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Occurrence of *Salmonella* and *Shigella* Species on Meat Contact Surfaces of Selected Butcher Shops in Wukari Metropolis, North-Eastern, Nigeria

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Abstract

Bacterial contamination of meat occurs in different stages ranging from surfaces of slaughter house, during transportation and surfaces in the butchers' shop such as tables, logs, hooks, weighing balances, aprons and knives and the clothes and hands of butchers. The aim of this research is to assess meat contact surfaces for *Salmonella* and *Shigella* species in selected butcher shops in Wukari. Samples were collected randomly using sterile swab sticks from 120 contact surfaces, including 60 samples from School gate, 60 samples from New market and 5 samples from Old market in selected butcher shops in Wukari. The samples were inoculated using the streak plate method onto prepared Salmonella-shigella agar and incubated at 37°C for 24 h. Colonies were sub cultured to obtain pure colonies which were identified using biochemical tests. Antibiotic susceptibility testing was done using the Kirby-Bauer disc-diffusion method after standardizing the inoculum using McFarland standards. The predominant bacterial isolate was *Salmonella* species (20.8%), while *Shigella* spp. (16.7%) was the least dominant bacterium. *Salmonella* spp. was 100% resistant to Ceporex, Augumentin, Peflacine, Ceftazidime. *Shigella* spp. was 100% resistant to Augumentin, Ceftazidime, Cetriaxone, and Ciprofloxacin. Both species had MRA index greater than 0.2. The results show high bacterial

contamination on meat contact surfaces in Wukari, including *Salmonella* and *Shigella*, with high antibiotic resistance to commonly used antibiotics. Strict hygiene practices, including regular hand washing, use of gloves, and proper sanitation of meat contact surfaces, should be implemented and enforced.

Keywords: Pathogens, Surface, Contact, Meat, Resistant

INTRODUCTION

Salmonellosis is a disease caused by *Salmonella* sp. that affects both humans and animals worldwide. Although the disease typically manifests as mild gastroenteritis in humans, life threatening systemic infections are very common, particularly among high-risk groups (Larock *et al*., 2015). Due to a major lack of coordinated national epidemiological surveillance systems and a poor healthcare system, the sources and routes of transmission of non-typhoidal salmonella remain unknown throughout Africa (Gunn *et al.,* 2014).

Salmonella is one of the primary sources of infection in food-producing animals, particularly chicken, and this has a direct impact on the global marketing of food-producing animals as well as animal-derived food items (Fagbamila *et al.,* 2017). *Salmonella* has been found in retail items such as chicken. *Salmonella* species is most commonly seen in retail chicken (Li *et al.,* 2020). Shigellosis, commonly known as bacillary dysentery, is a bacterial infection caused by Shigella. *Shigella sonnei* (about 70%) and *Shigella flexneri* (roughly 25%) are the most commonly implicated species, while other species are rarely implicated (Leting *et al.,* 2022).

The sources of these pathogens include the birds and animals including humans (Tagar and Qambrani, 2023). Foodborne pathogens mainly contaminate meat during preparation or processing and handling (Pakbin *et al*., 2021). During slaughter, dressing, and cutting involves the transfer of microorganisms from the exterior of the animal mainly from its hide and slaughter or sale surfaces. (Kanko *et al.,* 2023). The possible sources of these bacteria are skin of the animal, the meat contact surfaces (tables, logs, hooks, weighing balances and knives) and the clothes and hands of butchers (Bersisa *et al.,* 2019; Chutia *et al.,* 2019).

Meat handlers in Nigeria like their counterparts in other developing countries are yet to come to terms with these meat safety management protocols (Iwuagwu *et al.,* 2023).

Animals slaughtering and carcass handling in Nigeria also fall short of acceptable international standards; and that fresh meat sold to the public is contaminated at various degrees and stages. The display of meat in the market and the examination of meat with dirty hands by meat sellers and buyers with flies perching on them (Innocent *et al.,* 2023).

According to Food and Agriculture Organization of the United Nations (Sverdlik, 2017), most foods prepared in informal markets like butcher shops are in open-air public spaces either on foot or from mobile outlets, fixed outlets or removable outlets without enclosed space. This therefore portends increasing tendencies for meat contamination with ultimate compromise of consumers´ health and safety. Adesokan *et al.* (2021) reported that more than 91 million persons fall ill due to food-borne pathogens with 137,000 deaths recorded each year in Africa (Adesokan *et al.,* 2021).

Foodborne diseases occur commonly in developing countries, particularly Nigeria, because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, a lack of financial resources to invest in safer equipment, and a lack of education for food handlers (Endale and Hailay, 2013).

In spite of the increased consumer demand for food safety standards for raw meat, there are still poor hygiene and sanitary practices along the food production chain, which contribute to the unacceptable levels of bacterial load in meat. This research is of paramount public health concern considering the continued global emergence and re-emergence of food borne diseases. The aim of this research is to assess meat contact surfaces of in butchers' shops within Wukari metropolis.

MATERIALS AND METHODS

Study Area

This study was conducted within the metropolis of Wukari. Wukari lies within latitude 7˚51 N of the equator and 9˚47 E of the meridian with the land area of approximately 4,308km² (Abuh *et al.,* 2017). It is located in the southern part of Taraba State. It is bordered with Takum to the South, Donga to the East, Ibi to the North, at the west with Ukum local government area of Benue State and Gassol to the North-East. It is an important town in Taraba State, characterized with agricultural and commercial activities among other activities (Samuel *et al*., 2022).

Collection of Samples

Samples were collected randomly using sterile swab sticks from 120 contact surfaces including 5 samples from school gate, 60 samples from old market and 60 samples from new market from selected butcher shops in Wukari, The swabs were first moistened with normal saline solution and then rubbed gently and thoroughly over the surface to be sampled by swabbing a defined area of about 2 cm^2 of the surface especially areas with cracks, corners, or crevices as described by Azuamah *et al.* (2019). The samples were then transported in sterile containers to the Microbiology Laboratory Federal University Wukari, Taraba State for bacteriological assessment.

Isolation and Identification of *Salmonella* and *Shigella* species

Salmonella and Shigella species were isolated using selective media such as salmonella and shigella agar (SSA), xylose lysine deoxycholate agar (XLD), and xylose lysine tergito4 agar (XLT4). The bacteria were identified using biochemical tests such as catalase, oxidase, indole, citrate utilization test, hydrogen sulphide, methyl red, triple sugar iron test, nitrate reduction, glucose fermentation, and urease tests as reported by Iwuagwu *et al.* (2023). The isolates were further confirmed using Microgen GN-ID system which comprises two separate microwell test strips; GN A and GN B. Each strip contains 12 standardized biochemical dehydrated substrates (www.microgenbioproducts.com).

Preparation of McFarland 0.5 Standards for antibiotic susceptibility test

Approximately 85 ml of 1% H_2SO_4 was added to a 100ml volumetric flask. 0.5 ml of 1.175% $BaCl_2$ was added using a 0.5ml volumetric pipette and H_2O drop-wise to the H_2SO_4 while constantly swirling the flask. It was placed on a magnetic stirring bar in flask and place on magnetic stirrer for approximately 3 to 5 min (Khan, 2021).

Standardization of Inoculum

Twenty four h culture of the organism was suspended into test tube of sterile normal saline using sterile wire loop to form turbidity that match with 0.5 scale of McFarland's standard (1.5 × 108 cells/ml) (Khan, 2021).

Antibiotic Sensitivity Test (Kirby-Bauer Assay)

Antibiotic susceptibility testing was conducted following the Kirby-Bauer disc-diffusion test as earlier described (CLSI, 2023). Briefly, 3mL of sterile normal saline was used to emulsify an inoculum of each pure bacterial isolate. The density was thereafter adjusted to

0.5 McFarland standards. The mixture was then inoculated onto the Mueller-Hilton Agar (MHA) plates (Oxoid, England) using a sterile cotton swab dipped into the standardized suspension of bacterial cultures and the plates were left to dry. Antibiotic discs and were placed onto MHA plates. The discs contained Ceporex (30 μg), Ofloxacin (30 μg), Augumentin (30 μg), Peflacine (30 μg), Ceftazidime (30 μg), Gentamycin (10 μg), Ciprofloxacin (30 μg), Cetriaxone (30 μg) and Streptomycin (300 μg) (antibiotic Becton Dickinson and Company, Sparks, USA). Thereafter, the plates were incubated aerobically at 37°C for 24 h (Adesokan *et al.,* 2021). The zone of inhibition was measured in millimeters. The zone diameters were interpreted as susceptible, intermediate and resistant on the basis of the critical points recommended by Clinical and Laboratory Standards Institute in accordance with standards (CLSI, 2023).

RESULTS

The biochemical characterisation as presented in Table 1 showed that *Salmonella* species were positive to triple sugar iron test, hydrogen sulphide, nitrate reduction and glucose fermentation. *Shigella* species were positive to methyl red, glucose fermentation and citrate. The bacteria isolates were all gram-negative short rods and negative to indole, catalase, urease, oxidase.

The prevalence of the bacterial isolates is presented in Figure 1. The most predominant bacterial isolate was *Salmonella* species (60%), while *Shigella* spp. (40%) was the least dominant bacterium. Table 2 shows the antibiotic susceptibility test of the bacteria isolates. *Salmonella* spp. was resistant to Ceporex (CEP), Augumentin (AU), Peflacine (PEF), Ceftazidime (CTZ), and sensitive to Ofloxacin (OFX). *Shigella* spp. was resistant to Augumentin (AU), Ceftazidime (CTZ), Cetriaxone (TRX), and Ciprofloxacin (CPX), while being intermediate for Ceporex (CEP). Table 3 shows the resistance pattern of *Salmonella* and *Shigella* species.

Table 1: Biochemical test

S/N	Gram reaction	Indole	Catalase	CUT	Oxidase	H_2S	MR	TSI	NR	GF	Urease	Inference
1	-ve rod	-	-	+	-	+	-	+	+	+	-	*Salmonella* species
2	-ve rod	-	-	+	-	-	+		-	-	-	*Shigella* species

Key: CUT = Citrate utilisation test, MR = Methyl red, TSI = Triple sugar iron test, NR = Nitrate reduction, GF = Glucose fermentation, H_2S = Hydrogen sulphide, - = Negative result, + = Positive result

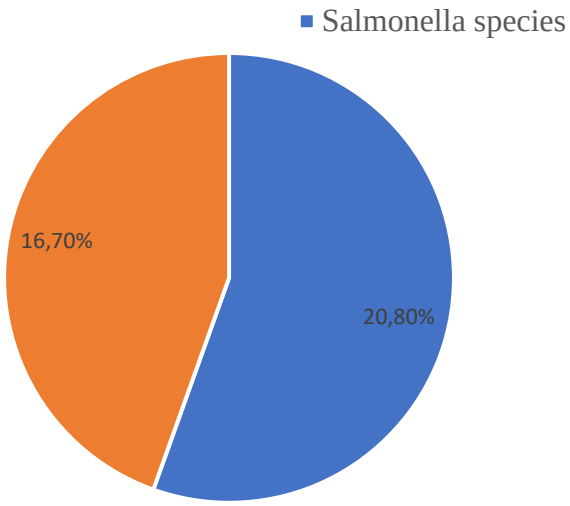

Figure 1: Prevalence of *Salmonella* and *Shigella* species isolated from meat contact surfaces

Table 2: Antibiotic susceptibility of *Salmonella* spp and *Shigella* spp isolated from meat contact surfaces

	Salmonella spp (n=25)			*Shigella* spp (n=20)		
Antibiotic	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
CEP	25(100)	0(0)	0(0)	0(0)	20(100)	0(0)
OFX	5(20)	3(12)	17(68)	6(30)	0(0)	14(70)
AU	25(100)	0(0)	0(0)	20(100)	0(0)	0(0)
PEF	25(100)	0(0)	0(0)	14(70)	6(30)	0(0)
CTZ	25(100)	0(0)	0(0)	20(100)	0(0)	0(0)
CN	17(68)	5(20)	3(12)	10(50)	3(15)	7(35)
CPX	25(100)	0(0)	0(0)	20(100)	0(0)	0(0)
TRX	17(68)	8(32)	0(0)	16(82)	4(20)	0(0)
S	14(56)	8(32)	3(12)	14(70)	6(30)	0(0)

Key: R= resistance, I= intermediate, S= susceptible, CEP= Ceporex, OFX= Ofloxacin, AU= Augumentin, PEF= Peflacine, CTZ= Ceftazidime, CN= Gentamycin, CPX= Ciprofloxacin, TRX= Cetriaxone and S= Streptomyci

Table 3: Resistance pattern of *Salmonella* and *Shigella* species

S/N	*Salmonella species*		*Shigella* species	
	Resistance pattern	Frequency	Resistance pattern	Frequency
1	CEP, AU, PEF, CTZ, CPX, S	1	CEP, AU, PEF, CTZ, CPX	1
2	CEP, AU, PEF, CTZ, CN, CPX, TRX	3	CEP, AU, CTZ, CPX, TRX	1
3	CEP, AU, PEF, CTZ, CPX, TRX, S	1	AU, CTZ, CN, CPX, CEP, TRX, S	1
4	OFX, AU, PEF, CTZ, CPX, S	1	AU, PEF, CTZ, CN, CPX, CEP, TRX, S	1
5	AU, PEF, CTZ, CN, CPX, CEP, S	1	OFX, AU, PEF, CTZ, CPX, CEP, TRX, S	1
6	OFX, AU, PEF, CTZ, CN, CPX, TRX, S	1	OFX, AU, PEF, CTZ, CN, CPX, CEP, TRX, S	1
7	AU, PEF, CTZ, CN, CPX, CEP, TRX, S	1		

Table 4: Multiple Drug Resistance (MRA) Index Pattern

Isolates	**No. of Multidrug Resistant Isolate**	**Classes of Antibiotic to Which Isolates Were resistant to**	**MAR Index**	**Percentage of Isolate With MRA Index Greater than 0.2**
***Salmonella* species**	1	5	0.5	5.6
	3	5	0.5	16.7
	1	5	0.5	5.6
	1	5	0.5	5.6
	1	5	0.5	5.6
	1	5	0.5	5.6
	1	4	0.4	5.6

***Shigella* species**	1	4	0.4	8.3
	1	4	0.4	8.3
	1	5	0.5	8.3
	1	5	0.5	8.3
	1	5	0.5	8.3
	1	5	0.5	8.3

Some of the isolates of *Salmonella* species were resistant to five different antibiotics, some against six antibiotics, some to seven, eight and nine antibiotics. The multidrug resistance index is presented in Table 4; out of the nine (9) isolated *Salmonella* species, eight (8) species had a MRA index of 0.5 with just one (1) species having 0.4. The percentage of isolate with MRA index greater than 0.2 ranges from 5.6 to 16.7. Out of the six (6) *Shigella* species isolated four (4) species had a MRA index of 0.5 and two (2) species had 0.4.

DISCUSSION

Salmonella and *Shigella* infections remain a major global public health problem, and meat is the primary source of transmission to humans (Ugbo *et al.,* 2023). This research showed that meat contact surfaces in Wukari are contaminated with *Salmonella* and *Shigella* species. This finding is consistent with the findings of Iwuagwu *et al.,* (2023) who reported *Salmonella* and *Shigella* species in meat contact surfaces in Abia State. This can be attributed to the poor hygienic and sanitary practices exercised during the slaughtering, transportation, and/or processing of the meat. This study shows that *Salmonella* sp. (60%) was the most prevalent bacteria isolate. This is lower compared to the prevalence of *Salmonella sp*. in other similar studies; Azage and Kibret (2017) 70% and Iwuagwu *et al.,* (2023) 62.68%.

The high prevalence of *Salmonella* species on the surfaces can be attributed to improper cleaning procedures, inadequate hygiene standards, and cross-contamination. The 100% resistant of *Salmonella* sp. to Ceporex (CEP), Augumentin (AU), Peflacine (PEF), Ceftazidime (CTZ), is not in agreement with the findings of Ugbo *et al.* (2023) who reported 50% to 100% resistance of *Salmonella* sp. to only ofloxacin and cefotaxime. *Shigella* sp. was resistant to Augumentin (AU), Ceftazidime (CTZ), Cetriaxone (TRX), and Ciprofloxacin (CPX).

Similarly, Garedew *et al.,* (2016) reported *Shigella* spp. to be resistant to Cetriaxone, ampicillin, amoxicillin. This is not in agreement with the findings of Dessale *et al.,* (2023) who reported *Shigella* sp. to be highly susceptible (87.5%, 7/8) to Ceftazidime, Ciprofloxacin, and ceftriaxone. All the isolates of *Salmonella* and *Shigella* exhibited resistance to one or more antibiotics. This could be due to indiscriminate use of antibiotics in the environment. The antibiotic resistance pattern recorded in this study might be due to the emergence of antibiotic resistant strains of *Salmonella* and *Shigella* species due to evolution or antibiotic pressure from the unrestricted use of antibiotics by the community and the routine practice of giving antimicrobial agents to domestic livestock as a means of preventing and treating diseases, as well as promoting growth that are subsequently transferred to humans though the food chain (Azage and Kibret, 2017).

Salmonella can grow in environments with high temperatures and moist conditions. Poor hygiene practices among workers and contaminated water sources also contribute to the spread of the bacteria. All the isolates in this research had Multiple Antibiotic Resistance index greater than 0.2. This is in agreement with the findings of Aondoackaa *et al.* (2024) who *Salmonella* and *Shigella* species from clinical samples to have MRA greater than 0.2. This may be as a result of previous exposure to antibiotics and development of resistance to commonly prescribed antibiotics, hence, antimicrobial susceptibility testing is imperative in selecting therapeutic options.

CONCLUSION

The results obtained from this study show that there was high bacterial contamination on meat contact surfaces in Wukari, including *Salmonella* (60%) and *Shigella* (40%), which exhibited a high antibiotic resistance to the commonly used antibiotics. This is a serious public health concern for meat consumers within the Wukari local government, as these bacteria are known pathogens that can cause foodborne illnesses. *Salmonella* and *Shigella* species have shown increasing resistance to antibiotics over the years. Contamination with antibiotic-resistant strains can complicate treatment and lead to a more severe and prolonged illness. It is therefore, recommended that Strict hygiene practices in butcher shops, including regular hand washing and proper clening of meat contact surfaces.

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