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Contamination and Critical Control Points (CCPs) along the processing line of sale of frozen poultry foods in retail outlets of a typical market in Ibadan, Nigeria

Victoria Olusola Adetunji* and Ismail Ayoade Odetokun

Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Nigeria.
Email: vadetunji@gmail.com; vo.adetunji@mail.ui.edu.ng

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ABSTRACT

Aim: Over the years, there have been considerable increases in the consumption of frozen poultry foods across Nigeria. Little attention has been paid to the microbial quality of these foods and hence constitutes a threat to public health. The contamination levels (*Enterobacteriaceae* and *Listeria* counts) and the presence of pathogenic *E. coli*, *Salmonella* and *Listeria* along the processing line of sale of frozen poultry foods were assayed in retail outlets.
Methodology and results: Bacteriological counts and bacterial isolation were carried out using standard plate methods, while the direct slide agglutination technique was utilized for serology. Bacteriological assay revealed extremely high counts (*Listeria* count (LC): 7.784±1.109 - 9.586±0.016 log cfu/cm^2; *Enterobacteriaceae* count (EC): 7.151±0.213 - 9.318±0.161 log cfu/cm^2), higher than stipulated by International Food Standard Agencies. The highest count for EC (9.318±0.161 log cfu/cm^2) and LC 9.586±0.016 log cfu/cm^2) was from the weighing scale and processing table. Averagely, LC (8.598±0.733 log cfu/cm^2) was higher than EC (8.145±0.936 log cfu/cm^2). Weighing scale had counts significantly different ($p < 0.05$) from all others for EC. But there were no significant differences in LC. Weighing scale and meat tables were critical control points (CCPs) in the processing line for sale of frozen poultry meats in the retail outlets. *E. coli* spp., *E. coli* O157:H7, *Salmonella* spp., *Salmonella* Enteritidis, *Listeria* spp. and *Listeria monocytogenes* were isolated along the processing line.
Conclusion, significance and impact of the study: Results of this study indicated that poultry meat are easily contaminated along the processing line of sale and may act as a potential risk to public health if counteractive measures are not applied to reduce microbial contamination during storage, sale and distribution to consumers.

Keywords: Frozen poultry foods, *Enterobacteriaceae* count, *Listeria* count, processing line of sale

INTRODUCTION

Various groups of foodborne pathogens exist, which if present in contaminated food products, can lead to significant impacts on human health and industrial economies (Sanders, 1999; Helms *et al*., 2003). The symptoms of foodborne illnesses, resulting from the consumption of pathogen contaminated foods, can range from mild to more severe indications such as diarrhea, fever, nausea, vomiting, abdominal cramps, dehydration, meningitis, endocarditis, kidney failure, and septicaemia (Darwin and Miller, 1999). *E. coli* O157:H7 infection has considerable economic impacts. Poultry producers, meat processors, meat distributors and wholesale and retail food outlets all incur direct and indirect costs. The cost of premature deaths, medical care and loss of productivity as a result of *E. coli* O157:H7 illnesses are usually very high. It was estimated that 3.2 million children die annually as a result of diarrheal diseases. Diarrhea and impaired nutritional status have also been reported in millions of children (CDC, 2005). Data on the incidence and prevalence of foodborne illness in Nigeria do not exist. This translates to the fact that more terrible and alarming cases of foodborne illnesses would be obtained in the countries as compared to the situations in first-world countries. Food handlers play a major role in ensuring food safety through the chain of production, storage and preparation (Hassanain, 2008). Mishandling and disregard for hygiene measures by handlers may result in food contamination which of course will have no desirable attendant consequences (Okojie *et al*., 2005).

The *Enterobacteriaceae* group of bacteria poses a great challenge to the production of raw and processed meat across the globe (Adetunji and Odetokun, 2011). Over the last two decades, bacterial infections caused by enterohemorrhagic *E. coli* have been on the increase (Schlundt, 2001). A multi-state outbreak of *E. coli* O157:H7 infections have been recorded (CDC, 2007). The occurrence of *Salmonella* can contaminate food

***Corresponding author**

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anywhere along the farm to fork continuum (Small *et al.*, 2006). Ready-to-eat products are typically contaminated during post-processing steps (Mbandi and Shelef, 2002). Post-processing contamination is largely contributed to poor handling practices (De-Cesare *et al*., 2003). All foodborne salmonellosis infections are non-typhoidal (Bailey and Maurer, 2005) and are major causes of foodborne illness (Hassanain, 2008). During 2003, *Salmonella* infections were responsible for 30% of 23,250 notifications of food borne diseases in Australia (OZFOODNET Working Group, 2003). *Listeria monocytogenes* has become one of the main pathogens transmitted by food (Porta *et al*., 2010). This organism causes severe non-enteric disease (meningitis, septicemia) in individuals that are immunocompromised and abortion in pregnant women. The economic implications of foodborne illnesses result in financial losses through litigations, medical expenses, expenses on product recall and disposal of contaminated products, and decreases in productivity (Whyte *et al*., 2004; Normanno *et al*., 2005).

The Hazard Analysis Critical Control Points (HACCP) concept is a systematic and scientific approach to process control (USDA, 1997). HACCP is used to describe an internationally recognized way of managing food safety and protecting consumers being a requirement of European Union (EU) food hygiene legislation that applies to all food business operators except farmers and growers (FDA, 1997). It is viewed as a means of preventing the occurrence of health and safety hazards in plants producing meat and poultry products. It does this by ensuring that controls are applied at any point (CCPs) in a food production system where hazardous situations could occur (USDA, 1997). The potential hazards that are reasonably likely to cause illness or injury in the absence of their control must be addressed in determining CCPs (Adetunji and Odetokun, 2011). Few studies exist on the isolation of pathogens of public health importance from retail poultry foods outlets in Nigeria. In addition reports on the critical control points along the processing line of sale of these frozen products are scarce. The aim of this study is therefore to assess microbial levels and culture, isolate and characterize some poultry-borne pathogenic organisms from samples obtained from retail frozen poultry outlets along the processing line of sales in a typical market while specifying their critical control points.

MATERIALS AND METHODS

This study was conducted between April and July, 2011. Sampling was carried out at the various poultry frozen foods retail shops located at Bodija market, Bodija, Ibadan. Thirty (30) swab samples (from a 2 cm^2 area) were collected randomly from poultry meat and retail facilities and equipments (Tables, weighing Scales, Knives, Covering Nylon and Carton) and stored on ice within the sterile swab casing in a cooler. Samples were then taken to the laboratory for microbial analysis.

All glass-wares used for this test were thoroughly washed and sterilized in Hot Air Oven (Elektro, HELIOS, Sweden) at 100 °C for 15-20 min. All media used were prepared according to manufacturers' specifications. The Barrow and Feltham, 1993 and FDA, 1995 protocols with a little modification were used for *E. coli* and *Listeria* isolation and identification while *Salmonella* spp. isolation was done according to Hendriksen, 2003. The direct slide agglutination technique using specific antisera was utilized for serotyping of the pathogenic strains. SPSS for Windows, version 17.0 (SPSS Inc. Chicago, IL) was used for statistical analysis. Repeated readings of replicate plates (n=4) were used to determine the microbial counts along processing line of sales and were expressed in mean±S.D. Microbial levels were analyzed by One-way analysis of variance (ANOVA). Tukey's multiple comparisons tests were applied as post-hoc when significant differences were determined. To compare significant differences between EC and LC at each sampled point on the processing line of sale, T-test was used. All statistical analysis was carried out at 0.05 level of error. Figure 1 was schemed using Microsoft excel (2007) functions.

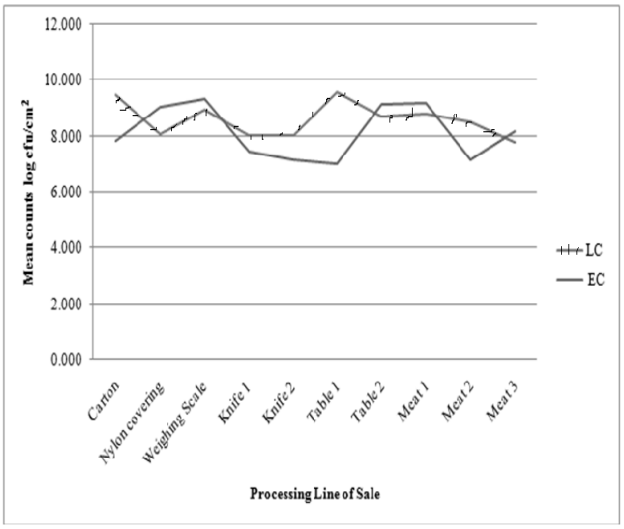

Figure 1: Comparison of the average counts (log cfu/cm^2) along the processing line of sales between *Enterobacteriacea* (EC) and *Listeria spp.* (LC).

RESULTS

Bacteriological analysis of the swabs samples obtained from poultry frozen food retail outlets, Bodija market, Ibadan had high bacteria counts for both *Listeria* (LC) and *Enterobacteriaceae* (EC). Mean log cfu/cm^2 of the counts obtained from the meat and facilities sampled were determined as given in Table 1. The mean counts obtained for LC ranged from 7.784±1.109 to 9.586±0.016 log cfu/cm^2 with lowest and highest from meat and table respectively. EC also ranged from 7.151±0.213 to 9.318±0.161 log cfu/cm^2. The

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highest count for EC (9.318±0.161 log cfu/cm^2) was from the weighing scale sampled. Averagely, LC (8.598±0.733 log cfu/cm^2) was higher than EC (8.145±0.936 log cfu/cm^2) (Figure 1). Critical Control Points (CCPs) noted along the processing line of sale were the weighing scale and meat tables (Figure 2).

Table 1: Mean $\log_{10}$ cfu/cm^2 *Enterobacteriaceae* and *Listeria* counts.

SAMPLES	*Listeria* Count mean ± SD (log cfu/cm^2) n=4	*Enterobacteriaceae* Count mean ± SD (log cfu/ cm^2) n=4
Carton	9.491±0.084$^{a\alpha}$	7.827±0.180$^{b\alpha}$
Nylon covering	8.079±1.100$^{a\beta}$	9.043±0.334$^{a\alpha}$
Weighing Scale	8.953±0.809$^{a\alpha}$	9.318±0.161$^{a\alpha}$
Knife1	8.017±0.088$^{a\beta}$	7.452±0.213$^{a\beta}$
Knife 2	8.039±0.056$^{a\beta}$	7.151±0.213$^{b\beta}$
Meat 1	8.808±0.119$^{a\beta}$	9.184±0.153$^{a\beta}$
Meat 2	8.519±0.000$^{a\beta}$	7.151±0.213$^{b\beta}$
Meat 3	7.784±1.109$^{a\alpha}$	8.182±0.199$^{a\alpha}$
Table 1	9.586±0.016$^{a\alpha}$	7.000±0.000$^{b\beta}$
Table 2	8.701±0.109$^{a\alpha}$	9.146±0.022$^{b\beta}$
Total	8.598±0.733	8.145±0.936

SD = Standard Deviation, n = numbers.
Values with the same superscripts (alphabets i.e ^{aa} or ^{bb}) between sample counts on the rows are not statistically significant at $p < 0.05$. Values with the same superscripts (symbols i.e $^{\alpha\alpha}$ or $^{\beta\beta}$) along sample counts on the columns are not statistically significant at $p<0.05$

Statistically at $p<0.05$, significant differences were noticed in the LC between the sample from Table 1 and samples from the following: Knife 1, Knife 2, Meat 1, Meat 2 and Nylon covering. Taking the sample collected from the weighing scale as a reference point, statistical differences at $p<0.05$ were noticed in a multiple comparison with all other samples except the carton and Meat 3 samples for EC. Using Tukeys HSD post hoc test, weighing scale had counts significantly different from all others for EC and no statistical differences were recorded for LC (Figure 1).

Isolated *E. coli* strains were subjected to various tests, the results of which are presented below (Table 2). Both strains isolated had same biochemical characteristics and were gram positive rods, pinkish in colour. For *Salmonella* spp., all ten strains were gram negative rods, catalase positive, coagulase negative and indole negative (Table 2) while 7 of the 10 samples subjected to *Listeria* count that produced esculin were used for microscopic examination with biochemical test carried out on them. All seven strains were Gram and catalase positive but negative with coagulase reactions. With TSI (Lab M, UK), the suspected *E. coli* spp. were glucose positive (yellow/acidic butt), lactose/sucrose positive (yellow slant), produced Hydrogen sulphide slightly and gave off gas bubbles from glucose. The suspected *Salmonella* strains were glucose positive (yellow/acidic butt), lactose/sucrose negative (red slant), formed hydrogen sulphide and also yielded gas from glucose.

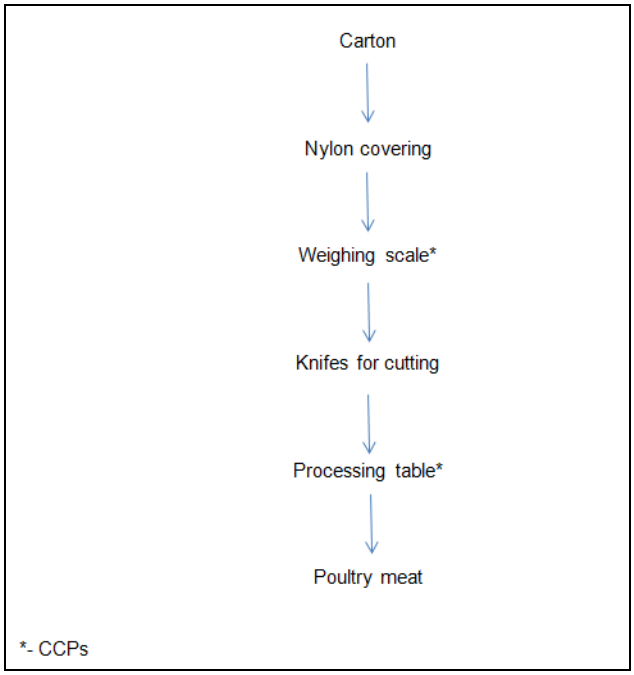

Figure 2: CCPs along the processing line of sale.

One of the two *E. coli* strains (Table 2) on which serology was carried out showed a clumping reaction indicating that it was an *E. coli* O157H7 strain (positive for antiserum). Using the O-antiserum, the following samples/strains were confirmed to belong to the genus *Salmonella*: SSM 1, SSM 2, SST 1 and SSW (Table 2). After the four positive *Salmonella* spp. were subjected to Factor-9 antiserum, only SSW isolated from the weighing scale produced a clumping reaction i.e. *Salmonella* Enteritidis. Of the seven presumptive *Listeria monocytogenes* strains, the strain isolated from the carton sample was confirmed to be *Listeria monocytogenes* (Table 2).

DISCUSSION

We found that both the EC and LC obtained from swab samples of chicken meat and facilities along the processing line of sale in retail outlets at the Bodija market, Ibadan were high. The average counts for

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Table 2: Results of microscopic examination, biochemical characterization and serology of the bacterial species.

Strains	Gram stain	Catalase	Coagulase	Indole	*E. coli* antiserum	Salmonella Poly-O/Factor-9	Listeria antiserum
ECN	-ve	+ve	-ve	+ve	-ve	NA	NA
ECS	-ve	+ve	-ve	+ve	+ve	NA	NA
SSC	-ve	+ve	-ve	-ve	NA	-ve/-ve	NA
SSK 1	-ve	+ve	-ve	-ve	NA	-ve/+ve	NA
SSK 2	-ve	+ve	-ve	-ve	NA	-ve/-ve	NA
SSM 1	-ve	+ve	-ve	-ve	NA	+ve/-ve	NA
SSM 2	-ve	+ve	-ve	-ve	NA	+ve/-ve	NA
SSM 3	-ve	+ve	-ve	-ve	NA	-ve/-ve	NA
SSN	-ve	+ve	-ve	-ve	NA	-ve/+ve	NA
SST 1	-ve	+ve	-ve	-ve	NA	+ve/-ve	NA
SST 2	-ve	+ve	-ve	-ve	NA	-ve/-ve	NA
SSW	-ve	+ve	-ve	-ve	NA	+ve/+ve	NA
LSC	+ve	+ve	-ve	ND	NA	NA	+ve
LSK 2	+ve	+ve	-ve	ND	NA	NA	-ve
LSM 1	+ve	+ve	-ve	ND	NA	NA	-ve
LSM 2	+ve	+ve	-ve	ND	NA	NA	-ve
LST 1	+ve	+ve	-ve	ND	NA	NA	-ve

Keys:- +ve: Positive, -ve: Negative, NA: Not applicable, ND: Not determined

Enterobacteriaceae and *Listeria* were far above international standards. In ready-to-eat (RTE) foods, EC is satisfactory when it is < 2log, marginal between 2 and 4log and unsatisfactory when > 4log (FSANZ, 2001). The high counts recorded from the meat, weighing scale and table are suggestive that there exists a perpetual microbial cross-contamination of the chicken meat, processing facilities and environment. The higher EC noticed on the nylon covering after the carton is possibly due to the poor hygienic handling of the frozen foods. Sometimes in the retail outlets, the carton and nylon coverings are left exposed when poultry meats are taken from them. Usually, all the meat sold were weighed before sales. This makes the weighing scale a focus for microbial accumulation, spread and cross-contamination, thus corrective actions must be applied at this point to control foodborne pathogens. The weighing scales are made up of stainless steel and these surfaces have been shown to facilitate adhesion of pathogenic foodborne bacteria. For instance, *Listeria monocytogenes* attached to stainless steel and other surfaces within 20 min of contact (Mafu *et al.*, 1990). Lower counts (EC and LC) were observed on knifes utilized for processing than on the tables. The highest LC obtained on the processing tables were expected. In most cases, the tables are left outside the retail outlets and are not thoroughly washed before daily use or sometimes left unwashed till the next day. These tables are made from wood that has a high possibility for bacteria adhesion and subsequent biofilm formation. When pathogenic organisms form biofilms, their resistance to cleaning and sanitation is increased. This is an important CCP demanding remedial actions.

There is the possibility that every chicken meat sold will be contaminated and this could facilitate diseases outbreaks and transmission if corrective actions are not applied along the processing line. Jimenez *et al.* (2003) stipulated *E. coli* as the predominant species during poultry slaughter while Kozacinski *et al*. (2006) indicated *Enterobacteriaceae* counts in their ground chicken meat samples as 1.7-3.7 log cfu/g. Presence of *Enterobacteriaceae* in meats indicate possible fecal contamination and/or exposure of meat to non-hygienic conditions (Temelli *et al*., 2011). This fact makes chicken meat a concern for suppliers, consumers and public health officials worldwide (Ruban and Fairoze, 2011). Proper cold chain in preserving the poultry meat, interrupted power supply, low level of hygiene in the retail shops as well as low level of education on the part of the retailers are some of the factors responsible for the high level of microbial contamination. Oyarzabal and Hussain (2010) stated that maintaining *Salmonella* organisms in poultry processing plants is still a problem despite the decreasing trend observed for the organisms over the last decade. In this study, *Salmonella* organisms were isolated from the samples using a standard procedure nonetheless counts were not carried out.

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Enumeration of *Salmonella* is difficult and expensive compared to estimating counts of many common bacteria (Cox *et al.*, 2011).

Following microscopic and biochemical examination, serological study carried out revealed that pathogenic *E. coli* O157:H7, *Salmonella* Enteritidis and *Listeria monocytogenes* were present in the samples collected. This is similar to findings by other researchers who isolated pathogenic *E. coli* O157:H7 (Doyle and Schoeni, 1987; Ingham *et al*., 2005), *Salmonella* Enteritidis (Bonyadian *et al*., 2007) and *Listeria monocytogenes* (Abo El-Enean *et al.*, 2008; Ahmed and Abd EL-Atti, 2010) from poultry samples. This indicates the possible microbiological risks to which consumers are faced with. *E. coli* O157:H7, pathogenic *Salmonella* spp. and *Listeria monocytogenes* must not be detected in 25 g of meat (FSANZ, 2001) but the detection of *Listeria monocytogenes* in ready-to-eat foods prepared specifically for 'at risk' population groups (the elderly, immunocompromised and infants) are considered as potentially hazardous (FSANZ, 2001). Also, the USDA (1996) has maintained a zero-tolerance policy on the detection of *Listeria monocytogenes* in ready-to-eat products. Thus, *Listeria monocyotogenes* contaminated foods are declared “adulterated” according to Federal Meat Inspection Act and the Poultry Inspection Act, 21 U.S.C. 601 (m) or 453 (g), respectively (U.S. CODE, 1994). *Listeria monocytogenes* is particularly significant for cold-stored, ready-to-eat foods as it is frequently found in the environment and can grow at refrigerated temperatures (Ahmed and Abd EL-Atti, 2010). Improved *Salmonella* enumeration methods are needed to develop more useful risk assessments (Cox *et al*., 2011).

CONCLUSION

To eradicate these pathogenic organisms along the processing line of sale, the whole procedures should be overhauled while the following specific corrective actions should be applied at the CCPs. The weighing scale should be thoroughly washed, cleaned and sanitized daily. Hot water at ≥ 85 °C should be introduced during cleaning and sanitation at the retail outlets. The weighing scale should be covered with disposable coverings at each weighing to prevent accumulation and further spread of pathogenic *E. coli, Salmonella* and *Listeria*. Also, knives used during the cutting process must be more than two and should be sterilized always in readily available hot water. Processing tables should not be left outside the shops while washing, cleaning and sanitation of the tables should be done daily and routinely. Washing of the tables should not be delayed till the next day. Alternative materials such as steel should be used to make processing table instead of wood that usually facilitates easy bacteria adhesion.

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

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