
Prevalence of antibacterial resistance of *Salmonella* serovars isolated from blood 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 H. I.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 determine the prevalence and antibiotic resistance profile of *Salmonella* isolates recovered from the blood samples of hospitalized subjects in Kano metropolis.

Study design:

Sample collection: 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.

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* was performed by slide agglutination test using polyvalent (O) and (H) *Salmonella* antisera. Antibiotic susceptibility studies were by the disc diffusion method using ampicillin, chloramphenicol, ciprofloxacin, nalidixic acid and Trimethoprim-sulfamethoxazole. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ampicillin, chloramphenicol, ciprofloxacin, nalidixic acid and Trimethoprim-sulfamethoxazole were also determined.

Results: *Salmonella* serovars isolated from the patients comprised of 39 *Salmonella typhi* (37.5%), 35 *S. typhimurium*, (33.7%), 14 *S. paratyphi C* (13.5%), 11 *Salmonella paratyphi B* (10.6%) and 5 *Salmonella paratyphi A* (4.8%).

Of the 104 isolates 97 (93.3%) were resistant to Ampicillin, 90 (86.5%) resisted Nalidixic acid, 34 (35.6%) resisted Chloramphenicol, 16 (35.4%) resisted Cotrimazole while none (0%) resisted Ciprofloxacin.

Conclusion: Most *Salmonella* serovars isolated from patients in Kano, Nigeria resisted Ampicillin. They also resisted Nalidixic acid, Chloramphenicol and Cotrimazole, in decreasing order. Ciprofloxacin remained effective against all the *Salmonella* isolates tested.

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].

Enteric fever occurs in all parts 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 loss, constipation and anaemia. Typhoid and paratyphoid are clinically indistinguishable [14].

Increasing antibacterial drug 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 existing antibacterial drugs in recent times among patients with *Salmonella* infection necessitates research by medical microbiologists to assess and report the patterns of antibacterial drug resistance among *Salmonella* serovar in our environment [15]. 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].

Majority of antibacterial drugs 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 antibacterial drugs 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 [16].

2. MATERIALS AND METHODS

2.1 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 Specimens

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

2.3 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.

2.4 Isolation and identification of salmonellae in blood specimens

2.4.1; 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 [17].

2.4.2; Purification of isolates

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

2.4.3; Biochemical characterization of *Salmonella*

Isolation and identification of organisms was carried out as described by ISO [18]; Habtamu et al. [13]; OIE [17]. A 24 h pure culture of each isolate was used to determine their gram stain reaction. The following biochemical tests were carried out: Indole test, triple sugar iron test, citrate test, methyl-red test, Voges-Proskauer test, lysine decarboxylase test, ornithine decarboxylase test, urease test, sugar (trehalose, sucrose, inositol, glucose, dulcitol, maltose, mannitol, melibiose, salicin, rhamnose and arabinose) fermentation test and motility test. Isolates were further characterized using commercially available identification system-Analytical Profile Index (API) 20 E test kit (Biomérieux, France).

2.5 Sero-typing 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 [19] by slide agglutination test.

2.6 Tests 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 [20].

2.6.1 Standardization of inoculum

This was done as described by CLSI [21].

2.6.2 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 by agar diffusion technique as described by Kirby-Bauer [22] and WHO [23]. This was carried out according to WHO protocol [23]. 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 namely; ampicillin (25µ), chloramphenicol (30µ), ciprofloxacin (25µ), nalidixic acid (30µ) and Trimethoprim-sulfamethoxazole (30µ) [23].

2.7 Determination of Minimum Inhibitory Concentration (MIC) of the isolates:

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 using agar dilution method [24].

2.8 Statistical analysis of results

Statistical Package for Social Science (SPSS) version 14 was used [25]. 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

From Table 1, out of 104 presumptive *Salmonella* isolates obtained in this study, *Salmonella typhi* was the predominant serovar with 39 (37.5%) followed by *Salmonella typhimurium* 35 (33.7%), *Salmonella paratyphi C* 14 (13.5%), *Salmonella paratyphi B* 11 (10.6%) and *Salmonella paratyphi A* with 5 (4.8%). However, the mean value between *Salmonella* serovars was also statistically significant ($p < 0.05$).

Table 1: The prevalence serovars of *Salmonella* from blood of patients presented to hospitals in Kano, Nigeria.

<i>Salmonella</i> serovars	No. detected	Percentage prevalent
<i>Salmonella paratyphi A</i>	5	4.8
<i>Salmonella paratyphi B</i>	11	10.6
<i>Salmonella paratyphi C</i>	14	13.5
<i>Salmonella typhi D</i>	39	37.5
<i>Salmonella typhimurium</i>	35	33.7
Total	104	100

$$t = 3.059 \quad p \text{ value} = 0.0377$$

S. = *Salmonella*; No. = Number; % = Percentage of total number of *Salmonella* isolated (104).

Of the 104 *Salmonella* isolates tested 97(93.3%) were resistant to Ampicillin, 90(83.5%) resisted Nalidix acid, 34(53.6%) resisted Chloramphenicol and 16(15.4%) resisted Cotrimazole. None of the isolates (0 %) was resistant to Ciprofloxacin (Table 2).

Table 2:Prevalence (%) of drug resistance by *Salmonella* serovars from blood of patients presented to hospitals in Kano Nigeria, against five antibacterial drugs used in the country.

Antibiotic	Disc potency (µg)		No. and % of Resistant Isolates
n= 104			
AMP	25		97(93.3)
CH	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; *S.* = *Salmonella*; *cfu* = Colonies forming unit; µg = 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 paratyphi* C, B and A. This finding is in consonance with studies by Akinyemiet *al.* [26] and Asmaet *al.* [27]. Contrary to the work conducted in Kano

metropolis (the study area) by Abdullahi[28] who revealed that *Salmonellatyphimurium* was the most predominant serovar. The highest susceptibility to *Salmonellatyphi* in the study area could probably be because *Salmonella* infections occur by ingesting organisms in contaminated food or water from contaminated hands (*Salmonellatyphi* is mainly water-borne, *Salmonellatyphimurium* and *Salmonellaparatyphi* are mainly food-borne). In the area where this research was conducted, residents experience serious problem of inadequacy of water supply that results in high consumption of unhygienic water. 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 [28].

In addition, in the study area many other reports indicated that multiple drug resistance (MDR) to *Salmonella typhi* was among the most frequent salmonellae responsible for high infection among carriers and high opportunistic infections among AIDS patients. There is also a diagnostic dilemma in the issue of chronic carriers; in the study area the *Schistosoma haematobium* is common, and it was reported that a chronic urinary carrier state results from localization of typhoid bacilli in schistosomiasis, the *Schistosoma-Salmonella* complex [29]. The patient will become a variant that has chronic foci of infection but whom *Salmonellatyphi* is shed intravascularly rather than in stool or urine [29]. Similarly, as few as 10^3 mean infective dose of *Salmonellatyphi* organisms is required to produce clinical or subclinical infection in humans but perhaps 10^5 - 10^8 salmonellae are required for other salmonellae in African countries. Furthermore, waterborne disease outbreaks are often unreported or underreported in developing countries because of the lack of systematic studies. In future and present Kano may not be an exception in this regard [30].

On the other hand, less infection with *Salmonella typhimurium* and *Salmonellaparatyphi* (mainly food-borne) could probably be because most food stuffs were washed properly or cooked before consumption and this helps on the decrease or total destruction of *Salmonella typhimurium* and *Salmonellaparatyphi* in food stuffs [31].

Of the five (5) antibacterials tested, most of the isolates (93.3%) resisted ampicillin 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.* [32]; Malla *et al.* [33]; Gautum *et al.* [34] and Abdullahi [28]. 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 [32]. It has been observed that antibacterial susceptibility of *Salmonella* isolates is not constant but dynamic and varies with time and environment. There is strong evidence that the use of antibacterials 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 [35].

Acquired antibacterial resistance is a growing worldwide problem due to the increasing use of antibacterials in humans, animals, and agriculture. In developing countries the situation is particularly serious for the following reasons: In many countries, antibacterials can be obtained

outside of recognized treatment centres, and taken without medical authorization or supervision. This leads to inappropriate use of antibacterial and their being taken at sub-optimal dosages and for an insufficient length of time [36]. Often the high cost of an antibacterial, result in an incomplete course being purchased, sufficient only to alleviate symptoms. Patients are not sufficiently informed about antibacterial and their use. Problems also arise when antibacterial 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. Antibacterial are often prescribed when they are not needed or for self-limiting infections, e.g. diarrhoeal disease and viral respiratory infections[37, 38].

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 antibacterial. In another instant the high resistant of *Salmonellaserovars* to commonly antibacterial 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 [39, 40].

5. Conclusion and Recommendation

The study revealed that among the *Salmonellaserovars* isolated, *Salmonellatyphi* was predominant followed by *Salmonellatyphimurium*, *Salmonellaparatyphi* C, B and A. However, the result of *in vitro* – antibacterial susceptibility testing of *Salmonella* isolates using disc diffusion technique, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) demonstrated that of the five (5) antibacterial tested, **most of the isolates (93.3%) resisted ampicillin.**

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[14].

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 its 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 [41].

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COMPETING INTERESTS

The authors declared that they have no competing interests exist.

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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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NHREC/21/08/2008n/AKTH/EC/216
OUR REF: AKTH/MAC/SUB/12^A/P3/1V/707 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: akthrb@hotmail.com



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

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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 institution.
- . 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. 3546, POST OFFICE ROAD, KANO

HMB/GEN/488/I

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 serous 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 Abdullahi Mas'ud
HAMISU ABDULLAHI MAS'UD
EXECUTIVE OFFICER
FOR: DIRECTOR GENERAL

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 harmed in 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)