Original Research Article

Prevalence and antimicrobial susceptibility pattern of Salmonella species in asymptomatic individuals in Makurdi metropolis

ABSTRACT

Aims: This study was aimed to determine the prevalence antimicrobial susceptibility pattern, and associated factors of *Salmonella* species among asymptomatic street food vendors in Makurdi metropolis of Benue state, North central Nigeria.
Study design: Mention the design of the study here.
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%),andchloramphenicol (76.7%) butless resistant to norbactin (4.1%), ciprofloxacin (4.1%), and gentamicin (17.1%). Also, 80.0% of *Salmonella* species were multidrug resistant. **Conclusion:**The findings showedthat 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.

Keywords: Antimicrobial resistance, Salmonella species, Makurdi, Prevalence

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* infectionsis known to be responsible for millions of infections each year. According to World Health Organization estimates, there are about 16 million newcases and 600,000 deaths from typhoid fever each yearworldwide[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 misuse 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 pandrug-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. MATERIAL 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, Carbolfuschin, 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.

2.2.2 Isolation and Identification of *Salmonella spp*

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

2.2.3 Antibiotic Susceptibility Testing

The antibiotic susceptibility test of theisolates was carried outusing 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: 10µg), Chloramphenicol (CHL: 10 µg), Cotrimoxazole (SXT: 10 µg), Norbactin (NOR: 10µg), 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. Theorganism which grew with characteristics including colorless colonies onSalmonella-Shigella (SSA) Agar, black metallicsheen on Bismuth Sulphite Agar, Gram negative, rod shape, nitrate-positive,Hydrogen sulphide-positive, andMethyl 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.

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)**

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, less resistant to norbactin (4.1%), ciprofloxacin (4.1%), and gentamicin (17.1%).

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)

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. Other lower results include those from research conducted in 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. 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 antibioticmay become susceptible if treatment with the antibiotic issuspended for a long time, is interesting because it makes the implicationfor public health management glaring for all.

4. CONCLUSION

In this study, the overall prevalence of *Salmonella* species was high. *Salmonell*a species was highly resistant to Tetracycline, Ampicillin and Chloramphenicol.Howeverless 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.

REFERENCES

1. Aubry P, Gaüzère BA. Coqueluche: Actualités 2018. *MedecineTropicale*. 2018.
2. Senthilkumar B, Prabakaran G. Multidrug resistant *Salmonella typhi* in asymptomatic typhoid carriers among food handlers in Namakkal district, Tamil Nadu. *Indian Journal of Medical Microbiology.* 2005 Apr 1;23(2):92-4.
3. Popa GL, Papa MI. *Salmonella spp*. infection-a continuous threat worldwide. *Germs*. 2021 Mar;11(1):88.
4. Akinyemi KO, Ajoseh SO, Fakorede CO. A systemic review of literatures on human *Salmonella enterica* serovars in Nigeria (1999-2018). *The Journal of Infection in Developing Countries.* 2021 Sep 30;15(09):1222-35.
5. World Health Organization: WHO.. *Salmonella (non-typhoidal)*. https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal).(2018, February 20)
6. Hoffman SA, Sikorski MJ, Levine MM. Chronic *Salmonella Typhi* carriage at sites other than the gallbladder. *PLoS Neglected Tropical Diseases*. 2023 Mar 23;17(3):e0011168.
7. Marami D, Hailu K, Tolera M. Prevalence and antimicrobial susceptibility pattern of *Salmonella* and *Shigella* species among asymptomatic food handlers working in Haramaya University cafeterias, Eastern Ethiopia. *BMC research notes*. 2018 Dec;11:1-6.
8. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection*. 2012 Mar 1;18(3):268-81.
9. Worku T, Jejaw A, Kannan S, Wondafrash B. Isolation and antimicrobial sensitivity patterns of enteric bacterial pathogens from asymptomatic food handlers, Jimma, Ethiopia. *American Journal of Health Research.* 2015;3(6):399-406.
10. Cheesbrough M. District laboratory practice in tropical countries. 2nd Ed.,Cambridge University Press, Cambridge,UK. 2006;50:75-77.
11. CLSI. Performance standards for antimicrobial disk susceptibility tests. 13th Edition. CLSI Standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
12. Beshiru AT, Igbinosa EO, Igbinosa IH. Prevalence of antimicrobial resistance andvirulence gene elements of *Salmonella serovars* from ready-to-eat (RTE) shrimps.*Front. Microbiol*. 2019;10:1613.
13.Ifeadike CO, Ironkwe OC, Adogu PO, Nnebue CC, Emelumadu OF, Nwabueze SA, Ubajaka CF. Prevalence and pattern of bacteria and intestinal parasites among food handlers in the Federal Capital Territory of Nigeria. *Nigerian Medical Journal*. 2012 Jul 1;53(3):166-71.

14. Maharjan A, Bhatta CP, Bhuyan KC. Antibiotic sensitivity pattern of *Salmonella* species isolated from blood culture.
15. Amare A, Eshetie S, Kasew D, Amare A, Abebe W, Moges F. Prevalence of *Salmonella spp*., *Shigella spp*., and intestinal parasites among food handlers working in University of Gondar student's cafeteria, Northwest Ethiopia. *Frontiers in Public Health*. 2024 May 1;12:1370338.
16. Legese H, Kahsay T, Gebrewahd A, Berhe B, Fseha B, Tadesse S, Gebremariam G, Negash H, Mardu F, Tesfay K, Adhanom G. Prevalence, antimicrobial susceptibility pattern, and associated factors of *Salmonella* and *Shigella* among food handlers in Adigrat University student's cafeteria, northern Ethiopia, 2018. *Tropical diseases, travel medicine and vaccines*. 2020 Dec;6:1-9.
17. Mama M, Alemu G. Prevalence, antimicrobial susceptibility patterns and associated risk factors of *Shigella* and *Salmonella* among food handlers in Arba Minch University, South Ethiopia. *BMC infectious diseases*. 2016 Dec;16:1-7.
18. Mobolaji OA, Olubunmi OF. Assessment of the hygienic practices and the incidence of enteric bacteria in food handlers in small businesses in an urban area in Abeokuta. *Int J Microbiol Res.* 2014;5(3):41-9.
19. Diriba K, Awulachew E, Ashuro Z. Research Article Prevalence and Antimicrobial Resistance Pattern of *Salmonella*, *Shigella*, and Intestinal Parasites and Associated Factor among Food Handlers in Dilla University Student Cafeteria, Dilla, Ethiopia.
20. Mengistu G, Mulugeta G, Lema T, Aseffa A. Prevalence and antimicrobial susceptibility patterns of *Salmonella serovars* and *Shigella* species. *J MicrobBiochem Technol*. 2014 Dec 10;6(S2):S2-006.
21. Origenes RW, Espinosa-Gelisanga R, Alturas SB. Street Food's Microbiological Load and Vendors' Food Hygiene and Safety Practices Compliance in the Schools of Cebu City. *International Journal of Science, Technology, Engineering and Mathematics*.;2(1):48-60.
22. Dagnew M, Tiruneh M, Moges F, Gizachew M. Bacterial profile and antimicrobial susceptibility pattern among food handlers at Gondar University Cafeteria, Northwest Ethiopia. *J Infect Dis* Ther. 2013 Apr;1(105):2332-0877.
23. Mengist A, Mengistu G, Reta A. Prevalence and antimicrobial susceptibility pattern of *Salmonella* and *Shigella* among food handlers in catering establishments at Debre Markos University, Northwest Ethiopia. *International Journal of Infectious Diseases*. 2018 Oct 1;75:74-9.
24. Mardu F, Negash H, Legese H, Berhe B, Tesfay K, Haileslasie H, Tesfanchal B, Gebremichail G, Belay G, Gebremedhin H. Assessment of knowledge, practice, and status of food handlers toward *Salmonella*, *Shigella*, and intestinal parasites: A cross-sectional study in Tigrai prison centers, Ethiopia. *PloS one*. 2020 Nov 3;15(11):e0241145.
25.Solomon FB, Wada FW, Anjulo AA, Koyra HC, Tufa EG. Burden of intestinal pathogens and associated factors among asymptomatic food handlers in South Ethiopia: emphasis on salmonellosis*. BMC research notes*. 2018 Dec;11:1-6.
26. Aklilu A, Kahase D, Dessalegn M, Tarekegn N, Gebremichael S, Zenebe S, Desta K, Mulugeta G, Mamuye Y, Mama M. Prevalence of intestinal parasites, *salmonella* and *shigella* among apparently health food handlers of Addis Ababa University student's cafeteria, Addis Ababa, Ethiopia. *BMC research notes*. 2015 Dec;8:1-6.
27. Ibrahim RA, Teshal AM, Dinku SF, Abera NA, Negeri AA, Desta FG, Seyum ET, Gemeda AW, Keficho WM. Antimicrobial resistance surveillance in Ethiopia: Implementation experiences and lessons learned. *African Journal of Laboratory Medicine.* 2018 Jun 1;7(2):1-4.
28. Ndihokubwayo JB, Yahaya AA, Desta AT, Ki-Zerbo G, Odei EA, Keita B, Pana AP, Nkhoma W. Antimicrobial resistance in the African Region: Issues, challenges and actions proposed. *African Health Monitor*. 2013 Mar;16:27-30.