Journal of Food Protection 87 (2024) 100220

Contents lists available at ScienceDirect

Journal of Food Protection

journal homepage: www.elsevier.com/locate/jfp

Research Paper

Levels of Indicator Bacteria and Characteristics of Foodborne Pathogens from Carcasses of Cattle Slaughterhouses in Korea

Serim Hong [1], Jin-San Moon [2], Soon-Seek Yoon [2], Ha-Young Kim [2,*], Young Ju Lee [1,*]

[1] College of Veterinary Medicine & Institute for Veterinary Biomedical Science, Kyungpook National University, Daegu, Republic of Korea
[2] Bacterial Disease Division, Animal and Plant Quarantine Agency, Gimcheon, Republic of Korea

ARTICLE INFO

Keywords:
Carcass
Cattle
Foodborne pathogen
Indicator bacteria
Slaughterhouse

ABSTRACT

The initial microbial contamination of carcasses during slaughtering adversely affects spoilage and shelf life and is of global concern for food safety and meat quality. This study evaluated the hygiene and quality using the prevalence of foodborne pathogens and the level of indicator bacteria on 200 carcasses, collecting 10 from each of 20 cattle slaughterhouses in Korea. The distribution of aerobic bacterial count in carcasses was significantly highest at 2.0–3.0 $\log_{10}$ CFU/cm^2 (34.1%), whereas the *Escherichia coli* count was significantly highest at under 1.0 $\log_{10}$ CFU/cm^2 (94.0%) ($P < 0.05$). *Clostridium perfringens* was most prevalent (60.0% of slaughterhouses; 17.5% of carcasses), followed by *Yersinia enterocolitica* (30.0% of slaughterhouses; 6.5% of carcasses), *Staphylococcus aureus* (15.0% of slaughterhouses; 4.0% of carcasses), *Listeria monocytogenes* 1/2a (5.0% of slaughterhouses; 1.0% of carcasses), *Salmonella enterica* subsp. *enterica* serovar Infantis (5.0% of slaughterhouses; 1.0% of carcasses), and Shiga toxin-producing *E. coli* O:66 (5.0% of slaughterhouses; 0.5% of carcasses). Although 28 *C. perfringens* isolates from 11 slaughterhouses were divided into 21 pulsotypes, all isolates showed the same toxinotype as type A and only carried the *cpa*. Interestingly, 83.3% of isolates from two slaughterhouses located in the same province showed resistance to tetracycline. Furthermore, 13 *Y. enterocolitica* isolates from six slaughterhouses were divided into seven pulsotypes that were divided into biotypes 1A and 2 and serotypes O:5 and O:8, except for isolates that could not be typed. Twelve (92.3%) isolates only carried *ystB*, but one (7.7%) isolate carried *ail* and *ystA*. Moreover, 46.2% of *Y. enterocolitica* isolates showed multidrug resistance against ampicillin, cefoxitin, and amoxicillin/clavulanic acid. This study supports the need for continuous monitoring of slaughterhouses and hygiene management to improve the microbiological safety of carcasses.

Livestock can easily become contaminated with foodborne pathogens originating from feces and intestinal contents spread over the carcass surface during the slaughter process; hence, bacterial contamination of carcasses is a concern for both food safety and meat quality (Barco et al., 2015; Durmuşoğlu et al., 2020). In particular, because slaughterhouses are the first stage of the food production process, specific attention is necessary to implement hygiene during the slaughter process (Nakamura et al., 2022). Kim et al. (2018) also reported that the microbial level of carcasses from slaughterhouses is an important aspect of hygiene management, and the quality and safety of carcasses can be evaluated using indicator microorganisms such as aerobic bacteria and *Escherichia coli*.

Cattle can be infected with various foodborne pathogens during the rearing period, and these infections can be asymptomatic; consequently, foodborne pathogens can eventually be transmitted to humans through cattle acting as carriers (Chlebicz & Śliżewska, 2018). Recently, the European Food Safety Authority (EFSA, 2022) reported that *Listeria monocytogenes*, Shiga toxin-producing *E. coli* (STEC), and *Salmonella enterica* were detected in beef and beef products at rates of 3.9%, 1.7%, and 0.2%, respectively. Moreover, the Centers for Disease Control and Prevention (CDC) (CDC, 2022, 2023c) reported human infections of *Salmonella enterica* and STEC caused by contaminated ground beef, emphasizing the importance of food safety. In particular, STEC produces toxins that can cause severe illness in humans (Davis et al., 2014), and cattle are the most common reservoir of STEC (Capps et al., 2021).

Camargo et al. (2019) have already reported that contamination of foodborne pathogens during cattle slaughtering can occur through the slaughter facility and carcass handling. Moreover, Jiang et al. (2022) and Dong et al. (2014) also reported that foodborne pathogens from

* Corresponding authors.
E-mail addresses: kimhy@korea.kr (H.-Y. Kim), youngju@knu.ac.kr (Y.J. Lee).

https://doi.org/10.1016/j.jfp.2024.100220
Received 4 October 2023; Accepted 8 January 2024
Available online 11 January 2024

carcasses were ultimately transmitted to humans via the processing stage. Therefore, several countries, including the United States, the European Union, Canada, and Australia, are verifying carcass safety management by monitoring indicator microorganisms and foodborne pathogens (Bohaychuk et al., 2011; EFSA, 2022; Karp et al., 2017; Wilhelm et al., 2011). Korea has also been continuously monitoring foodborne pathogens at slaughterhouses nationwide since 2000 (Moon et al., 2021), which involves collecting carcasses from 10 of approximately 70 cattle slaughterhouses nationwide for annual monitoring for the presence of seven pathogens: STEC, *Salmonella enterica*, *Staphylococcus aureus*, *Clostridium perfringens*, *Campylobacter* spp., *L. monocytogenes*, and *Yersinia enterocolitica.* Therefore, this study aimed to evaluate the hygiene and quality of cattle carcasses collected nationwide using the prevalence of foodborne pathogens and the level of indicator bacteria and to analyze the genetic characteristics of major foodborne pathogens.

Materials and Methods

Sample collection. Between 2020 and 2021, 200 carcasses (10 carcasses per slaughterhouse) were collected from 20 cattle slaughterhouses nationwide. According to the Ministry of Food and Drug Safety (MFDS) (MFDS, 2023a), a sterile sponge (Nasco, Fort Atkinson, WI, USA) hydrated with 10 mL of buffered peptone water (BPW; BD Biosciences, San Jose, CA, USA) was used to swab a composite 300-cm^2 surface area that included one flank site (100 cm^2), one brisket site (100 cm^2), and one rump site (100 cm^2) from each carcass cooled at 4 °C for 24 h after slaughter. All swab samples were transferred to the laboratory at 4 °C.

Bacterial count and isolation. Swab samples were inoculated into 30 mL of BPW and homogenized for 1 min using a stomacher (Stomacher 80 Biomaster, Seward, UK). To determine aerobic bacteria and *E. coli* counts, serially diluted (tenfold) aliquots of the swab sample were analyzed using the TEMPO® reader system (bioMérieux, Marcy l'Étoile, France) and Petrifilm plates (3M, St. Paul, MN), respectively, according to the manufacturer's instructions. The isolation of foodborne pathogens was performed according to the standard microbiological protocol notified by the MFDS (2023a). Briefly, to isolate STEC, *Campylobacter* spp., *S. aureus*, *C. perfringens*, and *Y. enterocolitica*, 1 mL of BPW was inoculated into each 9 mL of mEC with novobiocin (Merck, Darmstadt, Germany), Bolton broth (Oxoid, Basingstoke, UK) with laked horse blood (Oxoid), Tryptic soy broth (BD Biosciences) with 10% NaCl, Cooked meat medium (BD Biosciences), and Peptone sorbitol bile broth (Sigma-Aldrich, St. Louis, MO, USA), respectively, and incubated for 24 h at 37 h°C for *E. coli*, *S. aureus*, and *C. perfringens*, 48 h at 42 h°C for *Campylobacter* spp., and 48 h at 30 h°C for *Y. enterocolitica.* For *Salmonella enterica*, 10 mL of BPW was primarily incubated for 24 h at 37 h°C, and then, 0.1 mL of preenriched BPW culture was inoculated in 10 mL of Rappaport–Vassiliadis broth (Oxoid) and incubated for 24 h at 42 h°C. For *L. monocytogenes,* 1 mL of BPW was first inoculated in 9 mL of *Listeria* enrichment broth (BD Biosciences) and incubated for 24 h at 30 h°C, and then, 0.1 mL of broth was secondarily enriched in 10 mL of Fraser broth (BD Biosciences) for 48 h at 37 h°C. All enriched media were streaked on tellurite–cefixime–sorbitol MacConkey agar (Oxoid) for STEC, Baird–Parker agar (Oxoid) supplemented with egg yolk tellurite emulsion (Oxoid) for *S. aureus*, Tryptose–sulfite–cycloserine agar supplemented with egg yolk emulsion (Oxoid) for *C. perfringens,* Cefsulodin–irgasan–novobiocin agar (BD Biosciences) for *Y. enterocolitica*, Xylose lysine tergitol–4 agar (BD Biosciences) for *Salmonella enterica*, and Oxford agar (Oxoid) for *L. monocytogenes* followed by incubation for 24 h at 37 h°C. Modified campy blood–free agar (Oxoid) streaked for *Campylobacter* spp. was incubated for 48 h at 42 h°C. All suspected colonies were confirmed via PCR using an AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea) and an AccuPower® PCR PreMix (Bioneer, Daejeon, Korea) using primers listed in Table 1. PCR was performed using TaKaRa PCR Thermal Cycler Dice (Takara, Seoul, Korea).

Serotyping. STEC was serotyped using commercial antiserum (Joongkyeom, Gyeonggi-do, Korea) according to the manufacturer's instructions and confirmed as described by Iguchi et al. (2015). *Salmonella enterica* were determined using the commercial *Salmonella* O and H antiserum (Difco, Detroit, MI, USA) according to the Kauffmann–White scheme (Grimont & Weill, 2007). *L. monocytogenes* and *Y. enterocolitica* were also serotyped using the respective commercial antiserum (Denka Seiken, Tokyo, Japan) according to the manufacturer's instructions.

Antimicrobial susceptibility testing. To determine the minimum inhibitory concentrations (MICs) for *C. perfringens* and *Y. enterocolitica*, 15 and 14 antimicrobial agents were determined by broth microdilution method using the commercially available Sensititre® panels ANO2B (TREK Diagnostic Systems, West Sussex, UK) and CMV3AGNF (TREK Diagnostic Systems), respectively, according to the manufacturer's instructions. Based on the Clinical and Laboratory Standards Institute guidelines M100 (CLSI, 2020), the susceptibility and resistance of *C. perfringens* and *Y. enterocolitica* were interpreted according to MIC breakpoints for *Clostridium* spp. and *Enterobacteriaceae* (Von Altrock et al., 2010), respectively. Furthermore, *C. difficile* ATCC 700057 and *E. coli* ATCC 25922 were used as quality control strains for *C. perfringens* and *Y. enterocolitica*, respectively, according to the CLSI (2020).

Detection of toxin and virulence genes. Toxin genes encoding the α-toxin (*cpa*), β-toxin (*cpb*), ε-toxin (*etx*), Í-toxin (*iap*), enterotoxin (*cpe*), and necrotic enteritis B-like (*netB*) were detected via PCR as described previously (Baums et al., 2004; Keyburn et al., 2008; Yoo et al., 1997). Three virulence genes, *ail*, *ystA*, and *ystB*, were also detected via PCR as described by Platt-Samoraj et al. (2006).

Biotyping. The biotyping of *Y. enterocolitica* was performed based on biochemical tests using lipase, esculin, indole, xylose, trehalose, pyrazinamidase, β-D-glucosidase, and the Voges–Proskauer test (Weagant & Feng, 2017).

Pulsed-field gel electrophoresis (PFGE). According to the CDC PulseNet protocol (CDC, 2023b), DNA was digested using *Sma*I (Takara Bio Inc., Shiga, Japan) and *Asc*I (Thermo Fisher Scientific, Cleveland, OH, USA) enzymes for *C. perfringens* and *Y. enterocolitica*, respectively. Electrophoresis was performed using the CHEF-DR® III PFGE system (Bio-Rad Laboratories, Hercules, CA, USA), and PFGE banding profiles were analyzed using Bionumerics software version 8.0 (Applied Maths, Sint-Martens-Latem, Belgium). Relatedness was calculated using the unweighted pair group method with the arithmetic averages algorithm based on the Dice similarity index. Isolates that exhibited a coefficient of similarity of ≥85% were considered genetically closely related (Lee et al., 2014; Rusak et al., 2014).

Statistical analysis. Pearson's chi-square test with Bonferroni correction was performed using the Statistical Package for Social Sciences version 26 (IBM Corp., Armonk, NY, USA). Differences were considered significant at $P < 0.05$.

Results

Levels of aerobic bacteria and *E. coli*. The distribution of aerobic bacteria and *E. coli* counts in cattle carcasses are shown in Table 2. The aerobic bacterial count was significantly highest at 2.0–3.0 $\log_{10}$ CFU/cm^2 (34.1%), whereas the *E. coli* count was significantly highest at under 1.0 $\log_{10}$ CFU/cm^2 (94.0%) ($P < 0.05$).

Prevalence of foodborne pathogens. The prevalence of foodborne pathogens in cattle slaughterhouses and carcasses are shown in Table 3. Among the 200 carcasses, *C. perfringens* was the significantly highest prevalent pathogen (17.5%), followed by *Y. enterocolitica* (6.5%), *S. aureus* (4.0%), *L. monocytogenes* 1/2a (1.0%), *Salmonella*

Table 1
Primer sequences used in this study

Bacteria	Target gene	Sequence (5′–3′)	Size (bp)	Annealing (°C)	Reference
Campylobacter coli	Random	F: AGGCAAGGGAGCCTTTAATC R: TATCCCTATCTACAAATTCGC	364	54	On and Jordan (2003)
Campylobacter jejuni	Random	F: CATCTTCCCTAGTCAAGCCT R: AAGATATGGCACTAGCAAGC	773	54	On and Jordan (2003)
Clostridium perfringens	*cpa*	F: GTTGATAGCGCAGGACATGTTAAG R: CATGTAGTCATCTGTTCCAGCATC	402	55	Yoo et al. (1997)
Listeria monocytogenes	*Listeriolysin O*	F: GACATTCAAGTTGTGAA R: CGCCACACTTGAGATAT	560	55	Jung et al. (2003)
Salmonella enterica	*InvA*	F: TTTACGGTCTATTTTGATTTG R: TATGCTCCACAAGGTTAATG	443	54	Arnold et al. (2004)
Shiga toxin-producing *Escherichia coli*	*stx1*	F: TTCGCTCTGCAATAGGTA R: TTCCCCAGTTCAATGTAAGAT	555	50	Franck et al. (1998)
	stx2	F: GTGCCTGTTACTGGGTTTTTCTTC R: AGGGGTCGATATCTCTGTCC	118	50	Franck et al. (1998)
Staphylococcus aureus	*clf A*	F: CTTGATCTCCAGCCATAATTGGTGG R: GCAAAATCCAGCACAACAGGAAACGA	638	55	Mason et al. (2001)
Yersinia enterocolitica	Y1-Y2	F: AATACCGCATAACGTCTTCG R: CTTCTTCTGCGAGTAACGTC	330	62	Wannet et al. (2001)

Table 2
Distribution of indicator bacterial counts in 200 carcasses from 20 cattle slaughterhouses[a]

Count interval (log_{10} CFU/cm^2)	Percentage (%) of cattle carcasses sampled	
	Aerobic bacteria	*E. coli*
≤ 1.0	15 (8.1)^{C,D,E}	188 (94.0)^{A}
1.0–2.0	36 (19.5)^{B}	6 (3.0)^{B}
2.0–3.0	63 (34.1)^{A}	3 (1.5)^{B}
3.0–4.0	32 (17.3)^{B,C}	3 (1.5)^{B}
4.0–5.0	18 (9.7)^{B,C,D,E}	0 (0)^{B}
5.0–6.0	20 (10.8)^{B,C,D}	0 (0)^{B}
6.0–7.0	12 (6.5)^{D,E}	0 (0)^{B}
≥ 7.0	4 (2.2)^{E}	0 (0)^{B}

[a] Values with different superscript letters represent significant differences in the same column ($P < 0.05$).

Table 3
Prevalence of cattle slaughterhouses and carcasses with isolated foodborne pathogens[a]

Pathogen	Percentage (%) of positive samples[b]	
	Slaughterhouses (n = 20)	Carcasses (n = 200)
Clostridium perfringens	12 (60.0)^{A}	35 (17.5)^{A}
Listeria monocytogenes 1/2a	1 (5.0)^{B}	2 (1.0)^{C}
Salmonella Infantis	1 (5.0)^{B}	2 (1.0)^{C}
Shiga toxin-producing *Escherichia coli* O:66	1 (5.0)^{B}	1 (0.5)^{C}
Staphylococcus aureus	3 (15.0)^{B}	8 (4.0)^{B,C}
Yersinia enterocolitica	6 (30.0)^{A,B}	13 (6.5)^{B}
Yersinia enterocolitica O:5	3 (15.0)	3 (1.5)
Yersinia enterocolitica O:8	1 (5.0)	3 (1.5)
Yersinia enterocolitica O:untypable	3 (15.0)	7 (3.5)

[a] Values with different superscript letters represent significant differences in the same column ($P < 0.05$).

enterica subsp. *enterica* serovar Infantis (*S.* Infantis) (1.0%), and STEC O:66 (0.5%) ($P < 0.05$). In particular, *Y. enterocolitica* was divided into two serotypes O:5 and O:8, except the serotype that could not be identified.

Furthermore, *C. perfringens* was isolated from the carcasses of 12 (60.0%) of 20 slaughterhouses, and *Y. enterocolitica* and *S. aureus* were isolated from the carcasses of six (30.0%) and three (15.0%) slaughterhouses, respectively.

Characteristics of *C. perfringens* and *Y. enterocolitica*. The genetic relatedness of the two major pathogens *C. perfringens* and *Y. enterocolitica* are shown in Figure 1. A total of 28 *C. perfringens* strains were isolated from 11 slaughterhouses. These isolates were divided into 21 pulsotypes, and isolates from the same slaughterhouse were divided into two or more pulsotypes, except for two isolates from slaughterhouse B. However, all isolates showed the same toxinotype as type A. Moreover, 12 (42.9%) of 28 *C. perfringens* isolates showed resistance to tetracycline, and interestingly, five (83.3%) of six isolates from two slaughterhouses, located at Chungcheong province, showed resistance to tetracycline.

A total of 13 *Y. enterocolitica* strains were isolated from six slaughterhouses. Interestingly, 11 *Y. enterocolitica* strains were isolated from four slaughterhouses located in Jeolla province. All isolates were divided into seven pulsotypes. Three isolates from slaughterhouse O showed the same pulsotypes, while six isolates from slaughterhouse G were divided into four pulsotypes. All isolates were divided into two biotypes 1A and 2, except one isolate that could not be typed. Furthermore, 12 (92.3%) isolates only carried *ystB* encoding an enterotoxin, but one (7.7%) isolate carried *ail* and *ystA*, which encode an attachment invasion locus and enterotoxin, respectively. These isolates showed high resistance to ampicillin (61.5%), cefoxitin (53.8%), and amoxicillin/clavulanic acid (46.2%), and six isolates (46.2%) showed multidrug resistance to these three antimicrobial subclasses.

Discussion

Meat promotes the growth of various microorganisms that cause food poisoning (Terrell & Hernandez-Jover, 2023); therefore, the initial contamination of carcasses with microorganisms can have an adverse impact on spoilage and shelf life (Shao et al., 2021). According to the Livestock Products Sanitary Control Act in Korea (MFDS, 2023b), the hygiene quality of a cattle carcass is considered satisfactory when aerobic bacteria and *E. coli* counts are < 5.0 and < 2.0 log_{10} CFU/cm^2, respectively. In this study, although 164 (82.0%) of 200 carcasses fulfilled this criterion for aerobic bacterial counts, 36 (18.0%) carcasses showed aerobic bacterial counts exceeding 5.0 log_{10} CFU/cm^2. In contrast, 194 (97.0%) of 200 carcasses fulfilled the criterion for *E. coli* counts, and only six (3.0%) carcasses showed *E. coli* counts exceeding 2.0 log_{10} CFU/cm^2. Although several studies have evaluated the hygiene quality of carcasses based on average aerobic bacterial and *E. coli* counts (Bohaychuk et al., 2011; Nyamakwere et al., 2016; Serraino et al., 2012; Van Ba et al., 2018), it is more important to evaluate the hygiene quality of each carcass by determining whether it fulfills the microbiological standards. Moreover, Elder et al. (2000) first reported that cattle hides are the primary source of carcass contamination during slaughter, which was

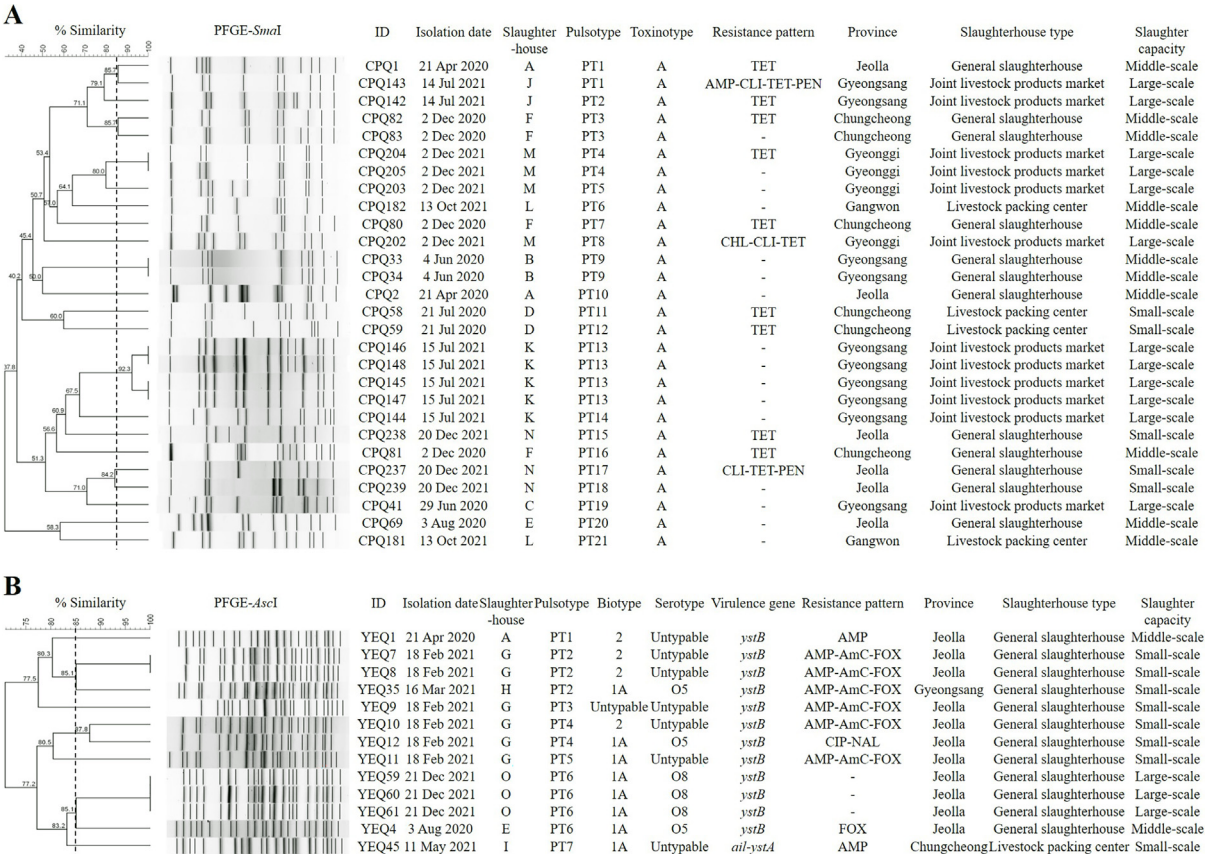

Figure 1. Dendrogram showing the genetic relationships among isolates characterized by PFGE profiles (A) *Clostridium perfringens*, (B) *Yersinia enterocolitica*. Isolates showing similarities of < 85% in PFGE were considered unrelated. Slaughterhouse types are divided into livestock packing center (slaughter, processing, and sale), joint livestock products market (slaughter and sale), and general slaughterhouse (slaughter only). Slaughter capacities (cattle/day) are divided into small-scale (≤ 90), middle-scale (91–150), and large-scale (≥ 151). Abbreviations: TET, tetracycline; AMP, ampicillin; CLI, clindamycin; PEN, penicillin; CHL. chloramphenicol; AmC, amoxicillin/ clavulanic acid; FOX, cefoxitin; CIP, ciprofloxacin; NAL, nalidixic acid.

later directly proven by Nou et al. (2003). Zweifel et al. (2014) also reported that improper handling of hides can eventually increase the counts of aerobic bacteria and *E. coli* in the carcasses. Therefore, for cattle slaughterhouses that do not comply with microbiological standards, it is necessary to strengthen the overall hygiene, including the hide removal process.

Although six of the seven foodborne pathogens tested in this study were isolated from carcasses, *C. perfringens* showed the highest prevalence (60.0% of slaughterhouses and 17.5% of carcasses). In China and Iran, the prevalence rates of *C. perfringens* in cattle carcasses from slaughterhouses were found to be 21.2% and 40.0%, respectively (Jiang et al., 2022; Saeid Hosseinzadeh et al., 2018). Carcass contamination by *C. perfringens* is known to occur through feces during the slaughter process (Jiang et al., 2022); therefore, cattle should be fasted over 12 h and showered before slaughter to reduce the possibility of fecal contamination (MFDS, 2023b). Nonetheless, *C. perfringens* food poisoning is the third most common bacterial food poisoning following that caused by pathogenic *E. coli* and *Salmonella enterica* in Korea (MFDS, 2023c). Moreover, the CDC (2023a) reported that *C. perfringens* causes almost one million cases of food poisoning annually in the United States.

In this study, 28 *C. perfringens* isolates from 11 slaughterhouses were divided into 21 pulsotypes through PFGE analysis, but all isolates showed the same toxinotype as type A. *C. perfringens* type A in one of seven toxinotypes (A–G) and cause diseases such as gas gangrene, enterotoxemia, and enteritis syndromes in both humans and animals (Uzal et al., 2015). Forti et al. (2020) also reported that *C. perfringens* type A only produces *cpa* of the six toxins, and all isolates in this study also only carried the *cpa* *C. perfringens* isolates showed a high resistance to tetracycline (42.9%) in this study. Interestingly, 83.3% of isolates from two slaughterhouses located in the same province showed resistance to tetracycline. Antimicrobial resistance genes are mobile genetic elements that can be transmitted between bacteria through horizontal gene transfer; therefore, it is believed that antimicrobials commonly used in the same province result in almost identical resistance patterns.

The second most frequent pathogen identified in this study was *Y. enterocolitica* (30.0% of slaughterhouses and 6.5% of carcasses). In Europe, human yersiniosis is the third most common foodborne zoonotic disease following campylobacteriosis and salmonellosis (EFSA, 2022). In general, pigs are recognized as a source of pathogenic *Y. enterocolitica* (Chlebicz & Śliżewska, 2018), whereas cattle may also be infected with *Y. enterocolitica*, although the prevalence is significantly lower than that in pigs (McNally et al., 2004). In this study, 18 of the 20 tested slaughterhouses slaughtered both cattle and pigs simultaneously, and all six slaughterhouses where *Y. enterocolitica* was isolated slaughtered cattle and pigs, although the workspace of these slaughterhouses are separated from each other. Therefore, additional research should be conducted into the hazard analysis of pig and cattle slaughterhouses operating together. Milnes et al. (2008) and McNally et al. (2004) reported that pathogenic *Y. enterocolitica* bioserotype 3/O:5,27 strains were shared between cattle and humans in the United Kingdom. Furthermore, Bonardi et al. (2018) reported that pathogenic *Y. enterocolitica* was isolated from the raw milk of cattle, which may cause yersiniosis in humans. Interestingly, in this study, 84.6% of *Y. enterocolitica* strains were isolated from four slaughterhouses located in the same province. For the aerobic bacterial counts, the distribution of slaughterhouse exceeding 5.0 $\log_{10}$ CFU/cm^2 were

the highest in the same province at 28.3% (data not shown), suggesting that the poor hygiene of these slaughterhouses is related to the contamination of *Y. enterocolitica*.

We found that three of 13 *Y. enterocolitica* strains isolated from six slaughterhouses were identified as serotypes O:5 and O:8, which are already known to be commonly associated with human disease (Fredriksson-Ahomaa et al., 2007; Sabina et al., 2011). Furthermore, eight and four *Y. enterocolitica* strains were identified as biotypes 1A and 2, respectively. Kot et al. (2010) reported that biotype 1A isolates from humans produce *Yersinia* heat-stable enterotoxin encoded by *ystB*, which appears to be the most appropriate virulence marker for determining the potential pathogenicity of *Y. enterocolitica* biotype 1A strains (Bancerz-Kisiel et al., 2017). Seven of eight biotype 1A isolates identified here also carried *ystB*; however, one biotype 1A isolate carried the chromosomal virulence marker *ail* and the enterotoxin-encoding *ystA*. Although *Y. enterocolitica* biotype 1A strains mostly lack the classical chromosomal virulence genes *ail* and *ystA* (Sabina et al., 2011), studies have shown that *ail* is highly conserved among *Y. enterocolitica* strains (Huang et al., 2010), and all human pathogenic *Yersinia* spp. carry *ail* in their chromosome (Joutsen et al., 2020). It has been reported that *Y. enterocolitica* biotype 2 also carries chromosomallyencoded virulence markers essential for virulence expression, and most cases of human yersiniosis in Europe belong to biotype 2 (Bancerz-Kisiel et al., 2015; Garzetti et al., 2014). Similarly, all biotype 2 isolates in this study carried *ystB*. Moreover, 46.2% of *Y. enterocolitica* isolates showed multidrug resistance, and interestingly, these isolates showed simultaneous resistance to ampicillin, cefoxitin, and amoxicillin/clavulanic acid. The identical resistance of these isolates is presumed to be due to the antimicrobial classes primarily used for cattle in Korea.

In this study, only 15.0% of slaughterhouses and 4.0% of carcasses were positive for *S. aureus*, whereas Hong et al. (2023) reported that the prevalence of *S. aureus* at pig slaughterhouses and in pig carcasses in Korea was 40.0% and 11.5%, respectively. Although the prevalence of *S. aureus* was lower in cattle carcasses than in pig carcasses, continuous systematic surveillance is required to prevent the human transmission of methicillin-resistant *S. aureus* (MRSA) because cattle-associated MRSA has been reported nationwide in Korea, including in cattle farms, slaughterhouses, retail markets, and bovine mastitic milk (Lee et al., 2020; Song et al., 2016).

In contrast to the prevalence of other pathogens, *L. monocytogenes* was only isolated from two carcasses (1.0%) from one slaughterhouse (5.0%) in this study. Human listeriosis was first reported in Korea in 2018 (Han et al., 2019), and large-scale outbreaks have not yet been reported.

S. Infantis is the fourth most common serovar in humans across Europe (Montoro-Dasi et al., 2023) and has also been frequently reported in patients in Asia, including in Korea (Iwabuchi et al., 2011; Kim et al., 2022; Liang et al., 2019). Mechesso et al. (2020) reported that the prevalence of *S.* Infantis in cattle carcasses was 6.7% from 2010 to 2012 in Korea but was not identified between 2013 and 2018. In this study, this serovar was isolated from two carcasses (1.0%) at one slaughterhouse (5.0%). Drauch et al. (2021) reported that *S.* Infantis was more resistant to disinfectants and could persist on farms despite cleaning and disinfection. However, continuous cleaning and disinfection are required for controlling the horizontal spread of *S.* Infantis throughout the food supply chain.

STEC is a well-known foodborne pathogen frequently isolated from cattle (Blankenship et al., 2020) and has been specifically found in cattle carcasses and feces from slaughterhouses in Korea (Kang et al., 2014; Lee et al., 2023). In this study, only one STEC isolate (O:66) was isolated. STEC is highly pathogenic in humans (Capps et al., 2021), and further studies on the pathogenicity of O:66 in humans are required.

Campylobacter spp. were not found in this study, and no recent studies of *Campylobacter* spp. in cattle slaughterhouses in Korea have been reported. However, *Campylobacter* spp. are also an important foodborne pathogen that can be transmitted to humans through carcasses, and continuous monitoring is required.

In summary, although the results of our study are not representative of all cattle slaughterhouses in Korea, they support the need for continuous slaughterhouse monitoring and hygiene management to improve the microbiological safety of carcasses.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This work was supported by the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea (grant number B-1543069-2024-25-01).

References

Arnold, T., Scholz, H. C., Marg, H., Rösler, U., & Hensel, A. (2004). Impact of *invA*-PCR and culture detection methods on occurrence and survival of *Salmonella* in the flesh, internal organs and lymphoid tissues of experimentally infected pigs. *Journal of Veterinary Medicine B, 51*(10), 459–463. https://doi.org/10.1111/j.1439-0450.2004.00808.x.

Bancerz-Kisiel, A., Platt-Samoraj, A., Szczerba-Turek, A., Syczylo, K., & Szweda, W. (2015). The first pathogenic Yersinia enterocolitica bioserotype 4/O:3 strain isolated from a hunted wild boar (Sus scrofa) in Poland. *Epidemiology and Infection, 143*(13), 2758–2765. https://doi.org/10.1017/S0950268814003872.

Bancerz-Kisiel, A., Szczerba-Turek, A., Platt-Samoraj, A., Michalczyk, M., & Szweda, W. (2017). A study of single nucleotide polymorphism in the *ystB* gene of *Yersinia enterocolitica* strains isolated from various wild animal species. *Annals of Agricultural and Environmental Medicine, 24*(1), 56–61. https://doi.org/10.5604/12321966.1230737.

Barco, L., Belluco, S., Roccato, A., & Ricci, A. (2015). A systematic review of studies on *Escherichia coli* and *Enterobacteriaceae* on beef carcasses at the slaughterhouse. *International Journal of Food Microbiology, 207*, 30–39. https://doi.org/10.1016/j.ijfoodmicro.2015.04.027.

Baums, C. G., Schotte, U., Amtsberg, G., & Goethe, R. (2004). Diagnostic multiplex PCR for toxin genotyping of *Clostridium perfringens* isolates. *Veterinary Microbiology, 100*(1–2), 11–16. https://doi.org/10.1016/S0378-1135(03)00126-3.

Blankenship, H. M., Carbonell, S., Mosci, R. E., McWilliams, K., Pietrzen, K., Benko, S., Gatesy, T., Grooms, D., & Manning, S. D. (2020). Genetic and phenotypic factors associated with persistent shedding of Shiga toxin-producing *Escherichia coli* by beef cattle. *Applied and Environmental Microbiology, 86*(20). https://doi.org/10.1128/AEM.01292-20.

Bohaychuk, V. M., Gensler, G. E., & Barrios, P. R. (2011). Microbiological baseline study of beef and pork carcasses from provincially inspected abattoirs in Alberta, Canada. *The Canadian Veterinary Journal = La Revue Veterinaire Canadienne, 52*(10), 1095–1100 http://www.ncbi.nlm.nih.gov/pubmed/22467964.

Bonardi, S., Le Guern, A. S., Savin, C., Pupillo, G., Bolzoni, L., Cavalca, M., & Pongolini, S. (2018). Detection, virulence and antimicrobial resistance of *Yersinia enterocolitica* in bulk tank milk in Italy. *International Dairy Journal, 84*, 46–53. https://doi.org/10.1016/j.idairyj.2018.04.003.

Camargo, A., Cossi, M., Silva, W., Bersot, L., Landgraf, M., Baranyi, J., Franco, B., & Luís Augusto, N. (2019). Microbiological testing for the proper assessment of the hygiene status of beef carcasses. *Microorganisms, 7*(3), 86. https://doi.org/10.3390/microorganisms7030086.

Capps, K. M., Ludwig, J. B., Shridhar, P. B., Shi, X., Roberts, E., DebRoy, C., Cernicchiaro, N., Phebus, R. K., Bai, J., & Nagaraja, T. G. (2021). Identification, shiga toxin subtypes and prevalence of minor serogroups of shiga toxin-producing *Escherichia coli* in feedlot cattle feces. *Scientific Reports, 11*(1), 8601. https://doi.org/10.1038/s41598-021-87544-w.

Centers for Disease Control and Prevention (CDC). (2022). *E. coli Outbreak Linked to Ground Beef*. Accessed September 19, 2023, from https://www.cdc.gov/ecoli/2022/o157h7-09-22/index.html.

Centers for Disease Control and Prevention (CDC). (2023a). *Prevent Illness from C. perfringens*. Accessed September 19, 2023, from https://www.cdc.gov/foodsafety/diseases/clostridium-perfringens.html.

Centers for Disease Control and Prevention (CDC). (2023b). *Pulsed-field Gel Electrophoresis (PFGE)*. Accessed January 18, 2023, from https://www.cdc.gov/pulsenet/pathogens/pfge.html.

Centers for Disease Control and Prevention (CDC). (2023c). *Salmonella Outbreak Linked to Ground Beef*. Accessed September 19, 2023, from https://www.cdc.gov/salmonella/saintpaul-07-23/index.html.

Chlebicz, A., & Śliżewska, K. (2018). Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: A review. *International Journal of Environmental Research and Public Health, 15*(5), 863. https://doi.org/10.3390/ijerph15050863.

Clinical and Laboratory Standards Institute (CLSI) (2020). *M100. Performance Standards for Antimicrobial Susceptibility Testing* (30th ed.). Wayne, PA: Clinical and Laboratory Standards Institute.

Davis, T. K., Van De Kar, N. C. A. J., & Tarr, P. I. (2014). Shiga toxin/verocytotoxin-producing *Escherichia coli* infections: Practical clinical perspectives. *Microbiology. Spectrum, 2*(4). https://doi.org/10.1128/microbiolspec.EHEC-0025-2014.

Dong, P., Zhu, L., Mao, Y., Liang, R., Niu, L., Zhang, Y., Li, K., & Luo, X. (2014). Prevalence and profile of *Salmonella* from samples along the production line in Chinese beef processing plants. *Food Control, 38*(1), 54–60. https://doi.org/10.1016/j.foodcont.2013.09.066.

Drauch, V., Kornschober, C., Palmieri, N., Hess, M., & Hess, C. (2021). Infection dynamics of *Salmonella* Infantis strains displaying different genetic backgrounds – with or without pESI-like plasmid – Vary considerably. *Emerging Microbes & Infections, 10*(1), 1471–1480. https://doi.org/10.1080/22221751.2021.1951124.

Durmuşoğlu, H., İncili, G. K., Demir, P., & İlhak, O. İ. (2020). Effects of workers' hand washing and knife disinfection practices on microbiological quality of small animal carcasses in slaughterhouse environment. *Journal of Food Processing and Preservation, 44*(12), 1–7. https://doi.org/10.1111/jfpp.14918.

Elder, R. O., Keen, J. E., Siragusa, G. R., Barkocy-Gallagher, G. A., Koohmaraie, M., & Laegreid, W. W. (2000). Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proceedings of the National Academy of Sciences of the United States of America, 97*(7), 2999–3003. https://doi.org/10.1073/pnas.97.7.2999.

European Food Safety Authority (EFSA) (2022). The European Union One Health 2021 Zoonoses Report. *EFSA Journal, 20*(12). https://doi.org/10.2903/j.efsa.2022.7666.

Forti, K., Ferroni, L., Pellegrini, M., Cruciani, D., De Giuseppe, A., Crotti, S., Papa, P., Maresca, C., Severi, G., Marenzoni, M. L., & Cagiola, M. (2020). Molecular characterization of *Clostridium perfringens* strains isolated in Italy. *Toxins, 12*(10), 650. https://doi.org/10.3390/toxins12100650.

Franck, S. M., Bosworth, B. T., & Moon, H. W. (1998). Multiplex PCR for enterotoxigenic, attaching and effacing, and Shiga toxin-producing *Escherichia coli* strains from calves. *Journal of Clinical Microbiology, 36*(6), 1795–1797. https://doi.org/10.1128/JCM.36.6.1795-1797.1998.

Fredriksson-Ahomaa, M., Stolle, A., & Stephan, R. (2007). Prevalence of pathogenic *Yersinia enterocolitica* in pigs slaughtered at a Swiss abattoir. *International Journal of Food Microbiology, 119*(3), 207–212. https://doi.org/10.1016/j.ijfoodmicro.2007.07.050.

Garzetti, D., Susen, R., Fruth, A., Tietze, E., Heesemann, J., & Rakin, A. (2014). A molecular scheme for *Yersinia enterocolitica* patho-serotyping derived from genome-wide analysis. *International Journal of Medical Microbiology, 304*(3–4), 275–283. https://doi.org/10.1016/j.ijmm.2013.10.007.

Grimont, P., & Weill, F. -X. (2007). *Antigenic formulae of the Salmonella servovars: WHO Collaborating Centre for Reference and Research on Salmonella*. Accessed May 20, 2023, from https://www.pasteur.fr/sites/default/files/veng_0.pdf.

Han, S.-H., Park, S.-H., Choi, S.-S., Jin, Y.-H., Kim, H.-S., Kim, J.-S., Park, J.-H., Ryu, J., Kang, M.-J., Jeon, S.-J., Hong, C.-K., Park, S.-Y., Oh, A.-R., Kim, Y.-J., Park, S.-H., Lee, J., & Oh, Y.-H. (2019). Food-borne outbreak of *Listeria monocytogenes* in school students in Seoul, Korea. *Journal of Food Safety and Hygiene, 5*(3). https://doi.org/10.18502/jfsh.v5i3.5691.

Hong, S., Kang, H. J., Lee, H.-Y., Jung, H.-R., Moon, J.-S., Yoon, S.-S., Kim, H.-Y., & Lee, Y. J. (2023). Prevalence and characteristics of foodborne pathogens from slaughtered pig carcasses in Korea. *Frontiers in Veterinary Science, 10*(1). https://doi.org/10.3389/fvets.2023.1158196.

Huang, Y., Wang, X., Cui, Z., Yang, Y., Xiao, Y., Tang, L., Kan, B., Xu, J., & Jing, H. (2010). Possible use of *ail* and *foxA* polymorphisms for detecting pathogenic *Yersinia enterocolitica*. *BMC Microbiology, 10*(1), 211. https://doi.org/10.1186/1471-2180-10-211.

Iguchi, A., Iyoda, S., Seto, K., Morita-Ishihara, T., Scheutz, F., & Ohnishi, M. (2015). *Escherichia coli* O-genotyping PCR: A comprehensive and practical platform for molecular O serogrouping. *Journal of Clinical Microbiology, 53*(8), 2427–2432. https://doi.org/10.1128/JCM.00321-15.

Iwabuchi, E., Yamamoto, S., Endo, Y., Ochiai, T., & Hirai, K. (2011). Prevalence of *Salmonella* isolates and antimicrobial resistance patterns in chicken meat throughout Japan. *Journal of Food Protection, 74*(2), 270–273. https://doi.org/10.4315/0362-028X.JFP-10-215.

Jiang, Y., Ma, Y., Liu, Q., Li, T., Li, Y., Guo, K., & Zhang, Y. (2022). Tracing *Clostridium perfringens* strains from beef processing of slaughter house by pulsed-field gel electrophoresis, and the distribution and toxinotype of isolates in Shaanxi province, China. *Food Microbiology, 101*(22). https://doi.org/10.1016/j.fm.2021.103887 103887.

Joutsen, S., Johansson, P., Laukkanen-Ninios, R., Björkroth, J., & Fredriksson-Ahomaa, M. (2020). Two copies of the *ail* gene found in *Yersinia enterocolitica* and *Yersinia kristensenii*. *Veterinary Microbiology, 247*(April). https://doi.org/10.1016/j.vetmic.2020.108798 108798.

Jung, B., Lim, H., & Jung, S. (2003). Development of differential media and multiplex PCR assays for the rapid detection of *Listeria monocytogenes*. *Korean Journal of Veterinary Research, 43*(2), 231–237.

Kang, E., Hwang, S. Y., Kwon, K. H., Kim, K. Y., Kim, J. H., & Park, Y. H. (2014). Prevalence and characteristics of Shiga toxin-producing Escherichia coli (STEC) from cattle in Korea between 2010 and 2011. *Journal of Veterinary Science, 15*(3), 369–379. https://doi.org/10.4142/jvs.2014.15.3.369.

Karp, B. E., Tate, H., Plumblee, J. R., Dessai, U., Whichard, J. M., Thacker, E. L., Hale, K. R., Wilson, W., Friedman, C. R., Griffin, P. M., & McDermott, P. F. (2017). National antimicrobial resistance monitoring system: Two decades of advancing public health through integrated surveillance of antimicrobial resistance. *Foodborne Pathogens and Disease, 14*(10), 545–557. https://doi.org/10.1089/fpd.2017.2283.

Keyburn, A. L., Boyce, J. D., Vaz, P., Bannam, T. L., Ford, M. E., Parker, D., Di Rubbo, A., Rood, J. I., & Moore, R. J. (2008). *NetB*, a new toxin that is associated with avian necrotic enteritis caused by *Clostridium perfringens*. *PLoS Pathogens, 4*(2). https://doi.org/10.1371/journal.ppat.0040026.

Kim, H. J., Kim, D., Kim, H. J., Song, S. O., Song, Y. H., & Jang, A. (2018). Evaluation of the microbiological status of raw beef in Korea: Considering the suitability of aerobic plate count guidelines. *Korean Journal for Food Science of Animal Resources, 38*(1), 43–51. https://doi.org/10.5851/kosfa.2018.38.1.043.

Kim, S. H., Sung, G.-H., Park, E. H., Hwang, I. Y., Kim, G. R., Song, S. A., Lee, H. K., Uh, Y., Kim, Y. A., Jeong, S. H., Shin, J. H., Shin, K. S., Lee, J., Jeong, J., Kim, Y. R., Yong, D., Lee, M., Kim, Y. K., Ryoo, N. H., … Shin, J. H. (2022). Serotype distribution and antimicrobial resistance of *Salmonella* isolates in Korea between 2016 and 2017. *Annals of Laboratory Medicine, 42*(2), 268–273. https://doi.org/10.3343/alm.2022.42.2.268.

Kot, B., Piechota, M., & Jakubczak, A. (2010). Analysis of occurrence of virulence genes among *Yersinia enterocolitica* isolates belonging to different biotypes and serotypes. *Polish Journal of Veterinary Sciences, 13*(1), 13–19.

Lee, W., Kim, M. H., Sung, S., Kim, E., An, E. S., Kim, S. H., Kim, S. H., & Kim, H. Y. (2023). Genome-based characterization of hybrid Shiga toxin-producing and enterotoxigenic Escherichia coli (STEC/ETEC) strains isolated in South Korea, 2016–2020. *Microorganisms, 11*(5), 2016–2020. https://doi.org/10.3390/microorganisms11051285.

Lee, H. H., Lee, G. Y., Eom, H. S., & Yang, S.-J. (2020). Occurrence and characteristics of methicillin-resistant and -susceptible *Staphylococcus aureus* isolated from the beef production chain in Korea. *Food Science of Animal Resources, 40*(3), 401–414. https://doi.org/10.5851/kosfa.2020.e20.

Lee, K. E., Lim, S. I., Shin, S. H., Kwon, Y. K., Kim, H. Y., Song, J. Y., & An, D. J. (2014). Distribution of *Clostridium perfringens* isolates from piglets in South Korea. *Journal of Veterinary Medical Science, 76*(5), 745–749. https://doi.org/10.1292/jvms.13-0430.

Liang, B., Xie, Y., He, S., Mai, J., Huang, Y., Yang, L., Zhong, H., Deng, Q., Yao, S., Long, Y., Yang, Y., Gong, S., & Zhou, Z. (2019). Prevalence, serotypes, and drug resistance of nontyphoidal *Salmonella* among paediatric patients in a tertiary hospital in Guangzhou, China, 2014–2016. *Journal of Infection and Public Health, 12*(2), 252–257. https://doi.org/10.1016/j.jiph.2018.10.012.

Mason, W. J., Blevins, J. S., Beenken, K., Wibowo, N., Ojha, N., & Smeltzer, M. S. (2001). Multiplex PCR protocol for the diagnosis of staphylococcal infection. *Journal of Clinical Microbiology, 39*(9), 3332–3338. https://doi.org/10.1128/JCM.39.9.3332-3338.2001.

McNally, A., Cheasty, T., Fearnley, C., Dalziel, R. W., Paiba, G. A., Manning, G., & Newell, D. G. (2004). Comparison of the biotypes of Yersinia enterocolitica isolated from pigs, cattle and sheep at slaughter and from humans with yersiniosis in Great Britain during 1999–2000. *Letters in Applied Microbiology, 39*(1), 103–108. https://doi.org/10.1111/j.1472-765X.2004.01548.x.

Mechesso, A. F., Moon, D. C., Kim, S.-J., Song, H.-J., Kang, H. Y., Na, S. H., Choi, J.-H., Kim, H.-Y., Yoon, S.-S., & Lim, S.-K. (2020). Nationwide surveillance on serotype distribution and antimicrobial resistance profiles of non-typhoidal *Salmonella* serovars isolated from food-producing animals in South Korea. *International Journal of Food Microbiology, 335*(September). https://doi.org/10.1016/j.ijfoodmicro.2020.108893 108893.

Milnes, A. S., Stewart, I., Clifton-Hadley, F. A., Davies, R. H., Newell, D. G., Sayers, A. R., Cheasty, T., Cassar, C., Ridley, A., Cook, A. J. C., Evans, S. J., Teale, C. J., Smith, R. P., McNally, A., Toszeghy, M., Futter, R., Kay, A., & Paiba, G. A. (2008). Intestinal carriage of verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica*, in cattle, sheep and pigs at slaughter in Great Britain during 2003. *Epidemiology and Infection, 136*(6), 739–751. https://doi.org/10.1017/S0950268807009223.

Ministry of Food and Drug Safety (MFDS) (2023a). *Processing Standards and Ingredient Specifications for Livestock Products*. Cheongju, Korea: Ministry of Food and Drug Safety.

Ministry of Food and Drug Safety (MFDS) (2023b). *Livestock Products Sanitary Control Act*. Cheongju, Korea: Ministry of Food and Drug Safety.

Ministry of Food and Drug Safety (MFDS). (2023c). *Food poisoning statistics*. Ministry of Food and Drug Safety: Cheongju, Korea. Accessed August 18, 2023, from https://www.foodsafetykorea.go.kr/portal/healthyfoodlife/foodPoisoningStat.do?menu_no=4425&menu_grp=MENU_NEW02.

Montoro-Dasi, L., Lorenzo-Rebenaque, L., Marco-Fuertes, A., Vega, S., & Marin, C. (2023). Holistic strategies to control *Salmonella* Infantis: An emerging challenge in the European broiler sector. *Microorganisms, 11*(7), 1765. https://doi.org/10.3390/microorganisms11071765.

Moon, J.-S., Kim, H.-Y., Hong, S., Eseul, K., & Kang, H. J. (2021). *Recent trends on detection of pathogenic E. coli from raw meat in Korea*. Accessed August 18, 2023, from http://submit.foodhygiene.or.kr/www/ThesisSearch/info_view.html?idx=2266.

Nakamura, A., Takahashi, H., Kondo, A., Koike, F., Kuda, T., Kimura, B., & Kobayashi, M. (2022). Distribution of psychrophilic microorganisms in a beef slaughterhouse in Japan after cleaning. *PLOS ONE, 17*(8), e0268411.

Nou, X., Rivera-Betancourt, M., Bosilevac, J. M., Wheeler, T. L., Shackelford, S. D., Gwartney, B. L., Reagan, J. O., & Koohmaraie, M. (2003). Effect of chemical dehairing on the prevalence of *Escherichia coli* O157: H7 and the levels of aerobic bacteria and *Enterobacteriaceae* on carcasses in a commercial beef processing plant. *Journal of Food Protection, 66*(11), 2005–2009. https://doi.org/10.4315/0362-028X-66.11.2005.

Nyamakwere, F., Muchenje, V., Mushonga, B., Makepe, M., & Mutero, G. (2016). Assessment of *Salmonella*, *Escherichia coli*, *Enterobacteriaceae* and aerobic colony counts contamination levels during the beef slaughter process. *Journal of Food Safety, 36*(4), 548–556. https://doi.org/10.1111/jfs.12275.

On, S. L. W., & Jordan, P. J. (2003). Evaluation of 11 PCR assays for species-level identification of *Campylobacter jejuni* and *Campylobacter coli*. *Journal of Clinical Microbiology, 41*(1), 330–336. https://doi.org/10.1128/JCM.41.1.330-336.2003.

Platt-Samoraj, A., Ugorski, M., Szweda, W., Szczerba-Turek, A., Wojciech, K., & Procajło, Z. (2006). Analysis of the presence of *ail*, *ystA* and *ystB* genes in *Yersinia enterocolitica* strains isolated from aborting sows and aborted fetuses. *Journal of Veterinary Medicine Series B, 53*(7), 341–346. https://doi.org/10.1111/j.1439-0450.2006.00969.x.

Rusak, L. A., Moura Falavina Dos Reis, C., Barbosa, A. V., Felipe Mercês Santos, A., Paixão, R., Hofer, E., Vallim, D. C., & Asensi, M. D. (2014). Phenotypic and genotypic analysis of bio-serotypes of *Yersinia enterocolitica* from various sources in Brazil. *Journal of Infection in Developing Countries, 8*(12), 1533–1540. https://doi.org/10.3855/jidc.4533.

Sabina, Y., Rahman, A., Ray, R. C., & Montet, D. (2011). *Yersinia enterocolitica*: Mode of transmission, molecular insights of virulence, and pathogenesis of infection. *Journal of Pathogens, 2011*, 1–10. https://doi.org/10.4061/2011/429069.

Hosseinzadeh, S., Bahadori, M., Poormontaseri, M., Dehghani, M., Fazeli, M., & Nazifi, S. (2018). Molecular characterization of *Clostridium perfringens* isolated from cattle and sheep carcasses and its antibiotic resistance patterns in Shiraz slaughterhouse, southern Iran. *Veterinarski Arhiv, 88*(5), 581–591. https://doi.org/10.24099/vet.arhiv.0009.

Serraino, A., Bardasi, L., Riu, R., Pizzamiglio, V., Liuzzo, G., Galletti, G., Giacometti, F., & Merialdi, G. (2012). Visual evaluation of cattle cleanliness and correlation to carcass microbial contamination during slaughtering. *Meat Science, 90*(2), 502–506. https://doi.org/10.1016/j.meatsci.2011.08.001.

Shao, L., Chen, S., Wang, H., Zhang, J., Xu, X., & Wang, H. (2021). Advances in understanding the predominance, phenotypes, and mechanisms of bacteria related to meat spoilage. *Trends in Food Science & Technology, 118*(PB), 822–832. https://doi.org/10.1016/j.tifs.2021.11.007.

Song, J. W., Yang, S. J., Shin, S., Seo, K. S., Park, Y. H., & Park, K. T. (2016). Genotypic and phenotypic characterization of methicillin-resistant *Staphylococcus aureus* isolated from bovine mastitic milk in Korea. *Journal of Food Protection, 79*(10), 1725–1732. https://doi.org/10.4315/0362-028X.JFP-16-067.

Terrell, G. C., & Hernandez-Jover, M. (2023). Meat and meat products. In *Food Safety Management*, pp. 141–184). Academic Press.

Uzal, F. A., McClane, B. A., Cheung, J. K., Theoret, J., Garcia, J. P., Moore, R. J., & Rood, J. I. (2015). Animal models to study the pathogenesis of human and animal *Clostridium perfringens* infections. *Veterinary Microbiology, 179*(1–2), 23–33. https://doi.org/10.1016/j.vetmic.2015.02.013.

Van Ba, H., Seo, H.-W., Pil-Nam, S., Kim, Y.-S., Park, B. Y., Moon, S.-S., Kang, S.-J., Choi, Y.-M., & Kim, J.-H. (2018). The effects of pre-and post-slaughter spray application with organic acids on microbial population reductions on beef carcasses. *Meat Science, 137*(November), 16–23. https://doi.org/10.1016/j.meatsci.2017.11.006.

Von Altrock, A., Roesler, U., Merle, R., & Waldmann, K.-H. (2010). Prevalence of pathogenic strains on liver surfaces of pigs and their antimicrobial susceptibility. *Journal of Food Protection, 73*(9), 1680–1683. https://doi.org/10.4315/0362-028X-73.9.1680.

Wannet, W. J., Reessink, M., Brunings, H. A., & Maas, H. M. (2001). Detection of pathogenic *Yersinia enterocolitica* by a rapid and sensitive duplex PCR assay. *Journal of Clinical Microbiology, 39*(12), 4483–4486. https://doi.org/10.1128/JCM.39.12.4483-4486.2001.

Weagant, S. D., & Feng, P. (2017). *BAM Chapter 8, Yersinia enterocolitica. Bacteriological Analytical Manual*. Accessed September 5, 2023, from https://www.fda.gov/food/laboratory-methods-food/bam-chapter-8-yersinia-enterocolitica.

Wilhelm, B., Rajić, A., Greig, J. D., Waddell, L., & Harris, J. (2011). The effect of hazard analysis critical control point programs on microbial contamination of carcasses in abattoirs: A systematic review of published data. *Foodborne Pathogens and Disease, 8*(9), 949–960. https://doi.org/10.1089/fpd.2010.0809.

Yoo, H. S., Lee, S. U., Park, K. Y., & Park, Y. H. (1997). Molecular typing and epidemiological survey of prevalence of *Clostridium perfringens* types by multiplex PCR. *Journal of Clinical Microbiology, 35*(1), 228–232. https://doi.org/10.1128/jcm.35.1.228-232.1997.

Zweifel, C., Capek, M., & Stephan, R. (2014). Microbiological contamination of cattle carcasses at different stages of slaughter in two abattoirs. *Meat Science, 98*(2), 198–202. https://doi.org/10.1016/j.meatsci.2014.05.029.