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INVESTIGATING THE PREVALENCE OF *SALMONELLA* SPP. FROM READY-TO-EAT BEEF (SUYA) SOLD AT THE UNIVERSITY OF CROSS RIVER STATE, CALABAR, NIGERIA

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Abstract

Meat is prone to contamination even when carefully handled properly, and *Salmonella* species remain the primary contaminant of meat at every stage. This study aimed to isolate *Salmonella* spp. from ready-to-eat beef sold (hawed) at the University of Cross River State (UNICROSS). Samples of ready-to-eat beef were randomly purchased from hawkers at three different locations in the university vicinity. Compared to those of the Centre for Food Safety, 33% of the samples from the wash and homogenized samples were unsatisfactory. Based on the biochemical analysis, all the samples tested positive for *Salmonella* spp. This study revealed that the ready-to-eat beef samples were contaminated with *Salmonella* spp. The study recommended that every stage of meat processing be closely monitored to minimize the rate of contamination. Advanced methods of identification should be used by future studies to reveal the particular serotype contaminating ready-eat beef in UNICROSS.

Keywords: Isolation; Characterization; Stick meat; *Salmonella* count, Public Health.

1.0 Introduction

It is widely reported that foodborne diseases are a great threat to humans worldwide (Adzitey *et al.*, 2020; Jaja *et*

al., 2020; Siriken, *et al.*, 2020; Sun *et al.*, 2021; Wang *et al.*, 2022). This can be attributed to the consumption of food, especially food that is reportedly undercooked and exposed to unhealthy

environments, as well as food prepared through questionable practices. Adley and Ryan (2016) stated that the process of producing food, which includes growth, harvest, transportation, and preparation, can be a source of infection for humans and animals when it occurs in unhygienic environments without proper temperature or environmental controls. Approximately 31 pathogens are known to be the main cause of foodborne diseases, 21 of which are bacteria, while five each are parasites and viruses (Adley & Ryan, 2016). Some of the predominant foodborne disease-causing bacteria include *Aeromonas hydrophila*, *Bacillus cereus*, *Clostridium botulinum*, *Brucella* spp., *Campylobacter* spp., *Vibrio cholerae*, *Enterobacter sakazakii*, *Escherichia coli*, *Listeria monocytogenes*, *Mycobacterium bovis*, *Coxiella burnetii*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Francisella tularensis* and *Yersinia* spp. (Ünüvar, 2018). Most of these foodborne diseases have been traced back to poor food hygiene practices and a lack of adherence to food safety protocols. One area of particular interest is meat contamination. Like in other food products, meat contamination can be traced through its host and/or the preparation processes involved. At every stage of the process, meat is prone to contamination, even when it is carefully and properly handled. Among the

bacteria causing meat contamination, *Salmonella* species remain the primary contaminants of meat at every stage. Nontyphoidal *Salmonella*, particularly *Salmonella enterica* and its serotypes, have been reported as the leading cause of meat and meat product contamination (Adley & Ryan, 2016; Bosilevac *et al.*, 2009; Ünüvar, 2018). This accounts for the high incidence of salmonellosis in the United States and other countries (Bosilevac *et al.*, 2009), including Nigeria. Typhoidal *Salmonella* has also been reported as a pathogen contaminating unhygienic meat (Kumar, *et al.*, 2022; Sodagari *et al.*, 2020; Thomas *et al.*, 2020).

At the Cross River University of Technology, the safety of beef cannot be guaranteed due to a lack of research on the assessment of *Salmonella* species in beef meat. Furthermore, the hawking of ready-to-eat beef is not regulated. Many individuals in the university vicinity are unaware of the preparation procedures involved in the hawking of meat. Interestingly, these hawked beef items are highly consumed by students, staff, and other workers at the university. Therefore, examining the safety of ready-to-eat meat is necessary to establish guidelines for the safe consumption of meat and to avoid outbreaks of foodborne diseases resulting from typhoidal or nontyphoidal *Salmonella* serotypes.

Thus, in this study, *Salmonella* spp. were isolated from ready-to-eat beef sold (hawed) at the University of Cross River State, Calabar.

2.0 Materials and methods

2.1 Area of the study

This study was conducted at the University of Cross River State. The university is located in Calabar, the capital city of Cross River State, Nigeria. The city is adjacent to the Calabar and Great Kwa Rivers and creeks of the Cross River (from its inland delta). Calabar is often described as the tourism capital of Nigeria, especially due to several initiatives implemented during the administration of Donald Duke as governor of Cross River State (1999–2007), which made the city the cleanest and most environmentally friendly city in Nigeria. Administratively, the city is divided into Calabar Municipal and Calabar South Local Government Areas. It has an area of 406 square kilometres (157 sq mi) and a population of 371,022 as of the 2006 census. Calabar is place where food vendors thrive for as a result of the high demand for fast food for students and other people in the city. It has often been described as a place for money because food vendors perform well. This is true for meat vending and hawking. Along almost every path, people selling grilled skewered meat,

especially beef kebab or stick meat, are popularly called Calabar.

2.2 Materials

The glassware and equipment used for this research included Petri dishes, glass slides, test tubes, a measuring cylinder, a syringe, beakers, a conical flask, an incubator, a refrigerator, a pressure pot, a Bunsen burner, a wire loop, a test-tube rack, stock bottles, a microscope, masking tape, foil paper, a plastic bag, ice pack cotton wool, a methylated spirit, a mortar and a pestle.

2.3 Media and reagents

The media and reagents used for this study included *Salmonella* Shigella Agar, Nutrient Agar, Peptone Broth, Simmons Citrate Agar, Urea, Kligler Iron Agar (KIA), Crystal Violet, Safranin Acetone, Hydrogen Peroxide Solution, Buffer, Dextrose, Indole Spot Reagent, and alcohol.

2.4 Sample collection

Three ready-to-eat beef samples were purchased from hawkers at three different locations at the University of Cross River State (UNICROSS), Calabar. The samples were collected through random sampling of various meat sticks by using sterile disposable tongs as outlined in the process hygiene criteria of Regulation EC 2073/2005 (Codex, 2005). The samples were wrapped in sterile aluminium foil to prevent contamination and transported to

Microbiology Laboratory, UNICROSS, for culture.

2.5 Sample preparation

The samples were aseptically washed and pounded to obtain washed and homogenized samples, respectively. This process resulted in two samples per sample (washed and rounded), yielding six samples as the total samples investigated. A sterile mortar and pestle were used to mash the washed beef kebab. One gram of the mashed suya was weighed and serially diluted using 1 ml of stock homogenate and 9 ml of distilled water. One milliliter of the washed samples was also serially diluted following procedures similar to those used for the mashed sample. This procedure was used to obtain a discrete colony (Adeleye *et al.*, 2022).

2.6 Plating of samples

The spread plate method was employed for the determination of the total viable count as described elsewhere by Chesbrough (2000). A tenfold serial dilution was performed for the samples in appropriate dilution tubes. Specifically, 0.1 ml of the homogenised sample was taken from tubes and spread onto sterile and freshly prepared *Salmonella* Shigella agar plates. Afterward, the plates were incubated at 37°C for 24 hours. Plates that did not show visible colonies after 24 hours were left for an additional 48 hours.

Discrete colonies were purified by sub-culturing on nutrient agar plates and subsequently identified using standard methods (Orpin *et al.*, 2019). Discrete colonies were removed after morphological observation and subsequently purified by restarting on nutrient agar plates before being stored on nutrient agar slants at 4°C for further biochemical characterization and identification.

2.7 Determination of the bacterial count

After the plates were incubated, the growth, number of colonies, morphology, and consistency of the isolates were examined, and the colony size was also measured. The discrete colonies were picked and sub cultured on a nutrient agar slant and incubated at 37°C for 24 hours. The sample was then refrigerated for further verification and confirmation via biochemical tests.

2.8 Biochemical test

The procedures adopted for Gram staining and other biochemical tests, as described by previous researchers, including Upula *et al.* (2021), were followed appropriately. The biochemical tests included catalase, coagulase, citrate, indole, urease, triple sugar iron (TSI), Voges–Proskauer (MR-VP), and motility assays.

2.9 Gram staining

A loopful of distilled water was placed on a grease-free slide, and a smear of the test isolate was prepared. The mixture was then allowed to air dry and heat fixed. The smear was flooded with crystal violet, allowed for 60 seconds and washed off with water. Lugol's iodine was applied, and the mixture was allowed to rest for 60 seconds before being rinsed off. The smear was decolorized with a few drops of acetone-alcohol for 3 seconds and immediately rinsed with water. Safranin was applied to the smear as a counterstain, which was allowed to stain for 30 seconds before rinsing off and blot-drying the slide. The preparation was then examined under a microscope using a 10100x oil immersion objective.

3.0 Results

3.1 Cultural characteristics of the colonies

All the colonies of the six samples cultured on Salmonella Shigella agar (SSA) were slightly pinkish, smooth, round, and slightly elevated. Only one colony was a nonlactose fermenter.

3.2 Biochemical characterization of the isolates

After confirming the Gram reaction of the isolates, many biochemical tests were conducted to aid in the identification of the isolates. These include citrate, oxidase, lactose, motility, indole, urease,

and triple sugar ions (KIA, H₂S and gas). All the isolates were bacilli (rod) and were negative according to oxidase, indole and urease tests. All the isolates were motile, while five isolates were lactose positive with only one nonlactose fermenter. The triple sugar ion density, as determined through KIA, showed a red slant and a yellow but with black deposits (hydrogen-sulfide formation) and gas. This suggested that all the samples studied tested positive for *Salmonella*. The biochemical characterization of the isolates is presented in Table 1.

3.3 Total Salmonella Count (TSC)

The total *Salmonella* count was determined by culturing the ready-to-eat beef samples on freshly prepared *Salmonella-Shigella* agar plates. The traditional method of counting bacterial colonies involved manually counting each colony on media. There was bacterial growth in all the samples analyzed. The counted colonies were not recorded based on the total *Salmonella* count until thorough identification of the colonies was complete, at which point the isolates were confirmed to be *Salmonella*. The TSCs of the washed beef samples were 0.5×10^5 CFU/ml for sample W1, 13.4×10^5 CFU/ml for sample W2 and 0.1×10^5 CFU/ml for sample W3. This implies that the highest number of colonies recorded in the washed samples was from sample W2 (13.4×10^5 CFU/ml),

while the number of colonies in the washed sample with the lowest TSC was from W3 (0.1×10^5 CFU/ml).

For the pounded samples, the TSC concentration was 0.1×10^5 CFU/g for sample P1, 0.2×10^5 CFU/g for sample P2, and 9.6×10^5 CFU/g for sample P3. The highest colony count was obtained from sample P3 (9.6×10^5 CFU/g), while the lowest colony count was obtained from sample P1 (0.1×10^5 CFU/g). The TSC data are presented in Table 2.

Table 1: Biochemical characterisation of the isolates

Isolate	Gram	Shape	Catalase	Citrate	Oxidase	Lactose	Motility	Indole	Urease	Triple Sugar Iron				Suspected Organism
										Slant	Butt	H ₂ S	Gas	
W1	-	Rod	+	+	-	+	+	-	-	R	Y	+	+	<i>Salmonella</i> spp.
W2	-	Rod	+	+	-	-	+	-	-	R	Y	-	+	<i>Salmonella</i> spp.
W3	-	Rod	+	+	-	+	+	-	-	R	Y	+	+	<i>Salmonella</i> spp.
P1	-	Rod	+	+	-	+	+	-	-	R	Y	+	+	<i>Salmonella</i> spp.
P2	-	Rod	+	+	-	+	+	-	-	R	Y	+	+	<i>Salmonella</i> spp.
P3	-	Rod	+	+	-	+	+	-	-	R	Y	+	+	<i>Salmonella</i> spp.

Key: W= Washed sample; P = Pounded sample; - = Negative; + = Positive; R/Y = Red/Yellow; ++ = Very strong positive

TABLE 2: Total *Salmonella* Count (TSC)

Sample Code	Total <i>Salmonella</i> Count
W1	0.5x10 ⁻⁵ cfu/ml
W2	13.4x10 ⁻⁵ cfu/ml
W3	0.1x10 ⁻⁵ cfu/ml
P1	0.1x10 ⁻⁵ (cfu/g)
P2	0.2x10 ⁻⁵ (cfu/g)
P3	9.6x10 ⁻⁵ (cfu/g)

Key: CFU/ml = colony forming unit per mil; cfu/g = colony forming unit per gram

4.0 Discussion

It is well documented that meat is a good medium for the growth of microorganisms, including *S. enterica*, because it is rich in proteins, lipids, and other nutrients that microorganisms use for growth (Prescott *et al.*, 2002). A publication in Meter Food (2017) revealed that meat has a temperature range of 35°C-37°C and an optimum pH of 6.5–7.5 in addition to water activity, which are favorable conditions for the growth of *Salmonella* spp. The World Health Organization reported that 2500 different *Salmonella* strains and salmonellosis caused by nontyphoid *Salmonella* serotypes have been identified (Nair *et al.*, 2015; WHO, 2013; Yin *et al.*, 2016). *Salmonella enterica* and *S. typhimurium* are the two most important serotypes of salmonellosis transmitted from animals to humans in most parts of the world (Switt *et al.*, 2009). This bacterium is widely distributed in domestic and wild animals. Salmonellosis in humans is generally contracted through the consumption of contaminated food of animal origin, including milk, meat, eggs, and

poultry, and other contaminated foods, such as condiments, green vegetables, chocolate, and drinking water (Reddy *et al.*, 2016; Rey-Matias *et al.*, 2016; Switt *et al.*, 2009; Zhu *et al.*, 2019).

This study was undertaken to investigate the presence of *Salmonella* species in ready-to-eat beef (stick meat) that was hawked in the vicinity of Cross River University of Technology (CRUTECH), Calabar. The study confirmed the presence of *Salmonella* spp. in all the samples studied. The total *Salmonella* count (TSC) was 0.5x10⁵ CFU/ml, 13.4x10⁵ CFU/ml, or 0.1x10⁵ CFU/ml for the washed samples. The TSCs for the homogenized samples were 0.1x10⁵ CFU/g, 0.2x10⁵ CFU/g, and 9.6x10⁵ CFU/g. Overall, the TSC of the washed samples was greater than that of the homogenized samples. Compared to the fewer than 10 colonies for satisfactory results proposed by the NAFDAC (2021), four plates were satisfactory, while two plates were not satisfactory. This finding suggested that some of the meat samples were poorly

processed and handled. These two unsatisfactory results are similar to those of a previous study in Bangladesh in which 3.15×10^4 , 2.68×10^3 , 4.46×10^3 and 1.19×10^4 CFU/gm TSC were reported from commercially available poultry feeds (Sultana *et al.*, 2017). Similarly, Adzitey *et al.* (2020) characterized *Salmonella enterica* and reported a microbial load of 3.36 log cfu/cm² with a 42.22% occurrence rate in the Tamale metropolis of Ghana.

The cultural and biochemical characteristics of the isolates revealed that they were members of the Enterobacteriaceae. In particular, all the isolates produced air bubbles, while five isolates produced hydrogen sulfide (H₂S) on the media. This suggested that the bacteria were *Salmonella* species. Interestingly, five isolates were lactose fermenters, while the other was a nonlactose fermenter. Although *Salmonella* species are generally known as nonlactose fermenters, many studies have isolated lactose-fermenting *Salmonella* strains. Several studies have reported strains of Lactose-Fermenting *Salmonellae* in the case of *S. anatum*, *S. newington*, *S. typhimurium*, *S. tennessee* and *S. seftenberg*, all of which belong to the nontyphoidal group (Blackburn & Ellis, 1973; McDonough, *et al.*, 2000; Falcao *et al.*, 1975; Falkow S, Baron, 1962). Although Manafi, Aliakbarlu and Dastmalchi (2020) identified typhoidal and nontyphoidal *Salmonella* (*S. enteritidis*, *S. typhimurium*, typhi and others), further verification, such as the use of Vitek, would clearly characterize the isolates in the present study. Manafiet *al.* (2020) reported 19 *Salmonella-positivity* isolates from meat samples via biochemical analysis and subsequently discovered seven additional *Salmonella-positivity* strains via multiplex PCR. Sallam *et al.* (2014) also reported inconsistencies between

biochemical and molecular methods. For example, Latif *et al.* (2014) reported a case of lactose-fermenting *Salmonella* Paratyphi A in a blood sample from a 27-year-old male with a 12-day history of fever.

Since the contamination of ready-to-eat meat can occur at any stage, contamination could result from poor hygienic practices by sellers or from processing processes (undercooking). Some of the meat vendors' hygiene practices are unsafe; hence, the high TSC in this study is worrisome and not surprising. Apart from the vendors themselves, many buyers could contribute to the contamination of these samples because meats are usually left uncovered for the buyers to select for their satisfaction. Such practices contribute to sample contamination (Jeffer *et al.*, 2021). Several studies have reported *Salmonella* cases across the globe. For example, Dallal *et al.* (2010) reported a 33% prevalence of *Salmonella* in beef and chicken samples in Iran. Similarly, 30% of the population was reported in Egypt (Sallam, 2014), 58% was reported in Vietnam (Nguyen *et al.*, 2016), 24.27% was reported in Iran (Manafi *et al.*, 2020), and 1.3% was reported in China (Ni *et al.*, 2018).

Overall, this study deduced, based on the lactose fermentation feature closely related to these features, *Salmonella* species are *S. typhimurium*, which has been isolated from meat and meat products, especially from undercooked meat (Kumar *et al.*, 2022; Yang *et al.*, 2010; Uzeh *et al.*, 2021; Wang *et al.*, 2022). One factor that contributed to the contamination of these meat samples was their condition. Dodd *et al.* (2017) explained that contamination results from warm meats being sampled and being sold at ambient temperature, thus providing a favorable environment for the growth of *S. enterica*.

Yang *et al.* (2010) also concluded that the predominant origin of nontyphoid *Salmonella* may have resulted from undercooked meat. These bacteria can be threatening, as cases of multidrug resistance have been reported (Bosilevac *et al.*, 2009; Yu *et al.*, 2017; Zhu *et al.*, 2019). This study revealed that all the meat samples tested positive for *Salmonella*. Some of the samples are safe for consumption, but a few are unsatisfactory. It is therefore necessary for food vendors to observe adequate hygiene practices to reduce the rate of contamination.

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