

Original Research Article

Prevalence and Antibiotic Resistance Profile of *Salmonella* serovars Isolated from Blood samples of Hospitalized Subjects in Kano Metropolis, Kano State, North-Western Nigeria

ABSTRACT

Aim: The aim of the study is to determine the prevalence and antibiotic resistance profile of *Salmonella* isolates recovered from the blood samples of hospitalized subjects in Kano metropolis.

Study design: Investigative

Place and duration of study: Samples were analyzed at the Medical Laboratory Science Department, School of Health Technology Kano, Kano state, Nigeria and Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. This work was carried out between May, 2011 and March, 2013.

Methodology: The blood specimens were cultured into thioglycollate broth and sub-cultured onto deoxycholate citrate agar (DCA), *Salmonella*-*Shigella* agar (SSA) and Brilliant Green agar followed by confirmation of presumptive colonies using different biochemical tests and analytical profile index 20E. Serologic identification of *Salmonella* were performed by slide agglutination test using polyvalent (O) and (H) *Salmonella* antisera. Antibiotic susceptibility studies were by the disc diffusion method using ampicillin (25μ), chloramphenicol (30μ), ciprofloxacin (25μ), nalidixic acid (30μ) and Trimethoprim-sulfamethoxazole (30μ). The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ampicillin ($\geq 32\mu\text{g/ml}$), chloramphenicol ($\geq 32\mu\text{g/ml}$), ciprofloxacin ($\geq 8\mu\text{g/ml}$), nalidixic acid ($\geq 32\mu\text{g/ml}$) and Trimethoprim-sulfamethoxazole ($\geq 4\mu\text{g/ml}$) were also determined. The result of antibiotic susceptibility testing using both techniques demonstrated that ampicillin, nalidixic acid and chloramphenicol were the least effective antibiotics. The relationship between the resistant rate of *Salmonella* isolates and antibiotics activities was not statistically significant ($p > 0.05$).

Results: *Salmonella* serovars isolated comprised of *Salmonella* typhi (predominant) with 39 (7.8%) followed by *Salmonella* typhimurium 35 (7.0%), *Salmonella* paratyphi C 14 (2.8%), *Salmonella* paratyphi B 11 (2.2%) and *Salmonella* paratyphi A was the least prevalent serovar with 5 (1.0%). Although, the relationship between different age groups was not significantly associated ($P < 0.05$), patients under age bracket of 21-30 years age group was found to be more susceptible to *Salmonella* infections and >40 years age group was the least susceptible. The result showed significant difference ($P < 0.05$) in infection rate, which was higher in males than the females' patients.

Conclusion: The high rate of resistance is hampering the use of conventional antibiotics, and growing resistance to newer antibiotics is aggravating the situation. Therefore, with this

emergence and continual increase in the drug resistance, determination of drug resistance along with timely diagnosis of *Salmonella* infection has become a matter of vital importance.

Keywords: *Salmonella* serovars, Patients, Blood samples, Antisera, Antibiotics, Resistant.

1. INTRODUCTION

Salmonellae are Gram negative, non-lactose fermenting and non-sporing bacteria. With exception of *Salmonella pullorum* and *Salmonella gallinarum*, all *salmonellae* are actively motile. They are also non-capsulated with exception of *Salmonella typhi* belonging to the family enterobacteriaceae [1, 2]. *Salmonella* infections in humans and animals have been recognized as a major public health problem [3]. *Salmonella*, a primary inhabitant of the gastrointestinal tract, is recognized as one of the most common causes of food borne infection worldwide, resulting in millions of infections and significant human death annually [4]. They are common contaminants of wide range of food, eggs, vegetables, and water. Additionally, they are carried by animals, rodents, pets, birds, reptiles and insects, usually without the display of any apparent illness [5]. The infections caused by *Salmonella* are considered one of the most widely spread water and food-borne zoonotic infection in developed as well as developing countries, though incidence varies between countries [6]. Food items such as poultry meat and other poultry products have been implicated as important sources for outbreaks of human *Salmonella* infection, with poultry alone, accounting for up to 50 percent of such outbreaks [7,8]. Non-typhoidal Salmonellosis is common in most parts of the world [9]. It is widely spread in Europe and North America [10,11]. Latin America, the Middle East and Africa [12], also in countries such as India [13], Japan [14] and the United States [15]. Several studies had documented isolation of non-typhoidal *Salmonella* from humans and poultry in different parts of Nigeria [16,17,18,19]. Outbreaks of Salmonellosis caused by *Salmonella Gallinarum*, *S. Pullorum*, *S. Typhimurium* and *S. Enteritidis* have also been reported [17,19,20].

Salmonellae can be divided into two major groups of clinical importance: Group 1 includes members of the genus that are involved as aetiologic agents of enteric fever i.e. *Salmonella typhi* and *Salmonella paratyphi*. Group II includes members of the genus that are involved as aetiologic agent of food poisoning (non typhoidal salmonellosis) i.e. *Salmonella typhimurium* and more recently serotype DT 104. Other members are *Salmonella enteritidis*, *Salmonella heidelberg*, *Salmonella agona*, *Salmonella newport*, *Salmonella hadar*, and *Salmonella dublin* [21, 22]. Enteric fever occurs in all part of the world where there is substandard water supply and sanitation. Some of the symptoms of typhoid fever are fever, vomiting, diarrhoea, headache, abdominal pain, body ache, breathlessness, weight lost, constipation and anaemia. Typhoid and paratyphoid appeared clinically indistinguishable [23]. Food poisoning salmonellosis is an infection caused by ingesting salmonellae in food that is contaminated by faeces of animals or humans directly or indirectly. Some of the symptoms of food poisoning salmonellosis are diarrhoea, vomiting, fever, and abdominal pain. These symptoms occur 12-36 hours after eating infected food [24].

When antibiotics first came in to use about 73 years ago, resistance was not a problem. From 1945 to the late 1980s, new antibiotics were developed faster than the bacteria developed resistance. While in the 1950s and 1990s improvements within these established classes were observed. It is now evident that the bacteria are winning. No new classes of antibiotics have been

developed in the last 10-18 years, while bacterial resistance to antibiotics has been increasing at an alarming rate. Bacteria resistance has now been reported against every currently available antibiotic [25]. Development of new antibiotics costs around £350 million or more, takes 7-10 years and its use may be restricted to delay resistance or reduce costs. For these reasons scientists and pharmaceutical companies have lost interest in the development of antibiotics and they now prefer to invest in other therapeutic areas [25].

Increasing antimicrobial resistance in *Salmonella* species has been a serious problem for public health worldwide. Widespread drug-resistant salmonellae and the associated complications in the treatment of infections have constituted a serious threat to the public health. In Nigeria, the increasing treatment failure with the empirical therapy in recent times among patients with *Salmonella* infection necessitates responsibility of medical microbiologists to assess and report the patterns of antibiotic resistance among *Salmonella* serovar in our environment [26]. The circumstances of occurrence and spread of antimicrobial resistance is complex; however, a major cause is the widespread use of antimicrobial agents in food animals, particularly in animal feed. Genetic analysis has indicated that the source of resistance is frequently a transferable plasmid [26].

Two 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 dwellers 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 [27].

Majority of antibiotic resistance studies on *Salmonella* from different parts of the world are on isolates obtained from stool samples of patients admitted into hospitals. Recent studies indicate that antibiotic resistance genes are growing problems as environmental contaminants. Therefore, this indicates the need to pay closer attention to isolation of *Salmonella* in blood and its community acquisition of multiple drug resistance [28].

2. MATERIALS AND METHODS

2.1 Study Design

2.1.1 Specimens

Venous blood (3mls) collected by competent hospital personnel from each patient who presumptively diagnosed for *Salmonella* infection and agreed to signed the consent form in the six (6) selected hospitals in Kano metropolis were used as specimens.

2.1.2 Test organisms

Salmonella serovars isolated in blood specimens collected from hospitalized subjects in the six (6) selected hospitals were used as the test organisms.

2.1.3 Patients

2.1.3.1 Inclusion criteria:

Patients (in and out) who patronized the six (6) 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) who agreed to signed the consent form were recruited for the study.

Any patient (in and out) who brought his blood specimen to the laboratory reception of one of the six (6) selected hospitals for widel test, malarial test and other related blood tests who agreed to signed the consent form were recruited for the study.

2.1.3.1 Exclusion criteria:

Patients (in and out) who patronized the six (6) 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) who not agreed to signed the consent form were excluded for the study.

Any patient (in and out) who brought his blood specimen to the laboratory reception of one of the six (6) selected hospitals for widel test, malarial test and other related blood tests who not agreed to signed the consent form were also excluded for the study.

2.1.4 Hospitals

The six (6) most patronized hospitals were randomly selected: that comprises of one (1) Teaching Hospital (Aminu Kano Teaching Hospital), three (3) specialist hospitals (Murtala Mohammed Specialist, Mohammed Abdullahi Wase Specialist and Sir Sunusi Specialist Hospital), one (1) General Hospital (Sheik Waziru Gidado General Hospital) and one (1) 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 Study area

Metropolitan Kano encompasses all the eight local governments of Dala, Fagge, Gwale, Municipal, Nassarawa, Tarauni, part of Kumbotso and Ungogo. Figure 1 this is in addition to part of local governments which was integrated in to local metropolis for planning purposes. It lies from Latitudes $11^{\circ} 52'N$ to $12^{\circ} 7'N$ and Longitudes $8^{\circ} 22.5'E$ to $8^{\circ} 47'E$ and is 1549ft above sea level. Kano metropolis is bounded by Minjibir LGA on the North East and Gezawa LGA to

the East. While, Dawakin Kudu LGA to the South East, Madobi and Tofa LGA's to the South West and lastly Dawakin Tofa LGA to the North West. The climate of Kano is typical dry and wet climate. Annual rainfall is about 850 – 870mm. The temperature is averagely warm to hot throughout the year at about 27⁰ C[29, 30].

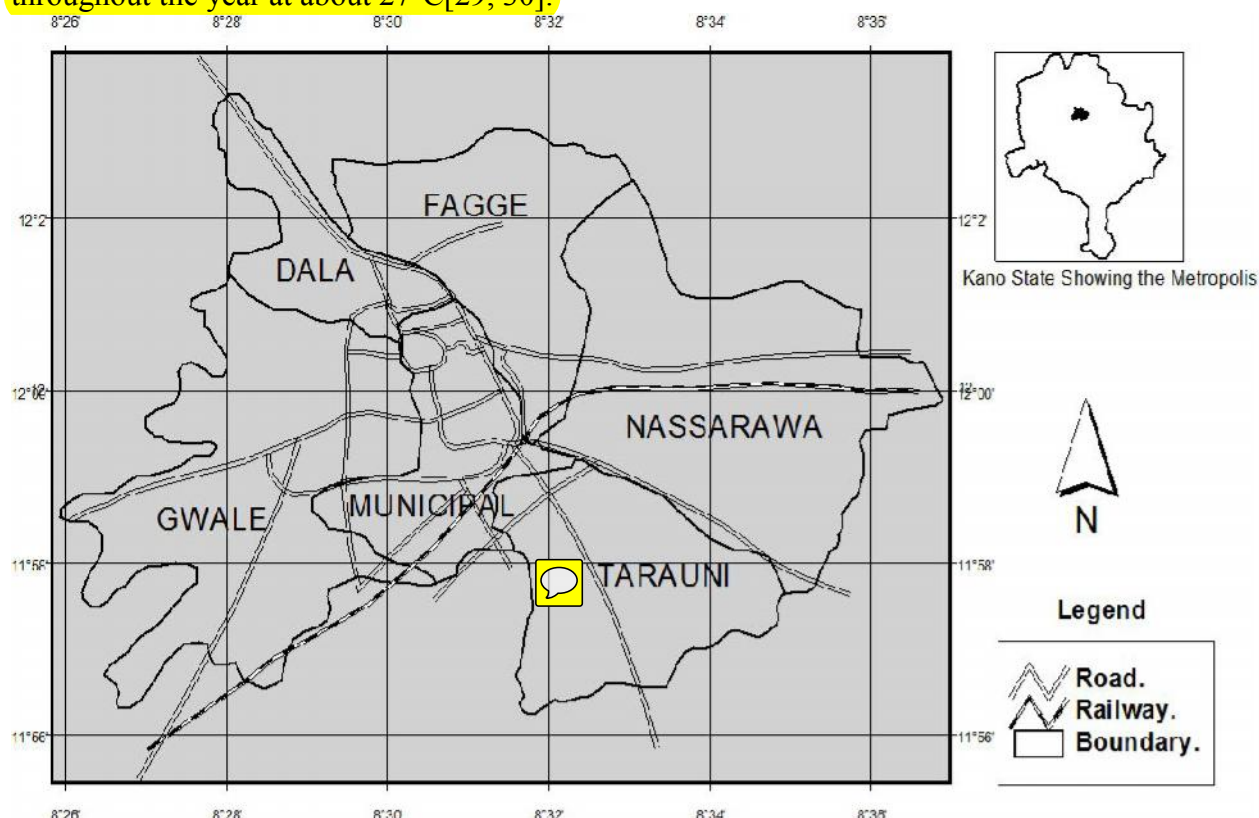


Figure 1: Map of Study area (Kano) indicating s (6) core metropolitan LGAs where study hospitals are located[30]

2.3 Ethical Consideration

Approval for the study was obtained from the Ethical/Medical Advisory Committees of the selected Hospitals and the State Ministry of Health/Hospital Management Board, Kano state.

2.4 Specimen Collection and handling

About five hundred (500) of blood specimens were collected from patients recruited for the study in Kano metropolis, blood collected from each patient with some or all clinical features of *Salmonella* infection was transferred into EDTA bottles. If daily is unavoidable blood samples were stored at 4⁰ C [31, 32].

2.5 Isolation and identification of salmonellae in blood specimens

2.5.1 Presumptive isolation of *Salmonella*

The cultured plates, SSA, BGA and XLD 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 [33].

2.5.2 Purification of isolates

The isolates were sub cultured onto SSA and nutrient agar for isolation of pure culture and subsequent biochemical characterization.

2.5.3 Biochemical characterization of *Salmonella*

Isolation and identification of organisms was carried out as described by ISO [34]; Habtamu et al. [20]; OIE [33]. 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 (Biomérieux, France).

2.6 Serotyping of isolates

Biochemically identified *Salmonella* isolates were further tested for somatic (O) and flagella (H) antigens with polyvalent *Salmonella* antisera (Oxoid, UK) according to Kauffmann White Scheme [35] by slide agglutination test.

2.7 Antimicrobial susceptibility using Disc diffusion technique:

In-vitro susceptibility of *Salmonella* isolates to various routine antimicrobial drugs was tested by the standard disc diffusion technique using guidelines established by NCCLS [36].

2.7.1 Standardization of inoculum

This was done as documented by CLSI [37]. Pure culture of identified *Salmonella* isolate (s) from an 18-hour plate culture was selected. Sterile wire loop was used to pick 2 to 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 sensitive 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.

2.7.2 Inoculation of test plates

This was carried out as described by NCCLS [36]. Optimally, within 5 to 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 37°C to allow for any excess surface moisture to be absorbed before applying the antibiotic discs.

2.7.3 Application of discs to inoculated agar plates

All positive cultures of *Salmonella* species isolated from blood samples were tested *in vitro* for susceptibility to different antibiotics by agar diffusion technique as described by Kirby-Bauer [38], NCCLS [39] and WHO [40]. This was carried out according to WHO protocol [40]. The susceptibility testing of *Salmonella* isolates were carried out using Mueller Hinton agar and were tested *in vitro* for susceptibility to five (5) different antibiotics namely; ampicillin (25µ), chloramphenicol (30µ), ciprofloxacin (25µ), nalidixic acid (30µ) and Trimethoprim-sulfamethoxazole (30µ) [39, 40].

2.8 Determination of Minimum Inhibitory Concentration (MIC):

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ampicillin ($\geq 32\mu\text{ml}$), chloramphenicol ($\geq 32\mu\text{ml}$), ciprofloxacin ($\geq 8\mu\text{ml}$), nalidixic acid ($\geq 32\mu\text{ml}$) and Trimethoprim-sulfamethoxazole ($\geq 4\mu\text{ml}$) were also determined using agar dilution method according to the criteria of the NCCLS [39] with modifications [39, 41].

2.9 Statistical analysis

Statistical Package for Social Science (SPSS) version 14 was used [42]. Descriptive statistics were used to categorical (frequency percentages) variables. Chi-square test analysis was used to determine association between the resistant rate of *Salmonella* isolates and antibiotics activities.

3. 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 34 representing 6.8% followed in that order by 11-20 years (28), 31-40 years (19), ≤ 10 years (15) and > 40 years (8) age groups, representing 5.6%, 3.6%, 3.0% and 1.6% respectively (Table 1).

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

Age group (Years)	No. examined	No. and % infected
≤10	49	15(3.0)
11-20	132	28(5.6)
21-30	167	34(6.8)
31-40	93	19(3.8)
≥40	67	8(1.6)
Total	500	104(20.8)

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

When the 104 infected patients were assessed in relation to sex, the prevalence rate of *Salmonella* infections was significantly higher ($P < 0.05$) in males than the females patients with 68 (14.0%) and 36 (7.0%) respectively (Table 2).

Table 2: The Distribution of *Salmonella* infections in Relation to Sex among Hospitalized Subjects in Kano Metropolis, Nigeria.

Sex	No. infected (%)	No. and % not infected	Total (%)
Males	68(14.0)	201(40.0)	269(54.0)
Females	36(7.0)	195(39.0)	231(46.0)
Total	104(20.8)	396(79.2)	500(100)

Fisher's exact test two sides p value = 0.0081

NO. = Number; % = Percentage of total number of specimens tested (500).

The result of *in vitro* – antibiotic susceptibility testing of *Salmonella* isolates using disc diffusion technique demonstrated that ampicillin was the least effective antibiotic with 97(93.3%) followed in that pattern by nalidixic acid (90), chloramphenicol (34) and cotrimoxazole (16) with 86.5%, 35.6% and 15.4% resistance respectively. The relationship between the resistant rate of

Salmonella isolates and antibiotics tested was not significantly associated ($p>0.05$). In this study, all the isolates tested were susceptible to ciprofloxacin (Table 3).

Table 3: Percentage Prevalence of Antibiotic Resistant Profile of *Salmonella* isolates tested using Disc Diffusion Technique

Antibiotic		Disc potency (µg)		No. and % of Resistant Isolates
n= 104				
CH	AMP	25		97(93.3)
		30	34(35.6)	
	COT	25	16(15.4)	
	CPX	30		0(0.0)
NA	30			90(86.5)

No. = Number; *n* = Number of isolates tested; % = Percent; μg = microgram;
AMP = Ampicillin; *CH* = Chloramphenicol; *CPX* = Ciprofloxacin; *COT* = Cotrimoxazole;
NA = Nalidixic Acid; % = Percentage of total number of *Salmonella* isolates tested (104)

From Table 3, out of 104 presumptive *Salmonella* isolates obtained in this study, the serovars of *Salmonella typhi* (predominant) with 39(7.8%) followed by *Salmonella typhimurium* 35(7.0%), *Salmonella paratyphi C* 14(2.8%), *Salmonella paratyphi B* 11 (2.2%) and *Salmonella paratyphi A* was the least prevalent serovar with 5 (1.0%). In addition, the MIC and MBC of antibiotics to *Salmonella* isolates showed that ampicillin was the least effective antibiotic with 92.3% followed in that pattern by nalidixic acid (81), chloramphenicol (32) and cotrimoxazole (17) with 77.9%, 30.8% and 16.3% resistance respectively. The relationship between the resistant rate of *Salmonella* isolates and antibiotics tested was not statistically significant ($p> 0.05$) (Table 4).

Table 4: Antibiotic Resistance Profile of the test *Salmonella* serovars (10^6 cfu/ml) against five(5) selected Antibiotics

Antibiotics and peak plasma/ μ g/ml					
<i>Salmonella</i> serovar	AMP	CH	COT	CPX	NA
(No. tested)	(≥ 32)	(≥ 32)	(≥ 8)	(≥ 32)	(≥ 4)
<i>Salmonella</i> paratyphiA (5)	5(100)	2(40.0)		1(20.0)	0(0.0)
<i>Salmonella</i> paratyphiB (11)	10(90.9)	3(27.3)	3(27.3)	0(0.0)	11(100)
<i>Salmonella</i> paratyphiC (14)	13(92.9)	4(28.6)	1(7.1)	0(0.0)	12(85.8)
<i>Salmonella</i> typhiD (39)	36(92.3)	14(35.9)	5(12.8)	1(2.6)	32(82.0)
<i>Salmonella</i> typhimurium (35)	33(94.3)	9(25.7)	7(20.0)	0(0.0)	21(60.0)
Total (n = 104)	96(92.3)	32(30.8)	17(16.3)	1(1.0)	81(77.9)

No. = Number; n = Number of isolates tested; % = Percent; S. = *Salmonella*; cfu = Colonies forming unit; μ g = microgram; ml = Milliliter AMP = Ampicillin; CH = Chloramphenicol; CPX = Ciprofloxacin; COT = Cotrimoxazole; NA= Nalidixic Acid; % = Percentage of total number of each *Salmonella* serovar tested.

4. DISCUSSION

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. Among the *Salmonella* serovars isolated, *Salmonella* typhi was predominant followed by *Salmonella* typhimurium, *Salmonella* paratyphiC, B and A. This finding is in consonance with studies by Akinyemi *et al.* [43] and Asmaet *al.* [44]. Contrary to the work conducted in Kano metropolis (the study area) by Abdullahi [45] who revealed that *Salmonella* typhimurium was the most predominant serovar. The highest susceptibility to *Salmonella* typhi in the study area could probably because *Salmonella* infections occurs by ingesting organisms in contaminated food or water from contaminated hands (*Salmonella* typhi is mainly water-borne, *Salmonella* typhimurium and *Salmonella* paratyphi are mainly food-borne). In the area where this research was conducted, residents experiences serious problem of inadequacy of water supply that result to high consumption of unhygienic water [45]. However, sewage contaminated water used for irrigation

contained high loads of *Salmonella typhi* (mainly water-borne) that can be transferred to consumer through contaminated farm products. There is also diagnostic dilemma in the issue of chronic carriers, in study area the *Schistosoma haematobium* is common, and it was reported that a chronic urinary carrier state result from localization of typhoid bacilli in schistosomiasis, the *Schistosoma-Salmonella* complex [46]. 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 [46]. Similarly, as few as 10^3 mean infective dose of *Salmonella typhi* organisms is require to produce clinical or subclinical infection in humans but perhaps 10^5 - 10^8 salmonellae is require 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. In future and present Kano may not be an exceptional in this regard [45].

On the other hand, less infection with *Salmonella typhimurium* 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 stuffs [47, 48].

In the present study, the highest infections recorded from 21-30 years age groups and least infection was recorded from patients with ≥ 40 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 [22]; Mashi [49] and Abdullahi [45].

The highest incidence in males and 21-30 years age group patients recorded in this study could probably be due to the fact that most of males in the study area were more prone to contaminations than their female counter part of the same age group, males are usually eat and drink outdoor and they do not recognize the state of the food or drink they eat and nature of the environment in which they are prepared. The high prevalence of *Salmonella* infection in males and 21-30 years age group is probably connected with water exposure by these individuals in their community, who are demographically and economically supposed to be the most active and productive of the populace in the communities and thus are more exposed to occupational hazard of farming, related water contact activities, contaminated environment, food and drink than children and elderly person [22]. Similarly, sharing of public toilet in school, market, bus stop by male would probably increase the level of infection among the male [45].

On the other hand females are less likely to be seeing eating, drinking and defaecating outdoor possibly because of culture and religious inclination. Moreover, females are less exposed to occupational hazard of farming, related water contact activities, contaminated environment, food and drink than their males' counterpart [22]. It was observed that males were responsible for 98% of activities involving contamination and water exposure [49].

On the other hand, the adults (≥ 40 age group) appreciate the quality of health they have, they are wise, matured enough, protect and maintain most their valuable physical asset health. In addition, the adults are not usually predisposed themselves various contaminated areas than youth [49]. Furthermore, the presence of little or no infection in older individuals may have been

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 an age dependent acquired immunity [50].

Of the five (5) antibiotics tested, ampicillin was the least effective antibiotic ($p < 0.05$) with 92.3% of the *Salmonella* isolates displaying the highest level of resistance, while ciprofloxacin gave the highest effective antibacterial activity against *Salmonella* isolates with 1.0% resistant rates. This work is in consonance with the findings of Hemalatha *et al.* [51]; Malla *et al.* [52]; Gautum *et al.* [53] and Abdullahi [45]. High levels of resistance of *Salmonella* serovars to the commonly available drugs like ampicillin, nalidixic acid and chloramphenicol could probably be due to antibiotic usage which is possibly the most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine. However, the rate of development of resistance appears to have accelerated in the past decade and today multiple resistant *Salmonella* constitute a global problem [51]. It has been observed that antibiotic susceptibility of *Salmonella* isolates is not constant but dynamic and varies with time and environment. 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 [45].

Abdullahi, [45] 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. Problems also arise when antibiotics sold in local markets are sub-standard or expired drugs. Guidelines regarding the selection of drugs, correct prescription, and information about drug 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 [45].

The property of multidrug 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 resistant of *Salmonella* serovars to commonly antibiotics like is probably due to some factors ranging from the use of fake drugs, abuse and misuse of those antibiotics found commonly in circulation among the general populace and health resources centers [54, 53].

5. Conclusion and Recommendation

From the result of this study, it can be concluded that, among the *Salmonella* serovars isolated *Salmonella typhi* was the most predominant with and *Salmonella paratyphi* A was the least. The males and 21-30 years age group were found to be more susceptible to salmonellosis. The risk of infection in reduces in older age group. The result of *in vitro* – antibiotic susceptibility testing of *Salmonella* isolates using disc diffusion technique, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) demonstrated that ampicillin was significantly ($P > 0.05$) the least effective antibiotic tested.

Mass campaign, public enlightenment should be given more attention on the effective personnel hygiene, food hygiene and environmental sanitation so as to reduced *Salmonella* infection in the community and Nigeria at large. Patients should be encouraged to patronized hospital immediately and be sent early to the laboratory for effective diagnosis and susceptibility test of organisms to ensure quick, specific and effective treatment of *Salmonella* infection. Government should provide good facilities in hospitals and schools as well as electricity in the city in order to reduce the level of poverty, illiteracy, unemployment, in security and crises in the society as well as mis and over diagnosis.

One of the means of overcoming the resistance problem in salmonellae is to discontinue the overuse and misuse of antimicrobial agents in food animals. Furthermore, because the resistance displayed by *Salmonella* serotypes reflects the environment in which the organism thrives, immediate action, including rigorous restriction of the use of β -lactam antibiotics and fluoroquinolones in food animals and humans and reinforcement of infection-control measures in clinical settings, should also be taken [23].

To reduce this antimicrobial resistance, public health reference laboratory with a tool to produce standardized antimicrobial susceptibility test results of antimicrobial 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 antimicrobial (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 antimicrobial agents with less side effects [55, 56].

COMPETING INTERESTS

The authors declared that they have no competing interests exist.

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