Microbiological Investigation of Ready-to-cook Pork *Bulgogi* on Korean Markets

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Abstract

In this study, ready-to-cook (RTC) pork *bulgogi* was investigated microbiologically to determine contamination levels. The investigation was conducted because of an increasing trend in the consumption of RTC meat products in Korea. Ninety marinated RTC pork *bulgogi* samples were collected from major retail outlets (M), department stores (D), and local markets (L) in Seoul, Korea from March to June 2011. This study examined total plate counts (TPC), *Escherichia coli*, and coliform bacterial counts, and the presence of *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7. The mean TPC values were 5.89, 6.08, and 5.89 Log CFU/g for M, D, and L, respectively. *E. coli* was not detected in any sample, but coliforms were present in 72 (80%) of the 90 samples collected. *B. cereus*, *E. coli* O157:H7, and *Salmonella* spp. were not detected; however, *S. aureus* and *L. monocytogenes* were detected in five (5.5%) and one (1.1%) of the 90 samples. Samples collected from M and D were contaminated with *S. aureus* and those from L with *L. monocytogenes*. These results demonstrate that the conditions under which RTC pork *bulgogi*s are handled and processed are unsanitary.

Key words: pork *Bulgogi*, ready-to-cook food, microbial safety, pathogens, *Staphylococcus aureus*, *Listeria monocytogenes*

Introduction

Pork *bulgogi* is a traditional Korean food. Thin slices of pork, usually arm picnic, shank, or ham, are marinated in a sauce containing soy sauce, onion, garlic, sesame oil and other seasonings. Pork *bulgogi* products can be contaminated with pathogenic bacteria during the handling or process if the raw materials used are contaminated or unhygienic conditions are applied. The ready-to-cook (RTC) product industry has expanded in Korea because of changes in customer consumption patterns. In Recent years, *bulgogi* is usually sold in markets as a RTC product. RTC foods, including *bulgogi*, are displayed open and exposed to contamination by bacterial pathogens; thus, several food-borne disease outbreaks have been associated with the consumption of contaminated RTC foods (Jo *et al.*, 2003). Consumers find RTC products very convenient because these products only require heating to prepare a meal. However, the safety of these prod-

ucts during distribution and sale is frequently not monitored, and RTC *bulgogi*, in particular, is exposed to a serious risk of contamination or spoilage by bacteria in the ingredients, such as vegetables, soy sauce, and raw meat (Beuchat, 1996; Nguyen-the and Carlin, 1994). The number of cases of food-borne disease caused by the consumption of contaminated RTC food has been increasing. In 2010, 271 cases of food poisoning were reported, and 7,218 cases of food poisoning have been reported in Korea. The most frequent causes of bacterial food poisoning in Korea are contamination with pathogens such as *Escherichia coli* (28 cases), *Salmonella* spp. (27 cases) and *Staphylococcus aureus* (19 cases) (KFDA, 2010a).

E. coli strains rarely cause disease, except certain strains involved in infections of the intestinal and urinary tracts of humans. Pathogenic *E. coli* can be divided into six pathotypes: enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), enteroinvasive (EIEC), and diffusely adherent (DAEC). Pathogenic *E. coli* has become a significant health concern especially *E. coli* O157:H7, having caused major outbreaks in food (Watterworth *et al.*, 2005).

S. aureus has been recognized as an indicator of food hygiene. *S. aureus* is a public health concern because of

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its ability to produce enterotoxins and grow in highly saline environments. In cured meats, such as cured ham, which have a high pH, the water activity (a_w) should be below 0.90 for preventing the production of staphylococcal enterotoxin (Gillespie, 2007; Gormley *et al.*, 2010).

Salmonella spp. are the most common pathogens associated with food-borne diseases, typhoid fever in humans, self-limiting enteritis, and fatal infections in animals. Red meat and poultry are good sources of *Salmonella*.

In addition, improper process and equipment could be associated with increasing the probability of cross contamination with *B. cereus* and *L. monocytogenes*. *B. cereus*, a facultative anaerobic, spore-forming, motile bacterium has been identified as the causative agent of 2 types of gastrointestinal diseases (Ankolekar *et al.*, 2009) and has been isolated from a wide variety of foods including spices (Choo *et al.*, 2007; Konuma *et al.*, 1988), ready-to-serve foods (Harmon and Kautter, 1991), and meat products (Smith *et al.*, 2004).

Listeria spp., particularly *Listeria monocytogenes* have been recognized as animal pathogens for over 70 years (Suihko *et al.*, 2002; Wesley, 1999). In the 1990s, *L. monocytogenes* was frequently isolated from all the major food products, such as unfermented dairy products (Ryser, 1999), cheese and meat products (Farber and Peterkin, 1999), and poultry and egg products. *L. monocytogenes* can survive in vacuum and gas-packed products and grow at refrigerated temperatures; this is a cause of concern for chilled meat products with an extended shelf-life.

The objective of this research was to evaluate the level of contamination of RTC meat products with pathogens on the major Korean markets, such as major retail outlets, department stores, and local markets. Pork *bulgogi* was chosen as the target RTC meat product and then the microbiological condition was investigated with the food hygiene indicator, such as TPC, *E. coli*, and coliforms as well as the presence of pathogens, such as *B. cereus*, *E. coli* O157:H7, *S. aureus*, *Salmonella* spp. and *L. monocytogenes*.

Materials and Methods

Sample collection

In total, 90 marinated pork *bulgogi* samples were collected from i.e., 3 samples each from 10 different major retail outlets (M), department stores (D), and local markets (L) located in Seoul, respectively, from March to June 2011 (Table 1). The collected samples were placed in sterile bags, transferred by refrigerated transport to the

Table 1. Types of samples used in this study

Location	Number of places ¹⁾	Number of samples
Major retail outlet	10	30
Department store	10	30
Local market	10	30
Total	30	90

¹⁾3 samples were collected from each place.

laboratory, and processed within 24 h of collection.

Food hygiene indicator bacteria

Testing for indicator organisms (total plate counts, *E. coli*, and coliform bacterial counts) has been introduced as a simple means of assessing the hygiene status of foods and helps ensure food safety (Park, 2004). The experimental procedure in our study was developed on the basis of the Korea Food Code (KFDA, 2010b). A 1:10 dilution sample of pork *bulgogi* was prepared by adding 25 g of meat sample obtained from a location to 225 mL 0.1% (w/v) peptone water; and the solution was homogenized in a stomacher (IUL instruments, Germany) for 2 min. Total plated counts, and *E. coli* and coliform bacterial counts were quantitatively assessed by inoculating the diluted samples on tryptic soy agar (TSA) plates and petrifilms (3MTM), respectively, and incubating 35°C for 24 h.

Isolation and characterization of pathogens

B. cereus

Twenty-five grams of meat samples obtained from each location was added to 225 mL 0.1% peptone water and homogenized for 1 min. The mixed samples were inoculated on mannitol-egg yolk-polymyxin (MYP, Oxoid) agar and incubated at 35°C for 24 h. We isolated the pink-red mannitol-negative colonies with surrounding lecithinase-positive zones of precipitation streak-cultured the isolated colonies on TSA plated plates, and maintained these as pure cultures. The strains on the TSA plate cultures were then, analyzed biochemically by using API 50 CH strips (Biomérieux, France).

E. coli O157:H7

Twenty-five grams of samples obtained from each location was added to 225 mL modified *E. coli* (mEC) medium with novobiocin as the antimicrobial supplement and incubated at 35°C for 24 h. The mixture was homogenized in a stomacher and mixed thoroughly. Inoculum were streaked on MacConkey sorbitol agar without cefixime and tellurite plates and incubated at 35°C for 16-

18 h. The sorbitol-negative colonies were then subcultured on eosin methylene blue (EMB) agar plates. Distinct *E. coli* colonies were isolated and streaked onto TSA plates for further characterization. The colonies that showed a green metallic sheen on EMB agar, because of rapid lactose fermentation, were isolated and considered as those of *E. coli*. We performed O157 latex agglutination test (DR0620, Oxoid) (Stampi *et al.*, 2004) for confirming the presence of *E. coli* O157 strains.

S. aureus

Twenty-five grams of samples, obtained from each location was added to 225 mL tryptic soy broth (TSB) with 10% NaCl and enriched at 35°C for 24 h and then inoculated on Baird-Parker agar plates enriched with egg yolk (EY) tellurite. The plates were incubated under aerobic conditions at 35°C for 24 h. Typical black and shiny convex-shaped colonies of *S. aureus* surrounded by clear zones of approximately 2-5 mm were isolated and cultured on TSA plates for further identification (Normanno *et al.*, 2005). The presumed *S. aureus* colonies were examined by Gram staining and catalase test. Gram- and catalase-positive isolates were further identified using a latex agglutination kit (PastorexTM Staph-Plus; Bio-Rad) and API Staph System (Biomérieux).

Salmonella spp.

Twenty-five grams of meat samples obtained from each location was added to 225 mL buffered peptone water (BPW), incubated at 35°C for 24 h, and homogenized for 1 min in a stomacher. After pre-enrichment, 0.1 mL of the culture solution was added to 10 mL Rappaport-Vassiliadis (RV) broth. The RV broth was then incubated at 42°C for 18 h. Loopfuls of the broth were streaked on *Salmonella-Shigella* (SS) agar and xylose lysine deoxycholate (XLD) agar plates. Red colonies with black centers on XLD agar were presumed as colonies of *Salmonella* spp. and were isolated and inoculated on TSA plates. Subsequently, the colonies were inoculated and streaked on triple sugar iron (TSI) slant. Because the inoculums could not ferment glucose and lactose, further study for gram staining, urease test and lysine decarboxylase test were not conducted.

L. monocytogenes

Twenty-five grams of meat samples obtained from each location was added to 225 mL Listeria enrichment broth (LEB) and incubated at 35°C for 18-24 h. After incubation, the broth was homogenized and inocula were

streaked on modified Oxford (MOX) agar plates with antimicrobial supplements and incubated at 37°C for 48 h. The presumed colonies of *L. monocytogenes* were further confirmed and characterized. Morphologically typical colonies of *L. monocytogenes* were verified by Gram staining, catalase reaction, motility test, hemolysis on 5% sheep blood agar, CAMP test with *S. aureus* ATCC 25923, and fermentation of sugars by API Listeria (Biomérieux) (Chaudhari *et al.*, 2004).

Statistical analysis

Analysis of variance (ANOVA) was performed on all data with the general linear model (GLM) procedure by using the program Statistical Analysis System (SAS version 9.1). The bacterial total plate counts in colony forming units (CFU)/g were transformed to logarithmic values (Log CFU/g) for statistical analyses. Mean values were determined using Tukey's studentized range (HSD) tests.

Results and Discussion

Distribution of food hygiene indicators in RTC pork *bulgogi*

Detecting pathogenic bacteria in food is very important, but this involves a relatively complex procedure. Therefore, testing for indicator organism has been introduced as a simple means of assessing the hygiene status of food samples and helps ensure food safety. We assessed the hygiene status of pork *bulgogi* from major retail outlets, department stores, and local markets by collecting and analyzing 90 *bulgogi* samples (30 samples from each type of location).

Table 2 shows the microbial distribution in samples obtained from major retail outlets. The mean value of total plate counts for 3 samples obtained at a particular place (total, 10 places) in each of the location groups ranged from 4.59 to 7.07 Log CFU/g. Total coliforms detected in samples obtained from 6 out of 10 major retail outlets ranged from 1.00 to 4.09 Log CFU/g. We observed similar coliform detection rates in samples obtained from department stores and local markets. Samples obtained from M3 (major retail outlets, place 3) showed the lowest total plate count and coliforms were not detected. However, samples obtained from M6 and M10 showed high total plate counts, even though the coliforms detected were 1.00 and 2.84 Log CFU/g, respectively. Samples obtained from the 10 department stores showed total plate counts and coliform counts ranging from 4.47 to 7.58 Log CFU/g and 2.23 to 4.25 Log CFU/g, respectively.

Table 2. Distribution of total plate counts, coliform, and *E. coli* counts in marinated meat from major retail outlets

Samples ¹⁾	Microbial count ²⁾		
	TPC ³⁾	Coliform	<i>E. coli</i>
M1	5.57±0.04 ^{bc}	2.51±0.30 ^b	ND ⁴⁾
M2	5.19±0.16 ^c	1.38±1.08 ^c	ND
M3	4.59±0.27 ^d	ND ^d	ND
M4	5.97±0.10 ^b	1.30±1.42 ^c	ND
M5	5.80±0.11 ^b	4.09±0.05 ^a	ND
M6	7.07±0.54 ^a	1.00±1.10 ^a	ND
M7	6.84±0.09 ^a	ND ^d	ND
M8	5.28±0.15 ^c	ND ^d	ND
M9	5.57±0.23 ^{bc}	ND ^d	ND
M10	7.00±0.16 ^a	2.84±0.24 ^b	ND

¹⁾M: major retail outlet²⁾Mean±SD (Log CFU/g) of samples³⁾TPC: total plate counts⁴⁾ND: not detected^{a-d}Means with different superscript within same row are significantly different ($p>0.05$).

Samples obtained from L1 to L10 showed the mean values of total plate counts and coliforms ranging from 4.57 to 7.34 and 2.13 to 4.15 Log CFU/g, respectively. *E. coli* were not detected in any samples. The total plate counts for most of the samples in our study were higher than that for raw meat. In the previous study, the counts of mesophilic microorganisms in raw pork meat ranged from 3.9×10^2 to 3.9×10^5 CFU/g, and coliforms were not detected. The average number of aerobic mesophilic bacteria of marinated meat product was 2.3×10^8 CFU/g, and the highest bacterial average was 3.1×10^8 (Björkroth, 2005). However, the study by Kwak *et al.* (1998) on pork *bulgogi* seasoning showed a high mesophilic total plate count of 5.97 Log CFU/g and a mean value of coliforms (logarithmic value for most probable number [MPN]/g) of 2.65 Log MPN/g. This result suggest that garlic, onion, soy sauce, and other ingredients in the seasoning used for pork *bulgogi* should have been the source of contamination and increased bacterial count.

The hygienic and sanitation level of 2 major retail outlets (M6 and M10), one department store (D6), and one local market was found to be low and should be improved. The hygiene status of marinated meat as observed by monitoring the indicator microorganisms, was significantly different among individual stores in each group ($p<0.05$), even though the total plate counts and coliform counts were almost similar among the groups ($p>0.05$).

Isolation and characterization of pathogens

Table 3. Distribution of total plate counts, coliform, and *E. coli* counts in marinated meat from department stores

Samples ¹⁾	Microbial count ²⁾		
	TPC ³⁾	Coliform	<i>E. coli</i>
D1	6.90±0.05 ^b	3.62±0.05 ^{abcd}	ND ⁴⁾
D2	5.83±0.15 ^c	ND ^f	ND
D3	5.59±0.20 ^c	3.25±1.02 ^{cd}	ND
D4	6.50±0.32 ^b	3.44±0.40 ^{bcd}	ND
D5	4.64±0.43 ^d	3.87±0.20 ^{abc}	ND
D6	7.58±0.08 ^a	3.13±0.21 ^d	ND
D7	4.47±0.07 ^d	4.25±0.07 ^a	ND
D8	5.91±0.24 ^c	4.13±0.06 ^{ab}	ND
D9	6.58±0.10 ^b	2.28±0.17 ^e	ND
D10	6.83±0.06 ^b	3.30±0.13 ^{cd}	ND

¹⁾D: department store²⁾Mean±SD (Log CFU/g) of samples³⁾TPC: total plate counts⁴⁾ND: not detected^{a-f}Means with different superscript within same row are significantly different ($p>0.05$).**Table 4. Distribution of total plate counts, coliform, and *E. coli* counts in marinated meat from local markets**

Samples ¹⁾	Microbial count ²⁾		
	TPC ³⁾	Coliform	<i>E. coli</i>
L1	6.12±0.17 ^{bc}	3.53±0.08 ^d	ND ⁴⁾
L2	5.62±0.31 ^d	3.90±0.13 ^{abc}	ND
L3	4.57±0.13 ^c	4.15±0.08 ^a	ND
L4	4.87±0.09 ^c	2.13±0.32 ^f	ND
L5	6.14±0.17 ^{bc}	ND ^g	ND
L6	5.69±0.09 ^d	2.26±0.22 ^f	ND
L7	6.09±0.04 ^c	3.03±0.26 ^c	ND
L8	6.49±0.11 ^b	4.08±0.05 ^{ab}	ND
L9	7.34±0.32 ^a	3.79±0.22 ^{bcd}	ND
L10	6.16±0.02 ^{bc}	3.61±0.14 ^{cd}	ND

¹⁾L: local market²⁾Mean±SD (Log CFU/g) of samples³⁾TPC: total plate counts⁴⁾ND: not detected^{a-g}Means with different superscript within same row are significantly different ($p>0.05$).

We evaluated the prevalence of selected bacterial pathogens (*B. cereus*, *E. coli* O157:H7, *S. aureus*, *Salmonella* spp., and *L. monocytogenes*) in marinated pork *bulgogi* collected from different types of retail shops located in Seoul, Korea. *B. cereus*, *E. coli* O157:H7, and *Salmonella* spp. were not detected in any samples (Table 5). We had two pink-red colonies with lecithinase-positive zones on the MYP agar, which were guessed as *B. cereus*, however, they were finally identified as *B. subtilis* according to their biochemical characterization found using API 50 CH strips.

Table 5. Prevalence of food borne pathogens in marinated pork *bulgogi*

Samples	Food borne pathogens				
	<i>B. cereus</i>	<i>E. coli</i> O157:H7	<i>S. aureus</i>	<i>Salmonella</i> spp.	<i>L. monocytogenes</i>
Major retail outlet	ND ¹⁾	ND	4/30 (13.3%) ²⁾	ND	ND
Department store	ND	ND	1/30 (3.3%)	ND	ND
Local market	ND	ND	ND	ND	1/30 (3.3%)
Total	ND	ND	5/90 (5.6%)	ND	1/90 (1.1%)

¹⁾ND: not detected²⁾Number of detected sample/total sample (%)

Three colonies that showed a green metallic sheen on EMB agar were isolated and investigated for their O157 latex agglutination ability for the purpose of evaluating the presence of *E. coli* O157 strains. According to their incompetence of agglutination, the isolates were finally concluded not to be *E. coli* O157:H7.

The presumed colonies on XLD agar were isolated and inoculated on triple sugar iron (TSI) slants. After incubation, it was confirmed that these bacteria could metabolize glucose and lactose. The presence of these characteristics confirmed that the inoculums were not *Salmonella* spp. A previous study had reported a high prevalence rate (20%) of *Salmonella* spp. in red meat from butcher shops in Gaborone, Botswana, which was attributed to inadequate temperature control, improper handling of raw meat, and inadequate environmental conditions (Mrema *et al.*, 2006). However, no *Salmonella* spp. was isolated from pork *bulgogi* in our study.

Examining 90 pork *bulgogi* samples, 5 samples (5.6%) tested were found to be positive for *S. aureus*. Four (13.3% of 30) of them were obtained from major retail

outlets, and the remaining one (3.3% of 30) was obtained from a department store; none were detected in samples from local markets. In the previous studies, *S. aureus* has been isolated from several foods, including meat and meat products, chicken, milk and dairy products, fermented food items, vegetables, and fish products (Wieneke *et al.*, 1993; Tamarapu *et al.*, 2001). Salted food products, such as ham, have been reported to be responsible for about 24% of all cases of *S. aureus* food poisoning (Qi and Miller, 2000). Comparing the results of *S. aureus* in this study with those of other studies, the prevalence of *S. aureus* detected is very highly regarded. Normanno *et al.* (2005) suggested that coagulase-positive staphylococci and *S. aureus* were the most predominantly detected bacteria in various kind food products, e.g., 26.1% from fresh meat and 48.1% from other meat products.

Characterization of isolated pathogens

Tables 6 and 7 show the characteristics of isolated *S. aureus* and *L. monocytogenes*, respectively. Samples showing the presence of *S. aureus* were positive for coagulase,

Table 6. Morphological and biochemical characteristics of *Staphylococcus aureus* isolated from marinated pork *bulgogi*

Characteristics	Results	Characteristics	Results
Gram stain	+ ¹⁾	D-Glucose	+
Shape	Irregular clusters	D-Fructose	+
Colony pigment (carotenoid)	+	D-Mannose	+
Egg-yolk lecithinase	+	D-Maltose	+
Catalase	+	D-Lactose	+
Aerobic growth	+	D-Trehalose	+
Growth on 10% (w/v) NaCl broth	+	D-Mannitol	+
Coagulase	+	Xylitol	- ²⁾
Hemolysis	+	D-Melibiose	-
Potassium nitrate	+	D-Raffinose	-
β-Naphthyl phosphate	+	D-Xylose	-
Sodium pyruvate (VP)	+	D-Saccharose	+
L-Arginine	+	Methyl-α-D-Glucopyranoside	-
Urease	+	N-Acetyl-Glucosamine	+
Acidification (aerobically)			

¹⁾Positive²⁾Negative

Table 7. Morphological and biochemical characteristics of *Listeria monocytogenes* isolated from marinated pork bulgogi

Characteristics	Results
Gram stain	+ ¹⁾
Shape	Rod
Catalase	+
Motility	+
β-Hemolysis	+
Differentiation <i>L. innocua</i> / <i>L. monocytogenes</i>	- ²⁾
Esculin hydrolysis	+
α-Mannosidase	+
Acidification	
D-Arabitol	+
D-Xylose	-
L-Rhamnose	+
Methyl-α-D-glucopyranoside	+
D-Ribose	-
Flucose-1-phosphate	-
D-Tagatose	-

¹⁾Positive

²⁾Negative

catalase, and hemolysis tests and negative for fermentation of xylitol, D-melibiose, D-raffinose, D-xylose, and methyl-α-D-glucopyranoside (Table 6). *L. monocytogenes* isolates hydrolyzed esculin on MOX agar and were positive for motility, α-hemolysis, and CAMP tests. The isolates were identified with 98.6% accuracy on the basis of the results of the fermentation of sugars detected by using the API kit (Table 7). The pathogens were finally confirmed by 16S rRNA gene sequencing and polymerase chain reaction (data not shown).

According to the Korea Livestock Products Sanitary Control Act, *E. coli* O157:H7 should not be detected in marinated meat. However, there are no specific standards for detecting food-borne pathogens in marinated meat. Therefore, microbial risk assessment should be strictly regulated and performed by evaluating the data scientifically.

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