Prevalence and Antimicrobial Susceptibility Pattern of *Salmonella species* in Asymptomatic Individuals in Makurdi Metropolis

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Authors' contributions

This work was carried out in collaboration among all authors. Author OJE. conceived and designed the study. Authors FR, NIH and IGA undertook the methodology of the study. Author ARH collected, analyzed the samples and did the statistical analysis under the supervision of Authors BBE and ICC furthermore, Author TSC drafted the original manuscript and Authors IIN and AAAD revised it. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: The aim of this study was to determine the prevalence and antimicrobial susceptibility pattern, of *Salmonella* species among asymptomatic individuals in Makurdi metropolis of Benue state, North central Nigeria.

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Study Design: Cross sectional study.

Place and Duration of Study: University of Agriculture, Makurdi in July 2023.

Methodology: A cross-sectional study was conducted among 300 randomly selected asymptomatic individuals in the Makurdi metropolis of Benue state, Nigeria from July 2023 to January 2024. Data were collected using a structured questionnaire. The stool specimens collected were examined for *Salmonella* using recommended culture methods. The antimicrobial susceptibility test was done using the disk diffusion technique. Data were described using descriptive statistical tools.

Results: The overall prevalence of *Salmonella* species was 48.7% (146/300). *Salmonella* species were more resistant to ampicillin (77.4%), tetracycline, (89.7%), and chloramphenicol (76.7%) but susceptible to norbactin (4.1%), ciprofloxacin (4.1%), and gentamicin (17.1%).

Also, 80.0% of *Salmonella* species were multidrug resistant.

Conclusion: The findings showed that the carrier rate of *Salmonella*, was average. Most isolates have also developed resistance to tetracycline, ampicillin and chloramphenicol. This development demands proper awareness campaigns and better hygiene and sanitary control measures to reduce the risk of infection.

1. INTRODUCTION

"A fecal-oral transmitted disease (FOTD) refers to the risk of contracting viral, bacterial, or parasitic infections through exposure to the feces of infected individuals or animals, whether they are symptomatic or asymptomatic" [1]. "*Salmonella* and *Shigella* species are the leading causes of food and water-borne gastroenteritis in humans, a significant global health concern" [2]. "The consequences of these infectious agents being transmitted are digestive disorders, especially diarrhea which may progress to dehydration, malnutrition and even death in extreme cases" [3].

"In developing countries, the incidence of *Salmonella spp.* infection is 540 cases per 100,000 people, compared to 0.2 cases per 100,000 in temperate regions. The highest infection rates are observed among children and adolescents aged 2 to 15 years in the most affected areas" [4]. "*S. Typhi,* among the *Salmonella* infections is known to be responsible for millions of infections each year. According to World Health Organization estimates, there are about 16 million new cases and 600,000 deaths from typhoid fever each year worldwide" [5]. "A key characteristic of these infections is the presence of healthy carriers for *Salmonella*. In fact, after recovering from typhoid fever, 2% to 5% of individuals continue to carry *S. Typhi*, which is intermittently shed in their stool and can lead to secondary infections" [6].

"The emergence of antimicrobial-resistant *Salmonella* and *Shigella* species presents a global challenge, particularly in developing countries where the abuse of antimicrobial agents in both humans and animals is more prevalent" [7]. "The development of resistance to commonly prescribed antimicrobial agents in bacteria is driven by drug target modification, alterations in bacterial cell permeability, and, most notably, the production of hydrolyzing enzymes known as β-lactamases" [7].

"The improper use of medications—including taking drugs without a valid prescription, self-medicating, and misuse—is also believed to contribute to the increase of antimicrobial resistance (Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria)" [8]. "The impact may be most severe in resource-limited settings, where second-line antibiotics for resistant bacteria are often either unavailable or too costly" [8].

There have been many studies on Salmonellosis conducted in Nigeria. Most of these studies are centered on food handlers, health facilities or hotels. There is limited data on Salmonella among asymptomatic individuals. This study was conducted to investigate the prevalence and antimicrobial susceptibility pattern among asymptomatic individuals in Makurdi metropolis, Benue state, Nigeria.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Media

Bacteriological media that were used in this study include: Nutrient agar (NA); Salmonella-

Shigella (SSA) Agar; Mueller-Hinton broth (MHB); Mueller-Hinton agar (MHA); Selenite F-Broth (SFB); Xylose Lysine Deoxychocolate agar (XLD);Triple Sugar Iron agar (TSI); Peptone water (PW) and Simmons Citrate agar (SCA).

2.1.2 Equipment

The equipment used in this study include: Autoclave, Incubator, Oven, Thermocycler, Microscope, and Electronic weighing balance.

2.1.3 Chemicals and reagents

The chemicals and reagents that were used in this study include: Acridine orange, Carbol fuschin, Crystal violet, Ethanol, Xylene solution, Pottasium hydroxide, Kovac's reagents, Ethydium bromide.

2.1.4 Study location

The study was conducted in Makurdi town, the capital of Benue State of Nigeria which was created in 1976 and is located on latitude $7^0 41$ N and longitude $8^0 37$ E on the Nigerian map. Random individuals made up of students in primary school, secondary school, University students and business men/women (from 8 different locations) were involved in the study.

2.2 Methods

2.2.1 Data and sample collection

Data related to the socio-demographic profile of all the participants and associated factors were collected using a questionnaire adapted from different kinds of literature [9]. The questionnaire included information on sex, age, educational status, income, and personal hygiene among others. After proper instruction, and verbal consent, each study participant was asked to bring 1 g of stool specimen in clean, dry, sterile container.

A total of 300 stool samples of random individuals who made themselves available were collected, and transported to the Microbiology Laboratory, University of Agriculture, Makurdi, for analysis.

2.2.1.1 Inclusion Criteria

Individuals who lived at the study location, were available at the time of data collection, and gave their consent were used for the study.

2.2.1.2 Exclusion Criteria

Individuals who used antibiotics and/or anthelminthic drugs and were not willing to give stool samples were excluded for the study and during sample collection.

2.2.2 Isolation and identification of *Salmonella spp*

Salmonella spp was isolated and identified using Gram staining, indole test, methyl red test, Urease test, Voges-Proskauer test, citrate test and oxidase test as described by [9].

2.2.3 Antibiotic susceptibility testing

The antibiotic susceptibility test of the isolates was carried out using the Kirby-Bauer disc diffusion method. For the standardization of the inoculum, a method as modified by Cheesbrough [10] "was used to prepare the McFarland 0.5 turbidity standard which was used to measure the density of bacterial cells. This was done by preparing a 1% solution of anhydrous barium chloride (BaCl2). 1% solution of sulfuric acid (H2SO4) was prepared. Thereafter, both barium chloride and sulfuric acid solutions were completely mixed together in a sterile conical flask to form a turbid suspension of BaSO4 in a specific proportion for each McFarland turbidity standard. Hence, the resulting mixture was stored in a foil-covered screw-cap test tube as a MacFarland standard at room temperature (25°C) when not in use".

"A sterile swab stick was soaked in the standardized bacteria suspension and streaked on the Mueller-Hinton agar plates and the antibiotics disc were aseptically placed at the centre of the plates and allowed to incubate at room temperature for 1 hour before re-incubating at 37ºC for 17 hours. The discs used include: Gentamicin (CN: 10 µg), Ampicillin (AMP: 10 µg), Chloramphenicol (CHL: 10 µg), Cotrimoxazole (SXT: 10 µg), Norbactin (NOR: 10 µg), Tetracycline (TET: 10 µg), and Ciprofloxacin (CIP: 10 µg). After incubation, the diameters of the zones of inhibition were measured to the nearest millimeter (mm) using a ruler and the result of the susceptibility test was interpreted using CLSI published guidelines for agar screen test" [11,12].

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of *Salmonella spp*

Out of the 300 samples collected and investigated, 280 (93.3%) samples were positive for several bacterial growth. The organism which grew with characteristics including colorless colonies on Salmonella-Shigella (SSA) Agar, black metallic sheen on Bismuth Sulphite Agar, Gram negative, rod shape, nitrate-positive, Hydrogen sulphide-positive, and Methyl red-positive was identified as *Salmonella spp.*

3.2 Occurrence of *Salmonella spp*

The occurrence of *Salmonella spp* was 48.7 % (146/300). Out of these samples, the highest frequency of occurrence was found between the ages 1-10 years, while ages 50 and above had the least frequency of occurrence as shown in Table 2.

3.3 Antimicrobial Resistance Profile

The Antibiotic Resistance of *Salmonella spp* isolates from stool of asymptomatic individuals in Makurdi metropolis is as given in Table 3. The result shows that the isolates of *Salmonella sp* were generally resistant to tetracycline (89.7%), ampicillin (77.4%), and chloramphenicol (76.7%). The isolates were however, susceptible to norbactin (4.1%), ciprofloxacin (4.1%), and gentamicin (17.1%).

3.4 Discussion

"The result of this study revealed a prevalence rate of 48.7% for *Salmonella spp* in the study area. This finding is similar to the 57.8% prevalence reported by Ifedike et al. [13] in Abuja, Nigeria, and similar to the 46.0% prevalence rate earlier reported" by Maharjan et al. [14] in Nepal. "It is however, higher than the 5.5% reported" by Amare et al. [15] in Ethiopia, 7.3% by Legese *et al* in Adigrat [16], 6.9% by Mama and Alemu in Arba Minch [17], and 5.5% reported by Mobolaji and Olubunmi [18] in Nigeria. "Results from researches conducted, showed varying prevalence rates including Ethiopia, Dilla (9.5%) [19], Addis Ababa" (10.5%) [20], and Nigeria (42.3%) [13]. The discrepancy may be attributed to differences in the research sites' environmental sanitation standards and the presence of suboptimal sanitary conditions.

"From our study, children within the age group (1–10) years, had the highest prevalence rate (57.7%) of *Salmonella sp.* This result is also in agreement with a report of 43.9% prevalence rate in a study by Origenes *et al* in Cebu City, India" [21]. It is believed that children form the most vulnerable group in environments with inadequate water supply and poor environmental hygiene due to their limited awareness.

Table 1. Sample distribution in Makurdi metropolis

SN	Location	No. Of sample collected	%
1	University of Agriculture Hostel	50	16.7
2	Anglican Secondary School, Wadata	35	11.7
3	St. Theresa Primary School, Wurukum	30	10.0
4	Makurdi International Nursery School, High Level	30	10.0
5	Mission Ward, North Bank	40	13.3
6	Benue Crescent, Waclata	40	13.3
7	School Lane, Wurukurn	40	13.3
8	Trade Centre	35	11.7
	TOTAL	**300**	**100**

Table 2. Age distribution of the study population

Age	No of Samples	Number (%) *Salmonella spp*
≤ 10	130	75(57.7)
11- 20	50	24(48.0)
21- 30	40	22(55.0)
31- 40	40	15(37.5)
41- 50	30	9(30.0)
> 50	10	1(10.0)
Total	**300**	**146(48.7)**

Table 3. Antimicrobial resistance profile of *Salmonella spp* from stool of asymptomatic individuals in Makurdi metropolis

Antibiotics	Disc Content (µg)	No. (%) resistance in *Salmonella spp* (n=146)
Ampicillin (AMP)	10	113(77.4)
Gentamicin(CN)	10	25(17.1)
Norbactin (NOR)	10	6(4.1)
Tetracycline (TET)	10	131(89.7)
Ciprofloxacin (CIP)	10	6(4.1)
Cotrimoxazole (SXT)	10	68(46.7)
Chloramphenicol (CHL)	10	112(76.7)

They tend to quickly quench their thirst without regard for the water source, especially if the water appears clear and colorless.

There was a high level of antibiotic resistance by isolates, to the routinely used antibiotics in this study area. This development as observed may be due to wrong and or inaccurate diagnosis as well as abuse of the available antibiotics, resulting in the development and spread of multi drug resistant strains of *Salmonella.*

The results of the antimicrobial susceptibility tests show that the isolates were highly resistant to tetracycline (89.7%), ampicillin (77.4%) and chloramphenicol (76.7%). This is similar to a study by Amare [15], that recorded *Salmonella spp* being resistant to Tetracycline, Amoxicillin/clavulanic acid, and Trimethoprim/Sulphamethoxazole (46.7%). It is also comparable with a study by Dagnew et al in Ethiopia, Gondar [22], Mengist *et al* in Debre Markos University [23], Mardu et al. in Tigrai [24], and Dilla [19], which indicates that antimicrobial resistance of Salmonella species is an increasing concern.

Salmonella isolates were less resistant to norbactin and ciprofloxacin (4.1%) which was different from previous reports in Ethiopia by Diriba et al., and Solomon et al. [19,25]. Most of the isolates were multidrug resistant (80.0%), which is comparable with the findings in Ethiopia [25], but lower than 100% resistance recorded in findings from a study by *Aklilu* et al. in Addis Ababa, Ethiopia [26]. "The high MDR rate of Salmonella isolates for the antibiotics currently used may limit antibiotic options for empirical therapy. These results suggested that antimicrobial resistance is still a major problem that has to be addressed. Antibiotic resistance has emerged as a major threat to public health, and this calls for unified global efforts to address the issue" [27]. "Antibiotic resistance is mostly caused by changes in bacterial genomes, inappropriate antibiotic use, poor drug regulation rules, incorrect drug prescriptions, and disobedience to prescriptions" [28]. An important observation that an organism that is previously resistant to a particular antibiotic may become susceptible if treatment with the antibiotic is suspended for a long time, is interesting because it makes the implication for public health management glaring for all.

4. CONCLUSION

In this study, the overall prevalence of *Salmonella* species was high. *Salmonella* species was highly resistant to Tetracycline, Ampicillin and Chloramphenicol. However less resistant to Norbactin and Ciprofloxacin. Among the total Salmonella isolates, multidrug resistance was documented in 80.0% of all bacterial isolates. The findings of this study highlight the importance of the rational use of antibiotics and other essential measures to combat the growing threat of antibiotic resistance. It is recommended to ensure access to potable water, improve laboratory diagnostic accuracy, and promote public education. Additionally, surveillance programs to track antimicrobial resistance patterns across other regions of the state and the country as a whole are advised.

5. RECOMMENDATION

Continuous health education and awareness programs will help educate people across all sectors about the dangers of microbial infections, as well as antibiotic abuse. Access to required facilities such as good and safe water, clean toilet will improve the personal hygiene of individuals. It is also advised that and constant epidemiological surveillance programs to track and monitor resistance patterns are deployed.

There are a few limitations to this study; first, characterization of the isolates to species level wasn't done due to lack of serological tests, financial constraints and lack of laboratory facilities in the study areas. Also, part of the socio-demographic data collected was lost due to an accident, making analysis for associated factors difficult. It is believed that the culture method and biochemical tests used was sufficient for identification to the genus level. In spite of these limitations, the information generated from this study is considered valuable as combined epidemiological and microbiological techniques were used to determine the prevalence and antimicrobial susceptibility pattern of *Salmonella spp*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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