Prevalence and risk of staphylococcal and coliform carcass contamination of chickens slaughtered in the informal market in Gauteng, South Africa

Oluwatola Adigun[1], Folorunso Oludayo Fasina [2,3], Awoke Kidanemariam[4], Nomakorinte Gcebe[5] and Abiodun A. Adesiyun[1]

[1]Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
[2]Food and Agriculture Organization of the United Nations, Dar es Salaam, Tanzania
[3]Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort, South Africa
[4]Bacteriology Laboratory, Agriculture Research Council, Onderstepoort Veterinary Research, Pretoria, South Africa
[5]Bacteriology Laboratory, Agriculture Research Council, Onderstepoort Veterinary Research, Pretoria, South Africa

Abstract

Purpose: The primary objective was to determine the prevalence of indicator microorganisms [*Staphylococcus aureus*, non-*S. aureus* staphylococci (NSAS), coliforms and aerobic bacteria] for contamination of chicken carcasses, carcass drip and rinse water from the informal chicken market in Gauteng, South Africa.

Design/methodology: Chicken swabs, chicken drips and rinse waters were collected from 151 chickens from 47 random outlets. Pre-tested questionnaires were administered to capture the risk factors for bacterial contamination. Standard microbiological procedures were conducted for isolation and enumeration of target bacteria.

Findings: NSAS (64% and 41%) and *S. aureus* (12% and 31%) were prevalent on carcasses and in carcass drip respectively. Coliforms (62%) and aerobic bacteria (85%) were detected in rinse water. Significant risk factors for contamination of carcasses with NSAS, *S. aureus* and coliform organisms were: evisceration of chickens on the same location used for sale, cleaning of display counter with dirty clothes/wipes, holding of differently-sourced chickens in the same cage prior to

slaughter, not cleaning the display table/counter and hands at all, washing knives in rinse water, high turnover of daily slaughter and length of time to display chickens.

Research limitation: The limitations of this research were the limited geographical coverage and small sample size.

Practical implication: The isolation of these indicator microorganisms suggests the potential presence of other chicken-borne pathogens not tested for in the study.

Social implication: The findings serve to inform policy on public health and street-vended food and can guide control on good sanitary practices.

Originality/value: This is the first comprehensive report on ready to eat chickens from the informal markets in Gauteng, South Africa.

Plain language summary

In South Africa, the informal chicken markets are well patronized and are located primarily in the townships. Chickens sold are cheaper, easily accessible and are the preferred meat-type but can also become sources of illnesses and food poisoning. For the first time from Gauteng, South Africa, we investigate some bacteria that can make these ready to eat chicken dangerous for human health, evaluate the likely sources of contaminations and report our findings.

Keywords: Staphylococcus aureus; Coliforms; poultry carcass; contamination; South Africa.

1. INTRODUCTION

Chicken meat is an excellent source of protein; however processed raw chicken may harbour microbial organisms (Bai et al., 2015; Dan et al., 2015), majority of which do not present food safety hazard. Chicken carcasses are often soiled with faecal matter, dust and dirt, hence, microorganisms can be detected on the carcasses post-slaughter. Ubiquitous bacteria exist in the breeder house environment and may be isolated from litter, dust and feathers, or may resides as normal micro-flora on chickens' skin, and in the respiratory and intestinal tracts (Clavijo, & Florez, 2018; Martins et al., 2013). During processing, damaged digestive tract can spill intestinal contents and contaminate chicken carcasses.

Pathogen contaminants in chicken meat from retail outlets may include *Salmonella* spp. (Bae et al., 2015; Cox et al., 2014; Donado-Godo et al., 2014; Niaullah et al.,2017; Ta et al., 2017), *Campylobacter* spp. (Rodrigo et al., 2007; Hu, Wang, & Li, 2015; Mansouri-Najand, Saleha & Wai, 2012; Sison et al., 2014), *Escherichia coli* and verocytotoxigenic *E. coli* (Rodrigo et al., 2007; Kagambega et al., 2012; Bai et al., 2015; Clavijo, & Flórez, 2018), *S. aureus* (Martins et al., 2013; Abdalrahman, Stanley & Wells, 2015), non-*S. aureus* staphylococci (Rodrigo et al., 2007; Oguttu et al., 2014; Osman et al., 2016). If improperly cooked, these chicken carcasses may cause foodborne infections and intoxications to humans (Fajó-Pascual et al., 2009; Ahlstrom et al., 2017; Huusko et al., 2017).

To minimise contamination, good sanitary practices should be observed and enforced at the level of processing and retailing of raw chicken meat (Kottawatta et al., 2017; Ramírez-Hernández, Varón-García, & Sánchez-Plata, 2017). In addition, retail supermarkets institute temperature control and good sanitary practices to avoid contamination (McLauchlin et al., 2017; Nhung et al., 2017). However, practices that take place in commercial and road side processing

plants/outlets can become points for contamination of carcasses with pathogens (Le Loir, Baron, & Gautier, 2003; Kottawatta et al., 2017; Lee et al., 2017). To minimise contamination further, most state or company authorities set standards and monitor processing operations routinely (Ramírez-Hernández, Varón-García, & Sánchez-Plata, 2017).

At some commercial operation and facilities, slaughter, processing and retailing take place at the same locations without control by health regulators (Rodrigo et al., 2007; Oguttu et al., 2015; Nhung et al., 2017). According to Grace et al. (2014), most vendors and people involved in informal markets are unlicensed, evade tax, and rarely comply with health and safety regulations. Chicken meat at the informal market is retailed freshly slaughtered and sold to customers at ambient temperature. During the above processes, the rinsing of carcasses may spread microorganisms throughout the entire carcass (Rodrigo et al., 2005).

To evaluate for microbes in carcasses, selected microorganisms have been used as indicators to assess hygienic practices at both processing and retail outlets selling raw chicken meat. These microorganisms include: *Enterobacteriaceae* (coliforms and *E. coli*) family, staphylococci and *S. aureus*, including the total aerobic bacteria (Gill et al., 2006; Lindblad et al., 2006; Ghafir et al., 2008; Matias, et al., 2010; Abdalrahman et al., 2015).

In South Africa, the informal chicken markets are well patronised and are located primarily in townships (Adigun et al., 2020). Community access these cheaper products compared with those from commercial outlets. In addition, the meat from the informal markets like the spent layer hens are preferred as delicacies among township populace and are easily accessible. Whereas the health authorities are concerned about the risks the informal outlets pose to the public, the current situation of sanitation and bacteriological quality of the chickens sold by the operators are unknown.

This study was undertaken, first to determine the prevalence and microbial load of selected indicator microorganisms (NSAS, and *S. aureus)* on chicken carcasses and carcass drip, and of coliforms and aerobic bacteria in rinse water. The strategy was to use the presence of the indictor microorganisms as a measure of the sanitary practices at the outlets in the informal chicken market and to estimate the risk of contamination by pathogens such as *Salmonella* spp. and *Campylobacte*r spp. amongst others.

2.MATERIALS AND METHODS

2.1 Location of informal chicken outlets and study strategy

Informal chicken markets/outlets (ICOs) in South Africa have been described by Adigun et al., (2020) and by the Department of Agriculture, Fisheries and Forestry [DAFF], (2018). Briefly described, the ICOs in South Africa are primarily small enterprises owned by individual/families < 20 persons. These outlets serve as unofficial destinations of spent layer hens, culled breeders and broilers from poultry farms nationwide. The uncontrolled, cross-province supplies of poultry to the informal outlets have implications for spread of poultry infectious diseases and limit the effort of the industry and the authorities for infection prevention (Adigun et al., 2020). At the ICOs, chickens kept in cages or tied in groups on the ground are removed for slaughter either in response to customer's request or slaughtered in large numbers and displayed either in rinse water or on counter tops. The average number of chickens slaughtered per day range from < 50 to > 1000 depending on the size of operation. Refrigeration or freezing facilities are unavailable in most places except in the Soweto operations and chilling of dressed chickens isn't practised. Pipe-borne water is rarely supplied to the outlets, being illegal operations, but water is transported to the outlets and stored in uncovered drums. In addition, organised disposal of waste water or solid waste is unavailable and some outlets dispose of offals, feathers and solid waste in bags or as environmental heaps that block drainage system (Adigun et al., 2020).

A comprehensive list of the ICOs in Gauteng province was obtained from stakeholders and the Veterinary Public Health Unit, Gauteng Department of Agriculture and Rural Development (GDARD). A pre-study assessment was made earlier to obtain comprehensive information on the throughput, processing methods, GPS locations and the average number of workers. A total of 61 consenting owners and outlets were recruited for the study.

2.2 **Determination of sample size and sample collection**.

The sample size for the study was estimated using the formula of Thrusfield (2007) below:

$$n_0 = \frac{\{1.96^2 \times P_{exp} \times (1 - P_{exp})\}}{d^2},$$

where P_{exp} is the expected prevalence and d is the desired precision.

A P_{exp} value of 50% and a d value of 8% were used,

$$n_0 = \frac{3.84 \times 0.5 \times 0.5}{0.0064} = 150$$

P_{exp} value of 50% was used to give equal chance to each sample for positivity or negativity and the d value of 8% was fixed to give ± 8% margin of error.

$n_0 = 150$ samples.

Therefore, the minimum sample size to be collected was 150. For this study, a total of 151 whole chicken carcasses and associated samples, each, were collected from the informal market outlets sampled.

The 151 samples originated from 47 out of the 61 consenting informal market outlets. The 61 outlets were initially categorised based on the criteria in the box below and the number of samples to be collected from each category was determined using proportional representation by size of outlet, daily throughput, operational practices and geographical locations:

Category 1: Large Operations with over 5 drums* or buckets for rinsing of carcasses and over 5 persons involved in the operation; Collected 5 samples from each outlet.

Category 2: Medium-sized Operations with 2-5 drums or buckets of water for rinsing carcasses and 2-5 persons involved in the operation: Collected 3 samples from each outlet.

Category 3: Small Operations with one bucket of water for rinsing carcasses and only one person running the operation: Collected 2 samples from each outlet.

Category 4: Home Operations with mechanical devise for de-feathering and 2-5 persons involved in the operation: Collected 3 samples from each outlet.

**Note that drums are large containers used by operators to hold water for rinsing of carcasses during daily operations. The size of the containers may vary from shop to shop and typically, water in these containers are used for whole day operations and are hardly changed in the course of the day.*

Simple random sampling was used to select samples to collect per operator until the maximum daily limit is reached per number of shop visited per day. Overall, 151 samples originated from six townships comprising Tembisa/Modise (8 outlets, 10 samples), Garankuwa (5 outlets, 18 samples), Alexandra (4 outlets, 20 samples), Germiston (5 outlets, 20 samples), Atteridgeville/Phomolong (7 outlets, 23 samples) and Soweto (18 outlets, 60 samples) (Table 1, Appendix 1).

Dressed whole carcasses, carcass drips (fluids drained and collected in plastic bags from rinsed dressed chickens) and samples of rinse water were collected for a total of 453 samples (151 each of carcass swabs, carcass drip and rinse water). These chickens were largely slaughtered at home in Soweto or in the shop (all the other locations).

Pre-tested questionnaire (Appendix 2a; Supplementary data) was administered concurrently to each consenting outlet's owner/vendor to capture demographic data and identify risk factors for bacterial contamination of carcasses at the time of sampling. The questionnaire comprised 19 questions and the risk factors investigated were the availability of refrigeration, type of birds slaughtered, average number of chickens slaughtered daily, evisceration method, method of de-feathering, carcass rinsing technique, location of carcass for sale, length of time (minutes) carcass is left exposed at ambient temperature, washing of hands after processing each carcass, clean table/counter. In addition, during the visit to each outlet for sample collection, the level of sanitation was assessed using a sanitation score (Appendix 2b; Supplemental data).

2.3 Sampling and processing

Samples were collected from spent layer hens (commercial hens at the end of egg production), culled breeders and broilers. Using aseptic techniques, the collected samples were: whole carcass in heavy-duty plastic bags, and rinse water (aliquots of water in buckets or drums used to rinse carcasses and equipment at the outlets) in sterile screw cap containers. These were transported on ice within 2 h of collection to the Poultry Pathology Laboratory of the University of Pretoria. At the laboratory, swabs of the internal and external carcass' surfaces were done and chicken drip (carcass fluid and drip which have collected in the plastic bags following transport of the carcass back to the laboratory) were drained into sterile bottles. All samples were transported to the Bacteriology Laboratory, ARC-Onderstepoort Veterinary Research (OVR) for further processing. Using approved laboratory protocols, *S. aureus* and NSAS (from carcass swab and carcass drip); Coliforms and Aerobic bacteria (total aerobic plate count, TAPC) (from rinse water) were isolated.

Serial dilutions (10-fold) of the carcass drip and rinse water samples were prepared with Ringer's solution (pH 7.3) for the enumeration of NSAS and *S. aureus*. All diluted samples were plated in duplicate on selective media and incubated at the appropriate temperature for 24-48 h and enumerated.

2.4 Determination of coliform count

A modified membrane filter technique (Rodrigo et al., 2005) was used to determine coliform count. Briefly, a 1:10 dilution (100 ml) of rinse water was made with Ringer's solution and vacuum-filtered with a 0.45 mm Millipore membrane filter (Millipore Corporation, Bedford, Massachusetts, USA). The filter was aseptically removed using sterile forceps and placed on Endo agar (Difco Laboratories, Detroit, Michigan, USA). The tests were conducted in duplicate and plates were aerobically incubated at 37°C and examined after 24 h for colonies with characteristic metallic green appearance. The colonies were counted and expressed as total coliform count per 100 ml**.**

2.5 Determination of total aerobic plate count (TAPC) of rinse water

Serial 10-fold dilutions of rinse water were made up to 10^{-5} in saline and 0.1 ml of each dilution was inoculated onto duplicate plates on nutrient agar (Oxoid Limited, Minnesota, USA) using the surface plating method (Food and Agriculture Organization [FAO], 1992; Rodrigo et al., 2005). The plates were incubated aerobically at 37°C for 24 h, and colonies on the plates between 30 and 300 colonies were counted and expressed as cfu/ml.

2.6 Enumeration of staphylococci in carcass drip

Serial 10-fold dilutions of carcass drip were made up to 10^{-8} with saline and inoculated onto Baird-Parker agar (BPA) (Oxoid Limited, Minnesota, USA) plates using 0.1 ml of the diluted samples and the plates were incubated aerobically at 37°C for 48 h. Colonies that appeared similar on each plate were counted and recorded. Representative colonies were Gram-stained. Isolates with characteristic appearance of staphylococci (greyish-black or black and Gram-positive cocci) were counted and expressed as the number of staphylococci per ml as described by Rodrigo et al. (2005). The determination of the counts of *S. aureus* and NSAS on each plate at the highest dilution with 30 – 300 colonies was made after the distinguishing tests (coagulase and DNAse tests) between *S. aureus* and NSAS were performed.

2.7 Detection of NSAS and *S. aureus* in carcass swabs

Standard methods (Food and Agriculture Organization [FAO], 1992), with slight modifications were used for the isolation and identification of NSAS and *S. aureus.* Carcass swabs were inoculated on BPA plates and streaked for isolation followed by aerobic incubation at 37°C for 48 h. Greyish-black or black colonies were tentatively classified as staphylococci. The numbers of suspect colonies on BPA plates were counted and representative colonies were picked to inoculate blood agar plates (BAP) which were aerobically incubated as described above. Suspect staphylococcal colonies on BPA plates were initially subjected to the following identification tests: Gram-stain, catalase, oxidase, indole and coagulase tests (Rodrigo et al., 2006). For coagulase production, a StaphTex latex agglutination test kit was used. Fermentation of mannitol and maltose was determined using the tube test as earlier described (Food and Agriculture Organization [FAO], 1992).

DNAse production was detected by inoculating DNAse agar (Oxoid, Basingstoke, UK) plates which were incubated at 37°C for 24 h after which agar was flooded with 1N Hydrochloric acid and left for a few minutes.

Susceptibility of the staphylococcal isolates to Polymycin B (300 Units) and Novobiocin (5 mcg) (Oxoid, Basingstoke, United Kingdom) was also determined on Mueller-Hinton agar using standard methods (Food and Agriculture Organization [FAO], 1992).

All isolates that were Gram-positive cocci, catalase-positive, indole-negative, oxidase-negative, fermenters of maltose and mannitol, coagulase-positive, Polymycin B-resistant, novobiocin-sensitive and DNAse-positive were identified as *S. aureus*. Isolates of NSAS were differentiated from *S. aureus* by being coagulase-negative, DNAse-negative and Polymycin B-sensitive. All the tests were performed on isolates recovered from carcasses and carcass drip.

2.8 Statistical analyses

Data from questionnaires (risk factors) were matched with laboratory results and entered into Microsoft Excel 2010. Continuous data were assessed for normality by plotting histograms, descriptive statistics and Anderson-Darling normality test. The unit of observation is individual shop from which the samples are collected and the unit of analysis is location (n = 6) within Gauteng, South Africa. Data were analysed descriptively as means with confidence intervals. Student t-test was used to calculate the *p*-value for proportions between two values and Kruskal-Wallis was used to calculate *p*-values for proportions among multiple values (Altman et al., 2000). One Way Analyses of Variance (ANOVA) was used to compare means for unequal sample size was used to compare the mean of counts (NSAS, *S. aureus*, coliforms and aerobic bacteria) across the six townships and ANOVA was for equal sample size was used for counts of NSAS and *S.*

aureus for each of the townships. Univariable and multivariable logistic regression analyses were performed to test the association between the dependent variables i.e. *Staphylococcus aureus,* NSAS (chicken carcass and carcass drip), coliforms and total aerobic bacteria and independent variables (n = 19). For logistic regression model for risk factors used in the study, the alternative reference factors for the variables include the following:

For NSAS and SA in chicken carcass swabs: Owners cleaned the table/counter with dirty cloths [Owners cleaned the table/counter with clean cloths], Eviscerate chickens [Did not eviscerate chickens], Processing and cutting chickens into pieces [Whole chicken carcasses sold] and the Number of chickens slaughtered daily (501 – 750) [Daily slaughter of 1 – 500 chickens].

For NSAS and SA in carcass drips: Disposal of solid waste in bags [Disposal of solid waste on the grounds of the outlet], Overall sanitary condition (fair) [Overall sanitary condition (poor)], Owners did not wash their hands at all after processing each chicken [Owners washed their hands after processing each chicken], Owners washed knives after processing each chicken [Owners did not wash knives after processing each chicken], Type of rinsing method (bucket) [Type of carcass rinsing method (stagnant water in drums)] and Types of birds slaughtered (culled breeders) [Type of birds slaughtered (spent layer hens/broilers)].

For aerobic bacteria and coliforms in rinse water: Length of time on table/counter (31 – 60 minutes) [Length of time on table/counter (30 minutes or less)] and Types of birds slaughtered (culled breeders) [Type of birds slaughtered (spent/broilers].

Continuous data were compared using Kruskal Wallis and Mann Whitney's test at p-value < 0.05 in NCSS statistical package, version 07.1.21.

2.9 Approval by the Ethics Committee

The protocol was approved by the Ethics Committee of the Faculty of Veterinary Science, University of Pretoria, South Africa prior to the commencement of the study (Approval number: V071-15).

3. RESULTS

3.1 Prevalence of NSAS and *S. aureus* on carcasses and chicken drip, and coliform and aerobic bacteria in rinse water.

S. aureus and NSAS

The overall prevalence of NSAS and *S. aureus* in carcasses were 64.2% and 11.9% ($p < 0.0001$) respectively, with NSAS being more significantly detected compared with *S. aureus* in samples from outlets (Table 1). Across the six townships, the prevalence of NSAS and *S. aureus* were statistically significantly ($p = 0.001$ and $p = 0.0001$ respectively; Table 1). In addition, 87.2% and 17.0% of all outlet-associated chicken carcasses presented with NSAS and *S. aureus* respectively and the difference was significant ($p < 0.00001$) (Table 1).

The prevalence of NSAS and *S. aureus* in the carcass drip were 41.1% and 31.1% respectively ($p = 0.07$; Table 2). Across the six townships, the differences in the prevalence were significant for NSAS ($p = 0.0002$) and *S. aureus* ($p = 0.0001$). The overall mean log10 NSAS and *S. aureus* cfu per ml of carcass drip were 6.08 and 6.66 respectively and across the six townships, the differences were statistically significant for NSAS ($p = 0.0001$) and *S. aureus* ($p = 0.0001$). The mean log10 NSAS cfu per ml of carcass drip ranged from 5.22 (Garankuwa) to 6.33 (Germiston) and for S. *aureus*, it ranged from 4.66 (Atteridgeville/Phomolong) to 7.01 (Soweto) (Table 2). A comparison of the mean log10 NSAS and *S. aureus* cfu per ml of carcass drip within each of the

six townships were not significantly for Garankuwa, and Germiston ($p > 0.05$) but significant for other locations assessed.

Coliform and aerobic bacteria

Coliforms and aerobic bacteria were detected in 61.6% and 85.4% of all the rinse water samples respectively with differences among locations being statistically significant ($p < 0.05$) except for Atteridgeville/Phomolong, Alexandra and Germiston (Table 3). Whereas 68.1% of the outlets' rinse water samples were coliform-positive, 87.2% were also aerobic bacteria-positive ($p = 0.03$). The prevalence of coliforms and aerobic bacteria varied considerably across the six townships for ($p = 0.0001$ and 0.02 respectively). Overall, the mean log10 coliform counts per 100 ml and aerobic bacteria counts per ml of rinse water was 5.42 and 7.37 respectively ($p < 0.0001$; Table 3). For the six townships, the mean log10 coliform counts per 100 ml ranged from 2.00 (Tembisa/Modise) to 6.01 (Germiston) while for aerobic bacteria, the mean log10 cfu per ml ranged from 4.81 (Atteridgeville/Phomolong) to 8.12 (Germiston) ($p < 0.0001$; Table 3).

3.2 Risk factors for isolation of NSAS and *S. aureus* from carcass swab and drip water, and coliform and aerobic bacteria from rinse water.

Based on risk analysis, processing and evisceration of chickens in the same location used for sale (OR = 21; $p < 0.01$), holding of differently sourced chickens in the same cage prior to slaughter (OR = 5; $p < 0.01$) and vendors not cleaning the display table/counter at all (OR = 3; $p = 0.05$) were associated with the risk of isolation of NSAS from chicken carcasses (Table 4). For *S. aureus*, owners who washed knives after processing with reused stagnant water and the high number of chickens (n = 501-750) slaughtered daily were associated with the risk of isolation of the organism (Table 4). Similar risk profiles were obtained for carcass drip samples (Table 4). For coliforms and aerobic bacteria, the time period that the carcass has spent on the display table/counter (31-60

minutes; OR = 100; $p < 0.01$); or > 60 minutes (OR = 5; $p = 0.03$), slaughtering of culled breeders (OR = 76; $p < 0.01$) and spent layer hens (OR = 11; $p = 0.01$) as well as not cleaning table at all (OR = 8; $p = 0.02$) were all significant risk factors.

4. DISCUSSION

Chicken carcasses processed in non-regulated outlets and wet markets have been reported with high prevalence of pathogens (Rodrigo et al., 2006; Medeiros et al., 2011; Cook et al., 2012; Donado-Godoy et al., 2014). In this study, the presence of NSAS, *S. aureus*, coliforms and aerobic bacteria associated with slaughtered chickens presented for sale at ICOs in South Africa was confirmed. The frequency of isolation and the counts were high compared with the standards for the food industry and demand attention of the country's food safety authorities as earlier recommended (Gill et al., 2006; Ghafir et al., 2008; Abdalrahman et al., 2015). Almost all the carcasses from all locations were contaminated with these indicator organisms for potential pathogens and the results are comparable with findings elsewhere (Kitai et al., 2005; Vaidya et al., 2005; Martins et al., 2013). In previous evaluation in the formal poultry processing plant, bacteria contamination were up to 2.0 cfu per ml in carcass and up to 5.0 cfu per ml on the conveyor belt, and Staphylococcus aureus was found in 24.1% of all product sampled from the processing plant (Geornaras et al., 1995; Van Nierop et al., 2005). The values determined for the informal ICOs based on this study were higher than for the formal industry but were within the acceptable limits for the meat industry (DAFF, 2010).

The prevalence of NSAS in both carcass swab and carcass drip was higher compared with *S. aureus.* This is consistent with the findings of others (Rodrigo et al., 2006; Chaves et al., 2018). Chicken drip are often drained by-products of rinse water used for cleaning many carcasses with

the potential for cross contamination. Considering that chicken drip in the current study was in direct contact with the carcass, and because previous relationship has been established between the indicator organisms like aerobic bacteria and salmonellae and Campylobacter, we can estimate the bacteriological quality of the chicken carcasses in this study (Ramírez-Hernández, Varón-García, & Sánchez-Plata, 2017). All the ICOs sampled carcasses, whether eviscerated or not, are all rinsed in contaminated bloody or blood-tinged stagnant water in buckets or drums, prior to packaging in bags. This could serve as an important risk factor for cross-contamination of carcasses.

The fact that *S. aureus* poses serious zoonotic and food safety concerns to human handlers, vendors and consumers of improperly cooked contaminated carcasses cannot be ignored. An earlier study (Hall et al., 2005) had reported the highest counts of microorganisms in rinse water compared with that from carcass swabs and chicken meat pieces. These findings agree with our study. The poor quality of water used at ICOs in South Africa was attributed to the fact that vendors often relied on non-potable water in unclean containers which has the potential to increase the likelihood of contamination with *S. aureus* as reported by Oguttu et al. (2014). The use of poor quality water to process chicken at these outlets which was assessed by the coliform count per 100 ml in the current study is in agreement with reports that coliforms, including fecal coliforms among others, are indicators of microbial water quality (Bae et al., 2013) and hygienic or sanitary practices in chicken processing and retail outlets (Ghafir et al., 2008; Gill et al., 2006).

The high TAPC per ml of rinse water detected across the outlets in the current study could be attributed to the fact that the rinse water was regularly in contact with human handlers and leakage of chicken intestinal and visceral contents into rinse water as observed in the current study. This agreed with published reports (Rosenquist et al., 2006; Pacholewicz et al., 2016a; Pacholewicz et

al., 2016b). It was worth noting that there was a wide variation in the median counts for NSAS (carcass drip), coliform and TAPC (rinse water). Similarly, the observed differences in samples and townships are probably a reflection of wide differences in the water quality and sanitary practices at the ICOs in these townships. These findings suggest that chicken meat purchased from the different ICOs investigated, which are non-regulated and use non-standardised operations, may pose significantly different health risks to consumers and that the management of these operations need to be improved.

In this study, many risk factors associated with NSAS, *S. aureus*, coliforms and aerobic bacteria found in chicken carcasses and carcass drip or rinse water have been identified for the ICOs in Gauteng province in South Africa. For contamination of carcasses and carcass drip with NSAS and *S. aureus*, the important risk factors were the practice of carcass evisceration, the high throughput and washing of knives in contaminated waters after processing each chicken. These risk factors have been reported to contribute the contamination of carcasses during processing, particularly in operations at informal market, wet market and pluck shops in developing countries. During evisceration, the possibility of contamination of rinse water and direct contamination of carcasses have been documented (Hue et al., 2010; Ramírez-Hernández, Varón-García, & Sánchez-Plata, 2017; Rodrigo et al., 2005). It is known that the evisceration process may spill and contaminate table/counter with transfer to the carcass, rinse water and chicken drip. The daily throughput chicken processing at outlets have been reported to increase the risk of carcass contamination, microbial load and prevalence of bacterial pathogens by others (Lues et al., 2007). Furthermore, a facility with higher throughput of chickens per day will have a high potential for spillage of intestinal contents and therefore a greater tendency for carcass contamination. In addition, the possibility exists that NSAS and *S. aureus* may originate from human handlers since

these organisms have been found on hands and finger tips of more than 50% of apparently healthy individuals (Le Loir, Baron, & Gautier, 2003; Lues et al., 2006). Failure to wash hands between processing of individual chicken, as confirmed in this study, may therefore extend contamination even to clean carcasses. Whereas the washing of knives after processing is supposed to reduce the risk of contamination of carcasses by *S. aureus*, the opposite occurred in the current study. The washing of knives was not compliant with sanitary practices as this was done using contaminated rinse water from the containers that were used to rinse chickens.

In our investigation rinsing carcasses with stagnant, often contaminated water, was also a risk factor (OR=2.9) for the isolation of *S. aureus*. A WHO report (World Health Organization [WHO], 1989) revealed that vendors are carriers of pathogens like *S. aureus* and staphylococci, and may eventually transfer these foodborne organisms to the processing water and subsequently to prepare foods. Furthermore, NSAS and *S. aureus* are part of the skin flora of chickens, of water with poor bacteriological quality or as contaminants transmitted by vectors such as flies (Lues et al., 2006; Firildak, Asan, & Gören, 2015). Thus, the level of NSAS detected in the current study may be an indication of poor sanitary practices at the ICOs in all the outlets sampled in Gauteng Province. The detection of NSAS in both carcass swabs and carcass drip could also have zoonotic significance for the handlers of the carcasses and clients who may especially be exposed to strains resistant to antimicrobial agents, including methicillin (Huber et al., 2011; Osman et al., 2016), which were not determined in the current study.

It is pertinent to emphasise that in developing countries such as South Africa, the use of indicator microorganisms to assess sanitary practices at the outlets of unregulated informal chicken market for the potential foodborne pathogens may be justified. This is because the approach has the advantages of being rapid and cost-effective. It has however also been reported that the use of

some indicator microorganisms used in poultry processing did not correlate with the presence of foodborne pathogens, highlighting the limitation of this approach. Carson et al. (1997) had reported that aerobic bacteria were not suitable as index organisms for salmonellae or *Campylobacter* on broiler carcasses.

5. CONCLUSION

The study provided evidence of bacterial food-borne contamination in informal food outlets in Gauteng Province, South Africa and identified some risk factors associated with potential food-borne contamination; authorities will need to define strategies to enforce sanitary laws and extend health service deliveries including food safety, to these communities. It is therefore recommended that the high prevalence and microbial load of selected indicator microorganisms in the current study reflect poor hygienic practices at the outlets studied; future studies should therefore be conducted to determine the occurrence of pathogens such as *Salmonella* spp. and *Campylobacter* spp., usually associated with chicken-borne outbreaks, in chickens from the informal market in Gauteng province.

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CONFLICT OF INTEREST

The authors declares no conflicts of interest

ORCID

Folorunso O. Fasina: https://orcid.org/0000-0003-3088-8752

Abiodun A. Adesiyun: https://orcid.org/0000-0001-9470-9421

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Table 1. Prevalence of non-*S. aureus* staphylococci and *S. aureus* in carcass swabs from informal chicken outlets

		Outlets (n) positive with bacteria in carcass skin swabs (%)			Carcass swab (n) positive for bacteria in carcass skin swab (%)			
Township	Outlet sampled (n)	Non-*S. aureus* staphylococci	*Staphyloccocus aureus*	*p-value*	Carcass sampled (n)	Non-*S. aureus* staphylococci	*Staphyloccocus aureus*	*p-value*
Atteridgeville/Phomolong	7	7 (100.0)	1 (14.3)	0.002	23	19 (82.6)	3 (13.0)	< 0.0001
Garankuwa	5	5 (100.0)	0 (0.0)	0.003	18	15 (83.3)	0 (0.0)	< 0.0001
Tembisa/Modise	8	6 (75.0)	0 (0.0)	0.003	10	6 (60.0)	0 (0.0)	0.004
Alexandra	4	4 (100.0)	3 (75.0)	0.32	20	10 (50.0)	4 (20.0)	0.05
Germiston	5	5 (100.0)	2 (40.0)	0.05	20	16 (80.0)	4 (20.0)	0.0002
Soweto	18	14 (78.0)	2 (11.1)	0.0001	60	31 (51.7)	7 (11.7)	< 0.0001
p-value		0.001	0.0001			0.0001	0.0002	
Total	47	41 (87.2)	8 (17.0)	< 0.0001	151	97 (64.2)	18 (11.9)	< 0.0001

*Student t-test was used to calculate the p-value for proportions/means between two values (*https://www.medcalc.org/calc/comparison_of_proportions.php*) and Kruskal-Wallis was used to calculate p-values for proportions among multiple values (*https://www.socscistatistics.com/tests/kruskal/default.aspx*), Altman et al., 2000)*

Table 2. Prevalence and counts of non-*S. aureus* staphylococci and *S. aureus* in carcass drips sampled from informal chicken outlets

	Outlets (n) positive with bacteria in carcass drip waters (%)				Carcass drip water (n) positive for bacteria in carcass drip (%)				Mean log_{10} count of bacteria per ml[a] of carcass drip (colonies forming units, cfu/ml)		
Township	Outlet sampled (n)	Non-*S. aureus* staphylococci	*Staphyloccocus aureus*	*p-value*	Drip water sampled (n)	Non-*S. aureus* staphylococci (NSAS	*Staphylococcus aureus*	*p-value*	Non-*S. aureus* staphylococci	*Staphylococcus aureus*	*p-value*
Atteridgeville/Phomolong	7	7 (100.0)	1 (14.3)	0.002	23	17 (73.9)	2 (8.7)	0.000	5.48	4.66	< 0.0001
Garankuwa	5	2 (40.0)	3 (60.0)	0.55	18	6 (33.3)	11 (61.1)	0.1	5.22	5.18	0.72
Tembisa/Modise	8	7 (87.5)	0 (0.0)	0.001	10	7 (70.0)	0 (0.0)	0.001	5.24	NA	NA
Alexandra	4	4 (100.0)	4 (100.0)	NA	20	11 (55.0)	9 (45.0)	0.53	5.27	5.07	0.07
Germiston	5	5 (100.0)	3 (60.0)	0.13	20	11 (55.0)	3 (15.0)	0.01	6.33	6.29	0.73
Soweto	18	6 (33.3)	9 (50.0)	0.32	60	10 (16.7)	22 (36.7)	0.01	6.62	7.01	< 0.0001
p-value		0.0002	0.0001			0.0001	0.0001		0.0001	0.0001	
Total	47	31 (66.0)	20 (42.6)	0.02	151	62 (41.1)	47 (31.1)	0.07	6.08	6.66	< 0.0001

[a] Detection limit was 10 colony forming units per ml since 0.1 ml was plated; NA = Not applicable.

Table 3. Prevalence and counts of coliforms and aerobic bacteria in rinse water sampled from informal chicken outlets

	Outlets positive with coliform & aerobic bacteria in carcass rinse waters (%)				Rinse water positive for coliforms & aerobic bacteria in carcass rinse water (%)				Mean log_{10} count of coliforms & aerobic bacteria per 100 ml of carcass drip (colonies forming units)		
Township	Outlet sampled (n)	Coliforms	Aerobic bacteria	*p- value*	Rinse water sampled (n)	Coliforms	Aerobic bacteria	*p- value*	Coliforms	Aerobic bacteria	*p- value*
Atteridgeville/ Phomolong	7	3 (42.9)	7 (100.0)	0.02	23	13 (56.5)	20 (82.6)	0.06	3.30	4. 81	< 0.0001
Garankuwa	5	2 (40.0)	5 (100.0)	0.05	18	14 (77.8)	18 (100.0)	0.04	4.17	6. 03	< 0.0001
Tembisa/Modise	8	3 (37.5)	8 (100.0)	< 0.01	10	3 (30.0)	9 (90.0)	< 0.01	2.00	4. 85	< 0.0001
Alexandra	4	3 (75.0)	4 (100.0)	0.32	20	5 (25.0)	11 (55.0)	0.06	4.75	5. 34	< 0.0001
Germiston	5	5 (100)	5 (100.0)	NA	20	20 (100.0)	20 (100.0)	NA	6.01	8. 12	< 0.0001
Soweto	18	16 (88.9)	12 (66.7)	0.11	60	38 (63.3)	51 (85.0)	< 0.01	4.98	6. 89	< 0.0001
p-value		0.0001	0.02			0.0001	0.0001		0.0001	0.0001	
Total	47	32 (68.1)	41 (87.2)	0.03	151	93 (61.6)	129 (85.4)	< 0.0001	5.42	7. 37	< 0.0001

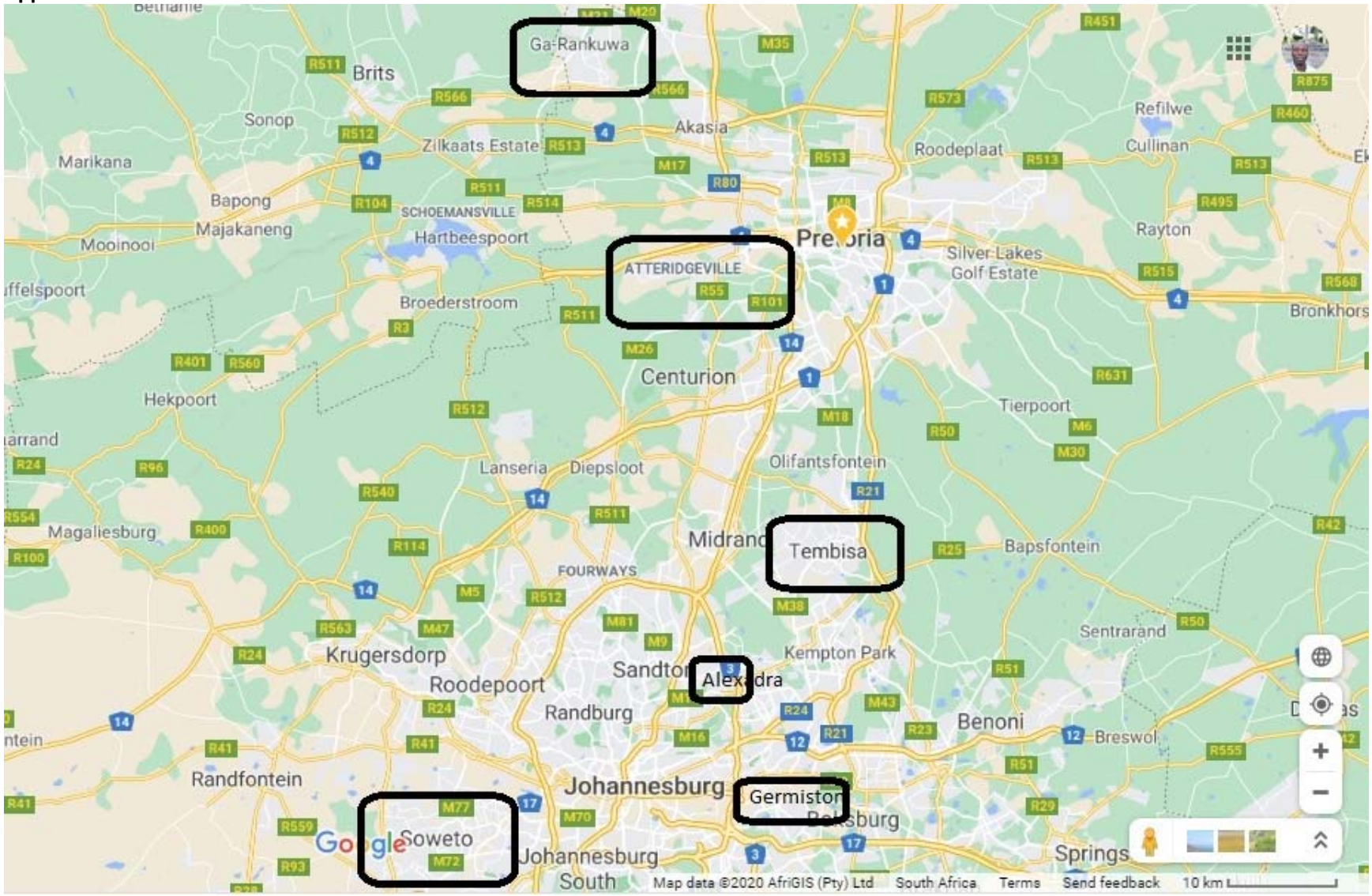
Table 4. Logistic regression model for risk factors for non-*S. aureus* staphylococci, *S. aureus*, coliform and aerobic bacteria in from chicken skin swab, and for aerobic bacteria and coliforms from 151 rinse water samples

Risk factors for non-S. aureus staphylococci and S. aureus from chicken skin swab, Gauteng, S. Africa				
Organism	**Variable***	**Odds ratio**	**(95%) CI**	***p*-value**
Non-*S. aureus* staphylococci	Owners cleaned the table/counter with dirty cloths	4.9	1.49-16.13	0.01
	Owners did not clean the table/counter at all	3.1	1.02-9.27	0.05
	Eviscerate chickens	21.4	4.80-95.38	<0.01
	Kept chickens in cages	5.4	2.00-14.51	<0.01
	Processing**	0.1	0.03-0.77	0.02
S. aureus	Eviscerate chickens	0.1	0.01-0.59	0.02
	No. of years in operation	0.03	0.07-2.36	<0.01
	Owners washed knives after processing	19.8	1.95-200.92	0.01
	No. of chickens slaughtered daily (501-750)	10.6	1.02-109.82	0.05
Risk factors for non-S. aureus staphylococci and S. aureus from 151 drip water samples, Gauteng, S. Africa				
Organism	**Variable***	**Odds ratio**	**(95%) CI**	***p*-value**
Non-*S. aureus* staphylococci	Disposal of solid waste	0.18	0.06-0.54	<0.01
	Eviscerate chicken (hand picking)	2.7	1.05-6.95	0.03
	Owners clean table with dirty cloths	1.5	0.44-5.29	<0.01
	Number of chickens slaughtered daily (501-750)	26.8	2.00-359.00	<0.01
S. aureus	Owners do not wash their hands at all after processing each carcass	7.31	1.30-41.18	0.02
	Processing	0.21	0.06-0.72	0.01
	Type of rinsing water	2.87	1.10-7.50	0.03
	Type of birds slaughtered (culled breeders)	0.04	0.01-0.25	<0.01
Risk factors for aerobic bacteria and coliforms from 151 rinse water samples, Gauteng, S. Africa				
Organism	**Variable***	**Odds ratio**	**(95%) CI**	***p*-value**
Aerobic bacteria	Length of time on table/counter (31-60 minutes)	5.6	1.31-23.95	0.02
Coliforms	Owners did not clean the table/counter at all	7.5	2.73-2.50	0.02
	Length of time on table/counter (31-60 minutes)	99.9	14.68-680.31	<0.01
	Length of time on table/counter (>60 minutes)	4.7	1.13-19.24	0.03
	Type of birds slaughtered (culled breeders)	76	8.28-697.25	<0.01
	Type of birds slaughtered (spent hens)	11.2	1.61-77.20	0.01

*Retained risk factors out of the 19 variables assessed

** Carcasses are cut into portions after evisceration and cleaning.

Appendices.



Appendix 1. Townships and locations where outlets were sampled for the study.
Map was adapted from Google Maps (https://www.google.com/maps/@-26.2233542,27.8875272,11z).

Description of sampled location*:

Alexandra: an informal township and part of the City of Johannesburg Metropolitan Municipality, Gauteng, South Africa. It is located near the upper-class suburb of Sandton. Alexandra (Coordinates: 26°6.23'S 28°5.77'E) has a human population of approximately 179,624 and a population density of 26,000/km^2 and a land area of 6.91 km^2.

Tembisa (Thembisa): is a large township situated to the north of Kempton Park on the East Rand, Gauteng, South Africa. It was established in 1957 when black people were resettled. It is located in the coordinates 26.0055°S 28.2102°E and has a human population of 463,109 and a human density of 11,000/km^2 and a land area of 42.80 km^2.

GaRankuwa: is a large settlement located about 37 km north-west of Pretoria. It has a total human population of 90,945 and a density of 1,700/km2. It is located in the coordinates 25°37'12"S 27°58'48"E and a total land area of 52.18 km^2.

Germiston: is a small city in the East Rand region of Gauteng, South Africa, It is part of the City of Ekurhuleni Metropolitan Municipality. It is located in the coordinates 26°13'4"S 28°10'2"E and covers a land area of 143.27 km^2. It has a human population of 255,863 and a population density of 1,800/km^2.

Atteridgeville: is a township located to the west of Pretoria, South Africa. It is bordered to the east of Saulsville, to the west of Proclamation Hill; to the north of Laudium and to the south of Lotus Gardens. It is located in the coordinates 25°46'24"S 28°04'17"E and has a human population of 64,425, population density of 6,500/km^2 and a total land area of 9.84 km^2.

Soweto: is a township of the City of Johannesburg Metropolitan Municipality in Gauteng, South Africa, bordering the city's mining belt in the south. Its name is an English syllabic abbreviation for **So**uth **We**stern **To**wnships. 200.03 km^2 and is located in the coordinates 26°15'58"S 27°51'57"E. it has a human population in excess of 1,271,628 and a density of 6,400/km^2.

* https://en.wikipedia.org/wiki/

Appendix 2a: Questionnaire for Owners of Informal Chicken Outlet and Sanitation Score Sheet used during visits to outlets

Serial #:_____ Area**:** _____**Date of Administration**: _____

1. **Name: _____(optional)**
2. **Address: _____(optional)**
3. **GPS coordinates of outlet: _____**
4. **Contact number(s): _____**
5. **Name of supplier:**

   i. **_____**

   _____

   ii.

	Types of birds slaughtered			
	Spent hens	Culled Breeders	Broilers	Others/combinations (list)
Yes				
No				

   iii. **Approximate number of each chicken type slaughtered daily:**

   a. Spent hens: _____ b. Culled breeders: _____ Broilers: _____

6. **Number of processing days per week: _____**

7. **Key steps in the operation at the outlet (List in sequence):**

Key steps in the operations of the outlets (sequence)			
Serial no.		Yes	No
	Kept chicken in cages		
	Pick chickens for slaughter		
	Slaughter with knives		
	Scalding		
	De-feathering		
	Eviscerate chickens		
	Processing		
	Packaging and retailing		
	Refrigeration		

8. **Type of de-feathering method used**

	Types of de-feathering method			
	Drum type	Tube type	Hand picking	Others (list)
Yes				
No				

9. **Type of rinsing method**

	Types of rinsing method		
	Running water/pipe	Stagnant water/Sink/Bucket	Briefly comment on the source of water(tap/river): _____ _____ _____ _____
Yes			
No			

10. **Number of years or months in operation:** _____

11. **Opening hours**

	Types of operation		
	Weekly	Daily	Briefly comment on the source of water: _____ _____
Yes			
No			

12. **Average cost of a whole chicken (Rand**): Spent hens:____, Culled breeders:_____, Broilers:_____

13. **Disposal of wastes from the outlet:**
    a. Liquid waste:_____
    b. Solid waste: _____

14. **Availability of refrigeration or freezing facilities at the outlet (tick**):

	Types of refrigeration method			
	Refrigerator	Freezer	Both	
Yes				
No				

15. **Location of carcass for sale:**
    a. Counter: _____ Refrigerator: _____ c. Freezer: _____d. Bucket_____

b. For outlets where carcasses are located on the counter for sale, how long do they remain there?
   i. Less than 30 min _____ ii. 30 min – 60 min _____ ii. Over 60 min: _____

16. Number and types of samples collected from the outlet?

a. *Chickens:* Spent hens: ____ No.:____ Culled breeders: ___ No.: ___ Broilers: ____ No.: ____
b. *Cloacal swabs*: Spent hens: ____ No.:____Culled breeders: ___No.: ___Broilers: ___No.:____
c. Post-evisceration carcass swab: _____
d. *Rinse water*:_____
e. *Drip water*: _____
f. *Whole carcass*_____

17. Any other comments/observations:

i. Do they wash the knives after processing each carcass? Yes.____ No.____
ii. Clean the table with clean cloths? Yes.____ No.____
iii. Clean the table with dirty cloths? Yes.____ No.____
iv. Wash hands with soap after processing each carcass? Yes.____ No____
v. Wash hand without soap after processing each carcass? Yes.____ No____
vi. Do not wash hand at all after processing each carcass? Yes.____ No____

18. Type and identification of samples collected at outlet:

<u>Codes</u>

a. Chicken-Spent (CS): _____
b. Chicken-Culled (CC): _____
c. Chicken-Broiler (CB) _____
d. Chicken-Spent Drip (CSD): _____
e. Chicken-Culled Drip (CCD): _____
f. Chicken-Broiler Drip (CB) _____
g. Water rinse/drain liquid in bags with chicken carcass (WR): _____
h. Chicken-Spent Cloaca (CSC): _____
i. Chicken-Culled Cloaca (CCC): _____
j. Chicken-Broiler Cloaca (CBC): _____

19. *Declaration and signature of owner or the person having charge of the animals/birds.*

I, (full name) _____, hereby declare ***that I have consented*** to participate in the study and the samples were collected by the authorized person mentioned above and that no relevant information was withheld from the authorized person.

Date: _____

Thank you for participating in the survey

***Appendix* 2b. Sanitation Score Sheet used for the Study**

	Score					
Category	**1 (worst)**	**2**	**3**	**4**	**5 (best)**	**NA***
1. Handlers of chickens at outlet						
1.1 Wore clean clothes with sleeves						
Clean clothes with sleeves						
Clean clothes without sleeve						
Dirty clothes without						
Dirty clothes with sleeves						
Dirty clothes with dirty sleeves						
1.2 Wore aprons						
Wore very clean aprons						
Wore clean aprons						
Did not wear aprons						
Wore moderately dirty clothes						
Wore very dirty aprons						
1.3 Had hair covered						
Yes						
No						
2 Cleanliness in cages or areas where live birds are kept						
Relatively clean and not crowded						
Relatively clean and crowded						
Relatively dirty –faeces etc- and crowded						
Relatively filthy and crowded						
Very filthy and very crowded						
3. Sanitation in slaughter area						
Kept very clean—Little blood/feathers/faeces						
Kept clean---some blood/feathers/faeces						
Moderately kept clean—Blood/feathers/lot of flies						
Poorly kept—blood/feathers/lot of faeces/few flies						
Very poorly kept—blood/feathers/faeces/many flies						
4. ***Sanitation in de-feathering or 'plucking' Area***						
Kept very clean—Little blood/feathers/faeces						
Kept clean---some blood/feathers/faeces						
Moderately kept clean—Blood/feathers/lot of faeces						
Poorly kept—blood/feathers/lot of faeces/few flies						
Very poorly kept—blood/feathers/faeces/many flies						
5. Sanitation in evisceration area						
Kept very clean—Little blood/feathers/faeces						
Kept clean---some blood/feathers/faeces						

Moderately kept clean—Blood/feathers/lot of faeces						
Poorly kept—blood/feathers/lot of faeces/few flies						
Very poorly kept—blood/feathers/faeces/ many flies						
6. Sanitation in rinsing of carcases						
Use of 3 rinsing bucket in sequence/Clean water						
Use of 2 rinsing buckets in sequence/Clean water						
Use of 1 rinsing bucket/bloody water/feathers/1carcass						
Use of 1 rinsing bucket/bloody water/feathers/ 2 carcasses						
Use of 1 rinsing bucket/bloody water/feathers/3 or more Carcasses						
7. Sanitation in packaging and sale areas						
Kept very clean—No blood/No feathers/No faeces/No flies						
Kept clean---No blood/ No feathers/No faeces/Few flies						
Moderately kept clean—Some Blood/Few feathers/Some faeces/Some flies						
Poorly kept—blood/feathers/lots of faeces/ few flies						
Very poorly kept—Some blood/Lots of feathers/Lots of faeces/Many flies						

NA: Not applicable/Not available