



Microbial hazards associated with pig carcasses and molecular detection of enterotoxigenic *Staphylococcus aureus* at different stages of the slaughter process

AA Adikwu^{1*}, EC Okolocha², II Luga¹ & EO Ngbede³

1. Department of Veterinary Public Health and Preventive Medicine, College of Veterinary Medicine, University of Agriculture, Benue State, Nigeria
2. Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
3. Department of Veterinary Microbiology, College of Veterinary Medicine, University of Agriculture, Benue State, Nigeria

*Correspondence: Tel.: +2348036058282; E-mail: alexadik@yahoo.co.uk

Copyright: © 2019 Adikwu *et al.* This is an open-access article published under the terms of the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Publication History:
Received: 03-09- 2018
Accepted: 15-01-2019

Abstract

Foodborne diseases have sparked a wave of continuous public health concern and the need for proactive measures to be taken in our communities. This study is aimed at determining the microbiological hazards associated with the pig slaughter process, assess the critical control points and screen for enterotoxin-producing *Staphylococcus aureus*. Three pig slaughter and processing facilities located within Makurdi town, Nigeria were utilized for the study. Swabs from carcasses during the slaughter process and the butchers' hands, water used for washing carcasses and meat samples were all processed using standard bacteriological procedures. A total of 241 samples consisting of 150 swabs from 50 carcasses, 32 hand swabs, 9 water and 50 meat samples were evaluated during the study period. Aerobic plate counts (APC) and total coliform bacteria counts (TCBC) were evaluated. The mean APC at the different sampling sites ranged from 7.89 to 8.18 logCFU/cm² while the mean TCBC ranged from 6.70 to 7.45 logCFU/cm². APC and TCBC were significantly different ($P < 0.05$) between the same stages of processing in different sampling locations, however, there was no statistically significant difference ($P > 0.05$) in samples obtained from the butchers' hands, although water samples generally had the highest mean APC (8.35 logCFU/ml). *Staphylococcus aureus* was isolated from 54 (22%) of the 241 samples with 8 (13.8%) of the isolates harbouring one or more enterotoxin genes (*sea*-17.5% and *sed*-2.5%). Bleeding, evisceration/splitting, transportation to retail outlets and all stages involving washing were identified as critical control points. Pork consumers in Makurdi were advised to properly cook pork before eating to prevent possible infection and/or intoxication.

Keywords: Aerobic plate count, Carcass, Contamination, Enterotoxin, Points, Pork, Total coliform bacteria count

Introduction

Pigs are among the abundant livestock potentials which Nigeria is endowed with and is one of the most preferred sources of animal protein in Makurdi and majority of the residents consume it. However, pork has been reported in many parts of the world to be a vehicle for spread of pathogens that cause food borne illnesses due to its contamination during the slaughtering process (Fahrion *et al.*, 2014; Self *et al.*, 2017; Nakao *et al.*, 2018). For proper food safety at various slaughter processes to be achieved, more sustainable meat inspection procedures, inspection of other animal products such as milk and eggs are necessary because these food-producing animals can harbour zoonotic bacterial pathogens (Nørrung & Buncic, 2008; Jöhler *et al.*, 2018). These zoonotic pathogens enter the food chain by direct or indirect contamination usually as a result of poor hygienic practices. Appropriate slaughter hygiene and risk-based preventive measures, is therefore important to ensure public health protection through enhancing the quality of meat (Zweifel *et al.*, 2005). Analysis of the pig slaughter process is of immense importance to logically estimate the risks involved in carcass contamination and to provide cogent preventive measures for possible infection. Abattoir-specific microbiological data on carcass contamination is also important (Spescha *et al.*, 2006; Milios *et al.*, 2014) because carcasses might be contaminated despite the absence of visible contaminants (Gill, 2004). Verification of slaughter hygienic conditions in most cases involves monitoring the microbial status of carcasses by determining indicator organisms like *Staphylococcus aureus*, Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp, Hepatitis A virus and Noroviruses from farms and carcasses at the end of slaughter (Brown *et al.*, 2000; Zweifel *et al.*, 2005; Ruby *et al.*, 2007; Macori *et al.*, 2018). Meat-borne diseases and intoxication is believed to be a major and widespread problem (Chugh, 2008). It is important that studies be carried out to assess levels of contamination and safety during the pork production process to assert the continuous need for results to support sustainable efforts towards quality management and food safety. The aim of this study was to assess the microbial quality of pork carcasses, assess the critical control points at different stages of the slaughter process in three pig abattoirs and screen for staphylococcus enterotoxins (SEs) genes.

Materials and Methods

Slaughter process, study area and design

The study was carried out in Makurdi, Nigeria and three slaughter houses; Wurukum, Kanshio and Modern market were used for sampling. Each slaughter house practiced similar procedures. The process begins approximately 5.45am and ends by 10am each day. Only pigs were slaughtered in each facility although the number of pigs slaughtered daily varied; about 5-10 pigs in Kanshio, 15-20 pigs in Modern market and 30-50 pigs in Wurukum. The slaughter process involves an antemortem inspection in the lairage before pigs head to the slaughtering bay next is sticking (bleeding) then singeing before the carcass is washed. Evisceration, splitting and decapitation followed after which the carcass is washed again then assembled in wheel barrows and transported out of the slaughter facility for onward movement to retail outlets. A cross-sectional study was carried out in the three slaughter houses from July, 2014 to May, 2015 to evaluate the bacteriological quality of the slaughter process. On each day of visit to the sites, five pig carcasses were sampled based on convenience. A total of 241 samples comprising: 150 swabs collected from 50 pig carcasses (50 after bleeding, 50 after singeing and 50 after evisceration/splitting of the carcass), hand swabs from 32 butchers palm, 9 water samples (three from each facility's water source) and 50 meat samples (one from each processed carcass).

Hazard is defined as a biological, chemical, or physical property that may cause a food to be unsafe for human consumption, this study focused on microbiological hazards mainly Aerobic bacteria, coliforms and *Staphylococcus aureus*. Hazard analysis was carried out by observing the steps taken before, during and after the slaughter process and assessing the potential microbial contaminants introduced. This was achieved by collecting carcass swabs, hand swabs, water and meat samples during the slaughter process and testing them microbiologically in the laboratory after collection. Consequently, results obtained ascertained the significant potential food safety hazard after quantifying the bacteriologic contaminants and comparing with internationally approved permissible standards.

Sampling procedure

Carcass sampling was carried out by the surface swabbing technique (using sterile cotton swabs)

from the medial thigh, dorsum and cervical regions of the carcass at three points during the slaughtering process. Point 1: After bleeding just before singeing. Point 2: After singeing and washing just before evisceration. Point 3: After evisceration and splitting just before washing. Water used for washing the carcass, swabs from the butchers' hands (palms) and meat (50g) at the end of the slaughtering process were also collected and analyzed. Using a sterile swab, 400 cm² subdivided into four areas of 100 cm² each; cervical region, medial thigh, dorsum and belly region were swabbed for point 1 and point 2 before evisceration. After evisceration and splitting (point 3) of the carcass, the belly region was replaced by the internal mid region of one of the last ribs on one half of the carcass. The butchers' palms were also swabbed with a sterile cotton swab as well. About 200 mls of water was collected aseptically from the water source (wells, river side or flowing water as the case may be) in a sterile sample bottle just before it was used to wash the carcass. About 50 g of pork was obtained from the sampled carcass at four different parts viz; the thigh, arm, abdominal and neck regions from each sampled carcass and pooled together into one sterile stomacher bag and homogenized for 60 seconds in 450 ml of 0.1% buffered peptone water (Oxoid, UK) in a stomacher.

Determination of aerobic plate counts (APC) and total coliform bacteria counts (TCBC)

Ten fold serial dilution of the stock suspension was prepared from the homogenized sample. One millilitre each of the homogenate was collected from the clear middle layer and used for serial dilution. APC and TCBC were determined by inoculating 0.1 ml from the test tubes containing 10⁵ and 10⁶

dilutions onto duplicate plates of nutrient agar (Oxoid, UK) and MacConkey agar (Oxoid, UK) plates respectively and incubated at 37°C for 24h (Ajogi *et al.*, 2005). Colonies on the nutrient agar plates were counted for APC and growths appearing pinkish on MacConkey agar were counted as TCBC. The respective averages calculated and expressed as logCFU/ml (Awosanya & Anifowose, 2011).

Isolation of Staphylococcus aureus

A loop-full from the enrichment broth (tryptone soya broth (Oxoid, UK) supplemented with 6.5% NaCl) was streaked onto Baird Parker agar supplemented with egg yolk tellurite (Oxoid, UK) followed by incubation for 24h at 37°C. Dark colonies with clear halo around them were presumptive *Staphylococcus* species. Dark colonies with clear halo around them were further sub-cultured onto nutrient agar to obtain pure colonies. The pure colonies were identified as *Staphylococcus aureus* based on the reaction on Gram staining, catalase, coagulase, DNase tests, haemolysis on 5% sheep blood agar plates and fermentation of sugars (glucose, mannitol, sucrose, maltose, raffinose and xylose) (Barrow & Feltham, 1993). The isolates were further confirmed using the Microgen Staph-ID® system (Microgen, Bioproducts, UK).

Detection of Staphylococcal enterotoxin genes

DNA was extracted from the *Staphylococcus aureus* isolates after overnight growth at 37°C in tryptone soya broth (Oxoid, UK) using the ZR fungal/bacterial kit (ZYMO, South Africa) according to the manufacturer's instruction. The enterotoxin genes (*sea*, *seb*, *sec*, *sed* and *see*) were amplified using the primers in Table 1 for the multiplex PCR and

Table 1: Target genes, corresponding primer sequences and amplicon sizes for detection of enterotoxin genes

Target gene	Primer sequence	Amplicon size (bp)
<i>sea</i>	SEA-3 CCTTTGGAAACGGTTAAAACG SEA-4 TCTGAACCTTCCCATCAAAAAC	127
<i>seb</i>	SEB-1 TCGCATCAAACACTGACAAACG SEB-4 GCAGGTA CTCTATAAGTGCCTGC	477
<i>sec</i>	SEC-3 CTCAAGAACTAGACATAAAAGCTAGG SEC-4 TCAAAAATCGGGATTAACATTATCC	271
<i>sed</i>	SED-3 CTAGTTTGGAATATCTCCTTTAAACG SED-4 TTAATGCTATAGATAAAGTTAAAACAAGC	319
<i>see</i>	SEE-3 CAGTACCTATAGATAAAGTTAAAACAAGC SEE-2 TAACTTACCGTGGACCCTTC	178

conditions

described by the Danish Technical Institute (DTU, 2014). Briefly, amplification of the target genes was performed in a total reaction volume of 23µL containing 12.5µL PCR master mix (1.5mM MgCl₂, 5 µL of 10 X PCR buffer, 2.5U Taq DNA polymerase, 200µM dNTP, 0.4µL of each primer (25µM), 4µL of PCR water, 2.5µL of Q-solution and 2µL template DNA. The PCR amplification was carried out with an initial denaturation of 94°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 53°C for 30 seconds and extension at 72°C for 30 seconds and a final extension at 72°C for 8 minutes (DTU, 2014). Ten µL (10µL) of the amplified product was electrophoresed in 1% agarose, stained with ethidium bromide (Thermo Fisher Scientific, USA) and visualized with a UV transilluminator (Enduro™).

Data analyses

The results of APC and TCBC were expressed as mean log CFU/cm² the analysis of variance (ANOVA) with Tukey’s post hoc test was used to analyze differences in bacterial counts between the different stages, sampling sites and abattoirs. The level of significance was set at α = 0.05 for the p-value.

Results

Aerobic plate count (APC) from pig carcasses, meat (pork), water and hand swabs

The mean aerobic plate counts ranged from 8.06 – 8.13 logCFU/cm², 8.11 logCFU/cm² and 8.14 logCFU/ml in Kanshio, Modern market and Wurukum respectively. The carcass after bleeding, just before singeing was more contaminated compared to after singeing and washing just before evisceration (8.06 logCFU/cm²), after evisceration

and splitting just before washing (8.09 logCFU/cm²). The results from individual locations showed higher contamination in carcasses sampled from modern market. Meat samples, hand swabs and water samples from Wurukum, Kanshio and Wurukum abattoirs were all contaminated as well (Table 2).

Total coliform bacteria count (TCBC) from pig carcasses, meat (pork), water and hand swabs

Carcasses from Wurukum appeared to be the most contaminated with coliforms followed by Kanshio and Modern market (Table 3). There was a significant reduction in TCBC after singeing and washing in all the abattoirs generally with an increase after evisceration in Wurukum abattoir. Samples obtained after bleeding and after evisceration showed a statistically significant difference (P<0.05) when compared among the three abattoirs/slaughter slabs studied. Water samples from Wurukum yielded the highest level of contamination followed by those from Kanshio and modern market. The butchers hands from the different abattoirs were also significantly different (P<0.05) with those from Kanshio being the most contaminated (Table 3).

Molecular Identification of Staphylococcus aureus enterotoxin genes

A total of 54 isolates from all the locations were positive for *Staphylococcus aureus* based on biochemical test and Microgen Staph-ID identification respectively (Table 4). Of the 40 isolates subjected to PCR, 7 isolates were positive for *sea* and 1 isolate positive for *sed* (Plate I). None were positive for *seb*, *sec* and *see* (Table 5).

Table 2: Aerobic plate counts (APC) of pig carcasses, meat (pork), hands and water samples collected from three abattoirs in Makurdi, Benue State, Nigeria

Location of abattoirs	Number of samples collected per location	Point 1 (logcfu/cm ²)	Point 2 (logcfu/cm ²)	Point 3 (logcfu/cm ²)	Meat (logcfu/g)	Water (logcfu/ml)	Hands (logcfu/cm ²)
Kanshio	70	8.06 ^{a1}	7.98 ^{a2}	7.98 ^{a3}	7.95 ^{ab}	8.22 ^a	8.26 ^a
Modern market	80	8.18 ^{b1}	8.18 ^{a2}	8.17 ^{a3}	8.19 ^a	8.21 ^b	8.02 ^a
Wurukum	91	8.16 ^{c1}	8.01 ^{b2}	8.12 ^{b3}	8.20 ^b	8.35 ^c	8.13 ^b
Total	241	24.40	24.17	24.27	24.34	24.78	24.41
Mean		8.13	8.06	8.09	8.11	8.26	8.14

*Columns with the same alphabets in superscript indicate significance while rows with the same numbers in superscript indicates significance. Analysis of variance (p<0.05 was considered significant)

*cfu–Colony Forming Unit

Table 3: Total coliform bacteria counts (TCBC) of pig carcasses, meat (pork), hands and water samples collected from three abattoirs in Makurdi, Benue State, Nigeria

Location of abattoirs	Number of samples collected per location	Point 1 (logcfu/cm ²)	Point 2 (logcfu/cm ²)	Point 3 (logcfu/cm ²)	Meat (logcfu/g)	Water (logcfu/ml)	Hands (logcfu/cm ²)
Kanshio	70	7.24 ^{a1}	7.08 ^{a2}	6.70 ^{a3}	7.25 ^a	7.00 ^a	7.79 ^a
Modern market	80	6.91 ^{b1}	6.91 ^{b2}	6.89 ^{b3}	6.90 ^b	6.85 ^b	7.59 ^a
Wurukum	91	7.45 ^{b1}	7.17 ^{c2}	7.28 ^{b3}	7.43 ^b	7.10 ^c	7.39 ^a
Total	241	21.60	21.16	20.87	21.58	20.95	22.77
Mean		7.20	7.05	7.07	7.19	6.98	7.59

*Columns with the same alphabets in superscript indicates significance while rows with the same numbers in superscript indicates significance. Analysis of variance (p<0.05 was considered significant)

*cfu – Colony Forming Unit

Table 4: Distribution of *Staphylococcus aureus* positive samples obtained from the three sampled abattoirs in Makurdi, Benue State, Nigeria

Sample points from all the abattoirs combined	Total no. of samples	No. positive for <i>S. aureus</i> based on biochemical test	No. positive for <i>S. aureus</i> based on Microgen test
Point 1	50	9 (18%)	3 (33.3%)
Point 2	50	8 (16%)	3 (37.5%)
Point 3	50	18 (36%)	5 (27.8%)
Meat	50	15 (30%)	8 (53.3%)
Water	9	1 (11.1%)	0
Hands	32	3 (9.4%)	1 (33.3%)
Total	241	54 (22.4%)	20 (37%)

*Point 1= after bleeding, *Point 2= after singeing and washing, *Point 3= after evisceration/splitting and washing

Table 5: Detection of enterotoxin genes in *Staphylococcus aureus* isolates obtained from the three abattoirs in Makurdi, Benue State, Nigeria

Enterotoxin genes	Frequency of occurrence
Sea	7
seb	0
Sec	0
sed	1
see	0

Key: sea - Staphylococcal enterotoxin A gene
 seb- Staphylococcal enterotoxin B gene
 sec – Staphylococcal enterotoxin C gene
 sed – Staphylococcal enterotoxin D gene
 see - Staphylococcal enterotoxin E gene

Critical control points

Bleeding, carcass washing after singeing, carcass washing after evisceration/splitting and transportation were considered to be critical control points in this study.

Discussion

The study applied the Hazard analysis and critical control point (HACCP) systems to describe the microbiological quality of pig carcasses and hygiene practices in some slaughter facilities in Makurdi. The concept was used to systematically study the slaughter and dressing procedures and to point out likely Critical control points (CCP) in the processing

line with significant contribution to meat quality. It was observed that the slaughter process practiced in all the slaughter facilities utilized for this study were similar, imbibing the same pattern of operation all through (Figure II). It was also observed that several procedures such as scalding, dehairing, polishing and chilling as recommended in a standard generic model for Pig slaughter (Pork Slaughter, 1996) were not practiced, for example splitting was performed almost immediately after -if not alongside- evisceration. These could be responsible for the high level of microbial contamination observed at Point 3 (After evisceration and splitting just before washing). Microbiological analyses showed that pigs presented for slaughter in all the study locations showed a relatively high level of contamination all surpassing the recommended permissible limits ($\geq \log_{10}$ 4.0 CFU/cm²) for human consumption (EU, 2004). Generally, the mean aerobic counts (APC) and total coliform counts (TCBC) obtained from all the sites were not statistically significant ($P > 0.05$) probably due to the similar practices carried out in all the slaughter sites. However, slight differences in the values obtained for the different slaughter stages (bleeding, singeing and evisceration/splitting) in the different sampling sites were observed. Factors such

as butchers' disposition with respect to hygienic practices, location of abattoirs and sources of water could be responsible for such differences observed, and also influenced the high level of contamination observed in the Modern market slaughter facility (mean APC: 8.18 logCFU/cm²). Potable water was observed to be scarce in all the abattoirs and where available, was obtained from poorly managed and polluted shallow wells. Pigs bled were left on the bare floor before singeing commenced and inadequate washing of eviscerated carcasses due to water shortage. The lower mean APC and TCBC at all the slaughter stages observed in Kanshio slaughter facility, (although still above the permissible limits), may likely be due to a flowing stream located close to the abattoir serving as their source of water for processing.

A general trend was observed in the APC and TCBC results from the three stages at the three abattoirs presenting a high microbial load after bleeding (point 1), a drop in level of contamination after singeing and washing (point 2) and a slight increase after evisceration/splitting/washing (point 3). Ineffective cleaning and lack of decontamination of vehicles and equipment used during animal transportation could lead to poor hygienic state of

animals presented for slaughter and further contamination in the lairages. Inappropriate handling techniques and unhygienic sticking/bleeding techniques as reported by Wilhelm *et al.* (2011); Bolton *et al.* (2002), Small *et al.* (2006) and Wheatley *et al.* (2014) have caused a profound effects on microbial levels and pose considerable risk to the meat quality. The high level of contamination and unhygienic practices observed in this study qualified this stage to be considered a CCP. The drop in APC and TCBC observed after singeing and washing (Point 2) is probably due to the effect of the heat generated by the flame on

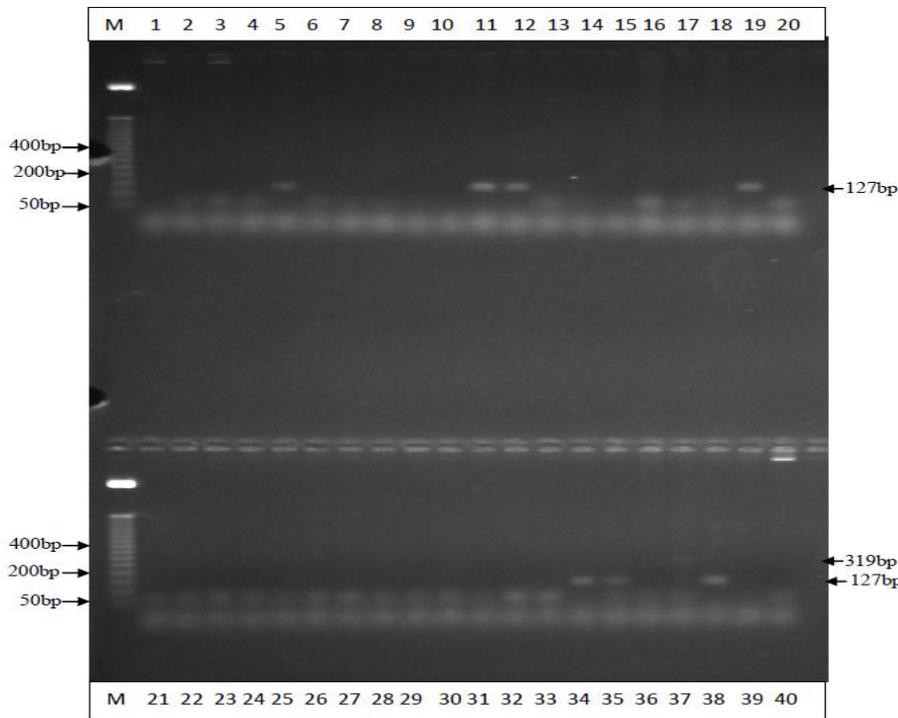


Figure I: Multiplex PCR result for detection of enterotoxin genes in *Staphylococcus aureus*. Lane M: 50bp molecular ladder. Lanes 1-40 are test isolates. Seven lanes showed amplification of the *sea* (127bp) and one *sed* (319bp) genes

the microbes. This agrees with similar studies carried out in pig slaughter houses, by Spescha *et al.* (2006) in Switzerland, Wheatley *et al.* (2014) and Pearce *et al.* (2004) both in Ireland. Evisceration (Point 3) has been frequently reported as a major source of contamination of pork carcasses (Pearce *et al.*, 2004; Zweifel *et al.*, 2005; Spescha *et al.*, 2006; Wheatley *et al.*, 2014). The findings of this study were not different, as depicted by a remarkable increase in the levels of APC and TCBC observed after the procedure. The main risk of carcass contamination during evisceration was the direct or indirect spillage of faecal material from rupture of the gut contents which was not followed by proper washing. Aside unhygienic practices, contamination risks could occur during the removal of contaminated pharynx, tonsils and tongue (Spescha *et al.*, 2006). The high level of contamination at point 3 might have resulted from contamination through the butchers hands, reuse of the water previously used for washing of the carcass. These findings confirmed evisceration as a CCP.

Water yielded the highest level of APC and TCBC in the different sampling locations. This is not surprising and could easily be attributed to the highly polluted water used and reused, and the seemingly unhygienic containers used to collect and store the water. This could also be a contributory factor to the presence of microbial contaminants detected after singeing and rise in levels of such contaminants after evisceration. These findings agree with those of Spescha *et al.* (2006) and Bello *et al.* (2011) who reported an increase in carcass contamination (in pigs and cattle respectively) after washing during slaughtering procedures in some abattoirs. The hazards observed necessitated every washing stage to be considered a CCP in all the slaughter houses except for that in Kanshio which was slightly different probably because of the flowing stream used as the source of water.

Findings from the butcher's palms indicated high levels APC and TCBC. These results agree with that of Nnachi *et al.* (2014) who also detected significant levels of contamination from meat handlers in Onitsha, Nigeria. The high level of contamination poses a high risk of cross-contamination not only between carcasses but also to humans, hence increasing the risk and transmission of zoonotic diseases. It is highly likely that butchers' hands may have become contaminated after handling live pigs during the slaughter process and engaging in other unhygienic activities before the evisceration stage. In this study, the meat samples were analyzed from all the finished carcasses destined for the market. A

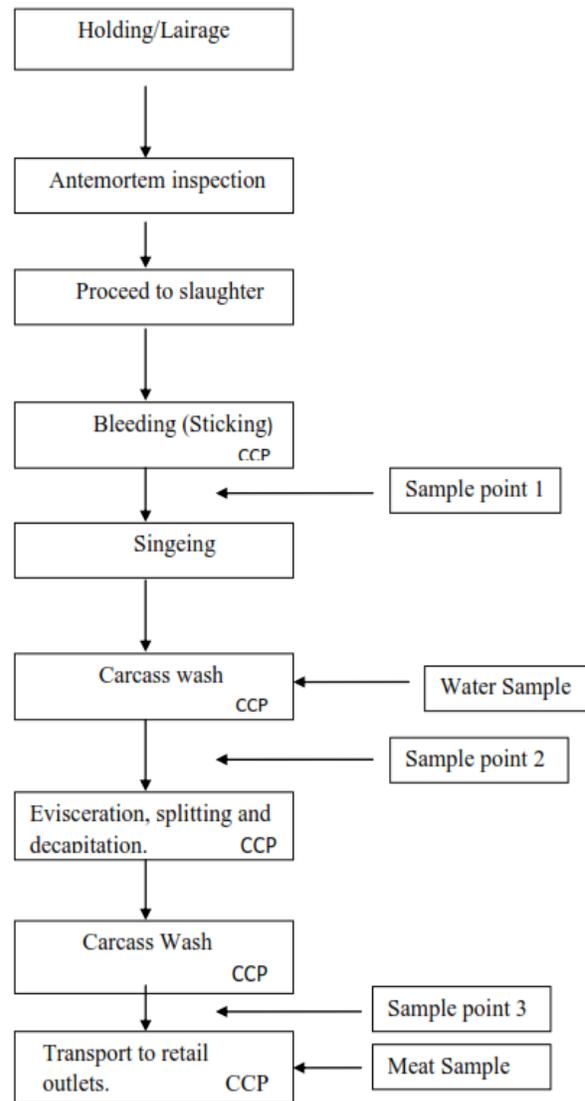


Figure II: Flow chart of the pig slaughter process practiced in Makurdi showing the critical control points (CCP) and the various sampling points

high level of contamination was observed showing mean APC and TCBC of 7.95 and 7.25 logCFU/g in Kanshio, 8.19 and 6.90 logCFU/g in Modern market and 8.20 and 7.43 logCFU/g in Wurukum. The results are higher than that of Bradeeba & Sivakumaar (2012) who reported TAC of 7.54 logCFU/g in pork in a study carried out in India and that of Hoque *et al.* (2008) who reported an average of 6.30 logCFU/g in Chevon in a study performed in slaughter yards in Bangladesh. The presence of bacterial pathogens in the water used and the hands of the meat handlers alongside the use of unclean wheel barrows to transport the finished carcasses through a distance of about 100 m to neighbouring retail outlets or into

vans waiting to convey the carcass to other destinations may have contributed to the high level of contamination of the meat. This poses a serious public health concern because it could lead to spread of infection to the consumers.

Although *Staphylococcus aureus* was detected at all the stages of the slaughter process, singeing considerably reduced detection. *Staphylococcus aureus* was also detected from water samples, butchers' hand swabs and pork being conveyed to the market. The detection of *Staphylococcus aureus* seemed to follow the same pattern as the APC and TCBC earlier discussed i.e. presenting an initial high load after bleeding, a drop after singeing and an increase after evisceration. The frequent isolation of *Staphylococcus aureus* after evisceration could be due to unhygienic removal of the visceral organs which may have led to puncture of the gut contents, performing the operations on the floor, the open nature of the slaughter slabs and cross-contamination from butchers. The efficiency of evisceration is said to be better controlled by corrective actions and appropriate training of personnel according to Standard Optimum Procedures and Good Manufacturing Practice (Prata *et al.*, 2013; Silva *et al.*, 2014; Wheatley *et al.*, 2014). *Staphylococcus aureus* was isolated from 30% of the meat sampled. This finding agreed with that of Ndahi *et al.* (2013) who reported 31.1% of *Staphylococcus aureus* isolation in raw meat from a study carried out in Zaria. *Staphylococcus aureus* was less detected in water (11.1%) and from butchers (9.4%). These findings suggest that the isolates seen in meat may not have originated solely from the animals, but could have been spread during the slaughtering process, or from the water used for washing the carcass, from butchers' hands or during further processing.

Staphylococcal food poisoning is a common food-borne disease resulting from ingestion of staphylococcal enterotoxins expressed by enterotoxigenic strains of *Staphylococcus* species (Hennekinne *et al.*, 2012). The wide range of source of infection cannot be overemphasized as outbreaks have been increasingly reported in milk, meat, fish and other foods (Arfatahery *et al.*, 2016; Huang *et al.*, 2017; Johler *et al.*, 2018). Twenty percent of the isolates yielded positive results for enterotoxin (*sea* and *sed*) genes. This finding slightly contrasts the report of Atanassova *et al.* (2001) which detected *sea* and *seb* probably because latter sampled salted and smoked uncooked pork, but agrees with the study of Balaban & Rasooly (2000), Kadariya *et al.*

(2014) and Argudin *et al.* (2010) who reported *sea* and *sed* as the most predominant gene carried by *Staphylococcus aureus*. Although this study screened *sea-see* genes, it is important to note that other staphylococcal enterotoxin genes such as *seg*, *sei*, *sem*, *sen*, *seo*, and *seu* exist and are pathogenic to humans (Ciupescu *et al.*, 2018). These toxins are resistant to environmental conditions such as freezing, drying, heat treatment and low pH (Hennekinne *et al.*, 2012). These properties especially the heat stability characteristic of *Staphylococcus aureus* toxins and the striking estimated low level of 0.1µg (Le Loir *et al.*, 2003) required to cause intoxication poses a significant threat to the public.

In conclusion, the operating procedures in the pig abattoirs in Makurdi are highly unsatisfactory owing to the very apparent unhygienic practices. Observation and evaluation of the slaughter process identified bleeding, evisceration/splitting, transportation to retail outlets and all stages involving washing as critical control points. The microbial counts surpassed the ≤4.0 (for APC) and ≤2.0 (for TCBC) recommended by the European Union standards. Potentially enterotoxigenic *Staphylococcus aureus* were also detected from the samples suggesting a risk of food-borne illness through intoxication of infection. It is therefore recommended that well designed standard abattoirs should be constructed to curb lapses in basic hygiene practices especially with emphasis of potable water supply. Also, development and implementation of HACCP for abattoir operations to enhance monitoring within processing plants is pertinent. This can detect high risk stages, increase controls alongside monitoring and other interventions were necessary.

Acknowledgement

We gratefully thank MacArthur foundation, Chicago, USA for sponsoring this research work, we also thank the entire staff of the Department of Public Health and Preventive Medicine, Ahmadu Bello University, Zaria and the staff of the Department of Public Health and Preventive Medicine, University of Agriculture, Makurdi.

Conflicts of Interest

The authors declare no conflicts of interest.

References

Arfatahery N, Davoodabadi A & Abedimohtasab T (2016). Characterization of toxin genes and

- antimicrobial susceptibility of *Staphylococcus aureus* Isolates in Fishery Products in Iran. *Scientific Reports*, doi:10.1038/srep34216.
- Ajogi I, Kabir J, Okolocha EC, Luga II, Dzikwi AA, Egege SC & Oni OO (2005). *A Manual for Clinics In Veterinary Public Health and Preventive Medicine*. Third edition, Hope in God Integrated Press, Zaria, Nigeria. Pp 11-14.
- Argudín MA, Mendoza MC & Rodicio MR (2010). Food poisoning and *Staphylococcus aureus* enterotoxins. *Journal of Toxins*, **2**(7): 1751–1773.
- Atanassova V, Meindl A & Ring C (2001). Prevalence of *Staphylococcus aureus* and *Staphylococcus* toxins in raw pork and uncooked smoked ham- A comparison of classical culturing detection and RFLP-PCR. *International Journal of Food Microbiology*, **68**(1-2): 105-113.
- Awosanya AEJ & Anifowose OR (2011). Pattern of microbial contamination of meat during meat display at the Bodija meat market, Ibadan, Nigeria. *Research Opinions in Animal and Veterinary Sciences*, **1**(7): 414-417.
- Balaban N & Rasooly A (2000). Staphylococcal enterotoxins. *International Journal of Food Microbiology*, **61**(1): 1–10.
- Barrow GI & Feltham RKA (1993). *Cowan and Steel's manual for the identification of medical bacteria*. Third edition, Cambridge University Press, Cambridge, United Kingdom. Pp 233.
- Bello M, Lawan MK, Kwaga JKP & Raji MA (2011). Assessment of carcass contamination with *E. coli* O157 before and after washing with water at abattoirs in Nigeria. *International Journal of Food Microbiology*, **150**(2-3): 184–186.
- Bolton DJ, Pearce RA, Sheridan JJ, Blair IS, Mc-Dowell DA & Harrington D (2002). Washing and chilling as critical control points in pork slaughter hazard analysis and critical control point (HACCP) systems. *Journal of Applied Microbiology*, **92**(5): 893–902.
- Bradeeba K & Sivakumaar PK (2012). Assessment of microbial quality of beef, mutton and pork and its environment in retail shops in Chidambaram, Tamil Nadu. *International Journal of Plant, Animal and Environmental Sciences*, **3**(1): 2231-2235.
- Brown MH, Gill CO, Hollingsworth J, Nickelson IR, Seward S, Sheridan JJ & Zink D (2000). The role of microbiological testing in systems for assuring the safety of beef. *International Journal of Food Microbiology*, **62**(1-2):7–1.
- Chugh TD (2008). Emerging and re-emerging bacterial diseases in India. *Journal of Biosciences*, **33**(4): 549–555.
- Ciupescu LM, Auvray F, Nicorescu IM, Meheut T, Ciupescu V, Lardeux AL, Tanasuica R & Hennekinne JA (2018). Characterization of *Staphylococcus aureus* strains and evidence for the involvement of non-classical enterotoxin genes in food poisoning outbreaks. *FEMS microbiology letters*, **365**(13): 139.
- DTU (2014). Multiplex PCR for the detection of the *mecA* gene and identification of *Staphylococcus aureus* from the community reference library of antimicrobial resistance of the Danish Technical University, Lyngby, Denmark. www.eurl-ar.eu/data/images/meca-pcr-protocol06.02.08.pdf, retrieved 17-03-2014.
- EU, (2004). Regulation (EC) no 852/2004 of the European parliament and of the council on the hygiene of foodstuffs. *Official Journal of the European Union*, 139:1-54.
- Fahrion AS, Jamir L, Richa K, Sonuwara B, Rutsa V, Ao S & Grace D (2014). Food safety hazards in the pork chain in Nagaland, North East India: Implication for human health. *International Journal of Environmental Research and Public Health*, **11**(1): 403-417.
- Gill CO (2004). Visible contamination on animals and carcasses and the microbiological condition of meat. *Journal of Applied Microbiology*, **67**(2): 413–419.
- Hennekinne JA, De Buyser ML & Dragacci S (2012). *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbiology Reviews*, **36**(4): 815–836.
- Hennekinne JA, Ke'rouanton A, Brisabois A & De Buyser ML (2003). Discrimination of *Staphylococcus aureus* biotypes by pulsed-field gel electrophoresis of DNA macro-restriction fragments. *Journal of Applied Microbiology*, **94**(2): 321–329.
- Hoque MA, Siddique MP, Habib MA, Sarker V & Choudhury KA (2008). Evaluation of sanitary quality of goat meat obtained from slaughter yards and meat stalls at late

- market hours. *Bangladesh Journal Veterinary Medicine*, **6**(1): 87-92.
- Huang LM, Hu LX, Yu H, Chen SC, Huang CP & Liu H (2017). Field epidemiological investigation on a foodborne outbreak caused by *Staphylococcus aureus* enterotoxin, in Hangzhou, 2014. *Zhonghua liu xing bing xue za zhi= Zhonghua Liuxingbingxue Zazhi*, **38**(12): 1642-1644.
- Johler S, Macori G, Bellio A, Acutis PL, Gallina S & Decastelli L (2018). Characterization of *Staphylococcus aureus* isolated along the raw milk cheese production process in artisan dairies in Italy. *Journal of Dairy Science*, **101**(4): 2915-2920.
- Karadiya J, Smith TC & Thapaliya D (2014). *Staphylococcus aureus* and Staphylococcal food-borne disease: An Ongoing challenge in Public Health. *BioMed Research International*, doi:10.1155/2014/827965.
- Le Loir Y, Baron F & Gautier M (2003). *Staphylococcus aureus* and food poisoning. *Genetics and Molecular Research*, **2**(1): 63–76.
- Macori G, Gilardi G, Bellio A, Bianchi D, Gallina S, Vitale N, Gullino M & Decastelli L (2018). Microbiological parameters in the primary production of berries: A pilot study. *Foods*, **7**(7): 105.
- Milios K, Drosinos EH & Zoiopoulos PE (2014). Carcass decontamination methods in slaughter houses. *Journal of the Hellenic Veterinary Medical Society*, **65**(2): 65-78.
- Nakao JH, Talkington D, Bopp CA, Besser J, Sanchez ML, Guarisco J, Davidson SL, Warner C, McIntyre MG, Comstock N & Xavier K (2018). Unusually high illness severity and short incubation periods in two foodborne outbreaks of *Salmonella* Heidelberg infections with potential coincident *Staphylococcus aureus* intoxication. *Epidemiology & Infection*, **146**(1): 19-27.
- Ndahi MD, Kwaga JKP, Bello M, Kabir J, Umoh VJ, Yakubu SE & Nok AJ (2013). Prevalence of antimicrobial susceptibility of *Listeria monocytogenes* and methicillin-resistant *Staphylococcus aureus* strains from raw meat and meat products in Zaria, Nigeria. *Applied Microbiology*, **58**(3): 262-269.
- Nnachi AU, Emele FE, Ukaegbu CO, Agah MV, Udu-Ibiam OE, Chukwu OS & Agwu MM (2014). Prevalence of methicillin-resistant *Staphylococcus aureus* in raw meat and meat handlers in Onitsha, Nigeria. *European Journal of Preventive Medicine*, **2**(1): 9-15.
- Nørnung B & Buncic S (2008). Microbial safety of meat in the European Union. *Meat science*, **78**(1-2): 14 – 24.
- Pearce RA, Bolton DJ, Sheridan JJ, McDowell DA, Blair IS & Harrington D (2004). Studies to determine the critical control points in pork slaughter hazard analysis and critical control point systems. *International Journal of Food Microbiology*, **90**(3): 331- 339.
- Pork Slaughter (1996). Generic HACCP model for pork slaughter: Submitted to USDA, Food Safety and Inspection Service by the International Meat and Poultry Alliance, Kansas, Missouri, USA. Pp 12 – 15.
- Prata CB, Lemos MVF, Prata LF & Caselani K (2013). Microbiological quality of cattle carcass during slaughter and occurrence of *E. coli* O157:H7 in beef. *Ars Veterinaria*, **29**(2): 93-97.
- Ruby JR, Zhu J & Ingham SC (2007). Using indicator bacteria and *Salmonella* spp. test results from three large-scale beef abattoirs over an 18-month period to evaluate intervention system efficacy and plan carcass testing for *Salmonella* spp. *Journal of Food Protection*, **70**(12): 2732-2740.
- Self JL, Luna-Gierke RE, Fothergill A, Holt KG & Vieira AR (2017). Outbreaks attributed to pork in the United States, 1998–2015. *Epidemiology & Infection*, **145**(14): 2980-2990.
- Silva FFPD, Horvath MB, Silveira JG, Pieta L & Tondo EC (2014). Occurrence of *Salmonella* species and generic *Escherichia coli* on beef carcasses sampled at a Brazilian slaughter house. *Brazilian Journal of Microbiology*, **45**(1): 17-23.
- Small A, James C, James S, Davies R, Liebana E, Howell M, Hutchison M & Buncic S (2006): Presence of *Salmonella* in the red meat abattoir lairage after routine cleansing and disinfection and on carcasses. *Journal of Food Protection*, **69**(10): 2342–2351.
- Spescha C, Stephan R & Zweifel C (2006). Microbial contamination of pig carcasses in different stages of slaughter in two European Union-approved abattoirs. *Journal of food protection*, **69**(11): 2568 – 2575.
- Wheatley P, Giotis ES & McKeivitt AI (2014). Effects of slaughtering operations on carcass

- contamination in an Irish pork production plant. *Irish Veterinary Journal*, **67**:1.
- Wilhelm B, Rajic A, Greig JD, Waddell L & Harris J (2011). The effect of hazard analysis critical control point programs on microbial contamination of carcasses in abattoirs: a systematic review of published data. *Foodborne Pathogens and Diseases*, **8**(9): 949–960.
- Zweifel C, Baltzer D & Stephan R (2005). Microbiological contamination of cattle and pig carcasses at five abattoirs determined by swab sampling in accordance with EU decision 2001/471/EC. *Meat Science*, **69**(3): 559–566.