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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 MA conceived and designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SOO, VJU and IHI were the consultants and mentor gave professional advice and proof reading of final draft. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The aims of the study were 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: The study is a descriptive cross-sectional study.
Place and Duration of Study: One milliliter of venous blood was collected from each patient with some or all clinical features of salmonellosis that sign a consent form and transfer into EDTA bottles. If daily is unavoidable blood samples were stored at 4℃. Samples were analyzed at the both Laboratories of the authors. This work was carried out between May, 2011 and March, 2013.
Methodology: The blood specimens were cultured in thioglycollate broth and sub-cultured onto

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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. 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 salmonellae isolates obtained in this study, twenty one were subjected to DNA extraction, real-time and multiplex polymerase chain reaction (PCR) using various primer sets targeting the specific sequences of the resistance genes.

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 Ciprofloxacine. Among the thirteen *Salmonella* isolates tested by real-time PCR, *Tem* gene was detected with a higher frequency (38.5%) compared to the *gyrB* gene (30.8%). In addition, four *Salmonella* isolates were found to harbour both *tem* and *gyrB* genes together with 30.8%. In this study, no *gyrA* gene amplicon was obtained, although one *Salmonella* Typhi strain was resistant to ciprofloxacin phenotypically. There was significant correlation between the presence of *tem* and/or *gyrB* and resistance to ampicillin and nalidixic acid. However, in this study, out of the eight *Salmonella* isolates tested by multiplex PCR technique, *sul2* gene was detected in two (25.0%) isolates and *catP* gene was detected in one (12.5%); while the amplification of these two genes failed in five *Salmonella* isolates (62.5%) and no isolate harboured both *sul2* and *catP* genes. There is no significant correlation between the presence of *sul2* /*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. Ciprofloxacine remained effective against all the *Salmonella* isolates tested. Almost all the genes tested were circulating within Kano metropolis, including *tem, gyrB, sul2* and *catP* genes with exception of *gyrA.* There was only significant correlation between the presence of *tem* and/or *gyrB* and resistance to ampicillin and nalidixic acid.

Keywords: 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 [9-12]. Outbreaks of salmonellosis caused by *Salmonella* serovars Gallinarum, Pullorum, Typhimurium and Enteritidis have also been reported [10,12,13].

Increasing antibacterial drug 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 drug resistance patterns of *Salmonella* serovar in our environment [14-16]. 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 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 drug 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 drug resistance in *Salmonella* [17,18].

With the emergence and continual increase in the multiple antibacterial drug 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

2.1 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 blood specimen to the laboratory reception of one of the six selected hospitals for widel test, malarial test and other related blood tests recruited to sign the consent form were used for the study.

Blood (1ml) collected from each patient diagnosed positive for salmonellosis was used as sample for the study.

2.3 Collection and Handling of Specimens

One milliliter of venous blood was obtained using sterile syringe from an antecubital vein of each patient recruited for the study and dispensed immediately into 10ml thioglycollate broth. Sterile bijou bottle that contained blood and 10ml thioglycollate broth was then labeled with specimen number, type of medium and date of dispensing [22].

2.4 Isolation and Identification of *Salmonellae*

2.4.1 Presumptive isolation of *Salmonella*

One milliliter of venous blood specimen was dispensed into 10ml thioglycollate broth and sub-cultured onto SSA, BGA and DCA agar everyday and incubated aerobically at 37℃ for 7 days [22]. 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 [22].

Bacterial isolates obtained were further sub-cultured by stabbing into nutrient agar slants and stored at 4℃ after aerobic incubation 37℃ 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℃ 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 [23], Habtamu et al. [13], and OIE [24]. 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) [25].

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 [25].

2.6 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].

2.6.1 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 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 [26].

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 37℃ to allow for any excess surface moisture to be absorbed before applying the antibacterial drug discs [26].

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 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 different antibacterial drugs; ampicillin (25µg), chloramphenicol (30µg), ciprofloxacin (25µg), nalidixic acid (30µg) and Trimethoprim-sulfamethoxazole (30µg) [39].

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 antibacterial discs on the inoculated plates. Within 30 minutes after applying the disc, the plate was inverted and incubated at 35℃ 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 [39].

Results were recorded as susceptible, intermediate susceptible or resistant based on the zones size of each antibacterial disc used [29,39].

2.7 Isolation of Genomic DNA of *Salmonellae*

Out of one hundred and four salmonellae isolated in this study, twenty one *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 isolates used comprise of seven *Salmonella* Typhimurium, six *Salmonella* Typhi A, three *Salmonella* Paratyphi B and C each and two *Salmonella* Paratyphi A.

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

Polymerase chain reaction (PCR) was carried out using various primer sets to detect the antibacterial drug resistance genes against to the five antibacterial drugs. The real-time and multiplex polymerase chain reaction (PCR) techniques were employed as described by Asma [21]. The primers used in the study include: Sulphur (*sul2*) F 5' TCA ACA TAA CCT CGG ACA GT 3' and Sulphur (*sul2*) R 5'-GAT GAA GTC AGC TCC ACC T-3' for trimethoprim-sulfamethoxazole resistant gene; Temoniera (*tem*) F 5'-GCA CGA GTG GGT TAC ATC GA-3' and Temoniera (*tem*) R 5'-GGT CCT CCG ATC GTT GTC AG-3 for ampicillin resistant gene; chloramphenicol acetyltranseferase P (*catP*) F 5'-CCT GCC ACT CAT CGC AGT-3' and chloramphenicol acetyltranseferase P (*catP*) R 5'-CCA CCG TTG ATA TAT CCC-3' for chloramphenicol resistant gene; Gyrase (*gyrA*) F 5'-TAC CGT CAT AGT TAT CCA CGA-3' and Gyrase (*gyrA*) R 5'-GTA CTT TAC GCC ATG AAC GT-3' for ciprofloxacin resistant gene, and Gyrase (*gyrB*) F 5′-GCGCTGTCCGAACTGTACCT-3′ and Gyrase (*gyrB*)[2] R 5'-TGATCAGCGTCGCCACTTCC-3′for nalidixic acid resistant gene [30,21].

2.9 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

3.1 Bacterial Isolation

Of the five hundred blood 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 Antibacterial Sensitivity of the *Salmonella* Isolates

The result of *in vitro* -antibacterial drug susceptibility testing 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 cotrimozaxole 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.

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 isolates were resistant to nalidixic acid (11 resistant isolates representing 100%) followed by ampicillin (10 resistant isolates representing 90.9%). However, only one *Salmonella* Typhi isolate was resistant to ciprofloxacin. The relationship between *Salmonella* serovars and antibacterial drugs tested was not statistically significant (P > 0.05) (Table 1).

3.5 Detection of Resistant Genes

The results of the determination of antibacterial drug resistance by targeting specific genes in *Salmonella* serovars tested by single PCR technique were shown in (Fig. 1). In this study, no *gyrA* gene amplicon was obtained, although one *Salmonella* Typhi strain was resistant to ciprofloxacin phenotypically. Meanwhile, *gyrB* genes (315bp) were amplified from 8 of the 13 *Salmonella* isolates (Fig. 1a), and *tem* genes (311bp) were amplified from 9 of the 13 isolates (Fig. 1b). (Fig. 2) revealed the result of determination of antibiotic resistance by targeting specific genes in *Salmonella* serovars tested by multiplex PCR technique. The conditions were optimized for amplification of *sul2* and *catP* genes. Out of eight (8) *Salmonella* isolates tested, two (2) amplicons of *sul2* genes (707bp) and one (1) amplicon of *catP* were amplified.

Summarily, *tem* and *gyrB* genes were detected in some of the *Salmonella* isolates. *Tem* gene has a higher frequency (5 isolates, 38.5%) compared to *gyrB* gene (4 isolates, 30.8%) In addition, all the four *Salmonella* isolates harbouring the *gyrB* gene also presented the *tem* gene. Only one *Salmonella* Typhi strain was resistant to ciprofloxacin phenotypically, but of the corresponding gene *gyrA* was not detected. There was significant correlation (r = 0.259) between the presence of the genes *tem* and/or *gyrB* and resistance to ampicillin and nalidixic acid.

Table 1. Antibacterial drugs resistance profile of *Salmonella* serovars isolated from blood of patients presented to hospitals in Kano Nigeria

Antibacterial drugs/ disc potency (µg)	*Salmonella* serovars (No. tested)				
	Paratyphi A (n=5)	Paratyphi B (n=11)	Paratyphi C (n=14)	Typhi D (n=39)	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; µg = microgram; AMP = Ampicillin; CH = Chloramphenicol; CPX = Ciprofloxacin; COT = Cotrimoxazole; NA = Nalidixic Acid; % = Percentage of total number of each Salmonella serovar tested

M 1 2 3 4 5 6 7 8 9 10 11 12 13 C+ C-
↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑

Fig. 1a. Gel electrophoresis of *gyrB* gene after single PCR test

Key: M= 1000bp DNA marker (A, E, J and N); C^+ = Positive control; C^- = Negative control; bp = Base pair

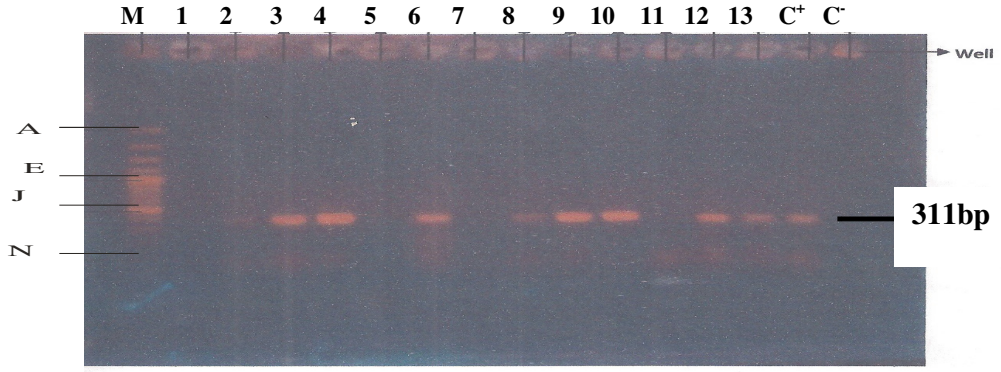

Fig. 1b. Gel electrophoresis of *tem* gene after single PCR test

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

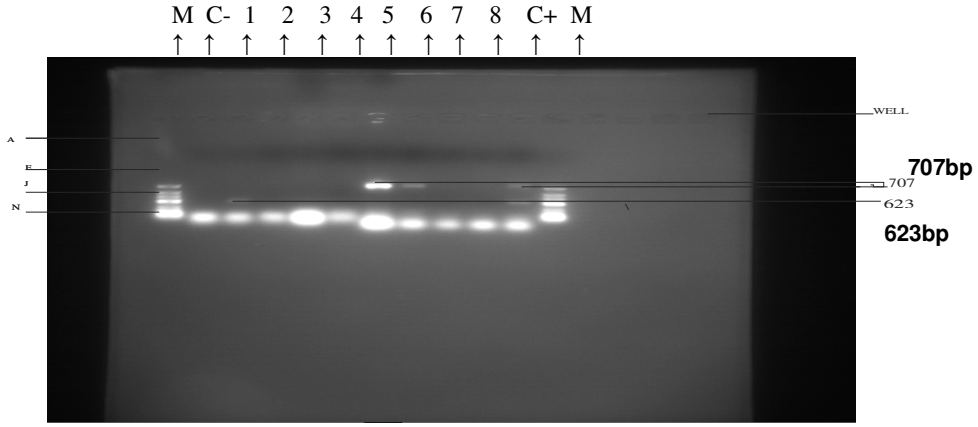

Fig. 2. Gel electrophoresis of *catP* and *sul2* genes after multiplex PCR test

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

In this study, out of the eight (8) *Salmonella* isolates tested by multiple PCR technique, the antibiotic resistance gene *sul2* was detected in two isolates (25.0%) and *catP* gene was detected in another isolate (12.5%). Five *Salmonella* isolates (62.5%) did not present any of the two genes. There is no significant correlation between the presence of any of the two genes (*sul2* and *catP*) and resistance to chloramphenicol and cotrimoxazole.

4. DISCUSSION

In this study, most of the *Salmonella* isolates from patients in Kano were significantly (X^2 11.03, P value = 0.001) resistant to ampicillin ($P< 0.05$) with 96 resistant strains representing 92.3% of the *Salmonella* isolates, 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 et al. [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 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 drug 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 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-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 of isolates did not contain any one of *sul2* or *catP.* Guerra et al. [43] also had similar experience with this study. Resistance to cotrimozaxole 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].

However, as observed in this study, the presence or absence of gene is related to the resistance, since the specific primers were designed and used to target specific resistance genes. If any of the targeted specific resistance genes is present an amplicon will be obtained after running the PCR or vice visa [41].

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

The result of *In vitro* – antibacterial drug susceptibility testing of *Salmonella* isolates using disc diffusion test demonstrated that most of the isolates (92.3%) was significantly ($P > 0.05$) resistant to ampicillin followed by nalidixic acid, chloramphenicol, and cotrimozaxole 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 *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.

6. RECOMMENDATIONS

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.

CONSENT

All authors 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

All authors 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 authors declared that they have no competing interests exist.

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