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Risk factors associated with *salmonella* prevalence among food vendors in southern Taraba, north-east, Nigeria

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Abstract

Humans are active and asymptomatic carriers of *Salmonella* and food vendors play a significant role in the effective transmission of the food pathogen. This research investigated the prevalence of *Salmonella* along with its associated risk factors among food vendors in Donga, Ibi, Takum, and Wukari towns. Using standard microbiological procedures and techniques, blood and stool samples was obtained from 200 mobile and stationed food vendors. Upon analysis, 22 out of the collected samples were positive giving a record prevalence of 11%. Six (6) (Donga 0; Ibi 3; Takum 1; Wukari 2) of the positive samples were of the Typhoidal serovar. Apart from Ibi and Takum which recorded 4 positive cases each from the 16 isolated Non-Typhoidal *Salmonella* (NTS) from food handlers, the highest (5) isolates were from Donga, while the lowest was from Wukari with a total of 3 positive isolates. Poor food-handling training as well as unprofessional food handling attitude and practices observed among food handlers were identified as significant risk factors associated with the prevalence of *Salmonella* infection among this population. Hence, deliberate efforts should be initiated by Local Health Authorities in regulating the activities of food vendors by way of periodic medical screening of food handlers. Also, community-driven health promotion practices including behavioral change communication geared towards preventing microbial contamination of foods should be considered.

Keywords: Food Handlers; Prevalence; Risk Factors; *Salmonella*; Typhoid; Taraba

1 Introduction

Of the most often reported foodborne pathogen currently inflicting diseases in humans worldwide is *Salmonella* specie which is responsible for Typhoidal *Salmonella* (TS), and non-typhoid *Salmonella* infection (NTS) [1]. Food handling techniques and practices are significant in the transmission of *Salmonella*. According to the United States Food Drug Administration (USFDA), poor hygienic practices of food handlers have contributed to the spread of *Salmonella* in foods [2]. Several foodborne outbreaks are associated with restaurants [3]. On a global scale, the [4] estimates that about 48 million people suffer from food-borne illness annually, from which over 120,000 are hospitalized with over 2000 fatal cases. Over 70% of reported cases of food-related gastroenteritis have been attributed to poor food-handling practices and attitudes in restaurants [5]. Similarly, poor hygienic circumstances of local food vendors and restaurants otherwise known as boukas have been identified by Fashae et.al; [6] as a major factor contributing to food-bourne *Salmonella* transmission. Contrastingly high incidence of food-borne Salmonellosis has been linked to large-scale production of commercial food in urban areas [7].

Like other gram-negative bacterial pathogens such as Escherichia coli, *Enterobacter*, *Klebsiella*, *Shigella*, and *Proteus*, the genus *Salmonella* belongs to the order *Enterobacteriales*, a member of the large family *Enterobacteriaceae* [8]. Members of the genus *Salmonella* consist of peritrichous motile, Gram-negative, non-spore-forming rod-shaped bacteria [9], with a 0.7-1.5 µm range in diameter and 2-5 µm in length [10]. *Salmonella* Species are facultative anaerobes because they do

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not require oxygen for growth [9]. *Salmonella* Species obtain their energy from oxidation and reduction reactions using organic sources hence, they are chemoorganotrophs. *Salmonella* predominantly lives in the intestinal tracts of humans and other warm and cold-blooded animals as their natural habitats [10, 11]. *Salmonella* is disseminated into natural habitats such as soil, water bodies, and food plants through fecal contamination from infected persons. *Salmonella* is classified into two main species; *Salmonella enteric*a and *Salmonella bongori* [12]. Serovars of *Salmonella* are classified into sub-species according to the absence or presence of the O (Somatic) and H (Flagella) antigens [13]. *Salmonella* serovars can be further subdivided by phage-typing based on the sensitivity of their cells to the activity of selected bacteriophages [14]. Out of the thousands of the known serovars of the *Salmonella* enterica specie, those belonging to the *Salmonella enterica* I sub-specie are the most medically important, being responsible for more of the reported cases of not only Typhoid fever but, most reported cases of food poisoning leading to acute gastroenteritis [15]. Pathogenic *Salmonella* serovars are classified as either typhoidal (TS) or Non-typhoidal (NTS). TS and NTS are responsible for remarkable cases of (Para) Typhoid fever and serious gastroenteritis respectively. TS is transmitted from an infected human to a non-infected human and the infections are limited to a man and do not occur in animals [16]. NTS is mostly transmitted from infected animals to humans by direct contact [17]. Nonetheless, consumption of foods or water contaminated with the pathogen may surge the risk of contracting the infection [18].

In humans, the pathogenesis of *Salmonella* infections depends on the strain, serovar, infectious dose the nature of contaminated food, and most importantly the host status because some serovars are more highly pathogenic in humans than other animals; and even strains of the same serovars exhibit disparity in their pathogenicity [10]. The symptomatic burden of *Salmonella* infection is prompt and more serious among immunosuppressed individuals, children, and persons with certain blood disorders [10]. *Salmonella* possess various structural and physiological abilities that enhance its virulence, thereby enabling them to inflict acute and chronic disease. One of the more important virulent factors required by *Salmonella* to accomplish pathogenesis is the type three secretion system (T3SS) encoded in the *Salmonella* pathogenicity islands (SPIs) [15]. Other features enhancing *Salmonella* pathogenesis include adhesions, flagella, virulence plasmid, and biofilm-related proteins [15]. Upon successful pathogenic processes, *Salmonella* exhibits rapid clinical manifestations, depending on the spectrum of infection caused. Following the incubation period of between 6 to 48 hours for the NTS pathogen, gastroenteric symptoms may emanate. NTS gastroenteritis is characterized by fever (38°C to 39°C), nausea, vomiting, bloody and mucopurulent (containing mucus or pus) diarrhea, and abdominal pain [11]. Since *Salmonella* gastroenteritis is a self-limiting infection, significant symptoms such as fever and diarrhea may only persist for a varied period of between 2 to 7 days without treatment. The non-specific systemic symptoms of enteric fever commence immediately after a typical period of incubation of between 10 to 14 days; however, patients may experience symptoms of gastroenteritis for a few days which usually resolves before the start of the actual systemic symptoms of typhoid fever [11]. Typhoidal symptoms if untreated may persist for four weeks leaving the patient emancipated and exhausted [19, 20]. Hence, this first-of-its-kind research in Sothern Taraba, North-East Nigeria sets out to investigate risk factors associated with the prevalence of *Salmonella* infection among food vendors.

2 Material and methods

This transverse study was conducted in various communities in Wukari, Takum, Ibi, and donga Local Government Areas of Taraba State to investigate risk factors associated with *Salmonella* infection. A total of 200 mobile and stationed food vendors who were not previously receiving treatment using antibiotics for at least 2 weeks were included in the study. Those comprised in this study participated either in the preparation or serving of various kinds of food which includes and not limited to Kunu, Pito, Rice, Tuwo, Fufu, yam pottage, and Beans. A structured questionnaire was used to gather demographic data such as age, sex, marital status, and educational status from a total of 200 participants.

2.1 Sample collection

5ml of blood and 1g of stool samples were obtained from all 200 participants included in the survey for Widal tests and Stool culture for detection of TS and NTS respectively. Blood samples were stored at a temperature of between 3-2 degrees Celsius using conditioned icepacks to keep them fresh during transportation to the laboratory for analysis. Samples were brought to room temperature before analysis.

2.2 Sample analysis

2.2.1 Widal Tests

A tube agglutination test was performed on serum from centrifuged blood samples collected to detect the presence of serum agglutinins (antibodies specific to Flagella “H” and Somatic “O” of *Salmonella* species) in the patient’s serum. Antigens O and H from killed bacteria were stained with red and blue color respectively for purpose of enhanced detection of agglutination.

Four sets of 8 test tubes were obtained and labeled "1-8" for purpose of detecting O, H, AH, and BH antibodies from samples. 1.9ml of isotonic saline was added to the first tubes of the 4 sets. Thereafter, 1.0ml of isotonic saline was pipetted to other tubes labeled "2-8" of all sets (O, H, AH, and BH). 0.1 ml of serum was added to tubes labeled "1" of all sets and mixed homogeneously. 1.0ml of the diluted serum from tubes labeled "1" in both sets was respectively transferred to tubes labeled "2" of all sets and mixed. Same 1.0ml of diluted serum from tubes labeled "2" was equally transferred respectively to tubes labeled "3" in all sets. This serial procedure was repeated for tubes labeled "4-7" in all O, H, AH, and BH sets. 1.0 ml of diluted serum in tubes labeled "7" in all sets were discarded to ensure an even volume of sample. Serum sample dilution achieved for Tubes No: 1 2 3 4 5 6 7 in all sets are 1:10 1:20 1:40 1:80 1:160 1:320 1:640 respectively. Also, antibody titers of ≥1:80 and ≥1:160 for O and H antigens respectively, as cut-off values for positive titers tubes labeled "8" in all sets were not added with diluted serum and were considered as saline control. A drop of prepared Widal test antigen O, H, AH, and BH were added to all test tubes labeled "1-8" from all sets and mixed homogeneously. All tubes were covered and following incubation for 16 hours, observation for agglutination was made on the sediments at the bottom of the tube.

2.2.2 Blood Culture

Positive samples from tube agglutination were subjected to blood culture for confirmatory results. 10ml of blood was added directly to Bact/Alert culture media in bottles and incubated at room temperature. After 8 hours, subculture was done on MacConkey Agar and incubated overnight at 37 degrees. Observations were made for fresh colonies with round, flat, shiny, and fragile nature with glowing appearance, and were subjected to further biochemical analysis.

Stool Culture: 1g of stool sample from all participants was collected using a sterile container containing sterile saline. Using the pour plate method, 1ml of stool sample was inoculated into previously prepared *Salmonella*-Shigella Agar (SSA) before solidification and stirred in a circular motion to enable a homogenous mixture. Following solidification of the media, incubation was done for 24 hours at 37°C. Colorless colonies with black pigment at the center were presumed as *Salmonella* specie.

2.2.3 Biochemical tests

All presumed *Salmonella* isolates were subjected to biochemical analysis.

3 Results

Table 1 indicates the morphological and biochemical characteristics of isolated and identified bacterial pathogens with emphasis on the morphological presentation specific to the type of culture media used. The age and sex distribution of food handlers including other demographic indices are presented in Table 2, while Table 3 presents the prevalence of *Salmonella* specie in the area sampled with corresponding risk factors associated with the occurrence.

Table 1 Characteristics of Isolates

ISOLATES	Culture Media	Morphological characteristics	Gram Stain	Biochemical Characteristics						Organism
				CAT	IND	MR	LAC	SUC	GLU	
A	MacConkey Agar	round, flat, shiny and fragile nature with glowing appearance	Negative bacilli	+	-	+	-	-	+	*Salmonella*
	Salmonella-Shigella Agar (SSA)	Colourless colonies with pigment at the middle								

Abbreviation: +; Positive, -; Negative, CAT; Catalase, IND; Indole, MR; Methyl Red, LAC; Lactose, SUC; Sucrose, GLU; Glucose.

The table below indicates that about 68.5% of the food handlers were in the age group of 18 - 40 years. 79.5% of the participants were females. 31% of food handlers had basic training in food safety. A total number of 177 of the 200 food handlers included in the survey were involved in domestic livestock rearing including Chickens.

Table 2 Demographic information of food vendors involved in the study

Demography of food handlers involved in the study											
Location	**Age Group (N=50)**		**Sex (N=50)**		**Food category (N=50)**		**Hand Washing practices**		**Formal Secondary Education**		**Total No of participants**
	18-40	41-60	F	M	Mobile	Stationed	Y	N	Y	N	
Wukari	32	18	43	7	42	8	29	21	24	26	50
Donga	28	22	38	12	40	10	32	18	11	39	50
Takum	36	14	35	15	38	12	36	14	19	31	50
Ibi	41	9	43	7	44	6	24	26	8	42	50
Total	137	63	159	41	164	36	121	79	62	138	200

Twenty-two (22) of the samples collected from participants and tested were confirmed positive for *Salmonella*, giving *Salmonella* carriage of 11%. Six (6) out of the 22 isolates were S. *Typhi* representing 27.3% while 16 were of the Non-Typhoidal Serovar representing 72.7%.

Apart from Ibi and Takum which recorded 4 positive cases each, the highest (5) cases from 16 positive Non-Typhoidal *Salmonella* isolates from food handlers was from Donga, while the lowest was from Wukari with a total of 3 positive isolates. S. *Typhi* was not isolated in Donga but 3 isolates were recorded for Ibi while Wukari and Takum recorded 2 and1 isolates of S. *Typhi* respectively. All 22 (100%) food handlers with positive cases of *Salmonella* were either involved directly or indirectly in domestic animal rearing. Most, (77%) *Salmonella*-infected food handlers were females. *Salmonella* was isolated more among mobile food vendors (64%) than those stationed (36%). *Salmonella* isolates from food handlers were predominant (82%) among those who lack training in food handling. Interestingly, 59% of the infected food handlers do not practice hand washing before serving food. The details of these results are presented in the table below.

Table 3 Prevalence data of *Salmonella* isolated from food handlers

Location	**No. s participants**	**Age Group**		**Sex**		**Food category Mobile (M) Stationed (S)**		**Hand washing when Handling food**		**Knowledge of microbial food contami-nation**		***Salmonella* isolates**		**TOTAL *Salmonella* prevalence**	
		18-40	41-60	F	M	M	S	Y	N	Y	N	TS (%)	NTS(%)	No	%
Wukari	50	4	1	4	1	3	2	2	3	1	4	2	3	5	10
Donga	50	5	0	4	1	4	1	4	1	2	3	0	5	5	10
Takum	50	4	1	3	2	3	2	1	4	1	4	1	4	5	10
Ibi	50	5	2	6	1	4	3	2	5	0	7	3	4	7	14
Total	200	18	4	17	5	14	8	9	13	4	18	6 (3%)	16 (8%)	22	11%

4 Discussion

Of the total 200 individuals included in this survey, a total of 22 were positive for either TS (6) or NTS (16) giving a collective prevalence of 11%. No participants had both TS and NTS cases simultaneously. This finding is consistent with that of [21] who discovered over 30% prevalence of Salmonellosis in Lagos Nigeria. The communal prevalence of *Salmonella* is not unconnected to pathogenic contamination arising from unhygienic food handling along the chain of production to consumption [3]. Lower isolation rates of TS when compared to NTS as experienced in this current study may be due to the type of sample analyzed and the higher incubation period. Typhoid is more isolated from bone

marrow than from blood samples [21]. Also, NTS has a rapid incubation period of fewer than 3 days while TS has an incubation period of over 10 days [22, 19].

Analysis from administered questionnaires in this current research indicates that level of education, sex, and age are socioeconomic and demographic determinants influencing food vendors' behavior and attitude particularly as it relates to safe and hygienic food handling.

Of the 200 respondents in this study, only 63 (31.5%) completed at least a secondary level of education from which only 4 (18%) of the 22 *Salmonella*-positive respondents had prior knowledge of microbial contamination of food. This is considerably low especially when microbial activity in food production is rapidly on increase even with standard operating procedures. This current finding supports that of [23] which gave that, the literacy level of food handlers, social status of individuals as well as environmental factors has a significant link with high rates of *Salmonella* infection. Divergently, an assessment of the food safety knowledge and attitudes of food handlers by [24] suggested that a higher level of education and food handling experience though significant does not necessarily lead to an excellent food handling attitude. Yul et al [5] has opined that the knowledge of food vendors on food safety does not necessarily result in proper food safety behavior. Hence, knowledge-based training on microbial contamination of food must be supplemented with behavioral change communication messages for improved results.

As seen in this study, more *Salmonella* isolates (64%) were from mobile food vendors than those stationed. Owing to availability and cost-efficient peculiarities, people in the area under investigation patronize mobile food vendors more when compared to stationed restaurants and fast food for their cost efficiency. Again from the demographic data gathered, more mobile food vendor lacks basic education and are deficient in basic safe food handling practices such as hand washing. If available, mobile food vendors often bring with them containers with water for repeated hand washing practices sometimes, before and after serving food, therefore, importuning *Salmonella* contamination. Nonetheless, Isolating *Salmonella* from stationed restaurants and fast-food joints usually patronized by the rich is feasible as seen in this study owing to food vendors' poor knowledge of microbial food contamination and lack of basic food handling training. There is an existing opinion that good knowledge of food safety does not necessarily translate into strict hygienic practices during the processing and handling of food products [23]. Arguments have also been presented that, the attitudes of food handlers may be a more significant factor contributing to the high incidence rates of food-borne Salmonellosis than socioeconomic and other environmental factors [3]. Therefore, food handlers must be trained in food handling knowledge and behavior.

Conspicuously, sex has emerged as contributing factor contributing to high *Salmonella* prevalence in the area under investigation. The prominence of Mary Mallon (Typhoid Mary) in historic *Salmonella* transmission [25] is a direct consequence of gender-related inequalities in health. Findings from this study have indicated that *Salmonella* is more prevalent in females (77%) than Males. Successful *Salmonella* infection in humans depends on exposure to the pathogen, the nature of contaminated food, and most importantly the host's immunological response. According to [21] Women are more exposed than men to *Salmonella*. From the demographic data of this study, women (80%) participated more than men in the food vending business and food production chain including poultry production. Also, *Salmonella* has shown dominance more in fruits than cooked foods and has easily been isolated from several types of unwashed fruits. Evidence has it that women more than men tend to consume fruits more (Peer et al., 2021). Hence the pathogen may be isolated more from women [21]. Contrastingly, estrogen sex hormones are higher in Females than Males and facilitate immunological response against *Salmonella* by the production and induction of cytokine. [26]. Rapid immunological response to *Salmonella* infection is contingent on the immunological competence of the individual.

Age is also a predetermining factor contributing to *Salmonella* transmission. Over 80% of food handlers, who were positive for Salmonellosis, were between the ages of 18 and 40. Although, this age group (18 – 40) was more in this survey with participation rates of 68.5%. This finding is consistent with that of [21]. Low prevalence of *Salmonella* observed among participants who are above 40 years of age may be a direct consequence of hands-on experience and food safety and handling trainings they may have gained over the years.

5 Conclusion

No gainsaying regarding the devastating global health burden due to *Salmonella* particularly among food handlers in developing countries such as Nigeria. This study has concluded that the prevalence of *Salmonella* infection is considerably high. This rate suggests the potential risks of food handlers disseminating the bacterium among the general population leading to a possible community outbreak. Deliberate efforts should be geared towards arresting the spread of *Salmonella*. Local Health Authorities should regulate the activities of food vendors through intermittent medical screening and assessment of individual food handling practices including hand washing. Moreover, many of

these risk factors associated with *Salmonella* prevalence in Southern Taraba, North-East, Nigeria can be eased through community-driven health promotion practices including behavioral change communication messages aimed at preventing microbial contamination of foods.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

We declare no conflict of interests in this review

Statement of ethical approval

The study was approved by the Review Board of the Tropical disease Unit, Microbiology Department Federal University Wukari. This research was conducted considering all ethical issues such as Justice, Beneficence, Non-Maleficence, and Autonomy were considered when conducting the research.

Statement of informed consent

Written consent was obtained from all participants in this study.

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