
Antibacterial resistance profile and PCR detection of antibiotic resistance genes in *Salmonella* serovars isolated from blood samples of hospitalized subjects in Kano, North-West, Nigeria.

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Authors' contributions

This work was carried out in collaboration between all authors. Author M.A. conceived and designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors S.O.O., V.J.U and I.H.I. were the consultants and mentor gave professional advice and proof reading of final draft. All authors read and approved the final manuscript.

ABSTRACT

Aim: The aim of the study was to determine the antibacterial resistance profile and detect the presence of antibacterial resistance genes of *Salmonella* isolates recovered from the blood samples of hospitalized subjects in Kano metropolis.

Study design:

Place and duration of study: Three (3)-milliliters of venous blood were collected from each patient with some or all clinical features of salmonellosis that signed a consent form and transfer into EDTA bottles. If daily is unavoidable blood samples were stored at 4 °C. Samples were analyzed at both the Medical Laboratory of the authors Science Department, School of Health Technology Kano, Kano state, Nigeria, Department of Microbiology, Ahmadu Bello University, Zaria, Biotechnology center, 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* was performed by slide agglutination test using polyvalent (O) and (H) *Salmonella* antisera. Antibacterial drug susceptibility studies were performed by the disc diffusion method using ampicillin, chloramphenicol, ciprofloxacin, nalidixic acid and Trimethoprim-sulfamethoxazole. Out of one hundred and four (104) salmonellae isolates obtained in this study, twenty one (21) *Salmonella* isolates were subjected to DNA extraction and real-time and multiplex after determining their antibacterial drug resistance using disc diffusion test using standard protocol. Polymerase chain reaction (PCR) detection of Antibacterial drugs resistance genes of *Salmonella* isolates were carried out using various primer sets for various antibacterial drugs resistance genes of the five

~~(5) selected antibacterial drugs. Various primer sets were employed for targeting the specific sequences of the resistance genes using real-time and multiplex polymerase chain reaction (PCR) techniques as described by Asma [37].~~

Results: Of the 104 isolates, 96 (92.3%) were resistant to Ampicillin; 81 (77.9%) resisted to Nalidixic acid; 30 (30.8%) resisted to Chloramphenicol; 17 (16.3%) resisted to Cotrimazole; while none (1.0%) resisted to Ciprofloxacin. ~~Amongst the thirteen (13) Salmonella isolates tested by real-time PCR, technique had one or both tem and gyrB gene/s. Tem gene was detected with a higher frequency of (38.5%) compared to the gyrB gene with (30.8%). In addition, four (4) Salmonella isolates were found to harbour both tem and gyrB genes together with (30.8%). Similarly, from the PCR result no amplification of gyrA gene was obtained (0.0%). There was significant correlation between the presence of any of the two genes (tem and/or gyrB) and resistance to ampicillin and nalidixic acid. However, in this study, out of the eight (8) Salmonella isolates tested by multiplex PCR technique, sul2 gene was detected in two (2) isolates with a higher frequency of (25.0%) isolate and compared to catP gene was detected in one (1) isolate with (12.5%); while the amplification of these two genes failed in five (5) Salmonella isolates (62.5%) were found not to harbour any one of the two genes representing 62.5% and. No Salmonella isolate was found to harbour both sul2 and catP genes together.~~ There is no significant correlation between the presence of ~~any of the two genes (sul2 and/-catP)~~ and resistance to chloramphenicol and cotrimoxazole.

Conclusion: Most *Salmonella* serovars isolated from patients in Kano, Nigeria resisted to Ampicillin. They also resisted to Nalidixic acid, Chloramphenicol and Cotrimazole, in decreasing order. Ciprofloxacin remained effective against all the *Salmonella* isolates tested. Almost all the genes tested were circulating within Kano metropolis. ~~They including tem, gyrB, sul2 and catP genes with exception of gyrA. There was only significant correlation between the presence of any of the two genes (tem and/or gyrB) and resistance to ampicillin and nalidixic acid.~~

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Key words: *Salmonella* serovars; samples from blood; antibacterial resistance; Kano-Nigeria.

1. INTRODUCTION

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 [1]. Non-typhoidal salmonellosis is common in most parts of the world [2]. It is widely spread in Europe and North America [3, 4]; Latin America, the Middle East and Africa [5]; also in countries such as India [6], Japan [7] and the United States [8]. Several studies have documented isolation of non-typhoidal *Salmonella* from humans and poultry in different parts of Nigeria [9,10,11,12]. Outbreaks of salmonellosis caused by *Salmonella* Gallinarum, *S. Pullorum*, *S. Typhimurium* and *S. Enteritidis* have also been reported [10,12,13].

Increasing antibacterial drugs resistance in *Salmonella* species has been a serious problem for public health worldwide. In Nigeria, the increasing treatment failure with the empirical therapy in recent times among patients with salmonellosis necessitates the need for frequent assessment and reporting of antibacterial drugs resistance patterns of *Salmonella* serovar in our environment [14,15,16]. The high rate of resistance is hampering the use of conventional antibacterial drugs, and growing resistance to newer antibacterial drugs is aggravating the situation. The circumstances of occurrence and spread of antibacterial drug resistance is complex; however, a

Comment [D2]: Are they serovars? Or species?
For serovar, the right form is "Salmonella serovars Gallinarum, Pullorum, Typhimurium and Enteritidis"

major cause is the widespread use of antibacterial drugs in food animals, particularly in animal feed. Genetic analysis has indicated that the source of resistance is frequently a transferable plasmid [15,11].

However, majority of antibacterial drugs resistance studies on *Salmonella* in the study area are based on the use of isolates obtained from stool samples of patients with *Salmonella* infection admitted into hospitals. Recent studies indicate that antibacterial drugs resistance genes are a growing problem indicating the need to pay closer attention on the isolation of *Salmonella* in blood and community acquisition of multiple antibacterial drugs resistance in *Salmonella* [17,18].

With the emergence and continual increase in the multiple antibacterial drugs resistance, determination of antibiotic resistance along with timely diagnosis of *Salmonella* infection has become a matter of vital importance. Blood culture is the only method that is routinely used for determination of antibacterial drugs 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 [19,18]. Another major disadvantage is that its detection rate is only about 30%. Therefore, these necessitate the need for development of some effective method that cannot only diagnose typhoid fever and other *Salmonella* infection at an early stage but also provide information to enable the physician to start focused treatment from the onset of disease [20].

Molecular methods can provide a major breakthrough in this regard. Polymerase chain reaction (PCR) for rapid, sensitive and specific diagnosis of *Salmonella* infections has already been developed. Therefore, this necessitate the used of PCR for targeting specific genes such as *gyrA*, *gyrB*, *tem*, *sul2* and *catP* genes in *Salmonella* isolates that can provide useful information about its genetic resistance in the study area and Nigeria at large [21].

2. MATERIALS AND METHODS

2.2 Hospitals

The six (6) most patronized hospitals were randomly selected: including 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.

2.3 Specimens

Blood (3ml) collected from each patient diagnosed positive for *Salmonella* infections in Kano, North West, Nigeria were used as samples for the study [22].

2.4 Test organisms

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

Comment [D3]: Where is the section 2.1 ?

Comment [D4]: This translation of number is really not necessary, please eliminate these through the manuscript

Comment [D5]: The method? How the blood was sampled? In what kind container? How the samples were transported to the lab? Were the samples directly used for subsequent analysis? If not, how thw samples were stored? For how long?

Comment [D6]: Why here you need a reference?

Comment [D7]: This part is not necessary.

2.5 Patients

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) recruited to signed the consent form were used 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 recruited to signed the consent form were used for the study.

Comment [D8]: This part should be combined with the section of Specimens

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 [22].

Comment [D9]: How did you inoculated and incubated the plates? Time and temperature? Aerobically or anaerobically? Did you inoculate directly with the blood sample? How many mL you used for inoculation? By spread or by striking?

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.

Comment [D10]: What methods you used for getting pure culture? In plates or tube? Temperature and time for incubation? How do you know a isolate is pure? After purification, how did you store your isolates?

2.5.3; Biochemical characterization of *Salmonella*

Isolation and identification of organisms was ~~were~~ carried out as described by ISO [23], Habtamu *et al.* [13], and OIE [24]. A 24 h pure culture of each isolate was used to determine their ~~g~~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.

Comment [D11]: Please give the medium, incubation temperature and time.

2.6 Sero-typing of the 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 [25] by slide agglutination test.

Comment [D12]: Please give the preparation procedure, including incubation condition and time, medium, etc. How the cells were collected? Did you wash the cells? Did you justified the cell concentration? How did you observed the results?

2.7 Test of antibacterial sensitivity of the *Salmonella* isolates.

In-vitro susceptibility of *Salmonella* isolates to various routine antimicrobial drugs was tested by the standard disc diffusion technique [1].

Comment [D13]: Please give the medium, incubation temperature and time. How did you read the results or define a isolate is resistant?

2.7.1 Standardization of inoculum

This was done as described by CLSI [26]. 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

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 antibacterial drug discs [26].

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 antibacterial drugs by agar diffusion technique as described by Kirby-Bauer [27] and WHO [28]. This was carried out according to WHO protocol [29]. The susceptibility testing of *Salmonella* isolates were carried out using Mueller Hinton agar and were tested *in vitro* for susceptibility to five (5) different antibacterial drugs namely; ampicillin (25µ), chloramphenicol (30µ), ciprofloxacin (25µ), nalidixic acid (30µ) and Trimethoprim-sulfamethoxazole (30µ) [39].

2.8 Isolation of genomic DNA of *Salmonellae*

Out of one hundred and four (104) salmonellae isolated in this study, twenty one (21) *Salmonella* isolates were subjected to DNA extraction after determining their antibacterial drug resistance using disc diffusion test using standard protocol [19, 21]. The twenty one (21) isolates used comprises of seven (7) *Salmonella* Typhimurium, six (6) *Salmonella* Typhi A, three (3) *Salmonella* Paratyphi B and C each and two (2) *Salmonella* Paratyphi A.

2.9 Polymerase Chain Reaction (PCR) Detection of Antibacterial drug Resistance Genes of *Salmonella* Isolates by Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) ~~detection of antibacterial drugs resistance genes of *Salmonella* isolates were was~~ carried out using various primer sets ~~to detect the for various~~ antibacterial drugs resistance genes ~~of against to~~ the five (5) selected antibacterial drugs. ~~Various primer sets were employed for targeting specific sequences of the resistance genes using The~~ real-time and multiplex polymerase chain reaction (PCR) techniques ~~were employed~~ as described by Asma [21]. ~~if present in the DNA of the twenty one (21) *Salmonella* isolates, conditions were optimized for amplification of each sequence using primer pairs.~~ The primers used in the study include: *sul2*¹⁶ F 5'-TCA ACA TAA CCT CGG ACA GT-3' and *sul2*¹⁶ R 5'-GAT GAA GTC AGC TCC ACC T-3' for trimethoprim-sulfamethoxazole ~~resistant gene~~; *tem*⁶ F 5'-GCA CGA GTG GGT TAC ATC GA-3' and *tem*⁶ R 5'-GGT CCT CCG ATC GTT GTC AG-3' for ampicillin ~~resistant gene~~; and *catP*⁴ F 5'-CCT GCC ACT CAT CGC AGT-3' and *catP* R 5'-CCA CCG TTG ATA TAT CCC-3' for chloramphenicol ~~resistant gene~~; *gyrA*¹¹ F 5'-TAC CGT CAT AGT TAT CCA CGA-3' and *gyrA*¹¹ R 5'-GTA CTT TAC GCC ATG AAC GT-3' for ciprofloxacin ~~resistant gene~~, and *gyrB*¹² F 5'-GCGCTGTCCGAACGTACCT-3' and *gyrB*¹² R 5'-TGATCAGCGTCGCCACTTCC-3' for nalidixic acid ~~resistant gene~~ [30, 21].

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Comment [D15]: Please give the full gene name.

2.10 Statistical analysis of results

Statistical Package for Social Science (SPSS) version 14 was used [31]. 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

Bacterial isolation. Please add the isolation results: how many specimens were samples from each hospital? How many bacterial isolates were obtained from each hospital? The % of *Salmonella* positive specimens, etc.

***Salmonella* identification by biochemical characterization.** Please give the results of biochemical characterization and identification results based upon these characters.

Serotyping of the *Salmonella* isolates. Please move the serotyping results here!

The result of *in vitro* —antibacterial drug susceptibility testing ~~of *Salmonella* isolates using disc diffusion test~~ demonstrated that of the five (5) antibacterial drugs tested, most of the 104 isolates (92.3%) was significantly ($P > 0.05$) resistant to ampicillin. They were also resistant to nalidixic acid, chloramphenicol, and cotrimoxazole in decreasing order with 77.9%, 30.8%, and 16.3% resistance rate respectively. Ciprofloxacin remained effective against all the *Salmonella* isolates tested with exception of one (1) *Salmonella* isolate representing 1.0% resistance rate (Table 1).

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Table 1: Disc diffusion test on *Salmonella* serovars isolated from blood samples of hospitalized patients in Kano metropolis, Nigeria.

Antibacterial drugs	Disc potency (µg)	No. and % of Resistant Isolates n= 104

AMP	25	96(92.3)
CH	30	32(30.8)
COT	25	17(16.3)
CPX	30	1(1.0)
NA	30	81(77.9)

$$\chi^2 = 11.03 \text{ } P\text{-value} = 0.001$$

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

From the result of disc diffusion test, Out of 104 *Salmonella* isolates tested, 36 *Salmonella* Typhi, 33 *Salmonella* Typhimurium, 13 *Salmonella* Paratyphi C and 5 *Salmonella* Paratyphi A were found to display a high resistant to ampicillin. In contrast, all *Salmonella* Paratyphi B tested isolates were resistant to nalidixic acid with (11 resistant isolates/strains representing 100%) followed by ampicillin with (10 resistant strains/isolates representing 90.9%) resistance rate respectively. However, only one *Salmonella* Typhi isolate was found to be resistant to ciprofloxacin. The relationship between *Salmonella* serovars and antibacterial drugs tested was not statistically significant ($P > 0.05$) (Table 2).

Table 2: Antibacterial drugs resistance profile of *Salmonella* serovars isolated from blood of patients presented to hospitals in Kano Nigeria.

Antibacterial drugs / Disc potency (μ g)	Salmonella serovar (No. tested)				
	S. Paratyphi A (n=5)	S. Paratyphi B (n=11)	S. Paratyphi C (n=14)	S. Typhi (n=39)	S. Typhimurium (n=35)
AMP(25)	5(100)	10(90.9)	13(92.9)	36(92.3)	33(94.3)
CH(30)	2(40.0)	3(27.3)	4(28.6)	14(35.9)	9(25.7)
COT(25)	1(20.0)	3(27.3)	1(7.1)	5(12.8)	7(20.0)
CPX(30)	0(0.0)	0(0.0)	0(0.0)	1(2.6)	0(0.0)
NA(30)	4(80.0)	11(100)	12(85.8)	32(82.0)	21(60.0)

No. = Number; n = Number of isolates tested; S = *Salmonella*; % = Percent; μ g = microgram;

AMP = Ampicillin; CH = Chloramphenicol; CPX = Ciprofloxacin; COT = Cotrimoxazole;
NA = Nalidixic Acid; % = Percentage of total number of each *Salmonella* serovar tested.

Detection of resistant genes. The results of the determination of antibacterial drugs resistance by targeting specific genes in *Salmonella* serovars tested by real-time PCR technique were shown in Fig. 1 plates Ia, Ib and Ic. The conditions were optimized for amplification of *gyrA*, *gyrB* and *tem* genes. In this study, no *gyrA* gene amplicon was obtained, although only one *Salmonella* Typhi strain was resistant to ciprofloxacin phenotypically. However, from the PCR result there was no *gyrA* gene amplicon obtained (plate Ia). Meanwhile, From plate Ib *gyrB* genes (315bp) were amplified out of thirteen (13) *Salmonella* isolates tested, eight (8) amplicons of *gyrB* genes (315bp) were amplified from 8 of the 13 *Salmonella* isolates (Fig. Ib), and Out of thirteen (13) *Salmonella* isolates tested, nine (9) amplicons of *tem* genes (311bp) were amplified from 9 of the 13 isolates (plate Fig. Ic).

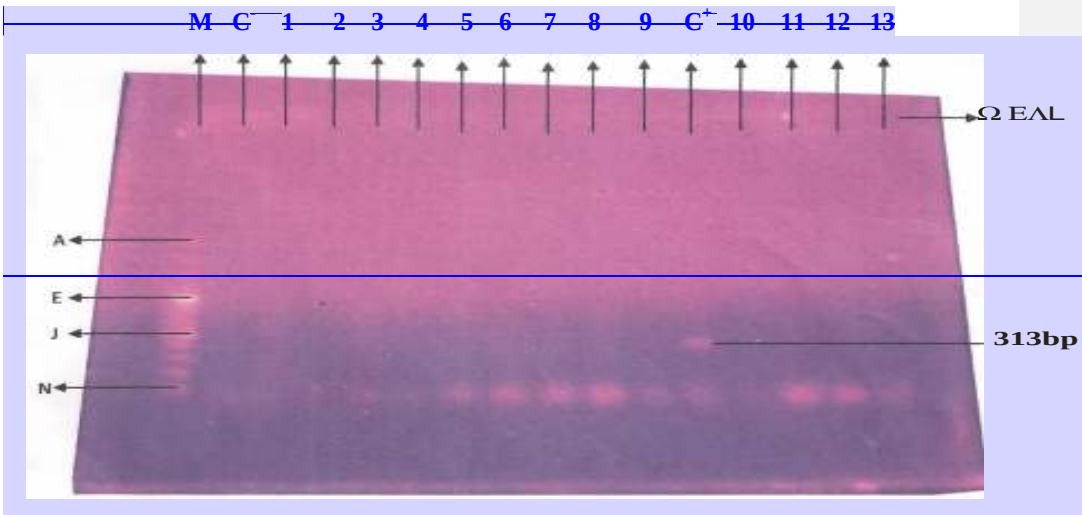


Plate Ia: Gel electrophoresis of *gyrA* gene after Real time PCR test.

KEY: M= 1000bp DNA marker (A, E, J and N); C⁺ = Positive control; C⁻ = Negative control;
bp = Base pair.

Comment [D17]: This figure can be deleted.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 C⁺ C⁻

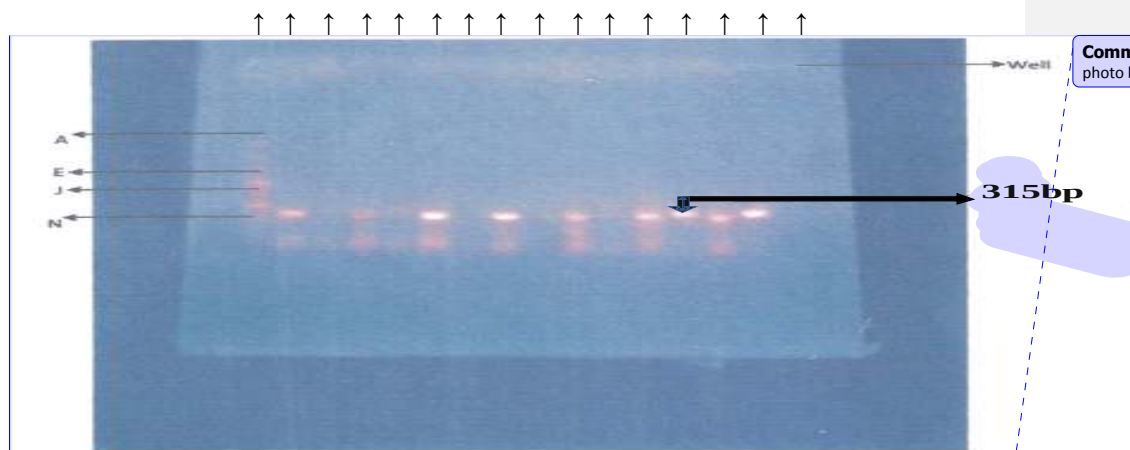


Plate Fig. Ib: Gel electrophoresis of *gyrB* gene after Real-time PCR test.

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KEY: M= 1000bp DNA marker (A, E, J and N); C⁺ = Positive control; C⁻ = Negative control;
bp = Base pair.

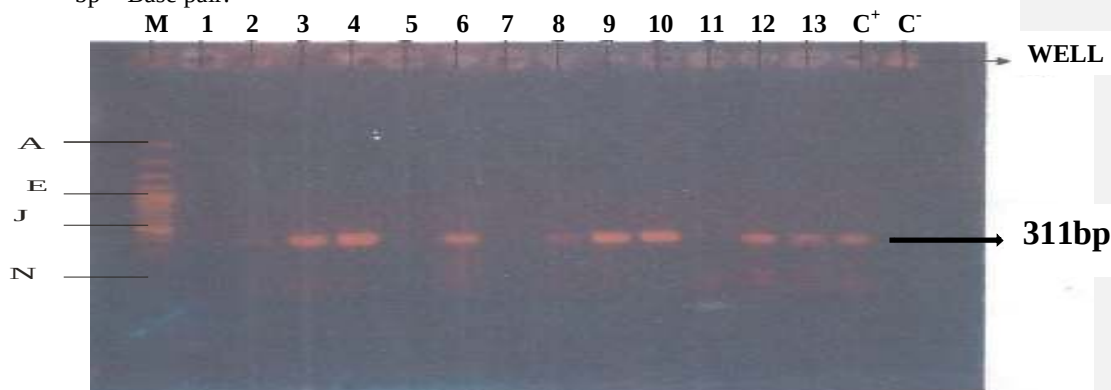
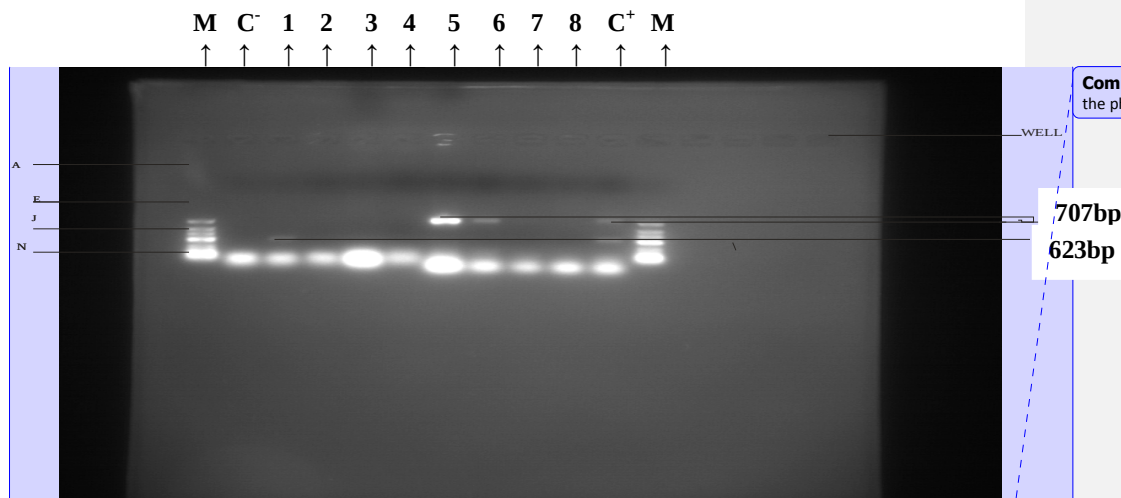


Plate Ic: Gel electrophoresis of *tem* gene after Real-time PCR test.

KEY: M= 1000bp DNA marker (A, E, J and N); C⁺ = Positive control; C⁻ = Negative control;
bp = Base pair.

Fig. 2 presented Plate II revealed the results of multiple PCR determination of antibacterial drug resistance by targeting to the specific resistance genes in *Salmonella* serovars tested by multiplex PCR technique. The conditions were optimized for amplification of *sul2* and *catP* genes. Out of the eight (8) *Salmonella* isolates tested, two (2) amplicons of *sul2* genes (707bp) in two isolates and one (1) amplicon of *catP* (623bp) in one were amplified.



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Plate H Fig. 2: Gel electrophoresis of *catP* and *sul2* genes after Multiplex PCR test.

KEY: M= 1000bp DNA marker (A, E, J and N); C⁺= Positive control; C⁻ = Negative control; bp = Base pair.

Summarily, The study revealed that, all the thirteen (13) *Salmonella* isolates tested by real time PCR technique had one or both *tem* and *gyrB* gene/s were detected in some of the *Salmonella* isolates. *Tem* gene has a higher frequency of (5 isolates, 38.5%) compared to *gyrB* gene with (4 isolates, 30.8%). In addition, all the four (4) *Salmonella* isolates were found to harbouring both *tem* and the *gyrB* genes also presented the *tem* gene together with 30.8%. Similarly, there was Only one *Salmonella* Typhi strain was resistant to ciprofloxacin phenotypically, but ; from the PCR result no amplification of the corresponding gene *gyrA* gene was not detected obtained (0.0%). There was significant correlation (r=?) between the presence of any of the two genes (*tem* and/or *gyrB*) and resistance to ampicillin and nalidixic acid (Table 3).

Comment [D21]: These two sentences are contradictory. 58.5% and 30.5% and not "all the thirteen isolates".

Comment [D22]: When you say "significant correlation", you should give the data.

Table 3: The distribution of *gyrA*, *gyrB* and *tem* genes in the various *Salmonella* serovars tested.

Antibacterial Resistance genes	No. Detected	% Detected
<i>gyrA</i>	0	0.0
<i>gyrB</i>	4	30.8
<i>tem</i>	5	38.5

<i>gyrB</i> & <i>tem</i>	4	30.8
No gene	0	0.0
Total	13	100

KEY: No. = Number; % = Percentage of total number of isolates tested (13).

In this study, out of the eight (8) *Salmonella* isolates tested by multiplex PCR technique, for the detection of antibiotic resistance genes, *sul2* gene was detected in two isolates a higher frequency of (25.0%) and compared to *catP* gene was detected in another isolate with (12.5%). Five (5) *Salmonella* isolates (62.5%) were found did not present to harbour any one of the two genes representing 62.5%. No *Salmonella* isolate was found to harbour both *sul2* and *catP* genes together. There is no significant correlation between the presence of any of the two genes (*sul2* and *catP*) and resistance to chloramphenicol and cotrimoxazole (Table 4).

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Comment [D24]: If this results are true, why you think these two genes are specific for the resistance of these antibiotics? This should be discussed latter.

Table 4: The distribution of *sul2* and *catP* genes in the various *Salmonella* serovars tested.

Antibacterial Resistance genes	No. Detected	% Detected
<i>sul2</i>	2	25.0
<i>catP</i>	1	12.5
Both genes	0	0.0
No gene	5	62.5
Total	8	100

KEY: No. = Number; % = Percentage of total number of isolates tested (8).

Comment [D25]: This table should be deleted and all the results have been presented in text.

Discussion

In this study ~~out of the five (5) antibacterial drugs tested using disc diffusion test~~, most of ~~the~~ *Salmonella* ~~serovars~~ isolated from patients in Kano, were significantly resistant to ampicillin ($P < 0.05$) with 96 resistant strains representing 92.3% of the *Salmonella* isolates ~~displaying the highest level of resistance~~, followed by nalidixic acid with 81 resistant strains representing 77.9%. *Salmonella* Typhi, *Salmonella* Typhimurium, *Salmonella* Paratyphi C and *Salmonella* Paratyphi A were found to display a high resistant to ampicillin. In contrast, all *Salmonella* Paratyphi B tested were resistant to nalidixic acid with 11 resistant strains representing 100%. Ciprofloxacin remained effective against all the *Salmonella* isolates tested with exception of one (1) *Salmonella* Typhi isolate representing 1.0% resistance rate. However, the relationship between *Salmonella* serovars and antibacterial drugs tested was not statistically significant ($p > 0.05$). This work is in consonance with the findings of Asma *et al.* [21], Hemalatha *et al.* [32], Malla *et al.* [33]; Gautum *et al.* [34] and Abdullahi [35].

The highest significant resistance ($P < 0.05$) of *Salmonella* isolates to ampicillin and nalidixic acid could probably be due to ~~the usage of~~ antibacterial drugs ~~usage~~ 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 [32, 10]. However, the rate of development of resistance appears to have accelerated in the past decade and today multiple resistant *Salmonella* constitute a global problem [36]. It has been observed that antibacterial drugs 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 bacterial pathogens for their antibacterial drug susceptibility profiles in different communities. There is strong evidence that the use of antibacterial drugs 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 antibacterial drugs in the field of veterinary medicine have resulted in the development of increasing number of bacterial strains possessing resistance to many antibacterial drugs. The property of multiple antibacterial drugs 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 antibacterial drugs. 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 antibacterial drugs, abuse and misuse of those antibacterial drugs found commonly in circulation among the general populace and health resources centers [36, 34].

Abdullahi [35] reported that acquired antibacterial drugs 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, antibacterial drugs can be obtained outside of recognized treatment centres, and taken without medical authorization or supervision. This leads to inappropriate use of antibacterial drugs and their being taken at sub-optimal dosages and for an insufficient length of time. Often the high cost of an antibacterial drug, results in an incomplete course being purchased, sufficient only to alleviate symptoms. Patients are not sufficiently informed about antibacterial drugs and their use [22, 35]. Problems also arise when antibacterial drugs sold in local markets are sub-

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sal2 *eatP* BOTH GENES NO GENE

standard or expired antibiotics. Guidelines regarding the selection of antibacterial drugs, correct prescription, and information about antibacterial drug resistance and how to minimize its spread are not communicated to those purchasing the antimicrobials. Antibacterial drugs are often prescribed when they are not needed or for self-limiting infections, e.g. diarrhoeal disease and viral respiratory infections [22, 35].

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 [37].

Broad spectrum antibacterial drugs are frequently used prophylactically, e.g. tetracycline. Laboratory facilities for accurate diagnosis and isolation of pathogens are often not available, resulting in an overuse and inappropriate use of antibacterial drugs [38]. Many countries do not have effective surveillance of important antibacterial drug-resistant bacteria. Training and facilities for performing standardized antibacterial drugs sensitivity tests are often lacking. Developing countries are often unable to afford costly second-line antibacterial drugs 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 [22].

The study presents genotypic identification of *gyrA*, *gyrB*, *tem*, *sul2* and *catP* genes among the 21 *Salmonella* isolates that were mostly resistant to ciprofloxacin, nalidixic acid, ampicillin, cotrimoxazole and chloramphenicol using the disc diffusion test. The genes responsible for antibacterial drug resistance were identified to be *tem* (ampicillin), *gyrB* (nalidixic acid), *catP* (chloramphenicol), and *sul2* (co-trimoxazole). It is noticeable that only 1(1.0%) *Salmonella* isolate was found resistant to ciprofloxacin; no amplicon of specific gene (*gyrA*) for ciprofloxacin resistance was obtained. In addition, four (4) *Salmonella* isolates were found to harbour both *tem* and *gyrB* genes together. There was a significant correlation between the presence of any of the two genes and resistance to ampicillin and nalidixic acid. These findings are in line with the works of Asma *et al.* [21] and Haque *et al.* [19].

Among the clinically and economically important antibacterial drugs resistance genes are those of *tem* and *gyrB* producing high level resistance to ampicillin and nalidixic acid, the most widely used antibacterial drugs in clinical and veterinary practices. The reservoir of resistant bacteria in food animals implies a potential risk for transfer of resistant bacteria, or resistance genes from food animals to humans [39, 40]. However, observed in this study diverse point mutations in the *tem* gene have contributed to the emergence of *tem*-type *Salmonella*, resulting in simultaneous high resistance to ampicillin [17]. Even though, nalidixic acid and ciprofloxacin are from the same group (quinolones), *Salmonella* isolates showed higher frequency of resistance to commonly use antibacterial drugs (nalidixic acid) than ciprofloxacin. This is presumably due to the readily available of and easy public access to nalidixic acid without proper medical prescription [41].

In addition, Reports have indicated that mutations in the QRDRs of the DNA gyrase genes can be found in *Salmonella* isolates expressing reduced susceptibility to fluoroquinolones [42,15]. This fact is possible since to acquire new properties bacteria must undergo a genetic change. Such a genetic change may occur by mutation or by the acquisition of new genetic materials

acquired by the transfer of resistance genes located in plasmids or transposons from one bacterium to another [22].

Five (5) of isolates did not contain any one of *sul2* or *catP*. Guerra *et al.* [43] also had similar experience with this study. Resistance to cotrimoxazole was exclusively mediated by *sul2* [43]. Very few *catP* genes have been detected previously in *Salmonella* isolates, but *catP* genes are also widespread among other Gram-negative bacteria [43].

The study also revealed that, some phenotypic resistance observed by disc method was seen to be genetically confirmed by the PCR. While some isolates showed antibacterial drug resistance phenotypically but not genetically. The discordance between phenotypes and genotypes in this study is not surprising, since several genes may confer a given phenotype and some isolates must have contained other genes that were not tested for in this study. Other reasons could be due to inoculum effect and substrate specificity which may affect the enzyme in an un-induced state at the time of testing with disc diffusion test. This creates a major challenge in laboratory routine susceptibility [19,44].

5. Conclusion and Recommendation

The result of *in vitro* – antibacterial drug susceptibility testing of *Salmonella* isolates using disc diffusion test demonstrated that ~~of the five (5) antibacterial drugs tested,~~ most of the isolates (92.3%) was significantly ($P > 0.05$) resistant to ampicillin followed by nalidixic acid, chloramphenicol, and cotrimoxazole in decreasing order. *Salmonella* Typhi, *Salmonella* Typhimurium, *Salmonella* Paratyphi C and *Salmonella* Paratyphi A were found to display a high resistant to ampicillin. In contrast, all *Salmonella* Paratyphi B tested were resistant to nalidixic acid with 11 resistant strains representing 100%. However, ciprofloxacin remained effective against all the *Salmonella* isolates tested with exception of one (1) *Salmonella* Typhi representing 1.0% resistance rate. However, the relationship between *Salmonella* serovars and antibiotics tested was not statistically significant ($P > 0.05$).

Almost all the genes tested were circulating within Kano metropolis. They included *tem*, *gyrB*, *sul2* and *catP* genes with exception of *gyrA*. There was only significant correlation between the presence of any of the two genes (*tem*, *gyrB*) and resistance to ampicillin and nalidixic acid.

Comment [D27]: Then, why you believe that these genes are related to the resistance?

~~One of the means of overcoming the resistance problem in salmonellae is to discontinue the overuse and misuse of antibacterial drugs 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 [28].~~

Comment [D28]: This conclusion has no relationship with your results

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 [45,46].

Comment [D29]: These are not conclusion related to your results. Also, no references are needed for conclusion.

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We thank all staff of the Management and Staff of Medical Laboratory Science Department, School of Health Technology, Kano, Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Biotechnology center Ahmadu Bello University Zaria, Kaduna State and Management and staff of all the six (6) selected Hospitals.

COMPETING INTERESTS

The authors declared that they have no competing interests exist.

REFERENCES:

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1. Ellaine S, Robert MH, Frederick JA, Robert VT, Marc-Alain W, Sharon LR *et al.* Emerging Infectious Disease. 2011;17(1).
2. Wilson JS, Hazel MS, Williams JN, Phiri A, French PN, Hart CA. Non-typhoid Salmonellae in United Kingdom Badgers: Prevalence and Spatial Distribution. *Applied Environmental Microbiology*. 2003; 69: 4312-4315.
3. Ling JM, Chan EW, Cheng AF. Molecular epidemiological analysis of *Salmonella* Enterica serotype Derby infections in Hong Kong. *Journal of Infection*. 2001; 42(2):145-153.
4. Wright JG, Tengelsen LA, Smith KE, Bender JB, Frank PK, Grendon JH, Rice DH, Thiessen AM, Gilbertson CJ, Sivapalasigam S, Barret TJ, Besser TE, Hancock DD, Angulo FJ. multidrug-resistance *Salmonella* Typhimurium in four animals' facilities. *Emerging Infectious Disease*. 2005;11(8):1235-1241.
5. Kariuki S, Revathi G, Kariuki N, Kiiru J, Mwituria J, Hart CW. Characterization of community acquired nontyphoidal *Salmonella* from bacteraemia and diarrhoeal infection in children admitted to hospital in Nairobi, Kenya. *BMC Microbiol*. 2006; 6(101):1-10.
6. Sahai S, Mahadevan S, Srinivasan S, Kanungo R. Childhood bacterial meningitis in Pondicherry, South India. *Indian Journal of Pediatrics*. 2001; 68:839-841.
7. Arai J, Tanabe Y, Miyake M. Acute encephalopathy associated with non-typhoid salmonellosis. *J. Child Neurol*. 2001;16:539-540.
8. Voetsch AC, Van Gilder TJ, Angulo FJ, Farley MM, Shallow S, Marcus R, Cieslak PR, Deneen VC, Tauxe RV. Food Net estimate of the burden of illness caused by non-typhoid *Salmonella* infections in the United States. *CID*. 2004; 127-134.
9. Muhammed M, Muhammed LU, Ambali AG, Mani AU, Azaad S, Barco L. Prevalence of *Salmonella* associated with chick mortality at hatching and their susceptibility to antimicrobial agents, *Veterinary Microbiology*. 2010;140:131-135.
10. Enabulele AS, Amune PO, Aborisade WT. Antibigrams of *Salmonella* isolates from poultry farms in Ovia North East local government area, Edo State, Nigeria. *Agriculture and Biology Journal of North America*. 2010;1(6):1287-1290.
11. Agbaje M, Davies R, Oyekunle MA, Ojo OE, Fasina FO, Akinduti PA. Observation on the occurrence and transmission pattern of *Salmonella* Gallinarum in commercial poultry

farms in Ogun State, South Western Nigeria. African Journal Microbiological Research. 2010;4(9):796-800.

12. Fasure AK, Deji-AgboolaAM, Akinyemi KO. Antimicrobial resistance patterns and Emerging fluoroquinolone resistant *Salmonella* isolates from poultry and asymptomatic poultry workers. African Journal of Microbiology Research.2012;6(11):2610-2615.

13. Habtamu MT, Rathore R, Dhama K, Rajesh KA. Isolation, Identification and Polymerase Chain Reaction (PCR).Detection of *Salmonella* species from materials ofPoultry origin. International Journal of Microbiological Research. 2011;(2):135-142.

14. Kruttgen, A., Razavi, S., Imohl, M. and Ritter, K. Real-time PCR assay and a synthetic positive control for the rapid and sensitive detection of the emerging resistance gene New Delhi *Metallo- β -lactamase-1* (bla (NDM-1)). Medical Microbiology and Immunology. 2011; **200**:137-141.

15. Lin-Hui Su, Cheng-Hsun Chiu, Chishih Chu, and Jonathan T. Ou. Antimicrobial Resistance in Nontyphoid *Salmonella* Serotypes: A Global Challenge. Clinical Infectious Diseases, 2004. **39**:546–551.

16. Kapil, A., Sood, S., Dash, N. R., Das, B. K. and Seth, P. Ciprofloxacin in typhoid fever. Lancet, 2009; Pp. 354- 156.

17. Okeke, N.I., Aboderin, O.A. andByarugaba, D. K. Journal of Emergent Infectious Diseases, 2007; **13(11)**: 1640-1646.

18. Shekhar, P., Rajat, P., Deepak, J., Neelam, S.Amit, R., and Sandeep, N. The baseline widal titre among the healthy individuals of the hilly areas in the Garhwal region of Uttarakh and Indian Journal of Clinical and Diagnosis Research. 2013; **7(3)**: 437–440.

19. Haque, A., Ahmed, I. and Qurcsbi, H. Early detection of typhoid by polymerase chain reaction. Annual Saudi Medicine, 2001; **19**: 337-340.

20. Poirel, L., Lagrutta, E., Taylor, P., Pham, J. and Nordmann, P. Emergence of *metallo- β -lactamase* NDM-1-producing multiple antibiotic-resistant *Escherichia coli* in Antimicrobiol Agents Chemotherapy, 2011; **4**: 4914-4916.

21. Asma, H., Abdul, H., Yasra, S., Aamir, A., Saira, B., Ayesha, T. and Mushkoor, M.. Identification of Drug resistance genes in clinical isolates of *Salmonella* Typhi for development of diagnostic multiplex polymerase chain reaction. Pakistan Journal of Medical Science, 2005; **21 (4)**: 402-407.

22. Cheesbrough, M. District laboratory practice in tropical countries, ECBS edition Combridge University Press, 2005; **2**: 182-187.

23. International Organization of Standardization (ISO) 6579. Microbiology general guidelines on methods for the detection of *Salmonella*. International organization of standardization, Geneva, Switzerland; 2002.

24. Office International des Epizooties (OIE). Fowl typhoid and Pullorum disease. In:Terrestrial manual. Office International des Epizooties, Paris, France.2012;3-5.

25. Kauffmann F. Serological diagnosis of *Salmonella* species, Kauffmann White Scheme Minkagarord, Copenhagen, Denmark;2008; 1974.

26. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. CLSI document M100-S20. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.

27. Kirby – Bauer, O. S. Antimicrobial sensitivity testing by agar diffusion method. American Medical Journal of Clinical Pathology, 2008; **44**: 493-493.

28. WHO. Manual for laboratory investigations of acute enteric infections.2004; WHO.

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CDO/83.

29. World Health Organization (WHO). Traditional Medicine. In: Aliyu M. and Chedi B. A. Z. (editors). Effects of the ethanolic stem bark extract of *Pterocarpus Erinaceus* (Fabaceae) on some isolated smooth muscles. *Bayero Journal of Pure and Applied Sciences*, 2008; **3(1)**: 34-38.
30. Eaves, D.J., Randall, L., Gray, D.T., Buckley, A., Woodward, M.J., White, A.P., et al. Prevalence of mutations within the quinolone resistance determining region of *gyrA*, *gyrB*, *parC*, and *parE* and association with antibiotic resistance in quinolone-resistant *Salmonella* Enterica. *Antimicrob Agents Chemotherapy*, 2004; **48**: 4012–4015.
31. Robert, H.C. and Jane, G.N.. Doing data analysis with Statistical Package for Social Science (SPSS) version 14.2005; Pp 344.
32. Hemalatha R., Vijayalakshmi P., Gyaneshwari, Rao M.V., Ramani A. Multidrug resistant *Salmonella* typhi in Hyderabad. *Indian Journal of Medical Microbiology*.2008;**17**: 39–41.
33. Malla, S., Kansaakar, P., Serichantalerg, S. *Journal of Nepal Medical Association*, 2008; **44**: 18-22.
34. Gautam, V., Gupta, N. K., Choudhary, U., Arora, D. R. Sensitivity Pattern of *Salmonella* serotypes in Northern India. *Brazilian Journal of Infectious Diseases*, 2008; **6**: 281-287
35. Abdullahi, M. Incidence and antimicrobial susceptibility pattern of *Salmonella* species in children attending some hospitals in Kano metropolis, Kano state, Nigeria. *Bayero Journal of Pure and Applied Sciences*, 2010; **3(1)**: 10 –15.
36. Pruden, A., Pei, R., Storteboom, H., et al. *Environmental Science and Technology*. 2006; **40**: 445-7450.
37. Guha, S., Jalan, B. Y., Dey, S., Easow, J. M., Wilson, G. and Shivananda, P. G. *Salmonella* bacteraemia in Pokhara: emergence of antibiotic resistance. *Nepal Medical College's Journal*, 2005; **7**: 23–25.
38. Kumurya, A. S. and Kawo, A. H. Quality assessment of some paediatric cotrimoxazole oral suspensions marketed in metropolitan Kano, Nigeria. *Biological and Environmental Sciences Journal for the Tropics*, 2010; **7(1)**: 26.
39. Heuer, O.E., Jensen, V.F., Hammerum, A.M. Antimicrobial antibiotic consumption in companion animals. *Emerging Infectious Disease*, 2005; **11(2)**: 344-345.
40. Gall, S. L., Desbordes, L., Gracieux, P., Saffroy, S., Bousarghin, L., Bonnaure-Mallet, M. and Jolivet-Gougeon, A. Distribution of mutation frequencies among *Salmonella* Enterica isolates from animal and human sources and genetic characterization of a *Salmonella* Heidelberg hypermutator. *Veterinary Microbiology*, 2009; **137**: 306-312.
41. Akinyemi, K. O., Smith, S. I., Oyefolu, A. O. and Coker, A. O. Multidrug resistance In *Salmonella* Typhi isolated from patients with typhoid fever complications in Lagos, Nigeria. *Journal of Public Health*, 2005; **119**: 321-327.
42. Cloeckaert, A., Chaslus-Dancla, E. Mechanisms of quinolone resistance in *Salmonella*. *Veterinary Research Journal*, 2001; **32**:291–300.
43. Guerra, B., Soto, S., Helmuth, R. et al. Characterization of a self-transferable plasmid from *Salmonella* Enterica serotype Typhimurium clinical isolates carrying two integron-borne gene cassettes together with virulence and antibiotic resistance genes. *Antimicrobial Agents and Chemotherapy*, 2002; **46**: 2977–81.
44. Nizami, T. Identification of flagellar or *ViaB* gene for PCR detection of *Salmonella enteric* serovar Typhi. *Diagnostics Microbiology of Infectious Diseases*, 2006; **62(2)**: 142-150.
45. Agada G. O. A., I. O. Abdullahi, M. Aminu, M. Odugbo, S. C. Chollom, P. R. Kumbish and E. J. Okwori. Prevalence and Antibiotic Resistance Profile of *Salmonella* Isolates from

commercial Poultry and Poultry Farm-handlers in Jos, Plateau State, Nigeria. British Microbiology Research Journal. 2014; **4(4)**: 462-479.

46. Akinyemi KO, Phillip W, Beyer W, Bohm R. *In-vitro* antimicrobial susceptibility Patterns of *Salmonella* Enterica serovars and emergence of *Salmonella* phage type DT071 in a suspected community-associated outbreak in Lagos, Nigeria. Journal of Infection in Developing Countries. 2007; 1(1):48-54.

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.

Consent of each patient was obtained through the consent form designed and distributed.

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NIHREC/21/08/2008a/AKTH/EC/216
OUR REF: AKTH/MAC/SUB/12^A/P3/IV/767 DATE: 30th April 2010

Dr. Abdullahi Ma'saud
Department of Microbiology

Through:
The Head of Department
Department of Microbiology
Ahmadu Bello University Teaching Hospital,
Zaria,
Kaduna State.

RE: ETHICAL APPROVAL

**RE: SEROLOGICAL AND PCR CHARACTERIZATION OF SALMONELLAE
AGENTS OF INVASIVE SALMONELLOSIS IN KANO METROPOLIS**

Refer to your application and your subsequent response to the Committee in respect of the above research proposal. The Committee considered your proposal and noted same as a prospective study.

In view of this, Ethical approval is hereby granted to conduct the research.

However, the proposal is subject to periodic reporting of the progress of the study.

Best regards,


Bara'atu Kabir (Mrs)
Secretary
For: Chairman, Ethical Committee
Email: akthurb@hotmail.com



KANO STATE
HOSPITALS MANAGEMENT BOARD
BOARD HEADQUARTERS
P.M.B 3540, Post Office Road, Kano

HMB/GEN/488/I/42

28/05/2010 (14/06/1431 AH)

Abdullahi Masa'ud,
Department of Microbiology,
Ahmadu Bello University, Zaria,
Kaduna State.

PROVISIONAL ETHICAL CLEARANCE.

Sequel to your application to conduct research on "Characterization, susceptibility studies and PCR detection of salmonella sereous isolated from blood samples of patients in Kano Metropolitan" and your subsequent appearance before the Hospitals Management Board's Ethical Committee, I am under instruction to inform you that provisional Ethical Clearance has been granted to conduct your research on the aforementioned topic based on the following condition:-

- . That informed consent must be obtained from all participant/client.
- . That all participant/client or patient must have a copy of duly completed and endorsed indorsed consent form.
- . That the blood sample should be collected by a well trained Medical Lab. Scientist of the respective insitution.
- . You are to submit one copy of the findings to Ethical Committee after completion for final approval, please.

Best regards,

ABDULLAHI TUKUR
PRIN. ASSISTANT SECRETARY
FOR: DIRECTOR GENERAL.



KANO STATE
HOSPITALS MANAGEMENT BOARD
BOARD HEADQUARTERS
P.M.B. 3548, POST OFFICE ROAD, KANO

HMB/GEN/488/1

21/7/2010 (10/8/1431AH)

The Chief Medical Directors,

✓ ~~MAWSH, MAWSH, WSGGH and SMSSH,~~
Kano.

INTRODUCTORY LETTER IN RESPECT OF:
ABDULLAHI MAS'UD (STUDENT)

I am directed to introduce the above named student from Department of microbiology, Ahmadu Bello University Zaria, who is to conduct a research on "Characterization, susceptibility studies and PCR detection of salmonella sereous isolated from blood sample of patients in Kano metropolitan".

In view of the above approval has been granted to him to conduct the research, you are requested to avail him all necessary assistance he may require. Attach herewith Ethical Committee's approval letter for further necessary action.

Accept my best regards, please.


HAMISU ABDUL MUHD
EXECUTIVE OFFICER
FOR: DIRECTOR GENERAL

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CONSENT TO PARTICIPATE IN RESEARCH WORK:

I'am Abdullahi Mas'ud a Ph.D student from Department of Microbiology, Ahmadu Bello University Zaria, wishing to conduct a Ph.D research titled "Characterization, susceptibility studies and PCR detection of Salmonella serovars isolated from blood samples of Patients in Kano metropolis".

I need a small quantity of your blood sample (3mls) for the research and the blood samples will be collected by competent hospital personnel. As such, you will not be harm on the process of blood collection. Also, information obtained during this study will be treated with utmost confidentiality and that the outcome of this research will help to improve diagnosis, treatment and prevention of typhoid fever in our community.

I will be glad if my request is duly granted. If you consent/assent to this request, please indicate below.

I(.....years old)
consent/assent to participate in this study.

.....

Signature and date

I(.....years old)
consent/assent for my son/daughter/warden to participate in this study.

.....

Signature and date

.....

Name, signature and date (for Researcher)