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Assessment of the Microbial Level for Livestock Products in Retail Meat Shops Implementing HACCP System

Jung-hyun Kim[1] and Dong-Gyun Yim*

Department of Health Administration and Food Hygiene, Jinju Health College, Jinju 52655, Korea
[1]*Department of Public Health, Graduate School of Public Health, Seoul National University, Seoul 08826, Korea*

Abstract

This study aimed to examine the microbial contamination levels in livestock products at retail stores. Beef, pork, and chicken samples from raw materials and final products were obtained between January and December 2015. All homogenized meat samples (25 g) were tested for the aerobic plate count (APC), coliform count (CC), and *Escherichia coli* count (*E. coli*). The highest APCs in meat samples, by month, at retail shops were obtained in September, followed by July, May, and October ($p<0.001$). However, APC was the highest in summer and the lowest in winter ($p<0.001$). Average APCs for beef, pork, and chicken samples were 2.90, 3.19, and 3.79 Log CFU/g, respectively ($p<0.05$). A comparison between different months revealed that, CC levels in meat samples ranged from 0 to 1.13 CFU/g, and the highest CC was obtained in August ($p<0.001$). By season, the highest CC was found in the summer, followed by autumn, and spring ($p<0.001$). All meat samples were negative for *E. coli*. The average $\log_{10}$APC and CC for all samples was 3.10 and 0.37 Log CFU/g, respectively. Furthermore, there was a direct correlation between the season and coliform presence ($p<0.001$). There was also a positive correlation between the APC and CC (r = 0.517, $p<0.001$). The microbiological APCs for livestock products were in most cases below 10^6 CFU/g.

Keywords: beef, pork, chicken, retail shops, microbial contamination

Introduction

Consumption and sale of meat products has constantly been increasing in Korea. In recent years, meat product sales increased by 136.9% in 2000 as compared to 1990 (45,644 (M/T)), by 43.26% in 2010 as compared to 2000 (154,914 (M/T)), and by 7.8% in 2013 (207,681(M/T)) compared to 2012 (KMIA, 2015). Considering this upward trend in the livestock product consumption in Korea, proper management of hygiene in addition to policies for ensuring food safety to protect public health and strengthen consumer confidence are in great demand. It is particularly important to manage livestock distribution processes to provide meat and meat products that are safe to consume (Lee *et al.*, 2010). Concerns surrounding meat or meat products harboring pathogenic microorganisms have increased in recent years, despite an improvement in

efforts to ensure the distribution of hygienic meat products (Bae *et al.*, 2011). Contamination commonly occurs during processing, when meat comes in contact with equipment at a slaughter facility (e.g., grinders, belts, saws), food handlers (e.g., hand contact, knives), and by exposure to air/water (Jay, 1992). Currently, establishing food safety program is the most efficient way to reduce microbial growth and contamination in food (Eisel *et al.*, 1997). Regulatory authorities have sought to improve the microbiological safety of meat by making it mandatory to implement 'hazard analysis and critical control points' (HACCP) systems (USDA, 1996). Korean regulatory authorities introduced HACCP systems in meat processing plants in 1997, slaughter houses in 2000, livestock product plants in 2001, milk processing plants, and meat sales and distribution points in 2004 (QIA, 2012). During the conversion of carcasses to retail cuts, microbial contamination is inevitable (Eisel *et al.*, 1997). Because the elimination of pathogens from raw meat is difficult or nearly impossible, the goals for HACCP systems for meat focus on reducing and preventing microbial growth (Eisel *et al.*, 1997). The currently recommended procedures for developing HACCP

*Corresponding author: Dong-Gyun Yim, Department of Public Health Administration and Food Hygiene, Jinju Health College, Jinju 52655, Korea. Tel: +82-55-740-1814, Fax: +82-55-743-3010, E-mail: tousa0994@naver.com

systems in the meat industry and the actions taken to control microbiological contamination are based on subjective assessments of microbiological data in production processes (Corlett, 1998). For effective control of bacterial contamination, a microbiological test of meat products is required (Gill, 1995). HACCP systems should be based on microbiological data that allow for estimation of the numbers of indicator organisms on meat products at various processing stages (Gill, 2000). The current approach to assessing microbial contamination of meat carcass at slaughterhouses and meat processing plants is to collect samples by excising or swabbing (Nutsch *et al.*, 1997). For raw meat products, safety and quality can be estimated using indicator microorganisms, and by subsequently obtaining the total aerobic plate count (APC), coliform count (CC), and *E. coli* (ECC) count (Jay, 1992). APC provides an estimate of overall bacterial populations. A higher APC is usually an indication of poorer quality and a reduced shelf life. The relationship between APC and concentration of foodborne pathogens in raw meats remains unclear (Jay, 1992). CC and ECC are generally indicators of fecal contamination and poor sanitation during processing. A high CC and ECC generally correlate with higher levels of foodborne pathogens originating from feces (Jay, 1992). However, data on seasonal and monthly variations in microbiological examinations of meat in different retail meat shops are limited. Especially, a microbiological assessment of different types of meat in HACCP-implementing retail shops has not been extensively performed. Therefore, this study aimed to determine the association of microbial contamination with sample types (raw or final products), season, month, meat types (beef, pork, and chicken), and prevalence to evaluate the microbiological hygiene of retail shops in Korea.

Materials and Methods

Sample collection

A total of 164 meat samples (66 from beef, 91 from pork, and 7 from chicken) were collected from randomly selected meat retail shops located in three administrative regions in Korea (Gyonggi, Gyeongsang, and Chungchong) over a one-year period during four different seasons. The sampling seasons were spring (early March to late May), summer (early June to late August), fall (mid-September to late November), and winter (early December to late-February). The selection of the retail shops was made based on their variety of processing lines (beef, pork, and poultry), and their work with both raw materials and final

products. Raw materials were un-processed meats or carcasses transported in refrigerator trucks from slaughterhouse and collected at the beginning of the production line, while final meat products were obtained from freshly packed commercial meat products with raw cut meats at the end of production line. Experienced technicians or quality control staff at the shops carried out the microbiological sampling. The HACCP system, including sanitation procedures, was adopted in all meat retail shops included in the study. After meat cutting and boning in the processing line, about 100 g of sample was taken from different portions of the whole meats and placed on pre-labeled styrofoam trays. All samples were collected aseptically with the use of a sterile knife and, placed in a sterile collection bag. In case of poultry samples, the whole chicken carcass was used for samples. Trays were vacuum-packaged with multilayer polyolefin and polyvinylidene chloride film. After refrigeration (4-5°C), samples were transported to the laboratory where about 100 g of sample was taken and analyzed within 24 h. All samples were trimmed using a stainless steel knife, which was sterilized with alcohol and flaming.

Microbiological analysis

Samples were analyzed to determine the APC, CC, and ECC, which serve as hygiene indicators, as described in the corresponding microbiological guidelines to the method specified in *Process Criteria and Ingredient Standard of Livestock Products* (QIA, 2013). For APC, 25 g samples were transferred aseptically into a sterile stomacher bag containing 225 mL of 0.85% sterile saline solution (NaCl, Difco Laboratories, USA) and homogenized in a stomacher (Stomacher® 400 Circulator, Seward, Ltd., UK) for 2 min at room temperature to achieve a 10^{-1} dilution. Homogenized microbial extracts were serially diluted in sterile distilled water. Each diluted 1 mL sample was plated individually and spread thoroughly. The APC was determined using plate count agar (Difco Laboratories, USA) incubated at 37±1°C for 48 h. The diluted 1 mL samples were also plated on 3M Petrifilm (3M, USA) to count coliforms and *E. coli*. The Petrifilm was also incubated at 37±1°C for 48 h. Blue colonies with bubbles were recorded and counted as *E. coli*, and the pink, or blue colonies with bubbles were counted as coliforms. All analyses were performed in triplicate, and results were expressed as the logarithm of colony-forming units per gram (Log CFU/g). In all cases, plate counts were determined and converted to $\log_{10}$ CFU values using standardized plate count rules (Vanderzant and Splittstoesser, 1992).

Statistical analysis

STATA version 12.0 was used for the statistical analysis. Student's *t*-test or ANOVA was used for comparison of the APCs and CCs by month, season, meat type, and product form and results are presented as the mean and standard errors. To compare the presence or absence of coliforms by season, meat types, and product form, data were analyzed by Chi-square test, and results are presented as frequencies (percentages) instead of means. Pearson correlation analyses were performed to evaluate the relationship between APC and CC. If necessary, logarithmic transformations were used for variables with skewed distributions. A two-sided with a *p*-value of <0.05 was considered statistically significant.

Results and Discussion

As shown in Table 1, samples were classified by month, season, meat types, and product form. Meat types comprised a majority of pork (55.5%), followed by beef (40.2%) and chicken (4.3%). For the product form, 78.7% of samples came from finished cuts, and the remainder (21.3%)

Table 1. General sample characteristics (n=164)

Sample		Number (%)	*p*-value
	Meat retail shops	164 (100.0)	
Month	January	10 (6.1)	<0.001
	February	6 (3.7)	
	March	4 (2.4)	
	April	14 (8.5)	
	May	15 (9.1)	
	June	9 (5.5)	
	July	21 (12.8)	
	August	12 (7.3)	
	September	3 (1.8)	
	October	16 (9.8)	
	November	22 (13.4)	
	December	32 (19.5)	
Season	Spring	33 (20.1)	0.046
	Summer	42 (25.6)	
	Autumn	41 (25.0)	
	Winter	48 (29.3)	
Livestock product	Beef	66 (40.2)	0.488
	Pork	91 (55.5)	
	Chicken	7 (4.3)	
Product form[1]	Raw material	35 (21.3)	0.626
	Finished product	129 (78.7)	

Results are shown as frequency (%).
[1]Raw materials were un-processed meats or carcasses transported in refrigerator trucks from slaughterhouse and collected at the beginning of the production line, while final meat products were obtained from freshly packed commercial meat products with raw cut meats at the end of production line.

came from raw materials. By month, the greatest number of samples were collected in December (18%), followed by November (13.4%), July (12.8%), October (9.8%), and September (1.8%). By season, the highest number of samples was collected in winter (29.3%), followed by summer (25.6%), fall (25%), and spring (20.1%).

APC at meat retail shops is presented in Table 2 and samples were categorized by month, season, livestock products, and product form. A significant difference was observed in the APC between months, seasons, and livestock products with the exception of product forms. By month, the highest APC was found in September, followed by July, May, and October, in descending order (*p*< 0.001). This high APC in September might be owing to neglect or failure in temperature management (<15°C) at meat retail shops, because the temperature drops in the

Table 2. Aerobic plate counts (APC) in samples form meat retail shops

		Mean±S.E	F-value / *p*-value
Month	January	2.40±0.00[J]	10.480/ <0.001
	February	2.40±0.00[J]	
	March	2.65±0.35[H]	
	April	2.40±0.00[J]	
	May	3.66±0.40[C]	
	June	3.21±1.03[F]	
	July	4.05±0.76[B]	
	August	3.28±0.79[E]	
	September	4.66±0.09[A]	
	October	3.41±0.81[D]	
	November	2.75±0.79[G]	
	December	2.47±0.16[I]	
Season	Spring	2.95±0.67[C]	13.151/ <0.001
	Summer	3.61±0.91[A]	
	Autumn	3.16±0.91[B]	
	Winter	2.44±0.14[D]	
Livestock product	Beef	2.90±0.74[C]	3.589/ 0.031
	Pork	3.19±0.89[B]	
	Chicken	3.79±1.12[A]	
Product form[1]	Raw material	2.91±0.85[B]	1.419/ 0.236
	Finished product	3.14±0.86[A]	

Results are shown as means±standard errors (SE). Means with different letters indicate a statistically significant difference (*p*< 0.05) by Duncan's multiple range test. P-values were calculated by ANOVA (unit: Log CFU/g). Logarithmic transformations were used for the APC.
[1]Raw materials were un-processed meats or carcasses transported in refrigerator trucks from slaughterhouse and collected at the beginning of the production line, while final meat products were obtained from freshly packed commercial meat products with raw cut meats at the end of production line.
[A-J]Means with different superscripts in the same column are significantly different (*p*<0.05).

early autumn as compared to the summer. Seasonal data revealed that the APC in meat samples was greatest in summer (3.61), followed by autumn (3.16), spring (2.95), and winter (2.44) ($p<0.001$). Similarly, Oh and Lee (2001) reported that microbial contamination of carcass surfaces in beef processing plants was the highest in summer and the lowest in winter. APCs at meat retail shops were 2.9, 3.19, and 3.79 CFU/g in beef, pork, and chicken, respectively ($p<0.05$). Chung *et al*. (1999) observed that the APC of pork samples taken from working tables at meat processing plants were higher than 10^3 CFU.

The level of coliforms in meat samples from retail shops is shown in Table 3. *E. coli* was not detected in any of the samples, and coliforms were rarely recovered from meat samples. Furthermore, there were significant monthly and season differences in the CC. CC was found to be

Table 3. Coliform counts (CC) in samples from meat retail shops

		Mean±S.E	F-value/ p-value
Month	January	-	
	February	-	
	March	-	
	April	0.27±0.72[E]	
	May	0.30±0.86[D]	
	June	-	5.165/
	July	1.00±1.26[C]	<0.001
	August	1.13±1.41[A]	
	September	-	
	October	1.07±1.25[B]	
	November	-	
	December	-	
Season	Spring	0.25±0.73[C]	
	Summer	0.83±1.23[A]	7.445/
	Autumn	0.42±0.93[B]	<0.001
	Winter	-	
Livestock product	Beef	0.30±0.78[C]	2.133/
	Pork	0.36±0.92[B]	0.122
	Chicken	1.03±1.28[A]	
Product form	Raw material	0.16±0.64[B]	2.501/
	Finished product	0.42±0.94[A]	0.116

Results are shown as means±standard errors (SE). Means with different letters indicate a statistically significant difference ($p<0.05$) by Duncan's multiple range test. P-values were calculated by ANOVA (unit: Log CFU/g). Logarithmic transformations were used for the APC.
[1])Raw materials were un-processed meats or carcasses transported in refrigerator trucks from slaughterhouse and collected at the beginning of the production line, while final meat products were obtained from freshly packed commercial meat products with raw cut meats at the end of production line.
[A-E]Means with different superscripts in the same column are significantly different ($p<0.05$).

highest in August, followed by October, July, May and April ($p<0.001$). Unexpectedly, coliforms were found in samples collected in October. CCs at meat retail shops were highest in summer (0.83), followed by autumn (0.42), and lowest in spring (0.25) ($p<0.001$). The CCs in meat retail shops were 0.30, 0.36, and 1.03 CFU/g in beef, pork, and chicken, respectively, which was much lower than what as reported by Oh and Lee (2001). However, there were no significant differences in meat types and product form.

As shown in Table 4, Chi-square testing was carried out to determine whether there was a relationship between the prevalence of coliforms and the season. A significant relationship was found between the two factors ($p<0.001$). There was no significant difference in the CC based on the product form; however, there was a statistically significant relationship between the presence of coliforms and the product form. Manios *et al*. (2015) have reported the CC increases in meat processing, from raw materials to the final products and have shown that fecal contamination of the final products originates primarily from raw materials, emphasizing the need for paying special attention to the hygiene levels in slaughterhouse and meat processing facilities.

The correlation between the APC and CC, as well as average microbial concentrations for APC and CC from samples, is shown in Table 5. We examined 127 samples with APC and 164 samples with CC. The average APCs and CCs for the samples were 3.10 and 0.37 Log CFU/g. Consistent with this result, the average APCs for retail cuts of beef samples in the study of Eisel *et al*. (1997) were approximately 3 CFU/g. We found a positive correlation between APCs and CCs (r=0.517***). Although coliforms cannot indicate the presence of *E. coli* O157, they may be useful as indirect indicators. Further studies are needed to confirm these findings.

The distribution of APCs and CCs in beef, pork, and chicken samples from retail shops are presented in Table 6. The APCs in the analyzed samples ranged from below the detection limit (<10^1 CFU/g) to <10^6 CFU/g. APCs in the range of 10^2 to 10^3 CFU /g and CCs from below 10^2 CFU/g ranging were found in 67.9% and 89.4% of the beef samples, respectively. The prevalence of APCs was lower than that found in other studies on the distribution of APCs and CCs in retail shops (Lee *et al*., 2007). Furthermore, a previous study reported APCs of ≤10^7 CFU/g in beef, pork, and chicken from retail shops (Jeon *et al*., 2011). The most commons APCs in pork and chicken samples were 10^2-10^3 and 10^4-10^5, followed by 10^3-10^4

Table 4. Correlation between coliforms and season, meat type, and product form

		Coliforms		χ^2/p-value
		Presence	Absence	
Season	Spring	4 (16.0)	29 (20.9)	19.626/<0.001
	Summer	14 (56.0)	28 (20.1)	
	Autumn	7 (28.0)	34 (24.5)	
	Winter	0 (0.0)	48 (34.5)	
Livestock product	Beef	9 (36.0)	57 (41.0)	4.328/0.115
	Pork	13 (52.0)	78 (56.1)	
	Chicken	3 (12.0)	4 (2.9)	
Product form[1]	Raw material	2 (8.0)	33 (23.7)	3.128/0.077
	Finished product	23 (92.0)	106 (76.3)	

P-values were calculated by Chi-square test.

[1]Raw materials were un-processed meats or carcasses transported in refrigerator trucks from slaughterhouse and collected at the beginning of the production line, while final meat products were obtained from freshly packed commercial meat products with raw cut meats at the end of production line.

Table 5. Correlation between aerobic plate count and coliform counts (unit: Log CFU/g)

Sample	Number	Mean±S.E	Correlations	
			APC	CC
Aerobic plate count	127	3.10±0.86[A]		0.517***
Coliforms counts	164	0.37±0.89[B]	0.517***	

Abbreviations: APC, aerobic plate count; CC, coliforms counts.

[A,B]Means with different superscripts in the same column are significantly different ($p<0.05$).

***$p<0.001$

Table 6. Distribution of aerobic plate count and coliform count for beef, pork, and chicken samples in retail shops

Livestock products	Bacteria	Distribution of bacterial count (CFU/g)						
		$<10^2$	10^2 –$<10^3$	10^3 - $<10^4$	10^4 - $<10^5$	10^5 - $<10^6$	10^6- $<10^7$	$>10^7$
Beef (%)	Aerobic plate count	1 (1.9)	36 (67.9)	10 (18.9)	5 (9.4)	1 (1.9)	-	-
(n=66)	Coliform	59 (89.4)	7 (10.6)	-	-	-	-	-
Pork (%)	Aerobic plate count	-	37 (52.9)	15 (21.4)	17 (24.3)	1 (1.4)	-	-
(n=91)	Coliform	80 (87.9)	8 (8.8)	3 (3.3)	-	-	-	-
Chicken (%)	Aerobic plate count	1 (0.8)	75 (58.6)	25 (19.5)	25 (19.5)	2 (1.6)	-	-
(n=7)	Coliform	4 (57.1)	3 (42.9)	-	-	-	-	-

CFU/g. Lee *et al.* (2007) suggested that pork samples showed higher coliform contamination than beef and chicken samples, which is consistent with our results. This may be due to the presence of feces during the slaughter process. The APCs and CCs of chicken samples were generally lower than those of beef and pork samples, which agrees with the report by Lee *et al.* (2007). Furthermore, Lee *et al.* (2007) reported APCs < 10^4 CFU/g and *E. coli* count< 10^2 CFU/g in 82% and 80% of the pork samples, respectively.

A microbial sampling program should include testing of incoming ingredients on a regular basis to estimate microbial levels in retail cuts. The wide distribution of APCs could mainly be attributed to the variability in the origin and extent of processing of the analyzed meat samples, (e.g., intact or processed meat) (Manios*et al.*, 2015). Most beef, pork, and chicken samples showed CCs of less

than 10^2. Microbial counts in the samples remained below the guidelines for maximum limit for meat (below 10^7 Log CFU/g) in retail meat shops (MFDS, 2015).

Upadhyaya *et al.* (2012) reported that the high overall prevalence of APCs in retail shops is related to the poor infrastructure, such as lack of dressing facilities, drainage, differentiation between clean and unclean operations, and a general lack of basic maintenance of hygiene and sanitation. It is suggested that contamination levels are further increased due to excessive handling of carcasses, by too many people, by keeping more than two kinds of meats in a shop without proper separation (Upadhyaya *et al.*, 2012).

Manios *et al.* (2015) indicated that the high contamination levels of meat may be the result of raw materials with high initial microbial load, poor hygiene practices during processing, and high temperatures (>15°C) by malfunction in the processing lines. In order to improve the hygi-

ene levels in butcher's shops, application of SSOP, systemic sanitation education of employees, hygienic control of utensils and equipment, and continuous monitoring of microorganisms will be required (Jeon *et al.*, 2011).

The most important factor contributing to microbial contamination of retail meat cuts was incoming raw materials. From this data, microbial limits were established for log10 APC as <6 CFU/g and for log10 CC as <4 CFU/g. Better quality and safer meat could be obtained by reducing microbial contamination during processing. When microbial reduction strategies are developed for operations, more attention should be paid to reducing microbial contamination in highly-contaminated parts of the meats. The microbial survey of retail shop indicated that environmental contamination, such as from food contact surfaces, floors, walls, and air, is probably not a significant source of overall microbial contamination. However, effective cleaning and sanitation programs and safe handling procedures are important for ensuring a safety, and high quality of products (Eisel *et al.*, 1997).

The present results show that APCs in meat samples from meat processing plants were highest in the summer and lowest in the winter. However, the highest APCs were found in September. Meat is extensively handled during boning and cutting, and meat surfaces can be exposed to unhygienic environments, making it susceptible to contamination (Currier *et al.*, 1986). Therefore, hygienic control of meat cutting at all operational stages in the processing line, in retail outlets, and in local markets is required for more effective control of pathogen spread and for improving the microbial safety of meat products (Choi *et al.,* 2013). Microbial testing for meat products is an important tool for identifying and monitoring potential hazards as part of HACCP and GMP programs (Eisel *et al.*, 1997). Analysis of the meat processing steps should be complemented by collection of microbial monitoring data in accordance with HACCP principles. The most common sampling method used in previous studies was swabbing (Belluco *et al.*, 2015), while a homogenized sampling method was used in this study. Although all samples were negative for *E. coli*, coliforms were detected in some meat samples from the retail shops. Minimizing the presence of bacteria in meat is vital, because *E. coli* and coliforms can cause serious public health problems (Lowe *et al.*, 2001).

In order to estimate microbial levels in ground beef and retail cuts, a microbial sampling program should include testing of incoming meats on a regular basis. When sampling incoming meats, a microbial sampling program should include sampling with whole-muscle tissues rather than swab sampling for the retail cuts.

Variables, such as the sampling and analytical methods used, area of meat sampled, and specific step of the processing line where samples are obtained could affect conclusions (Eisel *et al.*, 1997). Further studies should be performed to examine microbial contamination via contact surfaces and environmental sources and to determine the hygiene levels in meat processing plants and retail shops.

Conclusion

Our findings provide valuable basic data about the hygiene levels in retail shops. The data presented here can be used to monitor control points critical for the verification of sanitation control procedures. Our data show the presence and counts of indicator organisms such as aerobic and coliform bacteria in retail shops, and explain how bacterial loads are affected by factors, in slaughterhouses or meat processing plants. Quality control personnel in retail shops should be trained to carefully select raw materials from meat processing plants, as well as to avoid incorrect handling of products and ensure proper implementation of disinfection plans.

Acknowledgements

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