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ARTICLE

Evaluation of the Microbiological Status of Raw Beef in Korea: Considering the Suitability of Aerobic Plate Count Guidelines

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Abstract This study was conducted to analyze the microbiological contamination status of raw beef distributed in Korea, and evaluate the suitability of current aerobic plate count (APC) guidelines. We analyzed five years (2010-2014) of microbiological monitoring data obtained from the Ministry of Food and Drug Safety and investigated the microbiological status of raw beef collected from meat packing centers and meat shops in the Seoul/Gyeonggi, Gangwon, and Chungcheong regions in August 2015. From 2010-2014, most raw beef (>94%) displayed APC levels of $< 1.0 \times 10^6$ CFU/g. However, raw beef samples collected from all three regions in August 2015 had comparatively higher APC levels than those reported in previous years. To evaluate the relationship between the APC level and quality, changes in beef loin were evaluated during cold storage for 15 days at 4°C. On day 11, the mean APC level (4.7×10^6 CFU/g) conformed to current guidelines in Korea (1.0×10^7 CFU/g) and the pH value was 5.82. However, the sensory evaluation score for color and overall acceptability was under 3.0, meaning that the beef loin was not acceptable for eating. These results suggest that current APC guideline for raw beef should be lowered to 1.0×10^6 CFU/g to improve both the microbiological safety and palatability of raw beef.

Keywords aerobic plate count, beef, microbiological guidelines, monitoring, sensory evaluation

Introduction

Over recent years, meat consumption and production has continually increased. In Korea, annual meat consumption has increased from 36.8 kg/capita in 2009 to 52.3 kg/capita in 2016 (OECD, 2017). Meat is a great source of protein; however, it is easily perishable (Komba et al., 2012). Meat products are readily contaminated by a variety of food hazards, including biological, chemical, physical, and particularly microbial factors (Kim et al., 2016; Ji, 2002). Although the muscles of healthy animals do not contain microorganisms, meat can be contaminated during production and transportation (Ercolini et al., 2006). Contamination easily occurs during processing, via slaughter facility and food handling equipment (belts, grinders, saws, knives, etc.; Eisel et al., 1997). Regulations and guidelines exist to provide safe meat and meat products to consumers. However, despite efforts to serve the safest meat possible to consumers, worries surrounding the contamination of meat with pathogenic microorganisms have increased in recent years (Bae et al., 2011).

As increasing numbers of people adapt a Western-style diet, beef consumption has increased accordingly. As international trade in the meat industry grows, many countries have made efforts to ensure the availability of sanitary and safe meat (Oh and Lee, 2001). Monitoring the microbiological levels of raw meat products is an important facet of sanitary management. The quality and safety of raw beef can be evaluated using indicator microorganisms such as aerobic plate count (APC) and *Escherichia coli* (Jay, 1992). In the USA, the Food Safety and Inspection Service (FSIS) has conducted the Nationwide Microbiological Baseline study since 1992. Through this, FSIS can monitor the microbial contamination levels (APC, *E. coli*, *Salmonella*, etc.) of meat or carcasses, which are regulated by the US Code of Federal Regulations (9 CFR 310.25). The microbiological levels in food are also regulated by law in the European Union (EU; EC No. 2073/2005), and food products that violate regulations must be recalled. In Japan, the Ministry of Health, Labour and Welfare (MHLW) legislated the Food Hygiene Law to control *E. coli* and pathogen levels in meat products. Many countries control the meat hygiene by regulating or recommending of APC level in meat. Most countries regulate or recommend the APC level in meat at under 5×10^6 CFU/g or cm^2 (Table 1). However, the Korean APC guideline of 1.0×10^7 CFU/g is higher than that of other advanced countries (Table 1), and studies have reported that meat of this APC level displayed surface spoilage and off-odor (Gil, 1998; Oh and Lee, 2001; Young et al., 1988). Only the International Commission on Microbiological Specifications for Foods (ICMSF) provided the APC guideline level at 1.0×10^7 CFU/g or cm^2 in 1978. Therefore, the APC level recommended by the Ministry of Food and Drug Safety (MFDS) may not meet recent international meat freshness standards. Determination of a practical APC guideline for raw beef is required.

Therefore, this study was conducted to evaluate the microbiological contamination level of raw beef in Korea by analyzing MFDS surveillance results over five years (2010-2014) and monitoring microbiological contamination levels in raw beef from three regions in Korea. Furthermore, changes in beef quality and microbial levels during cold storage were determined to provide scientific evidence for changes to the APC guideline of raw beef.

Table 1. Comparison of the microbiological limits for carcasses and meat in different countries

Country	Products	Aerobic plate count	*E. coli*	Institute	Type
Korea	Carcasses (cattle, sheep)	$\leq 1.0 \times 10^5$ CFU/cm^2	$\leq 10^2$ CFU/cm^2	Ministry of Food and Drug Safety 2014-135	Guideline
	Carcasses (pigs)		$\leq 10^4$ CFU/cm^2		
	Carcasses (chicken, duck)		$\leq 10^3$ CFU/cm^2		
	Meat (cattle, sheep)	$\leq 1.0 \times 10^7$ CFU/g	$\leq 10^3$ CFU/g		
	Meat (pigs, chicken, duck)		$\leq 10^4$ CFU/g		
USA	Carcasses (steers, heifers, cows, bulls)	-	$\leq 10^2$ CFU/cm^2	Food Safety and Inspection Service, Electronic code of federal regulations	Standard
	Carcasses (broiler)	-	$\leq 10^3$ CFU/cm^2		
	Carcasses (swine)	-	$\leq 10^4$ CFU/cm^2		

Table 1. Comparison of the microbiological limits for carcasses and meat in different countries (Continued)

Country	Products	Aerobic plate count	*E. coli*	Institute	Type
Japan	Meat	≤5.0 × 10^6 CFU/g	Negative	FCOOP	Self -standard
	Poultry meat	≤5.0 × 10^6 CFU/g	-		
	Raw meat	≤5.0 × 10^5 CFU/g	Negative	Kinkicoop	Self-standard
	Meat	≤5.0 × 10^6 CFU/g	Negative		
	Meat	≤3.0 × 10^6 CFU/g	Negative	Kobecoop	Self -standard
	Raw meat	≤3.0 × 10^6 CFU/g	-	UCOOP	Self- standard
	Meat	≤1.0 × 10^7 CFU/g	-		
Australia	Meat	≤10^6 CFU/cm^2 or g	≤10^3 CFU/cm^2 or g	Meat Standards Committee AS 4696:2007	Guideline
European Union	Carcasses (cattle, sheep, horses)	≤5.0 Log CFU/cm^2	≤2.5 Log CFU/cm^2	Commission Regulation (EC) No 2073/2005	Standard
	Carcasses (pigs)		≤3.0 Log CFU/cm^2		
	Meat (MSM)[1)]	≤5.0 × 10^6 CFU/g	≤500 CFU/g		
ICMSF[2)]	Meat	≤1.0 × 10^7 CFU/cm^2 or g	-	International Commission on Microbiological Specifications for Foods	Guideline
FAO[3)]	Meat	≤10^5 CFU/cm^2	≤10^3 CFU/cm^2	United Nations Food and Agriculture Organization	Guideline

[1)]Mechanically separated meat.
[2)]International Commission on Microbiological Specifications for Foods.
[3)]Food and Agriculture Organization of the United Nations.

Materials and Methods

Analysis of the microbiological contamination of raw beef in Korea from 2010-2014

The APC level of raw beef loin (*Longissimus dorsi* muscles) is monitored in meat packing centers and meat shops throughout Korea by the MFDS. To analyze the microbiological contamination levels of raw beef in Korea for five years (2010-2014), the APC results for raw beef were obtained from the MFDS. The results are expressed as the distribution of APC (CFU/g).

Analysis of microbiological contamination in raw beef in Korea in August 2015

To monitor the microbiological sanitation status of raw beef throughout Korea, raw beef loin samples (n=21) were collected from meat packing centers (n=3) and meat shops (n=18) in three regions of Korea (the Seoul/Gyeonggi, Gangwon, and Chungcheong regions) in July and August 2015. One meat packing center and six meat shops were randomly selected in each region, and meats on display were purchased. Beef samples were transferred to the laboratory under refrigerated conditions for microbiological analysis.

Each meat sample (10 g) was placed into a sterile Stomacher bag, and sterile water (90 mL) was added to make a 10^{-1} dilution. The contents were homogenized using a BagMixer 400 Stomacher (Interscience, France) for 2 min, and further diluted using sterile water. Each diluent (1 mL) was plated onto APC or *E. coli* Petrifilm (3M Microbiology, USA). The films were incubated aerobically at 37°C for 48 h. Blue colonies with bubbles were counted as *E. coli*.

Evaluating changes in beef loin quality during storage at 4°C

To evaluate changes in beef loin quality during storage, the pH, microbiological, and sensory characteristics were investigated. Hanwoo (grade 1) beef loins (*Longissimus dorsi* muscles) were purchased from the local meat packing center 24 h postmortem and sliced to 1.5 cm thickness. Each sample was wrapped with low-density polyethylene film and stored at 4 ± 2°C for 15 d. The oxygen transmission rate of low-density polyethylene film was 35,273 cc/m^2/24 h at 1 atmosphere, 0.01 mm of thickness.

The pH was measured using a pH meter (Orion 230A, Thermo Fisher Scientific, Inc., USA) after 10 g of beef sample was homogenized with 90 mL of distilled water for 30 s.

For microbiological analysis, beef loin (10 g) was homogenized with sterile water (90 mL) using a BagMixer 400 Stomacher (Interscience, France) for 2 min, and 1 mL of diluent was plated onto APC Petrifilm (3M Microbiology, USA), which was incubated aerobically at 37°C for 48 h.

A 15-member sensory panel conducted the sensory evaluation of raw beef. The panelists were asked to score the raw beef for color, aroma, drip loss, and overall acceptability. The color, aroma, and overall acceptability were scored using a 9-point scale system from extremely like (9 points) to extremely dislike (1 point). Drip loss was the amount of water extracted from the meat; it was expressed from very high (9 point) to very low (1 point).

Statistical analysis

Analysis of variance was used for statistical analysis using SAS software, version 9.4 (SAS Institute Inc., USA). Duncan's multiple range test was used to compare means, with a 5% level of significance ($p<0.05$). All analyses were repeated at least 3 times and are expressed as means ± standard error (SE).

Results and Discussion

Analysis of the microbiological contamination of raw beef in Korea from 2010-2014

The APC and *E. coli* levels of raw meat are important microorganism indicators, which ensure the safety of meat by detecting microbial growth during transportation, storage, and retail sale (Ahmad et al., 2013). To maintain sanitary conditions, many countries recommend or regulate that the APC level of meat be $\leq 10^5$-10^7 CFU/g and the *E. coli* level be $\leq 10^2$-10^4 CFU/g (Table 1). In Korea, the MFDS recommends that the levels for raw beef in meat packing centers and meat shops be $\leq 1.0 \times 10^7$ and 1.0×10^3 CFU/g for APC and *E. coli*, respectively (Notice 2014-135, MFDS).

To analyze the microbiological contamination level of raw beef in Korea from 2010-2014, APC results were obtained from the MFDS. The distribution of APC in raw beef in meat packing centers and meat shops is shown in Table 2. In meat packing centers, 2,796 raw beef samples were investigated, and the microbial levels of 2,761 (98.75%) samples were $\leq 1.0 \times 10^6$ CFU/g. In meat shops, 4,335 raw beef samples were investigated, and the microbial levels of 4,106 (94.72%) samples were $\leq 1.0 \times 10^6$ CFU/g. Two (0.07%) and 22 (0.51%) samples exceeded the APC guideline level in meat packing centers and meat shops, respectively. This suggests that less than 1% of the beef in both meat packing centers and meat shops was contaminated, indicating successful management of beef contamination between 2010 and 2014. The contamination level in meat shops was higher than in meat packing centers, consistent with a study by Park et al. (2002), which reported that *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* were detected at 2.9, 10.2, and 10.2%, respectively in beef samples from meat shop. *L. monocytogenes*, and *S. aureus* were detected at 2.8 and 2.8%, respectively in beef samples from meat packing center. Importantly, 80% of beef served to the consumer passes through a meat shop (Ko et al., 2013). Therefore, the microbial contamination of raw beef in meat shops requires more careful control.

Table 2. Aerobic plate counts of raw meat from meat packing centers and meat shops across the country over five years (2010-2014)

Place	Distribution of aerobic plate counts (CFU/g)						
	$\leq 10^3$	$10^3 - \leq 10^4$	$10^4 - \leq 10^5$	$10^5 - \leq 10^6$	$10^6 - \leq 10^7$	$>10^7$	Total
Meat packing center	1,222[1)] (43.71)[2)]	933 (33.37)	492 (17.60)	114 (4.08)	33 (1.18)	2 (0.07)	2,796 (100)
Meat shop	1,279 (29.50)	1,209 (27.89)	988 (22.79)	630 (14.53)	207 (4.78)	22 (0.51)	4,335 (100)

[1)]Number of samples.
[2)]Percentage in the total number of samples (%).

Analysis of microbiological contamination in raw beef in Korea in August 2015

APC levels of raw beef from three regions of Korea were monitored in August 2015. The mean APC values of beef in meat packing center from the Seoul/Gyeonggi, Gangwon, and Chungcheong regions were 5.9×10^2, 4.4×10^3, and 1.0×10^2 CFU/g, respectively (Table 3). The mean values of *E. coli* of beef from meat packing centers in the Gangwon and Chungcheong regions were 6.3×10^1 and 3.0 CFU/g, respectively. No *E. coli* was detected in beef from meat packing centers in the Seoul/Gyeonggi region.

Table 3. Aerobic plate count and *E. coli* content of raw beef loin from meat packing centers in three regions in Korea (CFU/g)

Region	Aerobic plate count			*E. coli* content		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Seoul/Gyeonggi	5.9×10^2	5.8×10^2	6.0×10^3	ND[1)]	ND	ND
Gangwon	4.4×10^3	4.0×10^3	5.2×10^3	6.3×10^1	5.0×10^1	8.0×10^1
Chungcheong	1.0×10^2	5.0×10^1	1.3×10^2	3.0	ND	1.0×10^1

[1)]ND: Not detected.

APC and *E. coli* levels in raw beef from six meat shops are presented in Table 4. Microbial contamination can occur during transportation, storage, and treatment at meat shops (Ahmad et al., 2013). The maximum APC value detected in a meat shop was 1.2×10^7 CFU/g, and the minimum was 5.2×10^3 CFU/g. Only meat shop B in the Gangwon region exceeded the APC guideline level. *E. coli* only detected in three shops, at approximately 1.0×10^1 CFU/g.

According to previous studies, raw beef in Seoul, Korea had reported mean APC and *E. coli* values of approximately 1.0×10^5 and 1.0×10^1 CFU/g (Kim et al., 2005; Ko et al., 2013). According to microbial assessment performed in Korea from February-June 2007, 50% of beef samples showed 10^4-10^5 CFU/g of APC (Lee et al., 2007). However, in this study, only 16.6% of samples had APC levels of 10^4-10^5 CFU/g, and 66.7% displayed levels $\geq 1.0 \times 10^5$ CFU/g. Our results suggest that beef samples collected in 2015 had higher contamination levels compared to previous microbial assessments performed in Korea. This indicates that enhanced regulations are required to ensure the distribution of hygienic meat to the consumer.

Table 4. Aerobic plate count and *E. coli* content of raw beef loin from meat shops in three regions in Korea (CFU/g)

Region	Market place	Aerobic plate count			*E. coli* content		
		Mean	Minimum	Maximum	Mean	Minimum	Maximum
Seoul /Gyeonggi	A[1)]	4.2×10^6	4.0×10^6	4.4×10^6	ND[2)]	ND	ND
	B	8.3×10^3	7.5×10^3	9.4×10^3	ND	ND	ND
	C	1.8×10^5	8.5×10^4	3.0×10^5	1.3×10^1	ND	3.0×10^1
	D	1.0×10^7	1.0×10^7	1.1×10^7	ND	ND	ND
	E	1.3×10^5	7.6×10^4	2.2×10^5	ND	ND	ND
	F	6.8×10^5	3.1×10^5	9.6×10^5	ND	ND	ND
Gangwon	A	1.1×10^6	5.6×10^5	1.5×10^6	2.7×10^1	2.0×10^1	3.0×10^1
	B	1.2×10^7	7.7×10^6	1.4×10^7	ND	ND	ND
	C	1.1×10^5	6.4×10^4	1.9×10^5	3.0	ND	1.0×10^1
	D	1.6×10^4	1.1×10^4	2.3×10^4	ND	ND	ND
	E	1.3×10^6	1.1×10^6	1.5×10^6	ND	ND	ND
	F	2.8×10^4	1.5×10^4	4.1×10^4	ND	ND	ND
Chungcheong	A	1.1×10^6	5.8×10^5	1.6×10^6	ND	ND	ND
	B	7.5×10^3	5.5×10^3	7.4×10^3	ND	ND	ND
	C	6.1×10^5	5.3×10^5	6.8×10^5	ND	ND	ND
	D	5.2×10^3	3.8×10^3	7.2×10^3	ND	ND	ND
	E	2.2×10^4	1.9×10^4	2.5×10^4	ND	ND	ND
	F	4.5×10^5	4.1×10^5	4.7×10^5	ND	ND	ND

[1)]A: Supermarket, B-F: Butcher's shops.
[2)]ND: Not detected.

Evaluating changes in beef loin quality during storage at 4°C

Changes in beef quality and microbial levels were measured during cold storage to evaluate the practical APC level for beef. Table 5 shows the changes in pH, microbiological, and sensory evaluation of beef loin samples during storage for 15 days at 4°C. The pH of fresh meat is usually 5.5-5.8, and increases to 8.0 during meat spoilage (James, 1972; Shin et al., 2006). In Korea, meat is considered spoiled at pH values > 6.2 (Notice 2015-13, MFDS). The initial pH value of the beef loin was 5.63, and it had increased significantly by day 15 ($p<0.05$), exceeding 6.0 on day 14. This is consistent with previous studies (Kook and Kim, 2005; Shin et al., 2006). Increased pH is correlated with increases in microorganisms, and is accompanied by spoilage (Sutherland et al., 1975). Also it is related to the formation and accumulation of amine and ammonia (Maijala et al., 1993).

The initial APC of beef loin was 3.0 Log CFU/g (1.2×10^3 CFU/g), and it significantly increased through the storage period ($p<0.05$). Meat starts to decay at an APC of 6-7 Log CFU/g and meat with APC values of 8-9 Log CFU/g are not edible (Shin et al., 2006). In this study, at day 13, the APC of beef loin was 7.3 Log CFU/g (2.1×10^7 CFU/g), exceeding the guideline level (1.0×10^7 CFU/g) and it had started to decay.

The color, aroma, drip loss, and overall acceptability of the beef loin during cold storage were evaluated, and if the color, aroma, and overall acceptability score was below 3, the meat was considered inedible. Consumers choose beef through evaluation of its sensory traits, which are used to determine meat quality as well as healthiness (Verbeke and Viaene, 1999). As the storage period increased, the color, aroma, and overall acceptability score of the beef loin decreased ($p<0.05$). Drip loss did not show significant differences during storage. At day 11, the color and overall acceptability score were under 3 and aroma was 3.6. This means that the panels recognized the beef loin stored for 11 days was not acceptable for eating. Most international APC guidelines for raw beef are approximately 1.0×10^6 CFU/g (Table 1). Only Korea and the International Commission on Microbiological Specifications for Foods (ICMSF) recommend levels up to 1.0×10^7 CFU/g. This guideline was established in 2007 in Korea, and in 1978 by the ICMSF. Therefore, verification of the Korean APC guideline level for beef is needed. The APC level is highly correlated with the sensory qualities of meat. In this study, the APC level at day 11 was 6.7 Log CFU/g (4.7×10^6 CFU/g). Although this was below the APC guideline for beef in Korea, the sensory panel regarded it as unacceptable for eating. To support our results, Moon et al. (2013) reported that APC (R = −0.902, $p<0.01$) and sensory quality (R = −0.907, $p<0.01$) were highly correlated with the freshness of raw beef. Tang et al. (2013) revealed that an APC level of 1.0×10^7 CFU/g corresponded with spoilage and growth of APC on chilled pork, and this correlated closely with sensory changes during storage. Shin et al. (2006) detected meat spoilage when the pH was >6.0 and the APC was 6.8 Log CFU/g, and at that time, the sensory panel regarded the meat as unacceptable. Therefore, an APC value of 1.0×10^7 CFU/g is not adequate to indicate the freshness of raw beef. We suggest that this guideline be lowered to 1.0×10^6 CFU/g.

Table 5. Changes in pH, microbiological and sensory quality characteristics of raw beef loin (grade 1) during storage at 4°C

Trait		Storage (days)									
		1	3	6	9	10	11	12	13	14	15
pH		5.63 ± 0.015^{d}	5.63 ± 0.013^{d}	5.62 ± 0.006^{d}	5.66 ± 0.009^{d}	5.82 ± 0.006^{c}	5.82 ± 0.010^{c}	5.80 ± 0.012^{c}	5.84 ± 0.032^{c}	6.10 ± 0.027^{b}	6.32 ± 0.009^{a}
Aerobic plate count (Log CFU/g)		3.01 ± 0.188^{h}	3.55 ± 0.054^{g}	3.77 ± 0.019^{g}	5.94 ± 0.017^{f}	6.21 ± 0.117^{e}	6.67 ± 0.100^{d}	6.90 ± 0.022^{d}	7.33 ± 0.019^{c}	7.93 ± 0.075^{b}	8.25 ± 0.025^{a}
Sensory evaluation	Color	7.67 ± 0.289^{a}	7.78 ± 0.147^{a}	6.78 ± 0.222^{b}	5.00 ± 0.167^{c}	3.67 ± 0.289^{d}	2.89 ± 0.309^{e}	2.56 ± 0.294^{e}	2.78 ± 0.324^{e}	2.67 ± 0.289^{e}	1.56 ± 0.294^{f}
	Aroma	8.44 ± 0.176^{a}	7.33 ± 0.333^{b}	6.78 ± 0.278^{b}	5.78 ± 0.324^{c}	4.11 ± 0.389^{d}	3.56 ± 0.377^{d}	2.67 ± 0.167^{e}	2.22 ± 0.401^{e}	2.44 ± 0.294^{e}	1.22 ± 0.147^{f}
	Drip loss	1.67 ± 0.236^{a}	1.78 ± 0.222^{a}	1.56 ± 0.242^{a}	1.78 ± 0.278^{a}	1.67 ± 0.289^{a}	1.44 ± 0.242^{a}	1.56 ± 0.242^{a}	1.78 ± 0.324^{a}	1.78 ± 0.278^{a}	1.67 ± 0.289^{a}
	Overall acceptability	7.89 ± 0.261^{a}	7.67 ± 0.167^{a}	6.78 ± 0.401^{b}	5.56 ± 0.176^{c}	3.67 ± 0.167^{d}	3.00 ± 0.236^{de}	2.78 ± 0.147^{e}	2.33 ± 0.289^{e}	2.44 ± 0.242^{e}	1.44 ± 0.242^{f}

$^{a-h}$Means ± SE with different letters within same row differ significantly at $p<0.05$.

Conclusions

The prevention of microbiological contamination of beef in Korea was quite successful for the five-year period encompassing 2010-2014. However, we discovered that more recent raw beef for retail sale displayed a higher level of APC contamination, suggesting that this requires stricter control. When quality changes in beef loin was evaluated for 15 days at 4°C, although the APC was less than the accepted level in Korea, sensory panels considered the beef unacceptable at day 11. The present APC guideline for raw beef is higher than in other advanced countries and corresponds to the level at which the sensory characteristics of beef can decrease dramatically. Therefore, we suggest that present APC guideline be lowered to ensure meat safety and palatability. Strengthening the APC guideline similarly to the guidelines of other countries will provide safe beef to consumers in the domestic market and help ensure the safe sale of Korean beef in foreign markets.

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