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RESEARCH NOTE

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Enterobacteriaceae and Related Microorganisms Isolated from Rump of Raw Beefs

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Abstract In this study, 50 rump samples of raw beef obtained from local Korean supermarkets were analyzed to survey microbial distributions. As results, mesophilic microorganisms ranged from $(1.4\pm0.01)\times10^2$ to $(1.6\pm0.05)\times10^5$ CFU/g, and total coliforms ranged from 0 to $(1.3\pm0.04)\times10^4$ CFU/g. Major foodborne pathogens, including *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* spp., were not found among the samples. However, *Staphylococcus aureus* was isolated with 4% frequency. Other isolated microorganisms included *Enterobacter amnigenus* (4%), *Enterobacter cloacae* (24%), *E. coli* (24%), *Listeria innocua* (8%), *Staphylococcus saprophyticus* (56%), *Staphylococcus xylosus* (10%), and *Staphylococcus warneri* (8%).

Keywords: raw beef, rump, Enterobacteriaceae, foodborne pathogen, microorganism

Introduction

Raw beef itself is a good nutritional source for microorganisms and is highly susceptible to contamination by various microorganisms, including food poisoning bacteria. Among the food vehicles of food poisoning, poultry and red meat were reported as the most common for food poisoning outbreaks in England and Wales between 1992 and 2003 (1). For cases in Korea and Japan from 1971 to 1990, seafood was the most common vehicle, followed by meat and animal products (2). Recently, food poisoning incidents are occurring on a large scale and year-round regardless of season (3). According to statistics from the Korea Food & Drug Administration, the number of patients who suffered foodborne illness in 2004 was 10,388, and in 2005 it was 5,711 (3). Although patient numbers decreased in 2005, in July of that year, 4 consecutive food poisoning outbreaks occurred over the course of a few days (4). The principle factors for these outbreaks were associated with the consumption of raw foods such as *yukae*, shrimp, minced fish, and foods that were stored under abused conditions (4).

According to the Korean Food Standards Codex published by the Korea Food Industry Association (5), pathogenic bacteria such as *Salmonella* spp., *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Clostridium perfringens*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Bacillus cereus*, and *Yersinia enterocolitica* should not be isolated in foods. Likewise, in other countries, these microorganisms are under strict regulation and monitoring. In a study by Reid *et al.* (6), the authors reported 3.3% incidence of *E. coli* O157 and 2.2% incidence of *Salmonella* spp. in the rump area of slaughtered bovine; the authors indicated this area is at risk for cross-contamination during the dehiding process. Furthermore, Elder *et al.* (7) reported that beef cattle are likely to be contaminated by tools, equipment, air, and water during processing.

Seasoned raw meat is a distinct dish in Korea's food culture. A variety of raw meat dishes are made of beef, pheasant, fish, etc (8). Because heating process is not involved in cooking seasoned raw meat dishes, the dishes are likely to harbor various microorganisms. The objective of this study was to evaluate microbial distributions in the rump of beef, which is mostly used in *yukae* preparation. Using rump portions of raw beef, we determined mesophilic microorganism and coliform distributions, and also assessed for the presence of pathogenic bacteria, including *Salmonella* spp., *S. aureus*, *E. coli* O157:H7, and *L. monocytogenes*.

Materials and Methods

Sample The samples consisted of the rump area of first-grade Korean beef purchased from local supermarkets in the Daegu area and Gyeongsangbuk-do province. Immediately after collection, the 50 samples were transferred in an ice box and analyzed within 5 hr from purchase.

Analysis of mesophilic microorganisms and coliforms Each 10 g sample was taken aseptically and homogenized in a blender for 1 min in 90 mL of sterilized diluent (NaCl 0.85 g, peptone 0.1 g, KH_2PO_4 0.03 g, and Na_2HPO_4 0.06 g in 100 mL of distilled water), which was diluted to 1:1,000 using the serial dilution method. Using the pour plate method (9), a 0.1 mL amount of each diluted solution was incubated on plate count agar (PCA, Becton, Dickinson and Company, Sparks, MD, USA) for mesophilic microorganisms and on desoxycholate lactose agar (Becton, Dickinson and Company) plates for coliforms. The plates were incubated for 24 hr at 35°C. The colonies formed on the plates were counted and expressed as colony-forming units (CFU)/g of sample.

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Isolation of *Salmonella* spp. The isolation of *Salmonella* spp. was carried out using 2 enrichment steps (9). First, 10 g of each sample was aseptically taken, homogenized with a blender for 1 min in 90 mL of peptone water (peptone 10 g and NaCl 5 g in 1 L of distilled water), and incubated for 18 hr at 35°C. Following incubation, 0.1 mL of the culture was transferred into 10 mL of Rappaport Vassiliadis broth (Merck, Darmastadt, Germany) for a second enrichment, and then incubated for 24 hr at 42°C. One loop of the second enrichment broth was spread onto MacConkey agar (Becton, Dickinson and Company) plates and incubated for 24 hr at 35°C. Typical colorless lactose-nonfermenting colonies were transferred to triple sugar iron agar (TSI, Becton, Dickinson and Company) slants and incubated at 35°C for 24 hr. On these TSI agar slants, the colonies showing red slants and yellow butts with or without blackening were further identified by Gram-staining and an API 20E kit (bioMerieux, Marcy I'Etoile, France).

Isolation of *S. aureus* Tryptic soy broth (TSB, Becton, Dickinson and Company) with 10% NaCl was used for the enrichment of *S. aureus* (9). Ten g samples were aseptically taken and homogenized with a blender for 1 min in 90 mL of TSB with 10% NaCl. After 16-18 hr of incubation at 35-37°C, one loop of sample was transferred onto mannitol-salt-egg yolk (MSEY) agar (Becton, Dickinson and Company) plates and incubated for another 24 hr at 37°C. Yellow colonies, which were surrounded by an opaque zone due to the egg yolk reaction (+) and a yellow zone on the background due to the acid produced from mannitol fermentation, were identified using Gram-staining, catalase and oxidase tests, a coagulase test, and an API Staph kit (bioMerieux).

Isolation of *E. coli* O157:H7 Ten g samples were aseptically taken, homogenized with a blender for 1 min in 90 mL of modified EC with novobiocin (Merck), and incubated for 24 hr at 35°C (9). One loop of the enrichment broth was streaked onto sorbitol MacConkey (SMAC) agar (Becton, Dickinson and Company) plates and incubated for 24 hr at 35°C. Typical colorless colonies on the SMAC agar plates were transferred to eosin methylene blue agar (EMB, Becton, Dickinson and Company) plates and incubated for 24 hr at 35°C. On the EMB agar plates, blue-black colonies with a green metallic sheen were selected for *E. coli* O157:H7 confirmation. Gram-staining, O157 and H7 antisera (Becton, Dickinson and Company), and an API 20E kit (bioMerieux) were used for confirmation.

Isolation of *L. monocytogenes* The isolation of *L. monocytogenes* was carried out using 2 enrichment steps (9). First, 10 g of each sample was aseptically taken, homogenized with a blender for 1 min in 90 mL of UVM modified *Listeria* enrichment broth (Becton, Dickinson and Company), and incubated for 24 hr at 30°C. Then, 0.1 mL of enrichment broth was transferred into 9 mL of Fraser broth (Becton, Dickinson and Company) for the second enrichment, and incubated for 48 hr at 30°C. One loop of the Fraser broth was streaked onto Oxford agar (Becton, Dickinson and Company) plates. After 48 hr of incubation at 30°C, the plates were examined for typical *Listeria* colonies, represented as green colonies with a black zone. Suspect colonies were further identified using Gram-staining, catalase and oxidase tests, and an API Listeria kit (bioMerieux).

Results and Discussion

Distribution of mesophilic microorganisms and coliforms in raw beef The numbers of microorganisms associated with a food may be used to judge its microbiological safety and quality (10). For the raw beef analyzed in this study, mesophilic microorganisms ranged from $(1.4\pm0.01)\times10^2$ to $(1.6\pm0.05)\times10^5$ CFU/g (Table 1). The coliforms are used as an indicator of fecal contamination and include diverse genera and species (11). Here, total coliforms ranged from 0 to $(1.3\pm0.04)\times10^4$ CFU/g (Table 1). In raw beef for preparing *jangzorim*, Kim *et al.* (12) reported that the distribution of mesophilic microorganisms was 2.3×10 to 8.0×10^4 CFU/g, and no coliforms were isolated. In commercial meat, Lee and Park (13) reported that the distributions for mesophilic microorganisms and coliforms were 1.3×10^2 and 5.2×10^2 CFU/g, respectively. Waje *et al.* (14) reported the total bacteria count of 4.0×10^3 CFU/g and the coliforms count less than 10 CFU/g in beef. According to our microbial counts for mesophilic microorganisms and total coliforms, the results show ranges that are similar to other reports.

Isolation of *Salmonella* spp. Salmonellosis, caused by *Salmonella* spp., results in mild or acute gastrointestinal symptoms (15). *Salmonella* spp. were the most commonly implicated organisms in the England and Wales outbreaks from 1992-2003 (1). The majority of these *Salmonella* outbreaks were linked to the consumption of red meat and poultry (1). In Korea, the percentage of food poisoning from *Salmonella* spp. was 23.1% from 1971-1990, and 12.6-32.1% from 2000-2005 (2,3); seafoods and meats were the major food vehicles for outbreaks during 1971-1990 (2).

To isolate *Salmonella* spp., MacConkey agar plates and TSI slants were used. Suspect colonies of *Salmonella* spp. appear as colorless colonies on MacConkey agar because lactose, the sole carbohydrate, is not fermented (16). For TSI, lactose, sucrose, and glucose are present in a ratio of 10:10:1. Suspect colonies on TSI agar appear as red slants and yellow butts because the small amounts of acid produced by glucose fermentation are rapidly oxidized on the slant (16,17). Similarly, in this study, the isolated colonies appeared colorless on MacConkey agar, and on the TSI agar slants they appeared as yellow or red slants and yellow butts and produced gas. The colonies were

Table 1. Microbial counts of mesophilic microorganisms and total coliforms

	Microbial counts[1)] (CFU/g)	
	Mesophilic microorganism	Total coliforms
Minimum	$(1.4\pm0.01)\times10^2$	0
Maximum	$(1.6\pm0.05)\times10^5$	$(1.3\pm0.04)\times10^4$

[1)]n=50; Each value is mean±SD of triplicates.

Table 2. Average incidence of isolated microorganisms other than major foodborne pathogens in rump of beefs

Isolated microorganism	Average incidence[1)] (%)
E. amnigenus	4
E. cloacae	24
E. coli	24
L. innocua	8
S. saprophyticus	56
S. xylosus	10
S. warneri	8

[1)]n=50.

Table 3. Average incidence of *Salmonella* spp., *E. coli* O157:H7, *S. aureus*, and *L. monocytogenes* in rump of beefs

Isolated microorganism	Average incidence[1)] (%)
Salmonella spp.	0
E. coli O157:H7	0
S. aureus	4
L. monocytogenes	0

[1)]n=50.

confirmed as *Enterobacter amnigenus* and *Enterobacter cloacae* using an API 20E kit.

The genus of *Enterobacter* is widely distributed in nature, occurring in fresh water, soil, plants, and animal and human feces (18). *E. cloacae* is a pathogen associated with diverse disease, including neoplastic diseases, diabetes mellitus, chronic renal failure, and gastric ulcers (19).

Isolation of *S. aureus* *S. aureus* infection causes enterocolitis and dehydration (20). In Korea from 2000 to 2005, the number of *S. aureus* cases comprised 6.7-14.7% of the total cases of bacterial food poisoning (3). It is reported that nearly all outbreaks of staphylococcal food poisoning result from the direct or indirect contact of humans (20).

On the yellow background of MSEY agar plates, typical *S. aureus* colonies are shown as yellow colonies surrounded by opaque zones, and exhibit catalase (+) and oxidase (-) activities (21). Likewise, in this study, the isolated colonies were yellow with a yellow zone on the MSEY agar plates, and exhibited catalase (+) and oxidase (-) activities. Using an API Staph kit, the isolated colonies were identified as *S. aureus*, *Staphylococcus saprophyticus*, *Staphylococcus xylosus*, and *Staphylococcus warneri*. Among the samples, *S. aureus*, a major foodborne pathogen, was isolated with 4% frequency (Table 3). This result is similar with the report of Lee *et al.* (22), reporting the isolation of *S. aureus* from *yukae*. In this testing of raw beef samples, *S. saprophyticus* presented the highest rate of occurrence, with an average incidence of 56% (Table 2). Kloos and Schleifer (23) reported that *S. saprophyticus* was isolated from urine, and widely distributed in air, soil, dust, dairy products, and on the surfaces of animal carcasses. Although *S. saprophyticus* is regarded as non-pathogenic, some strains can cause urinary tract infections (23). *S. xylosus*, which is regarded as a non-pathogen, can cause acute pyelonephritis (24). *S. warneri*, recognized as a human pathogen, is associated with endocarditis and bacteremia (25).

Isolation of *E. coli* O157:H7 In many countries, *E. coli* O157:H7 has been the main cause of enterohemorrhagic *E. coli*-associated disease, causing bloody diarrhea, severe abdominal pain, haemolytic uraemic syndrome, and thrombotic thrombocytopaenic purpura (26).

Typical colonies of *E. coli* O157:H7 appear blue-black with a green metallic sheen on EMB agar plates (27). After overnight incubation on SMAC agar plates, colorless colonies appear because *E. coli* O157:H7 does not ferment sorbitol (28). In this study, isolated colonies were exhibited as violet colonies on SMAC agar plates and as blue-black colonies with a green metallic sheen on EMB agar plates. In subsequent agglutination tests, these colonies did not agglutinate with O157 and H7 antisera, and were isolated as *E. coli* with an API 20E kit (Table 2). *E. coli* was isolated with 24% frequency among the samples (Table 2). Because *E. coli* is the most widely used indicator of fecal contamination, its presence in raw beef indicates possible fecal contamination, as well as the potential presence of other enteric pathogens (11).

Isolation of *L. monocytogenes* Listeriosis is the disease caused by *L. monocytogenes* infection. The symptoms of infection include fever, severe headache, vomiting, and other influenza-type symptoms (29).

In this study, the isolated colonies were presented as typical *L. monocytogenes* bacteria, appearing as green colonies with black zones on Oxford agar plates, and having oxidase (-) and catalase (+) activities. However, the colonies were identified as *Listeria innocua* after testing with an API Listeria kit. *L. innocua* is non-pathogenic to humans and animals, and is isolated from soil, vegetables, bird droppings, and human and animal feces (30).

Based on these results, raw beef carries various microorganisms including hazardous microorganisms. It was not attempted to determine how the various isolated microorganisms infected the beef in this study. It is commonly regarded that (6,7,31) various procedures such as dehiding operations, slaughter, distribution, and handling influence carcass contamination, and contamination may occur through direct or indirect contact with personnel, tools, equipment, air, or water. Therefore, to ensure the microbial safety of raw meat, not only are hygienic production, distribution, and storage procedures necessary, but continuous monitoring for hazardous microorganisms is required.

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