RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v24i1.5

Alu *et al.*/*Sokoto Journal of Veterinary Sciences*, **24**(1): 35 - 46.

Evaluation of the bacteriologic quality of poultry carcasses during slaughter in Makurdi, Nigeria

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Publication History:
Received: 18-06-2025
Revised: 04-01-2026
Accepted: 06-01-2026

Abstract

This study was carried out to determine the microbiological hazards associated with the poultry slaughter process and to detect critical control points necessary for effective control of hazards. Three poultry slaughter slabs located in Makurdi were used, poultry carcasses from the slabs were examined at all the stages of slaughter process. The analyses were conducted by collecting swabs of each carcass after each stage during processing, the butchers' palm, table top, meat and water samples, assessing their microbiological qualities and presence of foodborne pathogen (*Salmonella* spp.) as indicator organism using standard microbiological procedures. A total of 249 samples were evaluated (October to December 2019) using the bacteriological analytical method. The results showed that mean total aerobic count (TAC) and mean Total Coliform Count (TCC) at the different sampling sites surpasses the recommended value with the mean Total Aerobic Count at point 2 and Table-top showing statistically significant difference between the different sampling sites. Seventeen (6.8%) of the 249 samples were positive for *Salmonella* spp., out of which 53% were from Wurukum market, 29% from Wadata market and 18% from Modern market. Antibiotic susceptibility testing showed that 82.4%, 76.5%, 64.7% and 52.9% were resistant to Colistine Sulphate, Ampicillin, Tetracycline and Ceftriaxone while 94.1%, 94.1%, 88.2% and 47.1% were highly susceptible to Ciprofloxacine, Imipenem, Enrofloxacine and Chloramphenicol. PCR confirmed the presence of *InvA* gene (11.8%) in 17 isolates. The high levels of TAC, TCC and isolation of *Salmonella* spp. with *InvA* gene capable of *invA*siveness indicates an unhygienic slaughter process practiced in Makurdi. which poses a risk to public health due to the potential of consumption of foof contaminated with *Salmonella* spp. with *InvA* gene. Evisceration, carcass wash, meat, table-top and butchers' palm are considered as critical control points during poultry processing in this study.

Keywords: HACCP, Makurdi, Microbiological hazards, Poultry, Salmonella

Introduction

The environment and processes used in chicken processing are critical to the quality and safety of poultry meat (Oladiran & Kabir, 2015). Producers, consumers and public health officials around the world are concerned about the microbiological purity and safety of commercially processed poultry products. With the rising global consumption of poultry meat and the wide range of poultry meat products and customer demand, guaranteeing the microbiological safety of poultry carcasses and cuts has never been more important (Rouger *et al.,* 2017). The microbiological quality of freshly processed poultry carcasses at the processing plant is determined by the level of contamination from live birds, the number and types of microorganisms introduced, contamination spread or cross contamination, technical design (type) of processing equipment, processing efficiency, temperature control, sanitary and hygienic practices in the plant (Rasschaert *et al.,* 2008). Meat can directly endanger customers' health if it contains microorganisms in toxic doses or dangerous amounts of their toxins (Barkocy-Gallagher *et al.,* 2003). Poultry products are recognized as major vehicles for transmitting *Salmonella* spp. to human with 40% cases attributable to consumption of poultry products (Chashni *et al.,* 2009; Ruban *et al*., 2010). Salmonella are pathogens of public health importance causing gastroenteritis and enteric fever in humans (Akond *et al*., 2012). Salmonellosis is one of the most common food borne diarrheal disease worldwide and remains a major public health problem in many parts of the world (Riyaz-UI-Hassan *et al.,* 2004). Majority of the over 2650 recognised serotypes of Salmonella infect both human and animals worldwide with signs ranging from fever, abdominal cramps, vomiting, diarrhoea and death (Guibourdenche *et al*., 2010; Scallan *et al.,* 2011; Issenhuth-Jeanjean *et al.,* 2014).

The importance of food as a vehicle for the transmission of salmonellosis has been well documented (Matsuoka *et al.,* 2004; Mullner *et al*., 2009; Dallal *et al.,* 2010). A variety of foods have been implicated as vehicles for transmitting salmonellosis to humans, including poultry, beef, pork, eggs, milk, cheese, fish, shellfish, fresh fruit, juice and vegetables (Acha & Szyfres, 2001; Mazurek *et al.,* 2004; Espi *et al.,* 2005; Varma *et al.,* 2005). However, poultry, egg, meat and dairy products continue to be the most common food vehicles of Salmonella infection (Rodrigue *et al.,* 1990; D'Aoust, 1994; Llewellyn *et al.,* 1998). Bacterial organisms generally are a major safety concern to the meat industry, where contamination can occur at multiple stage along the food processing chain either at the pre-harvest, harvest or post-harvest stages. Food handlers may re-contaminate a thoroughly processed or cooked meat for consumption (Oscar, 2013; Wagner, 2013). This is especially common in most developing countries where dressed carcasses are transported openly or kept in contaminated surfaces.

Despite the occurrence of Salmonella in raw and cooked poultry and poultry products as well as in beef in Nigeria (Mbata, 2005; Smith *et al*., 2012; Tafida *et al.,* 2013), very little information is available on the occurrence of Salmonella at poultry slaughter plants/houses even when it has been shown to be the main source of cross-contamination in many parts of the world (Wang *et al.,* 2013).

Most birds are processed in an open environment at a slaughter slab in the market with poor sanitary and hygienic conditions. It is not uncommon to see faecal contamination of carcasses from the gut during slaughter and processing of these birds. This may lead to contamination of the processing line, equipment and subsequently cross-contamination of non-infected birds and humans (Olsen *et al.,* 2003; Rostagno *et al.,* 2006).

For effective food safety management plan, it is important to continuously monitor the presence of Salmonella in various food sources especially poultry (WHO, 2010).

This study was designed to evaluate the bacteriologic quality of poultry carcass with Salmonella as the indicator organism during the slaughter process in three slaughter slabs in Makurdi using Hazard Analysis and Critical Control Point (HACCP), to detect *InvA* gene by PCR methods and also to determine the antimicrobial susceptibility of *Salmonella* spp. to commonly used antimicrobial agents.

Materials and Methods

Study area

The study was carried out at three poultry slaughter slabs in Makurdi, Benue State. Makurdi is the capital of Benue state and lies between latitude 7º30'N and longitude 8º35'E with an estimated population of 489,839 people based on the World Population (WPP, 2024). The physiographic characteristics span between 73 and 167m above sea level. The town is divided by the river Benue into the north and south banks, connected by two bridges: the railway bridge and the new dual carriage bridge.

Sampling procedure

Sampling was carried out at intervals using a convenient sampling method from three poultry slaughter slabs in Makurdi, located at Wadata market, Wurukum market and Modern market.

Two hundred and forty-nine samples (n= $z^2p(1-p)/d^2$ (Thrusfield, 2005), where p =20.2% (Fagbamila *et al.*, 2017), were screened for microbes and Salmonella from seven different matrices using convenient sampling and based on the willingness of butchers to cooperate. Sampling was carried out five times in each of the slaughter slabs within a period of two months. These include 51 carcass swabs after defeathering (Point 1), 51 carcass swabs after evisceration (Point 2), 51 carcass swabs after washing (Point 3), 51 meat samples, 15 water samples, swabs from the palms of 15 butchers, and 15 swabs of table-tops. Swabs were collected in a sterile swab container containing 10ml of 0.1% buffered peptone water. Water and meat samples were collected in sterile universal bottles, labelled, placed on ice, and immediately transported to the Veterinary Public Health and Preventive Medicine laboratory, Joseph Sarwuan Tarka University, Makurdi (JOSTUM) for analysis.

Enumeration of bacterial load

The total aerobic counts (TAC) and total coliform counts (TCC) were determined by plating 0.1ml from 10-5 and 10-6 dilutions on nutrient agar and MacConkey agar plates, and incubated at 37℃ for 24hours, and colonies were evaluated using the method described by Ajogi *et al.* (2005).

Isolation and identification of Salmonellae

Isolation of Salmonella was carried out according to the International Organization for Standardization (ISO, 2002) for isolation and characterisation of *Salmonella* spp.

Pre-enrichment was done by using the swab in the 10ml Buffered Peptone Water (Oxoid, UK) to swab the carcass, butchers' palm, table tops, water sample and 50g of the meat sample homogenized in a blender, and all the samples were kept in an ice pack for less than 2 hours before processing.

0.1ml of the pre-enriched broth was inoculated into 10ml of Rappaport-Vassiliadis (RV) broth for enrichment and incubated for 24 hours at 37℃. A loop full of culture from RV was then plated simultaneously onto Xylose Lysine Deoxycholate (XLD) medium and incubated at 37℃ for 24 hours (ISO, 2002). Suspected isolates were identified by the colour and morphology of the colonies on the agar plates (Salmonella colonies are typically pink to red with black centres).

Biochemical characterisation of isolates

Biochemical assays were carried out as described by Raafat *et al.* (2011). All isolates that gave the following reactions: urease negative, citrate positive, motile in motility medium, oxidase negative, lysine decarboxylase positive, Voges-Proskauer negative, gram negative, fermented glucose, mannitol, dulcitol and maltose but failed to ferment lactose, sucrose were considered to belong to the genus Salmonella.

Antimicrobial susceptibility tests:

The antimicrobial susceptibility test was carried out using the agar disk diffusion method as described by Bauer *et al.* (1966) and Clinical and Laboratory Standards Institute (CLSI) 2017. The diameters of the zones of inhibition were recorded to the nearest millimetre and classified as susceptible, intermediate and resistant. The following antimicrobials were used; Ciprofloxacin (5µg), Chloramphenicol (30µg), Tetracycline (30µg), Enrofloxacine (5µg), Ampicillin (10µg), Colistin sulphate (10µg), Nalidixic acid (30µg), Imipenem (10µg), Ceftriaxone (30µg) and Amoxicillin-Clavulanic acid (30µg)

The data obtained were presented in tables. The results of TAC and TCC were expressed as CFU/ml and the mean ± standard deviation (SD) values were calculated. Analysis of variance with Tukey post hoc test was used to determine the difference between the mean TAC and TCC at different points in the slaughtering process. The level of significance was set at $p \leq 0.05$. TAC and TCC values was also interpreted. Chi square (χ^2) and odds ratio (OR) were used to test the association and risk of contamination between presence of *Salmonella* spp. and sampling location.

Detection of the InvA gene using the polymerase chain reaction (PCR) technique

The genomic DNA was extracted using a DNA-easy kit (Qiagen, USA) following the manufacturer's instructions. Briefly, 200µl of bacterial suspension (XLD) was added to 200µl of Lysis buffer (AL) in a 1.5ml micro-centrifuge tube and mixed by pulse vortexing for 15 seconds, incubated at room temperature for 20 mins. 400µl of absolute ethanol was added to the sample and mixed by pulse vortexing for 15 seconds. The cells were washed twice with 500µl of Buffer AW1 (at 8000rpm) and 500µl of Buffer AW2 (at 14000rpm) and suspended in 40µl Buffer AE (Elusion Buffer). The extracted DNA samples were stored at -200C until required for PCR. Amplification of *invA* gene in Salmonella isolates was

performed by using a primer pair specific to that locus as described by Rahn *et al.* (1992). The PCR was carried out in a 25µl PCR mix comprising master mix of 12.5µL (Qiagen), 2.5µL of coral load, 1 µL of 10mM each of forward and reverse primer, 3 µL of nuclease-free water and 5 µL of DNA template. PCR amplification was performed in an Applied Biosystem 9700 thermocycler, programmed with the following cycling conditions initial denaturation at (94℃ for 3 minutes) followed by 40 cycles of denaturation at (94℃ for 30 seconds), annealing at (55℃ for 1 minute) and extension at (72℃ for 1 min), followed by a final extension at 72℃ for 10 minutes. The PCR products thus obtained were kept at 4℃ until required.

Eight microliters of the PCR product were electrophoresed in a 1.5% agarose gel stained using 10µl of 0.5µg/ml ethidium bromide at 70V for 1hour. A 100bp DNA ladder (Biolabs, UK) was used as molecular size marker. After appropriate migration, the Agarose gel was visualized under a UV trans-illuminator in a Bio-Rad gel documentation device and the results were documented.

PCR primers used

Target gene	Primer	Primer sequence	Size(bp)
InvA	*invA*F	GGTGGTTTTAAGCGTACTCTT	796
	*invA*R	CGAATATGCTCCACAAGGTTA	

Primers used was designed by Najmeh *et al.* (2023)

Results

Determination of Total Aerobic Count (TAC) and Total Coliform Count (TCC)

The mean total aerobic count (TAC) at the different sampling sites ranged from 1.76 x 10^8cfu/ml to 2.34 x 10^8cfu/ml in Wurukum market, 1.48 x 10^8cfu/ml to 1.60 x 10^8cfu/ml in Wadata market and 1.23 x 10^8cfu/ml to 1.58 x 10^8cfu/ml in Modern market (Table 1)

A high microbial contamination with mean total aerobic and total coliform counts surpassing the recommended (>10^6 cfu/g unsatisfactory for TAC and >10^4 cfu/g unsatisfactory for TCC, ISO 21528-2:2017) standards was detected from the different poultry slaughter slabs.

In general, the Wurukum market yielded the highest mean TAC at all points (Table 1), indicating the highest level of contamination however there was no statistically significant difference (p = 0.172) between point 1 of all the slaughter sites.

The meat samples had a mean TAC of 1.88 x 10^8cfu/g in Wurukum market, 1.89 x 10^8cfu/g in Wadata market and 1.47 x 10^8cfu/g in Modern market (Table 1) showing a clear significant difference (p = 0.03) between the mean TAC in Wadata market and Modern market slaughter sites, however the mean TAC for the three slaughter slabs showed no significant difference (p=0.405).

Water samples collected from all the slaughter sites studied had a high mean TAC. Modern market appeared to have the highest TAC (1.92 ×10^8cfu/ml) (Table 1) although, there was no statistically significant difference (p = 0.705) between the mean TAC across the different slaughter sites.

Palm swabs across the different slaughter sites indicate that butchers at the Modern market had the highest TAC (1.76 × 10^8cfu/ml) (Table 1) although there was also no statistically significant difference in the mean palm swab TAC across the different slaughter sites.

There was a significant difference (p = 0.027) in the TAC gotten from tabletop swabs across the different slaughter sites, with Wurukum market having the highest count (2.14 x 10^8cfu/ml) (Table 1). A total of 240 (96.4%) samples were positive for coliforms, with the water sample from Wurukum market showing the

Table 1: Total Aerobic Count (TAC) with p-values of samples collected from three different slaughter slabs at Makurdi, Benue State, Nigeria (Mean ± SD, 10^6)

Location	Sample Size n	Point 1	Point 2	Point 3	Meat	Water Sample	Butchers palm	Table Top	Market p-value
Wurukum Mkt	83	186.7±75.5	234.2±73.6	175.5±71.4	187.8±64.7	159.6±114.0	138.6±99.9	214.4±63.2	0.172
Wadata Mkt	83	151.1±70.5	160.4±72.4	147.5±64.6	189.2±100.9	140.8±79.8	119.4±81.6	102.2±47.6	0.593
Modern Mkt	83	122.7±72.5	158.6±91.1	151.3±112.9	147.0±83.8	192.4±94.9	176.2±106.2	207.2±75.3	0.807
Total	249	153.5±72.8	184.4±79.0	158.1±83.0	174.7±83.1	164.3±96.2	144.7±95.9	174.6±62.0	
P-value		0.058	0.025	0.104	0.405	0.705	0.649	0.027	

*Point 1= After Defeathering, *Point 2 = After Evisceration, *Point 3 = After washing

Table 2: Total Coliform Count (TCC) with p-values of samples collected from three different slaughter slabs at Makurdi, Benue State, Nigeria (Mean±SD, 10^6)

Location	Sample size(n)	Point 1	Point 2	Point 3	Meat	Water Sample	Butchers Palm	Table Top	Market P-value
Wurukum Mkt	83	102.5±91.7	113.5±97.2	89.3±87.7	94.8±92.5	135.6±121.7	104.2±112.4	129.0±123.1	0.062
Wadata Mkt	83	72.5±75.7	77.0±92.2	82.9±98.9	38.4±54.5	54.2±37.9	132.4±192.2	35.8±34.1	0.917
Modern Mkt	83	87.2±66.8	74.3±74.3	92.4±85.2	132.1±100.7	51.4±20.7	131.6±88.1	105.8±70.5	0.322
Total	249	87.4±78.1	88.3±87.9	88.2±90.6	88.4±82.6	80.4±60.1	122.7±130.9	90.2±75.9	
P-value		0.059	0.290	0.592	0.100	0.171	0.850	0.231	

*Point 1= After Defeathering, *Point 2 = After Evisceration, *Point 3 = After washing.

Table 3: Distribution of *Salmonella* spp positive samples obtained from the three poultry slaughter slabs in Makurdi, Benue State, Nigeria

Sample points from all sites combined	Total number of Isolates	Positives for *Salmonella* spp	Positives for *Salmonella* spp based on biochemical test	Positive for *Inv*Agene based on PCR
Point 1	51	7(13.7%)	0(0.0%)	
Point 2	51	10(19.6%)	1(2.0%)	
Point 3	51	12(23.5%)	7(13.7%)	
Meat	51	11(21.6%)	6(11.8%)	
Water	15	2(13.3%)	0(0%)	
Butcher's palm	15	2(13.3%)	2(13.3%)	1(6.7%)
Table Top	15	2(13.3%)	1(6.7%)	1(6.7%)
Total	249	46(18.5%)	17(6.7%)	2(0.8%)

*Point 1= After Defeathering, *Point 2 = After Evisceration, *Point 3 = After washing.

highest mean TCC (1.36×10^8 cfu/ml) and the least was observed in Table top at Wadata market. Among the stages, Wurukum Market appeared to be the most contaminated with a mean TCC of 1.02×10^8 cfu/ml, followed by Modern Market and Wadata market presenting a mean TCC of 9.24×10^7 cfu/ml and 8.29×10^7 cfu/ml, respectively. Between each location, there were statistically significant differences between point 1 and point 3 (p=0.033). A general trend as depicted by the mean values of all three points in all locations, showed a high mean TCC after evisceration (8.83×10^8cfu/ml), a slight reduction after washing (8.82×10^8cfu/ml). However, there was an increase in TCC after washing in Wadata market (5.9×10^7cfu/ml) and Modern market (1.8×10^7 cfu/ml) (Table 2). The meat samples showed no statistically significant difference among the three locations (p = 0.100) however, the highest mean TCC was observed in the Modern market (1.32×10^8 cfu/ml) and the least observed in Wadata market (3.8×10^7 cfu/ml). There was no statistically significant difference (p = 0.171) between the water samples analysed from all the locations however, the samples obtained from Wurukum market showed the highest mean TCC of 1.36×10^8 cfu/ml and subsequently Wadata market and Modern market showing a mean TCC of 5.4×10^7cfu/ml and 5.1×10^7cfu/ml, respectively. Hand swabs from all the locations were not statistically significant (p = 0.850) with those from Wadata market showing the highest mean TCC of 1.32×10^8 cfu/ml followed by Modern market and Wurukum market with mean TCC of 1.31×10^8 cfu/ml and 1.04×10^8 cfu/ml respectively. Swabs from Table top from all the locations showed no statistically significant difference (p = 0.231) with those from Wurukum market showing the highest mean TCC of 1.29×10^8 cfu/ml, 1.06×10^8cfu/ml in Modern market and 3.6×10^7 cfu/ml in Wadata market (Table 2).

Determination of the occurrence of Salmonella spp

Out of the 249 samples obtained from all the sampling points and slaughter sites, presumptive Salmonella isolates were 46 (18.5%) (Table 3). Further biochemical tests revealed a total of 17 isolates with characteristics consistent of *Salmonella* Spp. while confirmation using Polymerase Chain Reaction (PCR) to amplify *InvA* gene indicated that only two were *Salmonella* spp. The highest number of presumptive isolates were isolated from point 3 (after washing) (23.5%) followed by point 2 (after evisceration) (19.6%) and point 1 (after de-feathering) (13.7%). Eleven (21.6%) isolates were positive from meat

samples, two (13.3%) of water samples, two (13.3%) of butcher's palm and and two (13.3%) of table tops appeared positive for *Salmonella* spp. (Table 3).
In all the slaughter slabs *Salmonella* spp. was isolated most in Wurukum market followed by Wadata market and Modern market had the least isolation rate (Table 4).

Antimicrobial resistance of Salmonella isolates
Of the 17 *Salmonella* spp isolated from all locations in this study based on biochemical test and subjected to a panel of 10 commonly used antimicrobial agents, a high resistance level was observed against colistin sulphate; 82.4% (14/17), ampicillin 76.5% (13/17), tetracycline 64.7% (11/17) and ceftriaxone 52.9% (9/17). however, susceptibility was observed among 94.1% (16/17), 94.1% (16/17), 88.2% (15/17) and 47.1% (8/17) of ciprofloxacin, imipenem, enrofloxacine and chloramphenicol, respectively (Table 5).

PCR detection of isolates
Two of the Seventeen *Salmonella* spp. suspects screened for *InvA* gene detection using PCR produced a 796bp DNA fragment which confirms that the two isolates were Salmonella (Plate I).

Table 4: Distribution of *Salmonella* spp isolated from the poultry slaughter slabs within Makurdi and its environs, Benue State Nigeria (n = 249)

Slaughter slabs	Number tested	Number positive	Prevalence (%)
Wurukum Mkt	83	9	3.6
Wadata Mkt	83	5	2.0
Modern Mkt	83	3	1.2
Total	249	17	6.8

Table 5: Antimicrobial susceptibility profile of Salmonella isolated from poultry samples collected during the slaughter process at three (Wurukum, Wadata and Modern market) slaughter slabs in Makurdi, Benue State

Antimicrobial Class	Antibiotic	No. of susceptible isolates(%)	No. of intermediate isolates (%)	No. of resistant isolates (%)
Fluoroquinolone	Ciprofloxacin	94.1	5.9	0.0
Chloramphenicol	Chloramphenicol	47.1	17.6	35.3
Carbapenem	Imipenem	94.1	5.9	0.0
Beta-lactam	Amoxicillin/clavulanic acid	11.8	41.2	47.0
Cephalosporin	Ceftriaxone	0.0	47.1	52.9
Fluoroquinolone	Enrofloxacin	88.2	11.8	0.0
Polypeptide	Colistin Sulphate	0.0	17.6	82.4
Tetracycline	Tetracycline	0.0	35.3	64.7
Beta-lactam	Ampicillin	5.9	17.6	76.5
Quinolone	Nalidixic Acid	41.2	23.5	35.3

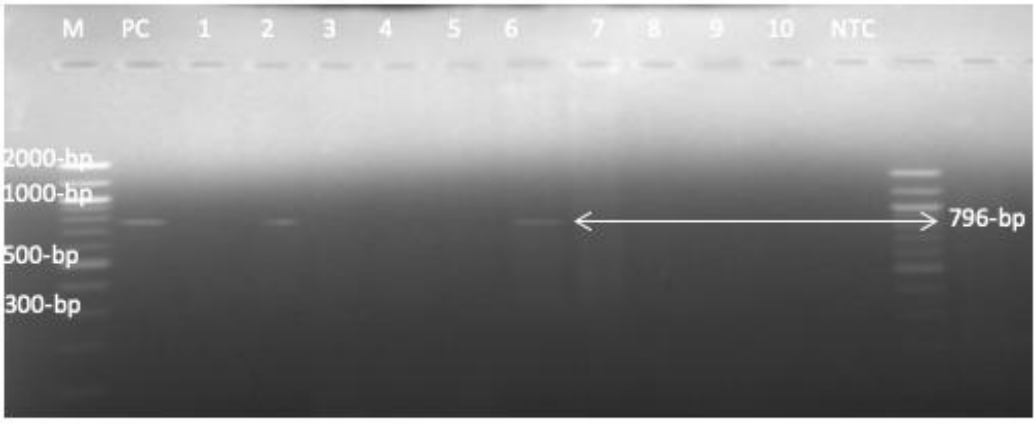

Plate I: Agarose gel electrophoresis showing PCR amplification of 796bp of inv A gene in two Salmonella isolates. Well labelled M; indicates molecular weight marker, PC; is Salmonella positive control, NTC; is Negative control, while wells 2 and 6 showed positive amplification of the gene

Discussion

There are very few poultry slaughtering facilities in Nigeria (Fagbamila *et al.,* 2017) and therefore live birds from different sources are sold and processed in an open environment (makeshift slaughtering slabs). One of the most important steps in developing a HACCP system is the establishment of Critical Control Points (CCPs). These are steps at which control can be applied to eliminate, reduce or prevent food safety hazards. In terms of fresh meat processing, safety hazards cannot be eliminated but can be prevented or reduced (Sheridan, 2000). Microbiological analyses showed that birds presented for slaughter in all the locations in this study showed a relatively high level of contamination all surpassing the recommended acceptable standard (ISO 21528-2:2017, ISO 6579-1:2017) for human consumption. A general trend was observed in the TAC and TCC results from the three slaughter stages in all the slaughter sites presenting a high level of contamination after defeathering (Point 1), a higher level after evisceration (Point 2) and a drop in the level of contamination after washing except for the TCC in Wadata market and Modern market. The mean total aerobic count (TAC) for point 2 (after evisceration) in all the sites were high with statistical significance when compared to other slaughter stages. The difference observed could be due to faecal contamination of carcass from the gut during slaughter and processing of the birds. The findings from this work is similar with a study carried out on poultry slaughter houses in United State of America and Jos, Nigeria (Olsen *et al.,* 2003, Rostagno *et al.,* 2006, Fagbamila *et al.,* 2017). Evisceration is one of the major source of contamination of poultry carcasses and this agrees with the work done by Orji *et al.* (2005) who isolated Salmonella (12.5%) from poultry droppings in Awka, south east Nigeria.

Washing might be expected to result in a wide spread distribution of microbiological contamination over carcasses. In this study, water yielded a high level of TAC in the different sampling locations and also a significantly high level of TCC as well, this result is not surprising and could easily be related to the lack of portable water at the slaughter slabs and the reuse of same water for several carcasses. The seemingly unhygienic containers used to collect and store the water may also serve as a source of contamination. These findings are in line with those of Spescha *et al.* (2006), Bello *et al.* (2011) and Fagbamila *et al.* (2017) where they reported an increase in carcass contamination after washing during slaughtering procedures in some abattoirs and slaughter slabs in Nigeria.

This study's observations and findings showed that swabs from the butchers' palms have high levels of TAC and TCC. These results agreed with that of Nnachi *et al.* (2014) who also detected significant levels of contamination from meat handlers in Onitsha, Nigeria. This also agrees with (Oscar, 2013; Wagner, 2013) who reported that food handlers may recontaminate a thoroughly processed or cooked meat meant for consumption. This high level of contamination poses high risk of cross contamination not only between carcasses but to humans also, hence increasing the risk and transmission of zoonotic diseases. This calls for registration and education of the meat handlers with emphasis on proper washing and disinfection of their hands and equipment before, during and after processing, the use of gloves when handling meat and provision of adequate water and sanitizers.

The TAC from table tops across the different slaughter slabs were high with statistical significance indicating absence of proper adherence to sanitary measures resulting from poor washing of table tops that would have been contaminated with intestinal contents from careless evisceration of poultry and this agrees with Rouger *et al.* (2017).

In the meat sample analysed, a high level of contamination was observed in the TAC and TCC. These findings are in disagreement with that of Bradeeba & Sivakumaar (2012) who reported lower TAC in a similar study and higher than that of Hoque *et al*.; (2008) who reported an average TAC in a study performed in slaughter yards. The presence of bacterial pathogens in the water used and the hands of the meat handlers as well as contaminated table surfaces may have caused such level of contamination of the meat.

Presumptive *Salmonella* spp. were found at all the stages of slaughter except after defeathering (point 1), carcass wash after evisceration yielded considerably increased detection. This is in agreement with Adeyanju & Ishola (2014) and Fagbamila *et al.* (2017) who reported cross contamination of poultry meat within the processing chain due to physical contact. However, presence of presumptive *Salmonella* spp. was detected at the different stages in the slaughter process, swabs from butchers' palm, table top and meat samples heading to the market. The detection of presumptive *Salmonella* spp. seemed to follow the same pattern as TAC and TCC presenting an initial load after evisceration and an increase after carcass wash. There seem to be a surge of 13.7% in the presumptive *Salmonella* spp. contamination at point 3 (carcass

wash) when compared to point 2 (2%) (after evisceration). The surge of presumptive *Salmonella* spp. contamination seen after carcass wash could be due to the carefree nature of removal of the visceral organs which may have led to puncture of the gut content, performing this procedure on contaminated table tops, the open nature of the slaughter slabs and cross contamination from butchers.

In this study, presumptive *Salmonella* spp. was isolated from 11.8% of meat sample. This finding agreed with that of Tafida *et al.* (2013) who reported 2.43% in raw meat from a study carried out in Zaria and Fagbamila *et al.* (2017) who reported 23.8% in intestinal samples in a study carried out in Jos. Low levels of *Salmonella* spp. were detected on butchers' palm (13.3%) and Table top (6.7%). This finding agreed with that of Fagbamila *et al.* (2017) who reported 19.1% from table swab in a study carried out in Jos. These findings suggest that the pathogen seen in meat may not have originated solely from the animals, but could have been spread during the slaughtering process, the dressing process, from butchers or from table tops.

The isolation of *Salmonella* from table tops in this study might be due to colonization resulting from biofilm formation by this pathogen on these surfaces, hence they are able to survive cleaning (Nidaullah *et al.,* 2017). The isolation of Salmonella from butchers' palm might be due to poor sanitary measures leading to colonization of their hands by pathogen.

The 6.8% overall isolation rate recorded for Salmonella in this study was lower than the 12.5% recorded from poultry droppings in Awka, south east Nigeria (Orji *et al.,* 2005), 22.6% reported by Adeyanju & Ishola (2014) in poultry processing plant in Ibadan, Nigeria and 20.2% in intestinal samples, table swab, defeathering water sample and carcass rinse sample from chicken slaughter slabs in Jos, North Central Nigeria (Fagbamila *et al.,* 2017). Variations in geographical location, type of samples collected, water source, detection method and possible additional contamination from handling of samples might be possible reasons for the differences in the prevalence of Salmonella in this study compared with those of other studies. Selective media (XLD) enrich for Salmonella do not guarantee complete specificity. Many non- salmonella organisms can survive and mimic its typical colony appearance resulting in false positive colony.

Increasing rates of antimicrobial resistance among Salmonella is a growing healthcare problem that needs to be monitored continuously. Salmonella isolates from this study were highly sensitive to Ciprofloxacin (94%), Imipenem (94%), and Enrofloxacin (88%). This is consistent with a study carried out by Obi & Ike (2015) who reported high sensitivity to Ciprofloxacin in a study carried out on chickens in Nsukka but in total disagreement with the reports of Salihu *et al.* (2014) that reported a high resistance to Ciprofloxacin (81.7%), this could be due to environmental differences or possibly differences in the strains of the bacteria but resistant to Ceftriaxone, Colistine sulphate, Tetracycline, Amoxicillin (47%) and Ampicillin. This is consistent with a study by Grema *et al.* (2018) on antimicrobial profile of Salmonella isolated from fish value chain in Zaria. But in contrast with the study by Obi & Ike (2015) who reported 100% resistance to Amoxicillin and Augmentin in a study carried out on chickens in Nsukka. The resistance to Tetracycline and Amoxicillin in this study could be attributed to long over use of these agents and related compounds in poultry feeds and or abuse by poultry farmer or local populace. A study by Mion *et al.* (2016) reported 72% sensitivity of *Salmonella* spp. isolated from poultry processing plants to Enrofloxacin which is similar to the sensitivity observed in the present study (Enrofloxacin 88%). Mion *et al.* (2014) also reported 100% sensitivity to chloramphenicol which is in contrast to the 47% sensitivity to chloramphenicol reported in this study. Differences in *Salmonella* spp. isolates, source of the isolates, geographical location, type and source of antibiotic tested might be responsible for the variations in sensitivity patterns observed.

inv A gene is a chromosomic sequence which is responsible for Salmonella virulence especially the *inv*Asion of host cells by the bacteria. The detection of the gene in the Salmonella isolated implies the organisms are virulent and will be able to penetrate host epithelia cells, causing infection. Fifteen out of the seventeen confirmed Salmonella isolates were not positive for inv A gene. The absence of the gene in the confirmed Salmonella isolates can lead to lack of *inv*Asiveness by those isolates. This is in agreement with the report of Oludairo *et al.* (2013) who reported three out of eight confirmed Salmonella not having inv A gene and Bacci *et al.* (2006) who reported that inv A gene was detected in 62 of the 63 strains of Salmonella screened, they submitted that all the strains carrying that genetic sequence can potentially *inv*Ade epithelial cells (Bacci *et al.,* 2006). This implies that the isolates that do not carry the gene may not be able to *inv*Ade epithelial cells and may not be virulent.

This finding is at variance with the reports of Oliveira *et al.* (2003), Zahreai *et al.* (2006), Trafny *et al.* (2006), Jamshidi *et al.* (2008), Nashwa *et al.* (2009) and Amini *et al.* (2010) who all detected and reported inv A gene in all Salmonella isolates they tested.

The implication of the presence of inv A gene in the isolates is that the organisms are actually able to cause infection in humans from which it was isolated from especially if host immunity is suppressed. They may also cause infection in chickens if the bacteria are contracted either from humans or contaminated objects (Table top) around them. This is especially important in slaughter slabs where there is human to human contact and human-to-animal contact. Stressed Salmonella cells, low pathogen load or overly selective conditions can reduce true recovery. Therefore, only a small portion of presumptive isolates may be confirmed as Salmonella after biochemical or molecular identification.

The operating procedures in the poultry slaughter slabs in Makurdi are highly unsatisfactory owing to poor management and severely unhygienic practices. High microbial contamination, with mean total aerobic and total coliform counts exceeding the recommended standards (> 10^6 cfu/g unsatisfactory for TAC and > 10^4 cfu/g unsatisfactory for TCC, ISO 21528-2:2017), was detected in different poultry slaughter slabs. Evisceration, carcass wash, meat, table top and butchers' palm are considered as critical control points during poultry processing in this study. *Salmonella* spp. was with an overall rate of 6.8% from the samples collected; the prevalence of *Salmonella* spp. was highest at carcass wash after evisceration, followed by butcher's palm and then meat. Butchers should be enlightened on the need to be careful during evisceration of carcass and thorough washing of their hands before processing of the slaughtered birds to reduce the prevalence of *Salmonella* spp. The ability to isolate *Salmonella* spp. with inv A gene which are known to enhance their virulence and pathogenicity makes this finding of great public health concern. The sensitivity test shows *Salmonella* spp. isolates were highly sensitive to ciprofloxacin, Imipenem, and Enrofloxacin and a high resistance to Colistin sulphate, ampicillin and tetracycline. Salmonellosis should be treated with Salmonella susceptible antimicrobials, the dosage and administration should be based on prescription by appropriate professionals in order to reduce the incidence of antimicrobial resistance. Public health authorities should organize campaigns to enlighten butchers and consumers on the mode of spread and transmission of Salmonella as this will help reduce the incidence.

Acknowledgement

The authors want to immensely appreciate the MacArthur Foundation Centre of Excellence in Veterinary Epidemiology, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria for funding this project. We are also very grateful to the staff of the Veterinary Public Health and Preventive Medicine Department, Joseph Sarwuan Tarka University Makurdi for the laboratory work.

Funding

The project was supported by the MacArthur Foundation Centre of Excellence in Veterinary Epidemiology, Ahmadu Bello University, Zaria, Nigeria.

Conflict of Interest

The authors declare no conflict of interest.

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