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Risk Factors and Occurrences of *Shigella* spp in Ready-To-Eat Vegetable Salads (Ayamutsa) Sold in Gombe Metropolis

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ABSTRACT

Shigella spp are pathogenic bacteria that cause shigellosis which is an ailment noted for significant disease burden especially in Africa. These bacteria are transmitted via contaminated food/water. In Gombe, ayamutsa is believed to pose health risks due to improper preparation and handling. Therefore, this research was aimed at investigating these ayamutsa for possible *Shigella* spp occurrence and also to identify risk factors responsible for the occurrence. 100 samples were inoculated on nutrient agar and SS agar. Identification was done using Gram's staining and biochemical tests. Antibiotic sensitivity tests were conducted based on CLSI guidelines, and a questionnaire was used to obtain data for determining the association between handling practices and contamination. Results revealed 10% of samples were contaminated with *Shigella* spp. The sensitivity test showed the isolates were sensitive to gentamicin, Augmentin, ceftriaxone, but resistant to ofloxacin and oxacillin. Questionnaire analyses revealed there is significant association between contamination and some handling practices. The findings from this study revealed that ayamutsa contamination with *Shigella* spp in Gombe metropolis poses a threat to public health and could also serve as a persistent source of *Shigella* spp spread in the community which could lead to an outbreak of shigellosis.

Keywords: Risk factors; *Shigella* spp; Occurrence; ready-to-eat Vegetable; Gombe

INTRODUCTION

The ready-to-eat vegetable salads have become highly popular food sources in numerous States of Nigeria. The collection of vegetables which constitute these salads include tomatoes, cucumber, carrots, cabbage and lettuce (Chukwuma, 2016). Despite the health benefits, consumption of such compounded vegetable salads has been linked with outbreaks of food-communicated ailments in numerous States nationally and internationally (Gourama, 2020), and non-existence or dearth of individual hygiene practices amongst food peddlers that deal in these vegetable salads is amongst the foremost factors which lead to food-borne infections after consuming these vegetable salads (Zenbaba et al., 2022).

Roughly a third amongst the total populace in industrialized nations could be afflicted by food-communicated contagions every 12 months, and these values are elevated in developing nations like Nigeria (Akhtar et al., 2014). The figures for ailments arising from food-communicated contagions linked to fresh fruits and vegetables keeps getting elevated globally (DeOliveira-Elias et al., 2018) and *Shigella* spp have been shown to be important in this instance (Correia et al., 2017).

Investigations into food-communicated ailments' risk features revealed how the greater percentage of outbreaks linked to food-communicated contagions could be ascribed to improper food preparation and processes by food handlers (Chen et al., 2024). Nevertheless, unsafe water sources, galley gears, zones with poor sanitary situations, eating uncooked vegetables, and proximity, present additional noteworthy risk features linked to these ailments (Gargiulo et al., 2022).

Characteristically, 1 to 2 out of every 10 ailments caused by enteric disease-causing bacteria, and half of all bloody diarrhoea/dysentery in kids, could be categorized as shigellosis which is the clinical manifestation of infection with *Shigella* spp. This illness is spread via the faecal-oral route, and has an infectious dose of only 10–200 microorganisms which makes infection relatively easy and quick (Percival and Williams, 2013).

In the year 2017 alone, a staggering 1,600,000 human mortalities resulted from diarrheal ailments and a third of those demises were amongst kids below 60 months in age. In defiance of treatment and prevention efforts, diarrhoea-associated ailments and demises persists in high numbers, particularly among populations of low- and middle-income countries (LMICs). *Shigella* spp contagions represent noteworthy sources of ailments and demises because of diarrhoea, and recurrent contagions possibly will lead to faltered growth and hypothetically influence impaired cognitive development among the youngsters of the developing nations (CIDID, 2024).

In Nigeria, a lot of information is still needed to ascertain the true prevalence of shigellosis. Nonetheless, prevalence for diarrhoea had been clocked at 18.8%, which also contributed to the diarrhoeal menace in Sub-Saharan Africa, where it accounts for an approximation of 150,000 human demises every 12 months amongst kids and other individuals, owing to poor hygiene and unsanitary situations (Kehinde-Peter and Umar, 2018).

In Gombe, there are undocumented reports that indicates and claims diarrhoea occurrences are partly due to contamination of the salads prepared and vended on the streets, hence this study was setup to investigate the issue and to also bridge or narrow the existing knowledge gap concerning the bacteriological safety of these ayamutsa.

MATERIALS AND METHODS

Study Area

This study was carried out within Gombe metropolis with locations for sampling and data collection ranging from Gombe Central Market, Bolari, Pantami, and Tudun Wada.

Sample Size

The sample size utilized in this research was 100. The actual population of ayamutsa vendors and hawkers could not be obtained at the time of this study, hence it was surmised that 100 samples were sufficient for the study because it has been documented that majority of statisticians have come to an agreement that the minimum sample size for obtaining any form of meaningful outcome is 100 (Piroska, 2022).

Sample Collection

The samples were collected by purchasing from the vendors in the same manner that regular consumers do, which involves the vendors putting the purchased ayamutsa in polythene bags and handing over to the consumer. Each collected sample was appropriately labelled and transported to the Microbiology Laboratory of Gombe State University for processing, experimentation, and analyses (Soncy et al., 2015).

Sample Homogenization

Nine (9) grams of each sample was mixed with 100 mLs of sterile distilled water separately and blended for 2 minutes. The blender was disinfected thoroughly after blending each sample (Najafi and Bahreini, 2012).

Isolation and Sub-Culture

Initially, spread plate technique was employed which encompassed taking 0.1mL from each homogenized sample and releasing it on the surface of nutrient agar in Petri dishes, separately for all the samples. Subsequently, a sterile glass rod was utilized to spread the inoculum evenly over the surface of the agar. The Petri dishes were then incubated in inverted positions at 37^{o}C for 24h (Ali et al., 2023). Subsequently, suspected *Shigella* spp were sub-cultured onto *Salmonella-Shigella* (SS) agar using streak plate method and incubated for another 24h at 37^{o}C (Shiwito Ango et al., 2023).

Identification

This was achieved using standard methods for determination of physical culture characteristics, Gram's staining/microscopy (Percival and Williams, 2013), and biochemical tests which included Kligler iron agar (KIA) test, catalase test, and motility-indole-urease (MIU) test (Dessale et al., 2023).

Antibiotic Susceptibility Assay

This was done using the guidelines of the Clinical and Laboratory Standards Institute. 24h old sub-cultures were employed in standardization of the inocula to 0.5 McFarland standard by utilizing direct colony suspension technique. Afterwards, these inocula were spread plated on Mueller Hinton agar Petri dishes, antibiotic discs were dispensed after 5 minutes, allowed to stand for 30 minutes, followed by incubation at 35^{o}C for 18h. Afterwards, the average zones of inhibition to the nearest millimetre were recorded and interpreted (CLSI, 2021).

Determination of Risk Factors

A questionnaire that was adopted and modified from the work of Iwu et al., (2017) was used to synchronously collect data from 257 respondents (100 ayamutsa sellers plus 157 vegetable sellers that also sell ayamutsa when opportune) during sampling. These data were then analyzed using chi-square test for association at 95% confidence limit and 5% margin of error (McHugh, 2013) using IBM SPSS version 25 software (Hinton, 2014).

RESULTS

Results of Isolation

The results of isolation revealed that 10% (n=10) of the samples presented growth of suspected *Shigella* spp based on physical observation of colonial morphology and appearances on nutrient agar and *Salmonella-Shigella* (SS) agar.

Results of Identification

Physical observation of culture characteristics on nutrient agar (Plate 1) revealed suspected *Shigella* spp as glistening, translucent, round-shaped colonies, while on SS agar (Plate 2), these colonies appeared colourless-opaque, round shaped, and smooth-textured.

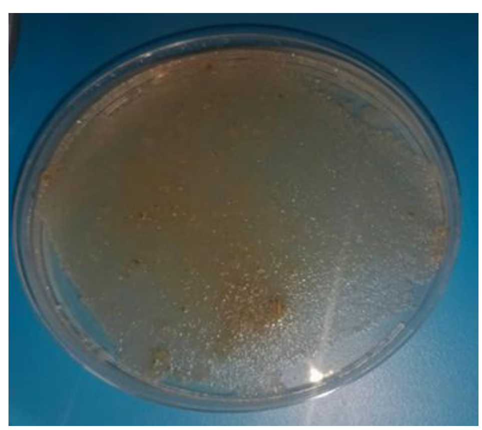

Plate 1: culture of suspected *Shigella* spp on nutrient agar

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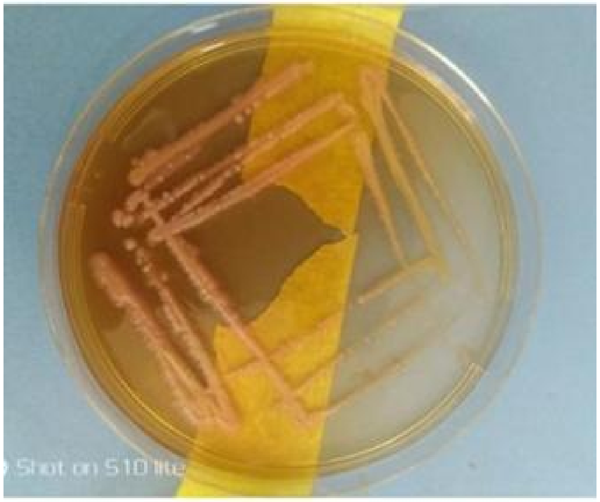

Plate 2: culture of suspected *Shigella* spp on SS agar

The results of Gram's staining (Plate 3) and microscopy (Plate 4) revealed that the suspected *Shigella* spp were Gram-negative and rod-shaped (bacilli).

Plate 3: Gram-stained slide

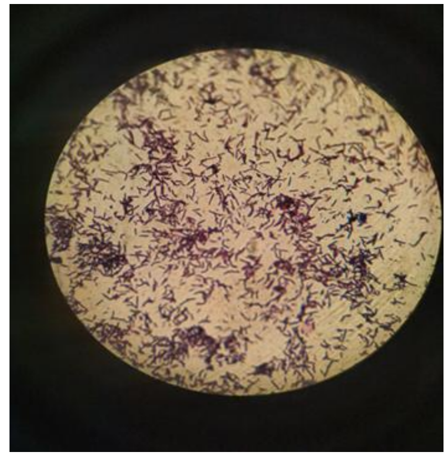

Plate 4: Microscopy

Results of Biochemical tests revealed that the suspected *Shigella* spp were catalase positive (Plate 5), KIA positive, and MIU negative (Plate 6).

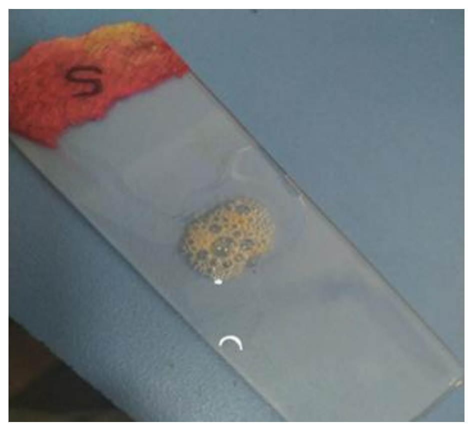

Plate 5: catalase test

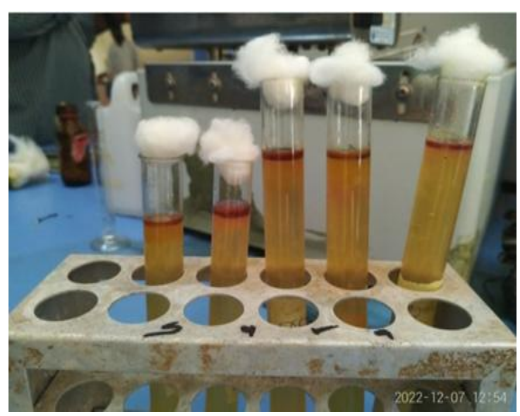

Plate 6: MIU test

The antibiotic susceptibility assay results (Table 1) revealed that the identified *Shigella* spp were resistant to cefuroxime, intermediately susceptible to erythromycin and ceftazidime, and sensitive to Augmentin, ceftriaxone, and gentamicin, based on the measured zones of inhibition (Plate 7).

Table 1: Results of antibiotic susceptibility assay

Antibiotics		**Zones of inhibitions (mm)**									
		S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
Gentamicin	10µg	22	26	28	22	29	22	23	25	29	27
Ceftriaxone	30µg	23	25	23	21	28	25	24	26	27	23
Augmentin	30µg	28	25	25	23	27	25	25	21	26	25
Cefuroxime	30µg	14	14	13	16	12	16	14	13	14	14
Erythromycin	5µg	18	15	16	17	18	15	14	15	19	14
Ceftazidime	30µg	14	12	13	12	11	12	14	12	11	13

Key: S1-S10=*Shigella* spp isolates

Plate 7: Antibiotic susceptibility test plate

Results of Risk Factors Determination

The chi-square test results (Table 2) revealed that lack of hand washing with soap (variable 7), type of material used for cleaning the containers (variable 10), repeated use of dish cleaning material (variable 11), and storing ayamutsa near raw food in the refrigerator (variable 14), were significantly associated with occurrence of *Shigella* spp in the samples.

DISCUSSION

The percentage of *Shigella* spp occurrence (10%) in the ayamutsa samples may seem low, but this actually presents a cause for alarm from the public health perspective considering the low infectious dose of the pathogen (Percival and Williams, 2013) which facilitates infection with relatively low numbers of this pathogen, and also considering the faeco-oral route of transmission and the dearth in availability of potable water in the study area (Gimba, 2024) which is a noteworthy risk factor for *Shigella* spp infection (CDC, 2023).

The occurrence of *Shigella* spp in ayamutsa as reported in this study agrees with the reports of Kabir et al. (2020), Adeleye et al. (2019), and Oyedele et al. (2020), who isolated *Shigella* spp from ready-to-eat vegetables in Katsina metropolis, Dutse, and south-western Nigeria, respectively.

The resistance to cefuroxime observed in all the *Shigella* spp isolated in this study could be attributed to antibiotic misuse/abuse that is rampant in Nigeria (Abibu et al., 2023) which has been documented to be responsible for development of antibiotic resistance by bacteria generally (Ventola, 2015). This issue needs urgent attention because the number and trend of antibiotic resistance by *Shigella* spp recovered from food and diarrhoeal stools have been reported to be on the increase in Nigeria since 2004 (Iwalokur et al., 2004).

The occurrence of cefuroxime resistant *Shigella* spp in ayamutsa as described in this study is in line with reports of Berihu et al.

(2024) who had isolated *Shigella* spp with high levels of antibiotic resistance from ready-to-eat fruit juices and salads in Northern Ethiopia, and also in line with the findings of Ogunremi (2021) who had isolated antibiotic resistant *Salmonella* spp from ready-to-eat fresh produce retailed in Umuahia, Nigeria.

Table 2: Results of chi-square test

VARIABLE ID	FREQUENCY								Total Respondents	Pearson Chi-Square	Significance (*p*-value)
	Strongly agreed/ Very common/ Yes/Very Likely		Agreed/Somewhat common/No / Somewhat likely		Partially agree/ Not very common/ Not sure/ Don't know		Not agreed/ I Don't know,				
	Yes	No	Yes	No	Yes	No	Yes	No			
1.	15	117	13	73	2	22	0	15	257	3.243	0.356
2.	11	69	13	106	4	33	2	19	257	0.520	0.914
3.	15	124	11	64	4	20	0	19	257	3.848	0.278
4.	11	81	3	45	10	75	6	26	257	2.931	0.402
5.	9	84	7	63	8	50	6	30	257	1.673	0.643
6.	5	45	13	122	7	27	5	33	257	3.385	0.336
7.	20	79	7	55	3	76	0	17	257	13.993	0.003
8.	22	115	6	82	2	20	0	10	257	6.031	0.110
9.	13	116	14	71	2	27	1	13	257	3.136	0.371
10.	11	78	6	115	9	23	4	11	257	17.002	0.001
11.	10	55	13	61	70	93	0	18	257	7.859	0.049
12.	11	129	19	96	0	1	0	1	257	4.864	0.182
13.	14	67	16	156	0	9	0	1	257	3.939	0.268
14.	19	130	7	93	0	3	4	1	257	25.322	0.000
15.	13	83	5	67	2	21	10	56	257	2.859	0.414
16.	10	65	12	115	6	28	2	19	257	2.081	0.556
17.	6	78	5	44	15	87	4	18	257	3.588	00.309
18.	11	68	10	100	2	31	7	28	257	4.461	0.216
19.	12	107	8	64	8	41	2	15	257	1.343	0.719
20.	7	88	5	67	3	26	15	86	257	2.660	0.447

The discovery in this study that certain unhygienic handling practices were significantly associated with *Shigella* spp occurrences in the ayamutsa was not unexpected because such practices have been known to create an avenue for transmission of pathogens from hands/or environment to foods (Schneider et al., 2015). These findings agree with the works of Abakari et al. (2018) who described how improper food handling could be responsible for occurrences of some pathogenic bacteria including *Shigella* spp in ready-to-eat vegetable salads in Tamale, Ghana.

The findings in this study further agree with the review of Garba et al. (2022) who described how mishandling contributed to contamination of vegetables with pathogenic bacteria, and also with the work of Younus et al. (2020) who highlighted that those foods typically handled, arranged, and sold at roadside eateries and other open sites are generally unhygienic.

The findings from this study have provided information that can be used for targeted health interventions particularly towards the ayamutsa vendors with the aim of educating them on hygienic food preparation and handling. This will improve the bacteriological safety of ayamutsa and reduce the public health threat it poses to the community.

CONCLUSION

At the time of this study, 10% of ayamutsa sold in Gombe metropolis were contaminated with *Shigella* spp that were resistant to ofloxacin and oxacillin, and these contaminations were found to be significantly associated with the handling practices of the vendors.

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