o}C for 24hours [14]. The cultured plates, SSA, BGA and DCA agar were examined for the presence of typical colonies of *Salmonella* based on cultural and morphological characteristics, that is, transparent colonies with black centre on SSA and pink colonies surrounded by a red medium on BGA, and small red translucent and or dome-shaped colonies, which may have central black spot due to hydrogen sulphide production [12].

Bacterial isolates obtained were further sub-cultured by stabbing into nutrient agar slants and stored at 4^{o}C after aerobic incubation 37^{o}C for 24 hours for subsequent analysis.

2.4.2; Purification of isolates

Presumptive culture of *Salmonella* stored in nutrient agar slant was sub-cultured onto SSA aerobic incubation 37^{o}C for 24 hours to observe for the colonial characteristics of *Salmonella* and isolation of pure culture for subsequent biochemical characterizations.

2.4.3; Biochemical characterization of *Salmonella*

Isolation and identification of organisms were carried out as described by ISO [15], Habtamu *et al*. [16], and OIE [17]. A 24 h pure culture of each isolate was used to determine their gram stain reaction. The following biochemical tests were carried out: Indole test, triple sugar iron test, citrate test, methyl-red test, Voges-Proskauer test, lysine decarboxylase test, ornithine decarboxylase test, urease test, sugar (trehalose, sucrose, inositol, glucose, dulcitol, maltose, mannitol, melibiose, salicin, rhamnose and arabinose) fermentation test and motility test. Isolates were further characterized using commercially available identification system-Analytical Profile Index (API) 20 E test kit (Biomerieux, France) following the manufacturer's guideline.

2.5 Sero-typing of the isolates

Serological identifications of presumptive *Salmonella* were performed by slide agglutination test. Presumptive isolates of *Salmonella* obtained from the series of biochemical tests were screened serologically with somatic O *Salmonella* Paratyphi A, B, C_1, *Salmonella* Typhimurium C_2 and *Salmonella* Typhi D.

An agglutination test was performed on a clean glass slide. The slide was divided into sections with a wax pencil and one small drop of physiological saline was placed in each test section on the slide. By using a sterile inoculating loop a portion of growth from the surface of TSI agar was removed and emulsified in each drop of physiological saline on the slide. It was then mixed thoroughly to create a moderately milky suspension. A bent inoculating loop was used to pick a small drop of antiserum and transferred to one of the suspensions; the second suspension served as the control (usually approximately equal volume of antiserum and growth suspension was mixed). The suspension and antiserum were mixed very well and then the slide was tilted back and forth to observe for auto-agglutination (agglutination is more visible if the slides is observed under a bright light and over a black background) [18].

If clumping appeared within 30 to 60 seconds the reaction is positive, the saline suspension (control) was examined carefully to ensure that it is even and does not show clumping resulting from auto agglutination. If auto-agglutination occurs, the culture is termed "rough" and cannot be serotype. When positive agglutination reaction was obtained in one of the antisera, the *Salmonella* serovars Paratyphi A, B, C_1, Typhimurium C_2 or Typhi D subgroup was confirmed, and no further testing with antisera needed to be conducted [18].

2.6 Test of antibiotic sensitivity of the *Salmonella* isolates.

In-vitro susceptibility of *Salmonella* isolates to various routine antibiotics was tested by the standard disc diffusion technique [2].

2.6.1 Standardization of inoculum

This was done as described by CLSI [19]. Pure culture of identified *Salmonella* isolate (s) from an 18-hour plate culture was selected. Sterile wire loop was used to pick 3 colonies of each *Salmonella* serotype and emulsified in 5 ml of sterile normal saline. The tube containing the bacterial suspension was inserted into a sensititre nephelometer (TREK Diagnostic systems, UK) after calibration. Adjustment was made with extra inoculum or diluents, if necessary, until 0.5 McFarland standards were obtained. Fifty microliter of the broth was further transferred into 5 ml of Mueller-Hinton broth (Oxoid, UK) in a tube [19].

2.6.2 Inoculation of test plates

Optimally, within 10 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton

swab was dipped into the standardized suspension in Mueller-Hinton broth. The dried surface of a 20 ml Mueller-Hinton agar plate in a 100 mm disposable plate (STERILIN, UK) was inoculated by streaking with the cotton swab over the entire sterile agar surface. The inoculated plates were air dried at 37ºC to allow for any excess surface moisture to be absorbed before applying the antibiotic discs [19].

2.6.3 Application of discs to inoculated agar plates

All positive cultures of *Salmonella* serovars isolated from blood samples were tested *in vitro* for susceptibility to different antibiotic by agar diffusion technique as described by Kirby-Bauer [20] and WHO [21]. This was carried out according to WHO protocol [22]. The susceptibility testing of *Salmonella* isolates were carried out using Mueller Hinton agar and were tested *in vitro* for susceptibility to five different antibiotics; ampicillin (25µg), chloramphenicol (30µg), ciprofloxacin (25µg), nalidixic acid (30µg) and Trimethoprim-sulfamethoxazole (30µg) [23].

The inoculated plates were air dried under aseptic condition to eliminate the liquid on the surface of medium, sterile forceps was used to place the antibiotic discs on the inoculated plates. Within 30 minutes after applying the disc, the plate was inverted and incubated at 35^{o}C for 18 hours. Meter ruler was then used on the underside of plate to measure the diameter of each zone of inhibition in millimeter. Zone diameter for ATCC 25922 was compared with NCCLS Published Limits; Interpretative chart was then used to interpret the zone sizes of Inhibition [23].
Results were recorded as susceptible, intermediate susceptible or resistant based on the zones size of each antibacterial disc used [22, 23].

2.7 Statistical analysis of results

Statistical Package for Social Science (SPSS) version 14 was used [24]. Descriptive statistics were used to categorical (frequency percentages) variables. Chi-square test analysis was use to determined association between the resistant rate of *Salmonella* isolates and antibiotics activities.

3. RESULTS

3.1 Bacterial isolation.

Of the five hundred stool specimens sampled from six selected hospitals studied, total of 126 bacterial isolates and 104 *Salmonella* positive specimens were recorded: 110 were collected from Murtala Mohammed Specialist Hospital (35 bacterial isolates and 5.8% *Salmonella* positive specimens were obtained), 100 from Aminu Kano Teaching Hospital (25 bacterial isolates and 4.4% *Salmonella* positive specimens were obtained), 90 from Mohammed Abdullahi Wase Specialist Hospital (20 bacterial isolates and 3.4% *Salmonella* positive specimens were obtained), 80 from Sir Sunusi Specialist Hospital (17 bacterial isolates and 2.6% *Salmonella* positive specimens were obtained), 60 from Sheik Waziru Gidado General Hospital (15 bacterial isolates and 2.2% *Salmonella* positive specimens were obtained) and 60 from Khadijat Memorial Private Hospital (14 bacterial isolates and 2.4% *Salmonella* positive specimens were obtained).

3.2 *Salmonella* identification by biochemical characterization.

Out of one hundred and twenty six (25.2%) bacterial isolates obtained from six selected hospitals studied, One hundred and eighteen (23.6%) presumptive *Salmonella* isolates were obtained from various biochemical characterization and identification test.

3.3 Sero-typing of the *Salmonella* isolates.

One hundred and four (20.8%) *Salmonella* isolates were obtained after serologic identifications of presumptive *Salmonella* isolates were performed by slide agglutination test.

3.4 The distribution of *Salmonella* infections in relation to age and sex

Although, the relationship between different age groups was not significantly associated ($P < 0.05$), patients under age bracket of 21-30 years were found to be more susceptible to *Salmonella* infections with 45 representing 9.0% followed in that order by 11-20 years (33), 31-40 years (27), ≤10 years (18) and >40 years (9) age groups, representing 6.6%, 5.4%, 3.6% and 1.8% respectively (Table 1).

Table 1: The distribution of *Salmonella* infections in relation to age among hospitalized patients in Kano metropolis, Nigeria.

Age group (Years)	No. examined	No. (%) infected
≤10	60	18(3.6)
11-20	140	33(6.6)
21-30	182	45(9.0)
31-40	85	27(5.4)
≥40	41	9(1.8)
Total	**500**	**132(26.4)**

KEY: *≥ = Greater than or equal to; ≤ = Less than or equal to; % = Percentage of total number of specimen tested (500).*

When the 132 infected patients where assed in relation to sex, the prevalence rate of *Salmonella* infections was significantly higher ($P > 0.05$) in males than the females patients with 83 (16.6%) and 49 (9.8%) respectively (Table 2).

Table 2: The distribution of *Salmonella* infections in relation to sex among hospitalized patients in Kano metropolis, Nigeria.

Sex	No. infected (%)	No. not infected (%)	Total (%)
Males	83(16.6)	203(40.6)	286(57.2)
Females	49(9.8)	165(33.0)	214(42.8)
Total	**132(26.4)**	**368(73.6)**	**500(100)**

Fisher's exact test two sides P value = 0.0081
KEY: *NO. = Number; % = Percentage of total number of specimens tested (500).*

3.5: The prevalence serovers of *Salmonella*

From Figure 1 out of 132 presumptive *Salmonella* isolates obtained in this study, *Salmonella* Typhi was the predominant serovar with 34(25.8%) followed by *Salmonella* Typhimurium 31(23.5%), *Salmonella* Enteritidis 25(19.9%), *Salmonella* Paratyphi C 17(12.9%), *Salmonella* Paratyphi A 15(11.4%) and *Salmonella* Paratyphi B was the least prevalent serovar with 12(9.1%) prevalence rate. However, the relationship between each *Salmonella* serovar obtained was also statistically significant ($P > 0.05$).

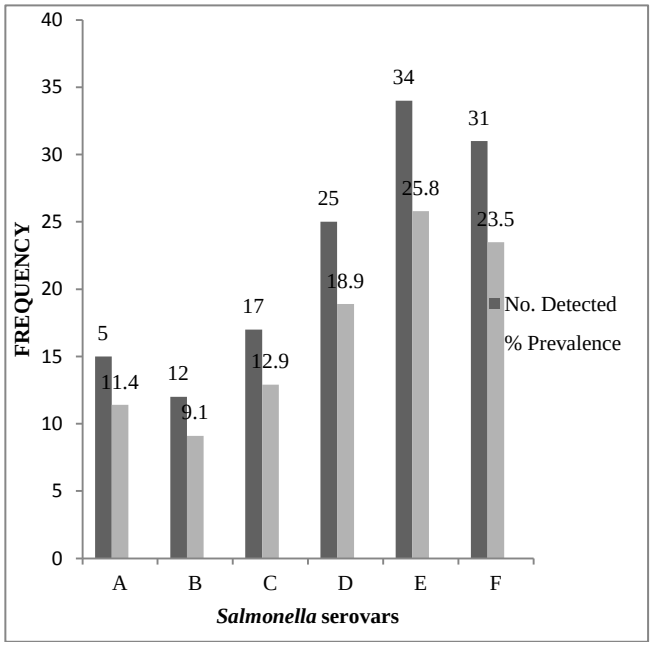

Figure 1: The prevalence serovers of *Salmonella* from blood specimens of hospitalized patients in Kano metropolis, Nigeria.
t= 3.059 P value = 0.0377
KEY: *S. = Salmonella; No. = Number; % = Percentage of total number of Salmonella isolated (104),* A = *S.* Paratyphi A, B = *S.* Paratyphi B, C = *S.* Paratyphi C, D = *S.* Enteritidis, E = *S.* Typhi, F = *S.* Typhimurium .

3.6 Antibiotic sensitivity of the *Salmonella* isolates.

Out of 104 *Salmonella* serovars isolated, 50 were triple, 54 double, and 18 single-antibiotic resistant phenotypes. Eighty two (62.1%) of the isolates displayed resistant to at least one or two antibiotics and fifty (37.9%) displayed resistant to three of antibiotics tested (Table 3).
Similarly, 50 isolates were found resistant to 1 of 3 first-line anti-*Salmonella* antibiotics (ampicillin, cotrimoxazole and chloramphenicol). In addition, 64 isolates were found resistant to 2 of 3 first-line anti-*Salmonella* antibiotics (ampicillin, cotrimoxazole and chloramphenicol), none of the isolates were resistant to all three first-line anti-*Salmonella* antibiotics (multi-antibiotics resistant salmonellae) (Table 3).

Table 3: Multiple antibiotic resistance patterns of *Salmonella* serovars isolated from blood specimens of hospitalized patients in Kano metropolis, Nigeria.

	Multiple antibiotic resistance patterns/phenotypes			
Single Resistance types	**Number with type**	**Number of antibiotics**	**Number of isolates with pattern**	**Resistance patterns/phenotypes**
AMP.	18	2	45	AMP., NA.
			11	AMP., COT.
			8	CH., NA.
		3	35	AMP., CH., NA.
			15	AMP., COT., NA.

KEY: *AMP = Ampicillin; CH = Chloramphenicol; CPX = Ciprofloxacin; COT = Cotrimoxazole; NA= Nalidixic Acid*

In this work, one hundred and fourteen (114) of the test *Salmonella* serovars exhibited multiple antibiotic resistances with five (5) different phenotypic resistant patterns. Forty five (39.5%) isolates have a MARI of 9.0 with two phenotypic patterns which differed in two different antibiotics (ampicillin and nalidixic acid). Thirty five (30.2%) isolates have a MARI of 7.0 with three phenotypic patterns which also differed in three different antibiotics (ampicillin, chloramphenicol and nalidixic acid). Fifteen

(13.2%) isolates have a MARI of 3.0 with three phenotypic patterns which differed in three different antibiotics (ampicillin, cotrimoxazole and nalidixic acid). Eleven (9.6%) isolates have a MARI of 2.2 with two phenotypic patterns which also differed in two different antibiotics (ampicillin and cotrimoxazole).

However, eight (7.0%) has a MARI of 1.6 with also two phenotypic patterns which also differed in two different antibiotics (chloramphenicol and nalidixic acid) (Figure 4).

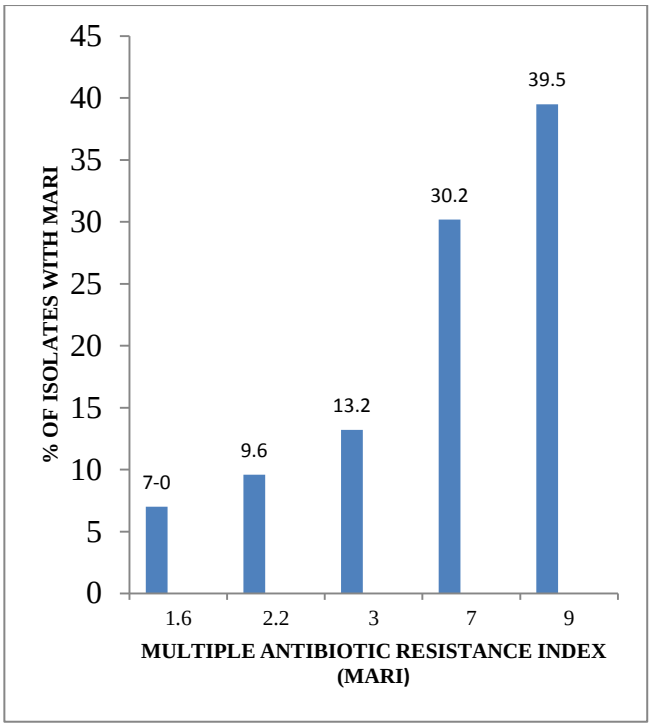

Figure 4: Multiple Antibiotic Resistances Indices (MARI) of *Salmonella* isolates from Kano metropolis, Nigeria.

The result of *in vitro* -antibiotic susceptibility testing demonstrated that of the five (5) antibiotics tested, most of the 132 isolates (89.4%) was significantly ($P > 0.05$) resistant to ampicillin. They were also resistant to nalidixic acid, chloramphenicol, and cotrimozaxole in decreasing order with 77.3%, 40.2%, and 26.5% resistance rate respectively. Ciprofloxacin remained effective against all the *Salmonella* isolates tested.

Table 5: Disc diffusion test on *Salmonella* serovars isolated from blood samples of hospitalized patients in Kano metropolis, Nigeria.

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Antibiotic	Disc potency (µg)	No. (%) of Resistant Isolates n= 132
AMP	25	118(89.4)
CH	30	53(40.2)
COT	25	35(26.5)
CPX	30	0(0.0)
NA	30	102(77.3)

$X^2 = 12.04$ P value = 0.001
No. = Number; n= Number of isolates tested; S = Salmonella; % = Percent; µg = microgram; AMP = Ampicillin; CH = Chloramphenicol; CPX = Ciprofloxacin; COT = Cotrimoxazole; NA = Nalidixic Acid; % = Percentage of total number of each Salmonella serovar tested.

4. DISCUSSION

In the present study, the highest infections were recorded from 21-30 years age groups and least infection was recorded from patients with ≥40 year age group. However the rate of infection among all the six (6) age groups was not significantly associated. In this study, male were more infected than their female counter part in the same age groups. In addition, the rates of infections between males and females patients were statistically significant ($P > 0.05$). This work is in consonance with the findings of Adkins and Santiago [25]; Abdullahi [11] and Mashi [26].

The highest incidence in males of 21-30 years age group patients recorded in this study is probably because most of the males in the study area were more prone to contaminations than their female counter part of the same age group. Males usually eat and drink outdoors and they do not recognize the state of the food or drink they eat and the nature of the environment in which the food and drink are prepared [26]. It was further observed that the high prevalence of *Salmonella* infection in males of 21-30 years age group is connected with water exposure by these individuals in their community, who are supposed to be demographically and economically active and productive individuals in the communities, because they are more exposed to occupational hazard of farming, related water contact activities, contaminated environment, food and drink than children and elderly persons [25]. Similarly, sharing of public toilet in school, market, bus stop by male would probably increase the level of infection among the male [11].

However, the low community awareness level of males of this age on the routes of *Salmonella* infection may increase the transmission potential of the disease since individuals would not care to protect them or take other cautions when exposure becomes necessary. It was further observed that even among informed males awareness level does not play important role in discouraging water activities, and practices have become necessary as they are the major means of subsistence; in term of irrigation, agriculture, gardening, outdoors business and other occupational hazards [27, 28].

Females are less likely to be found eating, drinking and defaecating outdoors. This is because of culture and religious inclination. Moreover, females are less exposed to occupational hazard of farming, related water contact activities, contaminated environment, contaiminated food and drink than their males' counterpart [27]. It was observed that males were

responsible for 98% of activities involving contamination and water exposure [26].

On the other hand, adults (≥40 year age group) appreciate the quality of health they have, they are wise, matured and therefore, protect and maintain most of their valuable physical health. In addition, the adults do not usually predispose themselves to various contaminated areas than youth [26]. Furthermore, the presence of little or no infection in older individuals may be connected with the less water contact activities thus reducing the risk of contracting infection. Another inference is the possibility of developed immunity by the older individuals who might have contracted the disease in their young age. Therefore, it can be concluded that such

insusceptibility could reflect on age dependent acquired immunity [29].

Various studies have been conducted by many researchers in different parts of the world establishing the significance of *Salmonella* serovars in the causation of *Salmonella* infection. Five (5) serovars of *Salmonella* were encountered in the present study. In this study, *Salmonella* Typhi was predominant followed by *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Paratyphi C, A and B. This finding is in consonant with studies by Asma *et al.* [30] and Akinyemi *et al.* [31]. This is contrary to the work conducted in Kano metropolis (the study area) by Abdullahi [10] who revealed that *Salmonella* Typhimurium was the most predominant serovar. The highest susceptibility to *Salmonella* Typhi in the study area could probably be because *Salmonella* infections occurs by ingesting organisms in contaminated food or water from contaminated hands (*Salmonella* Typhi is mainly water-borne, *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Salmonella* Paratyphi are mainly food-borne). In the area where this research was conducted, residents experienced serious problem of inadequacy of water supply which resulted to high consumption of unhygienic water. However, sewages and contaminated water used for irrigation contained high level of *Salmonella* Typhi (mainly water-borne) that can be transferred to consumer through contaminated farm products [10].

In addition, in the study area many other reports indicated that multiple antibiotic resistances (MDRs) to *Salmonella* Typhi were among the most frequent salmonellae which are responsible for high infection among carriers and high opportunistic infections among AIDS patients. There is also diagnostic dilemma in the issue of chronic carriers, in the study area, the *Schistosoma haematobium* is common, and it was reported that a chronic urinary carrier state is resulted from localization of typhoid bacilli in schistosomiasis, the *Schistosoma-Salmonella* complex [32]. The Patient will become a variant that has chronic foci of infection but whom *Salmonella* Typhi is shed intravascularly rather than in stool or urine [32]. Similarly, as few as 10^3 mean infective dose of *Salmonella* Typhi

organisms is required to produce clinical or subclinical infection in humans but perhaps 10^5 -10^8 salmonellae is required for other salmonellae in African countries. Furthermore, waterborne disease outbreaks are often unreported or under reported in developing countries because of the lack of systematic studies. Presently and, indeed, in the future Kano may not be an exception in this regard [10].

On the other hand, less infection with *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Salmonella* Paratyphi (mainly food-borne) could probably be because most food stuffs were washed properly or cooked before consumption and this help on the decrease or total destruction of *Salmonella* Typhimurium and *Salmonella* Paratyphi in food staffs [33, 34].

In this study, most of the *Salmonella* isolates from patients in Kano were significantly (X^2 12.04, P value = 0.001) resistant to ampicillin (P< 0.05) with 118 resistant strains representing 89.4% of the *Salmonella* isolates, followed by nalidixic acid with 102 resistant strains representing 77.3%. Ciprofloxacin remained effective against all the *Salmonella* isolates tested. However, the relationship between *Salmonella* serovars and antibiotics tested was not statistically significant (p > 0.05). Eighty two (62.1%) of the isolates displayed resistant to at least one or two antibiotics and fifty (37.9%) displayed resistant to three of antibiotics tested. Similarly, 50 isolates were found resistant to 1 of 3 first-line anti-*Salmonella* antibiotics (ampicillin, cotrimoxazole and chloramphenicol). Highest MARI was recorded from forty five (39.5%) isolates with MARI of 9.0 with two phenotypic patterns which differed in two different antibiotics (ampicillin and nalidixic acid). This work is in consonance with the findings of Asma *et al.* [35], Hemalatha *et al.* [36], Malla *et al.* [37], Gautum *et al.* 38] and Abdullahi *et al.* [27].

The highest significant resistance (P < 0.05) of *Salmonella* isolates to ampicillin and nalidixic acid could probably be due to the usage of antibiotics in the study area which is possibly the most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine [39, 14]. However, the rate of development of resistance appears to have accelerated in the past decade and today multiple resistant *Salmonella* constitute a global problem [40]. It has been observed that antibiotic susceptibility of *Salmonella* isolates is not constant but dynamic and varies with time and environment. This therefore demands the need for periodic screening of common pathogens for their antibiotic susceptibility profiles in different communities. There is strong evidence that the use of antibiotics can lead to the

emergence and dissemination of resistant salmonellae, which can then be passed onto people via food or through direct contact with animals. During recent years the wide spread use of antibiotics in the field of veterinary medicine have resulted in the development of increasing number of bacterial strains possessing resistance to many antibiotics. The property of multiple antibiotics resistance could be transferred through conjugation from resistant strains of salmonellae, to another by means of plasmid, which occur in cytoplasm of the donor bacterium and multiply independently of the chromosomal DNA. Thus a new bacterium with resistance factor emerges that is resistant to one or more antibiotics. In another instant the high resistance of *Salmonella* isolates to commonly used antibacterial drugs is probably due to some factors ranging from the use of fake antibiotics, abuse and misuse of those antibiotics found commonly in circulation among the general populace and health resources centers [40, 37].

Abdullahi [2] reported that, acquired antibiotics resistance is a growing worldwide problem due to the increasing use of antibiotics in humans, animals, and agriculture. In developing countries the situation is particularly serious for the following reasons: In many countries, antibiotics can be obtained outside of recognized treatment centres, and taken without medical authorization or supervision. This leads to inappropriate use of antibiotics and their being taken at sub-optimal dosages and for an insufficient length of time. Often the high cost of an antibiotic, results in an incomplete course being purchased, sufficient only to alleviate symptoms. Patients are not sufficiently informed about antibiotics and their use [11, 27]. Problems also arise when antibiotics

sold in local markets are sub-standard or expired antibiotics. Guidelines regarding the selection of antibiotics, correct prescription, and information about antibiotic resistance and how to minimize its spread are not communicated to those purchasing the antimicrobials. Antibiotics are often prescribed when they are not needed or for self-limiting infections, e.g. diarrhoeal disease and viral respiratory infections [11, 27].

Other overlapping problems are worsening the situation regarding typhoid fever and other *Salmonella* infections within Africa: the failure to control the spread of the *Salmonella* species involved, due to unclean water, poor sanitation, malnutrition, the failure to control resistant organisms and resistance genes so that, when infections occur, they produce more adverse consequences. It is perhaps obvious, if unaddressed, that poor and displaced persons in Africa are least likely to be able to access potable water, safe sanitation, and other factors to prevent faecal-oral infection and that public health facilities need to be strengthened to protect the poor [42].

Broad spectrum antibiotics are frequently used prophylactically, e.g. ampicillin. Laboratory facilities for accurate diagnosis and isolation of pathogens are often not available, resulting in an overuse and inappropriate use of antibiotics [41]. Many countries do not have effective surveillance of important antibiotic-resistant bacteria. Training and facilities for performing standardized antibiotics sensitivity tests are often lacking. Developing countries are often unable to afford costly second-line antibiotics to treat infections due to resistant organisms. This results in prolonged illness with longer periods of infectivity and to the further spread of resistant strains [11].

5. CONCLUSION

In addition, the frequency of *Salmonella* infections was highest among 21-30 year age group lowest in ≥40 year age group. However the rates of infection among all the six (6) age groups were not significantly associated. The prevalent

rate of *Salmonella* infections was significantly higher ($P > 0.05$) in males than the females patients with 83 (16.6%) and 49 (9.8%) respectively.

From the results of many studies conducted in different parts of the world, the *Salmonella* serovars that are frequently isolated in blood specimens of patients were also encountered in the present study. The study revealed that among the *Salmonella* serovars isolated, *Salmonella* Typhi was predominant followed by *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Paratyphi C, A and B.

The result of *in vitro* – antibiotic susceptibility testing of *Salmonella* isolates using disc diffusion test demonstrated that most of the isolates (89.4%) was significantly ($P > 0.05$) resistant to ampicillin followed by nalidixic acid, chloramphenicol, and cotrimoxazole in decreasing order. However, ciprofloxacin remained effective against all the *Salmonella* isolates tested. However, the relationship between *Salmonella* serovars and antibiotics tested was not statistically significant ($P > 0.05$). In addition, 50 isolates were found resistant to 1 of 3 first-line anti-*Salmonella* antibiotics (ampicillin, cotrimoxazole and chloramphenicol). Highest MARI was recorded from forty five (39.5%) isolates with MARI of 9.0 with two phenotypic patterns which differed in two different antibiotics (ampicillin and nalidixic acid).

6. RECOMMENDATION

Mass campaign, public enlightenment should be given more attention on the effective personnel hygiene, food hygiene and environmental sanitation. There is also need for reduced and appropriate consumption of antibiotics both for human and animals. Government should review existing antibiotic use policy to check abuse/misuse of antibiotics and ensure their correct

prescription; this would reduce the rate of emergence of *Salmonella* resistant in particular and other bacteria in general. Research on development of new antibiotics and vaccines for

treatment and prevention of *Salmonella* and other bacterial infections should be given more attention.
To reduce this antibiotic resistance, public health reference laboratory with a tool to produce standardized antibiotic susceptibility test results of antibiotic susceptibility tests are important for clinical treatment plans; adequate information must be provided to the health care providers. In addition, susceptibility testing help on providing guidance and monitor of treatment, narrower the spectrum of its antibiotic (the more proffered is it's use when one knows specifically the organism being treated), degree of susceptibility of organism can assist in determining the length of therapy (but not the only factor) and choice of cheaper antibiotic agents with less side effects.

CONSENT

"Author declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

"Author hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

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COMPETING INTERESTS

The author declared that I have no competing interests exist.

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