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#### Article history

<u>Abstract</u>

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### **Keywords**

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Bacteria and other microbial contamination of poultry meat remains a serious public health concern throughout the world, due to the nutritive value of the meat. A total of 200 raw broiler meat samples with skin on comprising 100 fresh and 100 frozen were purchased randomly from Korle-Gonno, Accra, Ghana. Aerobic plate and enterobacteria counts were determined by inoculating aliquots of homogenized samples after serial dilutions into plate count, MacConkey, violet red bile glucose agars and all incubated at 37°C for 24-48 h. Mean bacteria counts were expressed as colony forming units per gram (CFU/g) of the broiler meat and then converted to log<sub>10</sub> CFU/g. Salmonella sp. was isolated by enrichment in buffered peptone water, incubated at 37°C for 24 h. Aliquots were transferred into selenite cystine and Rappaport-Vassiliadis broths, incubated at 37°C and 42°C respectively for 24 h before a loopful of each broth was streaked on xylose-lysine deoxycholate agar plates and incubated at 37°C for 48 h. Enterobacteria and Staphylococcus aureus were also isolated on violet red bile glucose and Baird-Parker agars both incubated at 37°C for 24-48 h. Mean aerobic plate and enterobacteria counts ranging from 2.6-14.1  $\log_{10}$  CFU/g and 2.2-9.8  $\log_{10}$  CFU/g were found for the raw broiler meat samples from the cold stores and the local retail shops. Bacteria isolated were Proteus mirabilis (26.7%), Proteus vulgaris (25.1%), Klebsiella sp. (23.4%), Salmonella sp. (10.8%), coagulase negative Staph aureus (9.2%), and Escherichia coli (4.8%). From the study it is recommended that raw broiler meat and other meat products should be boiled thoroughly with safe water, kept, and served under good hygienic conditions to prevent microbial contamination because consumption of contaminated meat can lead to illness.

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# Introduction

Broilers are one of the domesticated animals or birds reared in most parts of the world for their meat consumption. Broiler meat is either whole carcasses or parts of the carcass or boned out meat of the species Gallus domesticus (Bhaisare et al., 2014). Broiler meat is an excellent source of good quality protein, but it is highly susceptible to microbial contamination. When it becomes contaminated it can lead to food borne infections in humans after consumption (Adu-Gyamfi et al., 2012; Bhandari et al., 2013). Poultry constitute about 30% of the world's total meat consumption and currently due to the rapid growth in consumer demand for poultry and poultry products over the past years have increased international trade in these foods (Thornton, 2010). Therefore standard methods of food safety and good quality should be maintained at all stages in the processing and storage. Broiler meat and other types of poultry products, have higher pathogenic and spoilage microbial counts than most other foods because contamination can occur at

several points during the processing until it gets to the final consumer (Temelli *et al.*, 2011).

Microbiological quality of broiler meat should be focused on keeping the quality or shelf life of the carcasses after slaughter of the birds. Keeping the quality is primarily concerned with the total number of microorganisms present which includes the aerobic plate counts, presence of bacteria and other microorganisms that can play key roles in the deterioration of the carcasses. Safety is also concerned with microorganisms that can cause food poisoning and other diseases in humans and animals after consumption of the carcasses. Over the past years many methods like the use of safe or quality water for washing during the processing have been used to control bacterial contamination, extend the shelf life of the raw broiler meat and other poultry products from factory level to the final consumer (Kaudia, 2001). Treatment at various stages such as refrigeration, heating, irradiation, application of chemical preservatives and management techniques like withdrawal of feed before catching, slaughtering and processing have been used to reduce bacterial contamination of raw broiler meat. This study investigated the aerobic plate and enterobacteria counts of raw broiler meat sold at cold stores and local retail shops at Korle-Gonno, Accra, Ghana. Isolation and identification of *Staphylococcus aureus, Salmonella* sp. and other enterobacteria were also performed from the samples.

### **Materials and Methods**

Before the start of the project, a preliminary survey of the various cold stores and local retail shops that sells raw broiler meat was conducted. This was carried out to gather information on the location of the cold stores, local retail shops, type of meat sold, sources of the meat sold, and also to get information on the hygiene and sanitary conditions of the cold stores and local retail shops environments (Pesewu et al., 2014a). Samples were collected between June-August, 2014. A total of 200 raw broiler meat samples (wings, breast, thighs and legs) with skin on comprising 100 fresh and 100 frozen (18-20°C) were purchased randomly spread across the entire Korle-Gonno, Accra, Ghana into sterile containers. All the selected cold store operators and the local retail shops interviewed confirmed that most of the raw broiler meats were imported from different countries outside Ghana. During the sampling, each sample purchased was given a specific number or code. At the laboratory, frozen samples were allowed to defrost at room temperature whilst the fresh samples were investigated on immediately. Sterile (90 ml) buffered peptone water were transferred into a sterile stomacher plastic bag containing 10 g of the raw broiler meat samples and homogenized for 2 min at 230 rpm in a stomacher (Seward, Norfolk, UK) at room temperature. Raw broiler meat samples (10 g) were also cut into small pieces with the aid of a sanitized kitchen knife and blended with sterile buffered peptone water (90 ml) for 5 min using a sanitized Waring Blender. The two different homogenized samples prepared by the two methods were subsequently serially diluted from 10-1 through to 10-5 according to the method of Pesewu et al. (2014b). Aerobic plate and enterobacteria counts were determined by the pour plate method. Aliquots (0.1 ml) of homogenized samples after serial dilutions were pipette into separate sterile Petri dishes and 15 ml of molten plate count agar (PCA: Oxoid Limited, Basingstoke, UK), MacConkey agar (MA: Becton, Dickinson and Company, Sparks, USA) and violet red bile glucose agar (VRBGA: Oxoid Ltd) each cooled to 45°C were added separately into the Petri dishes,

swirl to mix well ensuring that the sample and media are thoroughly mixed and also the media covers the Petri dishes evenly. All the plates were allowed to cool, solidify, inverted and incubated at 37°C for 24-48 h. Bacteria colonies growing on the agar plates were counted after incubation and the mean number of colonies in a particular dilution in a particular agar was multiplied by the dilution to obtain the aerobic plate count (Bhandari *et al.*, 2013). The results of the aerobic plate counts were expressed as the number of colony forming units per gram (CFU/g) of the raw broiler meat samples and then converted to  $\log_{10}$ CFU/g.

Salmonella sp. was isolated by enrichment in buffered peptone water aliquots of the homogenized samples after serial dilutions and incubated at 37°C for 24 h. Then 2 ml and 0.1 ml of the enrichment were respectively transferred into 20 ml of selenite cystine broth (SCB: Oxoid Ltd) and 10 ml of Rappaport-Vassiliadis broth (R-VB: Oxoid Ltd), incubated at 37°C (SCB) and at 42°C (R-VB) for 24 h (Cardoso et al., 2006; Abdellah et al., 2009). A loopful of each broth was streaked on the surface of xyloselysine deoxycholate agar (XLD: Oxoid Ltd) plates and incubated at 37°C for 48 h. Other enterobacteria were also isolated by inoculating aliquots of the homogenized serial diluted raw broiler meat samples onto the surfaces of VRBGA (Oxoid Ltd) plates and incubated at 37°C for 24h. Biochemical tests including catalase, oxidase, triple sugar iron (TSI), methyl red (MR), Voges-Proskauer (V-P), motility indole urease (MIU) and hydrogen sulphide (H<sub>2</sub>S) production were used for the identification of Salmonella sp. and other enterobacteria. Staph aureus was isolated by inoculating serial diluted homogenized raw broiler meat samples onto the surfaces of Baird-Parker agar supplemented with egg yolk tellurite emulsion (B-PA: Oxoid Ltd) plates and incubated at 37°C for 24-48 h. Colonies were identified using Gram staining, catalase and coagulase tests.

### **Results and Discussion**

Contamination of poultry products including raw broiler meat by pathogenic microorganisms more especially bacteria are greatest problems regarding world food industry. Mean aerobic plate and enterobacteria counts ranging from 2.6-14.1  $\log_{10}$ CFU/g and 2.2-9.8  $\log_{10}$  CFU/g were found for the raw broiler meat samples from the cold stores and the local retail shops (Table 1 and Figure 1 respectively). Mean bacteria colony counts of 19.94  $\log_{10}$  CFU/g have been found for chicken meat samples from selected supermarkets, local markets, and farms

Table 1. Mean aerobic plate and enterobacteria counts  $(\log_{10} \text{ CFU/g})$  of the raw broiler meat samples investigated

Sampling site	Sample size	Aerobic plate counts	Enterobacteria counts
Cold stores	100	14.1 ± 0.06	9.8 ± 0.48
Retail shops	100	2.6 ± 0.10	2.2 ± 0.00

in Accra, Ghana (Adu-Gyamfi *et al.*, 2012). Also, mean bacteria colony counts of 9.7  $\log_{10}$  CFU/g from raw retail chicken meat have been observed in Nigeria (Adesiji *et al.*, 2011). In Chitwan, Nepal mean bacteria colony counts of 27.9  $\log_{10}$  CFU/g from raw broiler meat samples from retail shops have been found (Bhandari *et al.*, 2013). The differences in mean bacteria colony counts may be due to the number of sample sizes in each study.

Bacteria isolated from the raw broiler meat samples investigated were Proteus mirabilis (26.9%), Proteus vulgaris (25.1%), Klebsiella sp. (23.4%), Salmonella sp. (10.8%), coagulase negative Staph aureus (9.2%), and Escherichia coli (4.8%) as presented in Figure 2. Various enteric bacteria including species of Proteus (86.7%) and Klebsiella (6.7%) have been found in chicken faeces (De Oliveira et al., 2004). Some of these bacteria can contaminate the raw broiler meat before and during the processing. However, the role of Proteus sp. in foodborne infections has been a debate over the past years, despite its potential as a pathogen. Proteus sp. is associated with food deterioration and hence its presence cannot be directly related to infections, but as a contaminating agent (Biranjia-Hurdoyal and Latouche, 2016). Proteus sp. is involved in the decomposition of carcasses and can be found in faeces, putrefied meat, sewer water, suppurating wounds, and others (Cardoso et al., 2006).

Most of the Klebsiella sp. and Salmonella sp. were isolated from the raw broiler meat samples from the cold stores. Klebsiella sp. is one of the bacteria that can be found in a lot of environments including the intestinal tract of humans and animals, and also in plants, soil, and water. Contamination of the raw broiler meat samples could be from any of the environments during the processing or even from the cold store keepers and their attendants. Salmonella sp. has been isolated from the gut contents (7.2%) of live birds from farms and carcasses/chicken parts (6.8%) from supermarkets, open markets, and cold stores in Ghana (Sackey et al., 2001; Adu-Gyamfi et al., 2012). Salmonella sp. is one of the most frequently isolated bacterial pathogens often associated with outbreaks of food-borne illness in most parts of the world especially in developing countries. Meat products



Figure 1. Aerobic plate counts (APC) of bacteria isolated from the raw broiler meat samples.



Figure 2. Percentage distribution of bacteria isolated from the raw broiler meat samples investigated.

in particular poultry are the most common sources of food poisoning by *Salmonella* sp. (Antunes *et al.*, 2003). But infection of food with *Salmonella* is not solely sufficient to cause food poisoning. Because the infected food should be moist and must be stored long enough under conditions such as storing for 24 h in a warm area that will allows heavy growth of the bacteria (Al-Mutairi, 2011).

The presence of coagulase negative *Staph aureus* on the raw broiler meat samples investigated could be due to handling from the processing site till it gets to the final consumer by the butchers, packers, transportation processes, cold store operators, and attendants. It could also be due to handling by the operators of the local retail shops and their selling attendants during the course of display and selling processes because *Staph aureus* is an opportunistic pathogen living in the nasopharynx and skin of up to 50% of normal people (Enright, 2003; Pesewu *et al.*, 2014c). *E. coli* was the least bacteria isolated from the raw broiler meat samples investigated but the presence of *E. coli* suggests or gives an indication of possible feacal contamination.

# Conclusion

Six different bacteria species were isolated and identified from the raw broiler meat samples investigated. It is recommended that raw broiler meat and other raw meat products should be boiled thoroughly with safe water, kept, and served under good hygienic conditions to prevent bacteria and other microbial contamination because consumption of contaminated meat and other food products can lead to illness including diarrhoea and typhoid fever.

### **Declaration of conflicting interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/ or publication of this article.

## **Ethical clearance**

Ethical Approval Number: (SAHS-ET./.10337093/AA/25A/2013-2014) was obtained from the Ethics Review and Protocol Committee, School of Biomedical and Allied Health Sciences (SBAHS), College of Health Sciences, University of Ghana before the start of the study.

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