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Microbiological quality of free-range chicken carcasses from a non-regulated slaughter facility in Kenya

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This study assessed the microbiological quality of meat from free rage-produced chicken processed in an informal slaughter facility. The total viable counts (TVC), total coliform counts, coagulase positive *Staphylococcus aureus*, *Streptococcus* species, *Salmonella* species and *Campylobacter* species were used as indicators. A cross-sectional sampling of chicken carcasses at informal slaughter facility was carried out. Whole carcass rinse fluid was prepared from 40 randomly obtained freshly dressed carcasses. Fluid samples were cultured in selective media to isolate and enumerate the specific bacteria. *S. aureus* was further identified by coagulase test, Streptococci by serotyping into Lancefield groups, Campylobacter by DNA analysis and Salmonella by biochemical tests and serology. Bacterial concentrations in the carcasses were calculated as colony forming units (CFU) per ml and CFU/cm^2. The mean carcass CFU/ml concentration was 1.59 × 107, 1.44 ×105, 3.2 × 104 and 1.06 × 104 for TVC, Coliforms, *S. aureus* and *Streptococci*, respectively. All the mean concentration values were higher than the limits recommended by the Codex Alimentarius Commission (CAC). Coagulase-positive Staphylococcus was isolated from 12 (30%) carcasses and Streptococci from 35 (87%). Majority Streptococci were Lancefield Group D (48.57%) followed by Group G (17.14%), and Group F (14.28%). Campylobacter genus was identified in 11 carcasses (27.5%) and *Campylobacter jejuni* in three (7.5%). On the other hand, Salmonella was not isolated from any carcass. The results of the study indicated that the low hygienic standard in non-regulated slaughter houses exposed the chicken meat to microbial contaminants which may pose a risk to the consumers. Improvement of slaughter infrastructure and capacity-building of slaughter personnel is therefore critically required to ensure food safety and enable access to high value markets.

Key words: Slaughter, free-range chicken, bacterial quality.

INTRODUCTION

Food spoilage and foodborne diseases are important economic and health concerns and raw poultry products are frequently contaminated by spoilage organisms and human pathogens. Contamination may occur exogenously

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or endogenously and the health of birds at farm level, transportation, slaughter facilities and processing are critical (Shahdan et al*.,* 2017; Rasschaert et al., 2020). Contaminated products undergo rapid spoilage especially when post-slaughter and processing preservation is inadequate, as a result of growth and metabolic activities of contaminant bacteria. Spoilage may lead to large economic losses, impacting on the economy of the poultry production sector (Rouger et al., 2017).

Bacterial contaminants may include pathogenic species and human infections results from handling raw products, undercooking or cross-contamination of other foodstuffs at retail or household level (Kennedy et al., 2011; Sirsat et al., 2014; Mkhungo et al., 2018). *Campylobacter* and *Salmonella* are the two most important human zoonotic gastrointestinal bacterial pathogens. Poultry meat is believed to be the main source of human *Campylobacter* infection worldwide (EU, 2017; Kuria et al., 2018; Carron et al., 2018) and an important source of non-typhoid salmonellosis (Antunes et al., 2016; WHO, 2018). Other important pathogens that may be associated with poultry foodborne disease include *Staphylococcus aureus,* verotoxigenic *Escherichia coli* and fecal *Streptococci* (Rouger et al., 2017; Svobodová et al., 2012; Vaidya et al*.,* 2005).

Free range chicken production in Kenya constitutes the largest proportion of the national poultry population, and serves as a source nutritional needs and income to resource-poor farmers in developing countries. However, production, transportation, slaughter and processing are carried out under poor sanitary conditions (King'ori et al*.,* 2010; Ipara et al., 2019). The poor hygienic processing conditions in informal facilities hinder the chicken products access to high value markets and expose consumers to health risks. Data on microbiological quality of chicken carcasses have been limited mainly to intensive production and conventional processing systems in Europe and North America and data on free range indigenous chicken processed under informal facilities has been scanty. Ensuring microbial safety in food products is important in the context of increasing production and consumption as well as safeguarding human health. Consequently, the Codex Alimentarius Commission (CAC) has set the limits for microbial organisms in foods (CAC, 1997). This study aims to assess the bacterial quality of meat carcasses from free range-produced chicken slaughtered in non-conventional facility in Nairobi, Kenya.

MATERIALS AND METHODS

Study area

The study comprised collection and laboratory analysis of chicken carcasses. Burma Maziwa market (Elevation: 1,795 M, Coordinates: S-01.2921°, E-036.8219°) in Nairobi County is one of several informal markets for indigenous free-range produced chickens. It receives chicken from several parts of the country and serves as a significant outlet of chicken destined to other smaller live bird-markets in the county. There are no chicken housing facilities and the chickens are held in wooden transport cages for short durations. Dressed carcasses are supplied to lower-class supermarkets and butcheries, and also homes. Slaughter facility consists of a single stone-walled room with concrete floor, a concrete work-bench and no running water. The slaughter method involves manual strangulation, dry-plucking and evisceration. Laboratory analysis was carried out at the faculty of Veterinary Medicine, University of Nairobi.

Sample collection

Five (5) freshly dressed chicken carcasses were randomly obtained from the slaughter house on each of 8 sampling days. A total of 40 carcasses were collected. The carcasses were put in sterile double polythene bags, labeled, put in a cool box and transported to the laboratory within 4 to 5 h. In the laboratory, the carcasses were weighed and then rinse-washed with 400 ml of buffered peptone water (pH 7.2) following the method described (NACMCF, 2007). The rinse fluid was then analyzed for the microorganisms of interest.

Enumeration of total viable counts (TVC) and coliforms

Tenfold serial dilutions of rinse fluid from each carcass were prepared in peptone water. One milliliter of each of the highest 4 consecutive serial dilutions (10^{-3} to 10^{-6}) was inoculated into Plate Count Agar (PCA) (HIMEDIA M091, India) in triplicates, using the pour plate overlay method. The plates were then incubated at 37°C for 48 h and the number of colonies formed per dilution recorded after 24 and 48 h of incubation. The mean CFU/ml and CFU/cm^2 were then calculated as described (Brichta-Harhay et al., 2008) and transformed into log CFU/ml and log CFU/$cm^{2.}$ Total coliforms were similarly enumerated, using Violet Red Bile Lactose Agar (VRBA) media (OXOID CM 0968, UK).

Enumeration and confirmation of coagulase positive *Staphylococcus*

Using spread plate method, 0.5 ml of each 4 consecutive 10 fold serial dilutions (10^{-1} to 10^{-4}) of the rinse fluid were inoculated in triplicates into mannitol salt agar (Oxoid CM 085, UK). The plates were incubated at 37°C for 24 h and the number of yellow colonies recorded per dilution. CFU/ml, CFU/cm^2 and the respective logs were then calculated. For confirmation of coagulase positive *S. aureus*, four suspect colonies per dilution were sub-cultured onto blood agar (OXOID CM0271, UK) and incubated at 37°C for 24 h. Golden yellow and cream white colonies were tested for catalase and coagulase activity.

Enumeration and serotyping of *Streptococci*

Sodium azide blood agar (OXOID CM 259, UK) was inoculated with 0.5 ml of each of the 4 consecutive 10 fold serial dilutions of the rinse fluid (10^{-1} to 10^{-4}), in triplicates, using the spread plate method. The plates were incubated at 37°C for 48 h and the number of pin point βhemolytic colonies recorded per dilution. CFU/ml, CFU/cm^2 and the respective logs were calculated. The colonies were purified by sub-culturing four β hemolytic colonies per plate onto blood agar plates and incubating for 24 h at 37°C. Characteristic βhemolytic colonies were serotyped into Lancefield groups using streptex* kit

(Oxoid, TSMX7829, UK), following the manufacturer's instructions.

Isolation of *Campylobacter* species

Campylobacter blood-free medium containing antibiotics and supplement (mCCDA, Oxoid CM739, UK) was used for isolation of thermophilic *Campylobacter* spp. All samples were cultured directly on the media (OIE, 2008) within 6 h of collection. Tenfold serial dilutions of the rinse-wash fluid were prepared in peptone water and 0.5 ml of 4 consecutive serial dilutions, 10^0 to 10^{-3}, inoculated into the media using the spread plate method. Inoculated plates were incubated at 42°C, for 48 h in candle extinction jar (Kuria et al., 2018). Suspect *Campylobacter* colonies were then selected for further analysis by Gram stain, catalase and oxidase biochemical tests. The Gram stain was performed using reagents prepared according to WHO (2009) method. Suspect isolates were confirmed by DNA analysis.

Campylobacter DNA analysis

DNA extraction

A loopful of suspect *Campylobacter* colonies was harvested and suspended in 200 µl of sterile distilled water in labeled 0.5 ml Eppendorf tubes. The tubes were then heated in boiling a water bath at 100°C for 10 min, cooled immediately on ice for 5 to 10 min and then centrifuged (Eppendorf, Gerãtebau, West Germany) at 11,000×g for 5 min. The supernatant was stored at -20°C and used as DNA templates. Confirmation of *Campylobacter* genus was done using PCR analysis for 16S rRNA gene (Linton et al., 1997) and identification of *Campylobacter jejuni* and *Campylobacter coli* by multiplex PCR using species specific primers. The primers, C412F and C1228R; C412F and C1228R; ENg03F and ENg04R for *Campylobacter* genus, C*. jejuni* and *C. coli*, respectively were based on nucleotides sequences of monospecific probes from DNA fragments library (WHO, 2009).

DNA amplification

Amplification of *Campylobacter* genus DNA was performed in a 25 µl reaction volume per sample. Briefly, aliquots of 12.5 µl of Taq Master Mix (Qiagen GmbH, Limburg, Netherlands), 10 pmol of each primer (Bioneer, Inc. USA), 5 µl of DNA template and 7.3 µl of molecular grade water (Qiagen GmbH, Limburg, Netherlands) were put in labeled sterile PCR tubes, and placed in a thermocycler (MJ Research, Watertown, MA, USA). The samples were subjected to initial denaturation temperature of 95°C for 10 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 59°C for 90 s, extension at 72°C for 60 s and a final extension of 72°C for 10 min. Amplification of *C. jejuni* and *C. coli* species DNA was performed in a 50 µl multiplex reaction volume per sample as follows: briefly, 25 µl Taq PCR Master Mix (Qiagen GmbH, Limburg, Netherlands), 5 µl of DNA template, 60 pmol of *C. coli* primers (Bioneer, Inc. USA), 25 pmol of *C. jejuni* primers (Bioneer, Inc. USA) and 18.3 µl of molecular grade water (Qiagen GmbH, Limburg, Netherlands) were put into labelled PCR tubes. The PCR protocol included initial denaturation temperature of 94°C for 5 min; 2 cycles of 1 min at 94°C, 1 min at 64°C, and 1 min at 72°C; 2 cycles of 1 min at 94°C, 1 min at 62°C, and 1 min at 72°C; 2 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C; 2 cycles of 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C; 2 cycles of 1 min at 94°C, 1 min at 56°C, and 1 min at 72°C; 30 cycles of 1 min at 94°C, 1 min at 54°C, and 1 min at 72°C; and a final extension step of 10 min at 72°C.

Agar gel electrophoresis

Amplicons were analysed by gel electrophoresis in agarose (Ultra PURE™, BRL, and Gaithersburg, MD) containing ethidium bromide (77 µl/100 ml) and submerged in 1x Tris-acetate buffer solution. Electrophoresis of *Campylobacter* genus and species amplicons was performed in 1.3 and 1% agarose gel, respectively. The PCR products were mixed with the loading dye (4:1) and loaded into the gel wells. A 100 bp DNA molecular ladder was used as size reference. Genomic DNA from *C. jejuni* (Kenya Medical Research Institute (KEMRI) 4529 and 4478 and *C. coli* (KEMRI 4443 and 4543) were used as positive control in all the PCR assays. Electrophoresis was conducted at 100 V for 1.5 h after which the amplicons were viewed and photographed under UV-transilluminator (VilberLourmat, Germany).

Isolation of *Salmonella* species

Salmonella was isolated by incubating 200 ml of the rinse wash fluid for 18 to 20 h at 37°C for pre-enrichment followed by inoculation of 1 ml of the broth into 10 ml of tetrathionate broth (HIMEDIA M032, INDIA) for selective enrichment (ISO 6579: 2002). The broth was incubated at 37°C for 18 to 24 h. A loopful of the broth was streaked onto XLD agar (OXOID CM 0469, UK) and incubated at 37°C for 24 h. *Salmonella* suspect isolates were subjected to Gram stain, Urea and Triple Sugar Iron biochemical tests. Positive suspects were stored in 10% skimmed milk at 4°C before serotyping. Slide agglutination tests were then carried out using Mast Assure™ Salmonella Antisera (Mast Group, Merseyside, UK) for the determination of type O, H and Vi antigens. A sample was considered positive if at least one morphologically characteristic colony was confirmed positive through the biochemical tests and serology.

Data analysis

The prevalence at 95% confidence interval of the contaminant microorganisms was computed using open epidemiologic software (www.openepi.com-free Microsoft software).

RESULTS

The prevalence and concentration of the contaminant bacteria, with the concentration compared to CAC-recommended concentration limits, are summarized in Table 1. Total viable counts and coliforms were recorded from all 40 carcasses. Coagulase positive *Staphylococcus* was isolated from 12 (30%, 95% CI: 17.38-45.42) carcasses and *Streptococcus* colonies from 35 (87.5%, 95% CI: 74.45-95.27%). *Streptococcus* Group D (*Enterococcus*) was isolated from 17 of the 35 samples (48.57%). Two (2) samples had mixed contamination with Groups D and F and Groups D and G, respectively. Group B was isolated from 1 sample (2.85%), Group F from 5 (14.28%), Group G from 6 (17.14), while 4 samples (11.42%) could not be classified. The concentration of all the contaminants was higher than the CAC recommended limits. Suspect *Campylobacter* colonies were detected in 20 (50%) of the 40 samples. DNA analyses confirmed *Campylobacter* genus in 11 samples (27.5%, 95% CI: 15.39-42.7); C*. jejuni* in three (7.5%) and *C. coli* in none. Primers for *Campylobacter* genus (C412F and C1228R) and primers

Table 1. The prevalence and concentration of some contaminant microorganisms in free-range chicken meat carcasses from an informal slaughterhouse in Kenya.

Contaminant	Prevalence at 95% CI	Mean CFU/ml	Mean log CFU/ml	Mean CFU/cm^2	Mean log CFU/cm^2	CAC limits[a] (CFU/g or ml)
Total viable count	40/40 (100%)	1.59 x 10^7	6.66	3.62x10^6	6.04	5x 10^6
Coliforms	40/40 (100%)	1.44 x10^5	3.32	3.22 x10^4	3.85	2 x10^3
S. aureus	12/40 (30%)	3.2x10^4	1.38	7.78x 10^3	1.21	10^3
Streptococci spp.	35/40 (87.5%)	1.06x10^4	2.37	2.41x10^3	1.99	10^2 /100 g

[a]Codex Alimetarius commission (CAC) recommended limits.

Table 2. Prevalence of *Campylobacter spp, Staphylococcus aureus, Streptococcus spp* and *Salmonella spp* in the indigenous chicken carcasses from a non-regulated slaughter facility in Kenya.

Sample size	Parameter analyzed	Presumptive	Confirmed	Prevalence	95% CI (Mid-P Exact)
40	*Campylobacter* genus	20	11	27.5%	15.39 - 42.7%
40	*C. jejuni*		3	7.5%	1.94 – 19.07%
40	*C. coli*		0	0%	0.0 -7.215%
40	Other *Campylobacters*		8	20%	9.75 – 34.45%
40	*Staphylococcus aureus*		12	30%	17.38- 45.42
35	*Streptococcus spp*		35	87.5%	74.45 – 95.27%
35	*Salmonella spp*	0	0	0%	0.0 - 8.20%

for *C. jejuni* (ENg03F and ENg04R) generated amplicons of 812 and 773 bp, respectively. *Salmonella* genus was not isolated from any of the carcasses (Table 2).

DISCUSSION

CAC has set acceptable concentration limits for microorganisms in foods in order to ensure quality and safeguard human health (CAC, 1997). We observed that chicken carcasses from unregulated slaughter facilities in Nairobi have high microbial load, which include potential pathogens. The mean concentration of TVC, coliforms, coagulase positive *S. aureus* and *Streptococcus s*pp. were higher than the acceptable limits. This was an indication of low hygiene standards of the slaughter facility and/or the slaughter process.

Total Viable Counts (TVC), also referred to as Aerobic Plate Count (APC) and total coliforms in a food sample informs on microbiological quality of the production process. Presence of these organisms in large numbers in raw poultry indicates unsanitary slaughter and processing practices (Svobodová et al*.,* 2012; Vaidya et al*.,* 2005). The presence of coliforms in the poultry meat is an indication of fecal contamination, exogenous or endogenous, and presents a public health risk of food borne diseases (Ruban and Fairoze, 2011; Temelli et al., 2011). Among the coliforms, *E. coli* is one of the most important cause of foodborne diarrhorea disease (WHO 2015). Specific pathogens *S. aureas*, *Streptococcus* spp. and *Campylobacte*r spp. were identified in the meat carcass. Apart from directly causing foodborne disease, the meat may be a source of cross contamination to other carcasses and foodstuffs during processing, at retail or household level (Sirsat et al., 2014; Kennedy et al., 2011; Rasschaert et al., 2020).

S. aureus related food poisoning as the third largest cause of food related illnesses worldwide resulting from the contamination of food by preformed *S. aureus* enterotoxins (Kornacki, 2010; Thaker et al., 2013; Akbar and Anal, 2013). The organism is a natural flora in the skins of animals and humans and frequently contaminates raw foods of animal origin (FSANZ, 2005). However, humans are the main reservoir for *S. aureus* involved in human foodborne disease despite the wide-spread association of *S. aureus* with animals (Loir et al., 2003). The organism is also a significant cause of poultry disease and may thus contaminate carcasses and consequently other foods (Pepe et al., 2006). In this study, 30% of the chicken carcasses were contaminated with coagulase positive *S. aureus* and all contaminated carcasses had more than the maximum permitted limit. The high contamination rates raise public health concern since poultry meat has been linked to staphylococcal food poisoning (Loir et al., 2003; Armstong et al., 2002).

The standard provided for *Streptococci* in foods and packed water is 10^2 CFU/100 ml. In this investigation, *Streptococcus* spp. were isolated from 35 out of 40

carcasses (87.5%) with the mean CFU/cm^2 and CFU/ml much higher than the maximum permitted limit. In the study, the *Streptococci* isolates were Lancefield Groups D, B, D, F, and G. Presence of Group D *(Enterococcus)* in carcasses was indication of feacal contamination, but may also originate from the urinary tract of humans (Poulsen et al., 2012). Molecular evidence of enterococci spread from animals to humans has been documented (Donabedian et al., 2003). Group G are part of the normal microbiota of the gastrointestinal tract and skin in animals especially cattle and potentially zoonotic. They may cause a variety of infections in humans including bacteraemia (Liao et al., 2008). Groups F and B are inhabitants of the upper respiratory mucosa and genital and gastrointestinal tracts, respectively in humans (Al-Charrakh et al., 2011; Hanna and Noor, 2020) and their presence in the carcasses may be an indication of contamination from slaughter personnel.

Prevalence of *Campylobacter* genus in the carcasses was 27.5% with *C. jejuni* contributing 7.5%, the balance being contributed by non-identified species. The public health risk is significant considering that *Campylobacter* spp. infections cause human gastro-enteritis more frequently than other enteric pathogens (WHO, 2015). Poultry meat accounts for approximately half of all food borne campylobacteriosis in humans (Hoffmann et al., 2017).

Negative results of *Salmonella* genus isolation from the meat carcasses were obtained in this study. In spite of contradictory results elsewhere (Zhao et al., 2016; Bailey and Cosby, 2005), this study alleviates fears of the chicken produced under free range production systems being common sources of human non-typhoidal infections. Indigenous free-range chicken have resistance potential to *Salmonella* (Msoffe et al., 2006). Further, *Salmonella* prevalence in poultry is age related (Beal et al., 2004) and the long duration to maturity may provide an opportunity for infection clearance ahead of slaughter.

CONCLUSION AND RECOMMENDATION

Chicken meat carcasses processed in an informal slaughterhouse were found contaminated by spoilage and pathogenic microorganism beyond the Codex Alimentarius limits. The high microbial contaminant load, which included potential human pathogens, may have been a consequence of contamination from the birds, slaughter facility or the slaughter personnel. The study recommends a need to sensitize consumers on proper handling of meat carcasses to avoid cross-contamination of other foodstuffs as well as adequate cooking. Currently, there are no regulated slaughterhouses for free-range chicken in the country. Establishment of regulated facilities accompanied by capacity building of slaughter personnel is also critically important in order to safeguard consumer health and to enable farmer's access to high value markets.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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