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ARTICLE

Monitoring of Microbial Contaminants of Beef, Pork, and Chicken in HACCP Implemented Meat Processing Plants of Korea

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Abstract This research was to evaluate microbial contamination levels in meat samples at hazard analysis critical control point (HACCP)-implemented processing plants that produce beef, pork, and chicken. During a period of about a year, a total of 178 samples (76 from beef, 89 from pork, and 13 from chicken) were obtained from raw materials (21.3%) and final products (78.7%). All samples were determined for each 25 g homogenized one. Samples were analyzed to determine the total aerobic plate count (APC), coliform count (CC), and *E. coli* count (ECC). By month, APC levels were the highest in September and the lowest in February ($p<0.001$). In comparison among season, APC levels in meat samples were the highest in the summer and the lowest in winter ($p<0.001$). By month, the highest CC prevalence was found in August, followed by October and then July ($p<0.001$). By season, the highest CC was obtained in summer, followed by autumn and then spring ($p<0.001$). All samples were negative for ECC. There was a direct correlation between the product form and coliform presence ($p<0.001$). In addition, there was a positive correlation between the APC and CC (r=0.261). The APCs in analyzed samples ranged from below $<10^1$ CFU/g to $<10^7$ CFU/g. In conclusion, the month and season had significant effects on microbial contamination levels at HACCP implemented processing plants. Interrelationships between (i) the product form and coliform, (ii) the APC and CC were revealed.

Keywords beef, pork, chicken, meat processing plants, microbial contamination

Introduction

In Korea, the consumption and sales of meat products are increasing. Specifically, sales of meat products increased by 136.9% in 2000 compared to 1990 (45,644 [M/T]), by 43.26% in 2010 compared to 2000 (154,914 [M/T]), and by 4.25% in 2011 compared to 2010 (161,495 [M/T]) (Korea meat industries association, KMIA, 2015). These statistics show that consumers' demand for meat products continues to increase. In

recent years, there has been growing concern about meat products carrying pathogenic microorganisms, despite enhanced efforts in meat and processed meat hygiene (Bae et al., 2011). Contamination can occur during processing, by contact with facility equipment (e.g., grinders, belts, saws), by contact with food handlers (e.g., hand contact, knives), and exposure to other environmental sources (e.g., air, water) (Kim and Yim, 2016). Regulatory agencies require meat-packing plants to implement hazard analysis critical control point (HACCP) systems for meat production processes to reduce the numbers of pathogenic organisms (USDA, 2016). However, since the complete elimination of pathogens from raw meat is difficult or impossible, the goal of HACCP for meats focuses on reducing and preventing microbial growth (Kim and Yim, 2016). Presently, the HACCP system is used in many regulated food industries. In Korea, 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 (Kim and Yim, 2016). Monitoring of microorganisms in meat products is an important step in HACCP programs (Kim and Yim, 2017), and the proper storage of meat products is crucial to controlling contamination of meat by spoilage organisms (Kim and Yim, 2017). It has been suggested that HACCP systems at meat processing plants should be based on microbiological data, with estimates of the numbers of indicator organisms on meat products at various stages of processing (Gill et al., 2000). For raw meat products, safety and quality can be estimated by the use of indicator microorganisms, including total aerobic plate count (APC), coliform count (CC), and *Escherichia coli* count (ECC) (Kim and Yim, 2016). APC provides an estimate of overall bacterial populations. A higher APC usually relates to poorer quality and a reduced shelf life. The relationship between APC and the concentration of foodborne pathogens in raw meats is unclear. CC and ECC provide an estimate of fecal contamination and poor sanitation during processing. High CC and ECC generally correlate with higher levels of foodborne pathogens originating from feces (Kim and Yim, 2016). Meat can be further contaminated or cross-contaminated by various pathogenic bacteria after the slaughter process, such as during chilling, cutting, deboning, and slicing processes (Duffy et al., 2001). Thus, all processing conditions are important factors that can affect microbiological quality. To improve the safety of final meat products, more information is needed at the point where carcasses or meat cuts are contaminated in meat processing plants (Choi et al., 2013). However, data on seasonal variation and meat type in microbiological examinations of meat in different meat processing plants are limited. In particular, a microbiological assessment of meat in HACCP-implementing meat processing plants has not been conducted. Thus, the objective of this study was to investigate the microbial contamination of meat, and determine its association with month, season, sample type (raw or final products), meat type (beef, pork, or chicken), and prevalence in order to evaluate the microbiological hygienic level of HACCP-implementing meat processing plants in Korea. In addition, potential relationship between coliforms and season, meat type, and product form were studied by elucidating the relevant hygiene aspects.

Materials and Methods

Sample collection

A total of 178 meat samples (76 from beef, 89 from pork, and 13 from chicken) were collected from five randomly selected meat processing plants located in three administrative regions in Korea (Gyonggi, Gyeongsang, and Chungchong) over a one-year period during four different seasons. The samples from meat processing plants were taken at 10 medium sized abattoirs. The selection of the plants was made based on their capacities to process large amounts of meat (approximately 30 tons of meat per day), their variety of processing lines (beef, pork, and poultry), and their work with both raw materials and final products. Raw materials were collected at the beginning of the production line, while final meat products were obtained from

freshly packed commercial meat. Collection was dependent on the cooperation of the owners. Experienced technicians or quality control staff at the plants carried out the microbiological sampling. The HACCP quality control system, including sanitation procedures, was adopted in all meat processing plants included in the study. After meat cutting and boning in the processing line, about 100 g of sample was taken from different portions (rump, midline, and brisket) in beef and pork of the whole meats and placed on pre-labeled styrofoam trays. The whole carcass was used for chicken samples. All samples were collected aseptically with the use of a sterile knife and placed in a sterile collection bag. In the poultry processing plants, the whole chicken carcass was used for samples. Trays were vacuum-packaged with multilayer polyolefin and polyvinylidene chloride film. After refrigeration (4-5℃), samples were transported to the laboratory where about 100 g of sample was taken from each carcass and analyzed within 24 hours. All samples were trimmed using a stainless steel knife that was sterilized with alcohol and flaming.

Microbiological analysis

Samples were analyzed to determine the total APC, CC, and ECC, which serve as hygiene indicators. For APC, 25 g samples were transferred aseptically into a sterile stomacher bag (STOMACHER® 400 CIRCULATOR, Seward, Ltd., UK) containing 225 mL of 0.85% sterile saline solution (NaCl, Difco Laboratories, USA) and homogenized for 2 min at room temperature to achieve a 1/10 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℃ 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℃ 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 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 type, and product form, data were analyzed by Chi-square test and results are presented as frequencies (percentages) instead 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 are classified by month, season, meat type, and product form. Meat types consisted of 50% pork, 42.7% beef, and 7.3% chicken. For product form, 78.7% of samples came from finished products and the remainder (21.3%) came from raw materials. By month, the greatest number of samples was collected in December (18%), followed by November (12.4%), July (11.8%), August (10.1%), and then September (3.4%) ($p<0.001$). By season, the greatest number of samples was collected in summer (28.7%), followed by winter (27%), fall (24.7%), and then spring (19.7%) ($p<0.05$).

Table 2 shows the APC in meat samples at meat packing plants. A statistically significant relationship between APC and

Table 1. General sample characteristics (n= 178)

Sample		Number (%)	*p*-value[a]
Plants	Meat packing plants	178 (100.0)	
Month	January	7 (3.9)	<0.001
	February	9 (5.1)	
	March	6 (3.4)	
	April	14 (7.9)	
	May	15 (8.4)	
	June	12 (6.7)	
	July	21 (11.8)	
	August	18 (10.1)	
	September	6 (3.4)	
	October	16 (9.0)	
	November	22 (12.4)	
	December	32 (18.0)	
Season	Spring	35 (19.7)	0.040
	Summer	51 (28.7)	
	Autumn	44 (24.7)	
	Winter	48 (27.0)	
Animal	Beef	76 (42.7)	0.917
	Pork	89 (50.0)	
	Chicken	13 (7.3)	
Product form	Raw material	38 (21.3)	0.496
	Finished product	140 (78.7)	

Results are shown as frequencies (%).
[a] Indicates that *p*-value for trend <0.05.

month, as well as APC and season, was found. By month, the highest APC was found in September, and then August, June, and July in descending order ($p<0.001$). This high APC in September might be due to a failure in temperature management (<15℃) in the meat processing plants. However, the APCs in meat samples were greatest in summer, followed by spring, fall, and winter ($p<0.001$). The APCs in meat processing plants were 3.62, 4.48, 3.57, and 2.46 CFU/g in spring, summer, autumn, and winter, respectively. This is similar to a report by Oh and Lee (2001), which noted that microbial contamination of carcass surfaces in beef processing plants were the highest in summer and the lowest in winter. These findings are in agreement with those reported by Kim and Yim (2016). The APC was also associated with meat type and product form, even though these results were not statistically significant. The APCs in meat processing plants were 3.59, 3.73, and 3.46 CFU/g in beef, pork, and chicken, respectively. This result was similar to a report by Kim and Yim (2016), which showed that the APCs of pork samples taken from work tables at meat processing plants were higher than 10^3 CFU/g. Gounadaki et al. (2008) reported the presence of *Listeria monocytogenes*, *Salmonella* spp., or *Staphylococcus aureus* was associated with elevated APCs and specific groups of spoilage microorganisms, and suggested the potential association between the general hygiene level and the presence of pathogenic bacteria. Manios et al. (2015) indicated that high contamination levels in meat may be the

Table 2. Aerobic plate counts (APC) in samples from meat packing plants

Sample		Mean±SE	*p*-value[a]
Month	January	2.47±0.19^{J}	<0.001
	February	2.40±0.00^{K}	
	March	2.56±1.55^{I}	
	April	3.31±0.98^{G}	
	May	4.25±0.67^{E}	
	June	4.48±0.29^{C}	
	July	4.34±0.52^{D}	
	August	4.64±0.72^{B}	
	September	5.05±0.56^{A}	
	October	3.41±0.81^{F}	
	November	3.28±0.80^{H}	
	December	2.47±0.16^{J}	
Season	Spring	3.62±1.12^{B}	<0.001
	Summer	4.48±0.56^{A}	
	Autumn	3.57±0.97^{C}	
	Winter	2.46±0.15^{D}	
Animal	Beef	3.59±1.12^{B}	0.614
	Pork	3.73±1.00^{A}	
	Chicken	3.46±1.08^{C}	
Product form	Raw material	3.39±1.00^{B}	0.140
	Finished product	3.71±1.06^{A}	

Results are shown as mean±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).
[a] Indicates that *p*-value for trend <0.05. Logarithmic transformations were used for the APC.
[A-K] Means with different superscripts in the same column are significantly different ($p<0.05$).

result of raw materials with a high initial microbial load, poor hygiene practices during processing, or high temperatures (>15℃) in the processing lines.

Table 3 shows the level of coliforms in meat samples from meat packing plants. *E. coli* was not detected in any samples, and coliforms were rarely recovered from meat samples. There were significant monthly and seasonal differences in the CC. The CC was found to be greatest in August, followed by October and then July ($p<0.001$), which is in agreement with the results of previous study (Kim and Yim, 2016). The CCs in meat processing plants were 0.24, 0.82, and 0.45 CFU/g in spring, summer, and autumn, respectively ($p<0.001$), which is much lower than the counts presented by Oh and Lee (2001). The CCs in meat processing plants were 0.31, 0.44, and 0.55 CFU/g in beef, pork, and chicken, respectively.

Coliforms were only found in the spring, summer, and fall. Chi-square testing was performed to determine whether there was a relationship between the detection of coliforms and the season, as shown in Table 4. The results showed that there was a significant relationship between the two factors ($p<0.001$). There were no significant differences in APCs according to the meat type or product form (data not shown). 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 ($p<0.05$)

Table 3. Coliform counts (CC) in samples from meat packing plants

Sample		Mean±SE	*p*-value[a]
Month	January	-	<0.001
	February	-	
	March	-	
	April	0.27±0.72[F]	
	May	0.30±0.86[E]	
	June	-	
	July	1.00±1.26[C]	
	August	1.14±1.49[A]	
	September	0.43±1.05[D]	
	October	1.07±1.25[B]	
	November	-	
	December	-	
Season	Spring	0.24±0.72[C]	<0.001
	Summer	0.82±1.27[A]	
	Autumn	0.45±0.96[B]	
	Winter	-	
Animal	Beef	0.31±0.82[C]	0.544
	Pork	0.44±1.01[B]	
	Chicken	0.55±1.05[A]	
Product form	Raw material	0.14±0.62[B]	0.066
	Finished products	0.46±0.99[A]	

Results are shown as mean±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).
[a] Indicates that *p*-value for trend <0.05. Logarithmic transformations were used for CCs.
[A-F] Means with different superscripts in the same column are significantly different ($p<0.05$).

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

Sample		Coliforms		χ^2/ *p*-value[a]
		Presence	Absence	
Season	Spring	4 (14.3)	34 (22.7)	19.062/<0.001
	Summer	16 (57.1)	42 (28.0)	
	Autumn	8 (28.6)	38 (25.3)	
	Winter	0 (0.0)	36 (24.0)	
Meat type	Beef	10 (35.7)	66 (44.0)	0.993/0.609
	Pork	15 (53.6)	74 (49.3)	
	Chicken	3 (10.7)	10 (6.7)	
Product form	Raw material	2 (7.1)	36 (24.0)	3.993/0.046
	Finished products	26 (92.9)	114 (76.0)	

p-values were calculated by Chi-square test.
[a] Indicates that *p*-value for trend <0.05.

(Table 4). Previous studies have shown that the CC increases during meat processing, from raw materials to the final products (Manios et al., 2015). Manios et al. (2015) suggested that fecal contamination of the final products originates primarily from raw materials, emphasizing the need for special attention to hygiene in the slaughterhouse and meat processing facility.

Table 5 represents the correlation between the APC and CC. One hundred and seventy-eight samples with APC and 160 samples with CC were examined. The average APCs and CCs for samples were 3.65 and 0.39 CFU/g. We examined the correlation between these counts, and found a positive correlation between the two types of bacteria (r=0.261***). Although coliforms cannot directly indicate the presence of *E. coli* O157, they may be useful as indirect indicators. More data are needed to confirm these findings.

The distribution of APCs and CCs in beef, pork, and chicken samples from meat processing plants are presented in Table 6. The APCs in analyzed samples ranged from below the detection limit ($<10^1$ CFU/g) to $<10^7$ CFU/g. The prevalence of aerobic bacteria in samples with APCs ranging from 10^2-10^5 CFU/g in beef was 35.8%. The most commons APCs in pork were 10^4-10^5 and 10^2-10^3, followed by 10^3-10^4 and 10^5-10^6 CFU/g. The wide distribution of APCs can 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. Counts in all samples remained below the guidelines for maximum microbiological limits for meat (ministry of food and drug safety, MFDS, 2018). Kim et al. (2000) found that the percentage of pork samples with APCs less than 10^4 CFU/cm^2 and ECs less than 10^2 CFU/cm^2 were 82% and 80%, respectively.

Interestingly, the APCs and CCs of chicken samples were generally lower than those of beef and pork samples, which agrees with a report by Lee et al. (2007). Coliforms in meat may be attributable to the presence of feces during the slaughtering process. Although all samples were negative for *E. coli*, coliforms were detected in some of meats from the plants. Minimizing the presence of bacteria in meat is essential, because *E. coli* and coliforms can cause serious public health problems (Lowe et al., 2001). According to our study, it is crucial that sanitary operation in which meat are processed are strictly controlled to prevent microbial contamination of the meat.

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

Sample	Number	Mean±SE	Correlations	
			1	2
Aerobic plate count	178	3.65±1.06	1.00	0.261***
Coliform count	160	0.39±0.94		1.00

*** $p< 0.001$.

Table 6. Distribution of aerobic plate count (APC) and coliform count (CC) for beef, chicken, and pork samples in meat packing plants

Animal samples	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 (%) (n=76)	APC	1 (1.5)	24 (35.8)	13 (19.4)	24 (35.8)	4 (6.0)	1 (1.5)	-
	CC	68 (89.5)	7 (9.2)	1 (1.3)	-	-	-	-
Pork (%) (n=89)	APC	-	25 (30.5)	18 (22.0)	33 (40.2)	6 (7.3)	-	-
	CC	76 (85.4)	9 (10.1)	4 (4.5)	-	-	-	-
Chicken (%) (n=13)	APC	-	6 (54.5)	-	5 (45.5)	-	-	-
	CC	10 (76.9)	3 (23.1)	-	-	-	-	-

Conclusion

The present study provides essential information about the hygienic levels at HACCP implemented meat processing plants through the assessment of microbiological data. Data from this study can be used to identify critical control points for the verification of hygiene control procedures. It could be concluded that the prevalence of APC in samples from meat processing plants was highest in August and the CC was found to be highest in August. Correlation between (i) the product form and coliform, (ii) the APC and CC were revealed. Further study should be required for microbiological assessment of meat cut at all operational stages such as the slaughterhouse, processing line, and retail outlets.

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