frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 31 March 2023
DOI 10.3389/fvets.2023.1158196

OPEN ACCESS

EDITED BY
Om P. Dhungyel,
The University of Sydney, Australia

REVIEWED BY
Piera Anna Martino,
University of Milan, Italy
Yumiko Okada,
NIHS, Japan
Nelson Phiri,
Eden University, Zambia

*CORRESPONDENCE
Ha-Young Kim
✉ kimhy@korea.kr
Young Ju Lee
✉ youngju@knu.ac.kr

SPECIALTY SECTION
This article was submitted to Veterinary Infectious Diseases, a section of the journal Frontiers in Veterinary Science

RECEIVED 03 February 2023
ACCEPTED 14 March 2023
PUBLISHED 31 March 2023

CITATION
Hong S, Kang HJ, Lee H-Y, Jung H-R, Moon J-S, Yoon S-S, Kim H-Y and Lee YJ (2023) Prevalence and characteristics of foodborne pathogens from slaughtered pig carcasses in Korea. *Front. Vet. Sci.* 10:1158196. doi: 10.3389/fvets.2023.1158196

Prevalence and characteristics of foodborne pathogens from slaughtered pig carcasses in Korea

Serim Hong[1], Hye Jeong Kang[2], Hye-Young Lee[2], Hye-Ri Jung[1], Jin-San Moon[2], Soon-Seek Yoon[2], Ha-Young Kim[2]* and Young Ju Lee[1]*

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

The introduction of bacteria into slaughterhouses can lead to microbial contamination in carcasses during slaughter, and the initial level of bacteria in carcasses is important because it directly affects spoilage and the shelf life. This study was conducted to investigate the microbiological quality, and the prevalence of foodborne pathogens in 200 carcasses from 20 pig slaughterhouses across Korea. Distribution of microbial counts were significantly higher for aerobic bacteria at 3.01–4.00 $\log_{10}$ CFU/cm^2 (42.0%) and 2.01–3.00 $\log_{10}$ CFU/cm^2 (28.5%), whereas most of *Escherichia coli* showed the counts under 1.00 $\log_{10}$ CFU/cm^2 (87.0%) ($P < 0.05$). The most common pathogen isolated from 200 carcasses was *Staphylococcus aureus* (11.5%), followed by *Yersinia enterocolitica* (7.0%). In total, 17 *S. aureus* isolates from four slaughterhouses were divided into six pulsotypes and seven *spa* types, and showed the same or different types depending on the slaughterhouses. Interestingly, isolates from two slaughterhouses carried only *LukED* associated with the promotion of bacterial virulence, whereas, isolates from two other slaughterhouses carried one or more toxin genes associated with enterotoxins including *sen*. In total, 14 *Y. enterocolitica* isolates from six slaughterhouses were divided into nine pulsotypes, 13 isolates belonging to biotype 1A or 2 carried only *ystB*, whereas one isolate belonging to bio-serotype 4/O:3 carried both *ail* and *ystA*. This is the first study to investigate microbial quality and the prevalence of foodborne pathogens in carcasses from slaughterhouses nationally, and the findings support the need for ongoing slaughterhouse monitoring to improve the microbiological safety of pig carcasses.

KEYWORDS

foodborne pathogen, pig, carcass, slaughterhouse, microbial quality

1. Introduction

Foodborne illness is an important public health problem that causes an estimated 600 million illnesses and 420,000 deaths annually worldwide (1). In particular, food-producing animals are the major reservoirs of many foodborne pathogens such as Shiga toxin-producing *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Campylobacter* spp. *Yersinia* spp., and *Listeria monocytogenes* (2–4), and contamination of carcasses with foodborne pathogens can occur at several stages within the food production chain (5).

The slaughter stage has been a major focus of food safety interventions. Namely, the introduction of bacteria into slaughterhouses can lead to microbial contamination at several processing steps during slaughter. The initial opening of the carcass and the removal of highly contaminated organs, such as the intestines, pluck set and tonsils (6), increase the risk of microbial spread to carcass surfaces. Moreover, insufficient disinfection of cutting knifes or machinery can lead to cross-contamination between carcasses (7).

Recently, Bae et al. (8) reported the first study tracking foodborne pathogens in pigs and related pork products at all points along the pork supply chain, including farms, slaughterhouses, meat processing plants, and retail stores, in Korea. In particular, *Y. enterocolitica* and Shiga toxin-producing *E. coli* were the major pathogens isolated from carcasses in slaughterhouses. Moreover, Im et al. (9) reported that *S. aureus*, *Salmonella* spp., and *C. perfringens* were isolated from edible pig intestines in 11 pig slaughterhouses in Korea. According to the United States Department of Agriculture, evaluation of the hygiene status in pig slaughterhouses mainly targets *Salmonella* and other major pathogens (10). In Korea, foodborne pathogens surveillance at the slaughter stage has been conducted nationwide since 2010 to ensure food safety and reduce risks to human health. This study aimed to report on microbiological quality and distribution of foodborne pathogens in pig slaughterhouses nationwide and identify the genetic relationships and characteristics of major foodborne pathogens.

2. Materials and methods

2.1. Sample collection

In total, 200 carcasses were collected from 20 pig slaughterhouses across Korea between 2020 and 2021. In addition, pigs are produced mostly from three-way hybrids: Landrace, Yorkshire, and Duroc, and slaughtered at a live weight of ∼110 kg. According to the Ministry of Food and Drug Safety (MFDS) protocol (11), a sterile sponge (Nasco, Fort Atkinson, WI, USA) hydrated with 10 ml of buffered peptone water (BPW; Difco, BD Biosciences, San Jose, CA, USA) was used to swab a 300 cm^2 surface area composite that included one belly site (100 cm^2), one ham site (100 cm^2), and one jowls site (100 cm^2) from each carcass cooled at 4°C for 24 h after slaughter. Ten carcasses were collected from each slaughterhouse, and all swab samples were transferred to the laboratory under 4°C conditions.

2.2. Bacterial count and isolation

Swab samples were inoculated in 30 ml of BPW and homogenized for 1 min using a stomacher (Stomacher 80 Biomaster, Seward, UK). To determine the aerobic bacteria and *E. coli* counts, aliquots containing serially diluted (10-fold) swab samples were performed using the TEMPO® reader system (bioMérieux, Marcy l'Étoile, France), and Petrifilm plates (3M, St. Paul, MN, USA), respectively, according to the manufacturer's instructions. The isolation of foodborne pathogens was performed according to the standard microbiological protocol notified by the MFDS (11). Briefly, to isolate Shiga toxin-producing *E. coli*, *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°C for *E. coli*, *S. aureus*, and *C. perfringens*; 48 h at 42°C for *Campylobacter* spp.; and 48 h at 30°C for *Y. enterocolitica*. For *Salmonella* spp., 10 mL of BPW was primarily incubated for 24 h at 37°C, and then 0.1 ml of pre-enriched BPW culture was inoculated in 10 ml of Rappaport–Vassiliadis broth (Oxoid) and incubated for 24 h at 42°C. For *L. monocytogenes,* 1 mL of BPW was also primarily inoculated in 9 ml of Listeria enrichment broth (BD Biosciences) and incubated for 24 h at 30°C, and then 0.1 ml of broth was secondarily enriched in 10 ml of Fraser broth (BD Biosciences) for 48 h at 37°C. All enriched media were streaked on Tellurite–Cefixime–Sorbitol MacConkey agar (Oxoid) for Shiga toxin-producing *E. coli*, 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* spp., and Oxford agar (Oxoid) for *L. monocytogenes* followed by incubation for 24 h at 37°C. Modified campy blood–free agar (Oxoid) streaked for *Campylobacter* spp. was incubated for 48 h at 42°C. All suspect colonies were performed using polymerase chain reaction (PCR) with specific primers (Table 1) (12–16, 19, 20), and MALDI-TOF mass spectrometry (bioMérieux). If the same species isolates from the same origin showed the same antimicrobial susceptibility patterns, then only one isolate was randomly selected.

2.3. Serotyping

*Salmonell*a spp. was serotyped using commercial *Salmonella* O, H-phase 1 and H-phase 2 antisera (Difco, Detroit, MI, USA) according to the Kauffmann–White scheme (17). *L. monocytogenes* was carried out using commercial antisera (Denka Seiken, Tokyo, Japan) against the serovars 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4c, 4d/4e, and 4b/4e following the manufacturer's instructions. *Y. enterocolitica* was serotyped using commercial antisera polyvalent group O:1–2, O:3, O:5, O:8, and O:9 (Denka Seiken) following the manufacturer's instructions.

2.4. Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of 17 and 14 antimicrobial agents for *S. aureus* and *Y. enterocolitica*, respectively, were determined by the broth microdilution method using the commercially available Sensititre® panels EUST (TREK Diagnostic Systems, West Sussex, UK) and CMV3AGNF (TREK Diagnostic Systems), respectively, following

TABLE 1 Primer sequences used in this study.

Bacteria	Target gene	Sequence (5′-3′)	Size (bp)	Annealing (°C)	References
Campylobacter coli	Random	F: AGGCAAGGGAGCCTTTAATC	364	54	(12)
		R: TATCCCTATCTACAAATTCGC			
Campylobacter jejuni	Random	F: CATCTTCCCTAGTCAAGCCT	773	54	(12)
		R: AAGATATGGCACTAGCAAGC			
Clostridium perfringens	*cpa*	F: GTTGATAGCGCAGGACATGTTAAG	402	55	(13)
		R: CATGTAGTCATCTGTTCCAGCATC			
Listeria monocytogenes	*Listeriolysin O*	F: GACATTCAAGTTGTGAA	560	55	(14)
		R: CGCCACACTTGAGATAT			
Salmonella spp.	*InvA*	F: TTTACGGTCTATTTTGATTTG	443	54	(15)
		R: TATGCTCCACAAGGTTAATG			
Shiga toxin-producing *Escherichia coli*	*stx1*	F: TTCGCTCTGCAATAGGTA	555	50	(16)
		R: TTCCCCAGTTCAATGTAAGAT			
	stx2	F: GTGCCTGTTACTGGGTTTTTCTTC	118	50	(16)
		R: AGGGGTCGATATCTCTGTCC			
Staphylococcus aureus	*clf A*	F: CTTGATCTCCAGCCATAATTGGTGG	638	55	(17)
		R: GCAAAATCCAGCACAACAGGAAACGA			
Yersinia enterocolitica	Y1-Y2	F: AATACCGCATAACGTCTTCG	330	62	(18)
		R: CTTCTTCTGCGAGTAACGTC			

the manufacturer's instructions. MICs were interpreted according to the Clinical and Laboratory Standards Institute guidelines M100 (18), and *Y. enterocolitica* followed breakpoints in *Enterobacteriaceae*. *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used as quality-control strains for *S. aureus* and *Y. enterocolitica*, respectively.

2.5. Detection of toxin and virulence genes

PCR amplification was performed to detect toxin genes in *S. aureus* and virulence genes in *Y. enterocolitica* as described by Van Duijkeren et al. (21) and Platt-Samoraj et al. (22), respectively. The toxin genes included those encoding enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, and *ser*), leukotoxin family (*lukED*), exfoliative toxins (*eta* and *etb*), toxic shock syndrome toxin (*tsst-1*), and panton–valentine leukocidin (*pvl*), and the virulence genes included attachment invasion locus (*ail*), *Yersinia* stable toxin A (*ystA*), and *ystB*.

2.6. *S. aureus* protein A typing and biotyping

Staphylococcus aureus protein A (*spa*) typing was performed using as described by Shopsin et al. (23) using Ridom StaphType (Ridom GmbH, Wurzburg, Germany; www.spaserver.ridom.de). The biotyping of *Y. enterocolitica* was performed using lipase, esculin, indole, xylose, trehalose, pyrazinamidase, and Voges–Proskauer biochemical tests according to methods described by Weagant et al. (24).

2.7. Pulsed-field gel electrophoresis

According to the Centers for Disease Control and Prevention PulseNet protocol (25), DNA was digested by *Sma*I (Takara Bio Inc., Shiga, Japan) for *S. aureus* and by *Asc*I (Thermo Fisher Scientific, Waltham, MA, USA) for *Y. enterocolitica*. Electrophoresis was performed using the CHEF-DRIII pulsed-field gel electrophoresis (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 arithmetic averages algorithm based on the Dice similarity index, and a similarity coefficient of 90% was fixed to assemble PFGE clusters.

2.8. Statistical analysis

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

TABLE 2 Distribution of microbial counts in 200 carcasses from 20 pig slaughterhouses.

Count interval (log_{10} CFU/cm^2)	No. pig carcass (%)	
	Aerobic bacteria	*E. coli*
≤ 1.00	0 (0)^{C}	174 (87.0)^{A}
1.01–2.00	6 (3.0)^{B,C}	11 (5.5)^{B}
2.01–3.00	57 (28.5)^{A}	12 (6.0)^{B}
3.01–4.00	84 (42.0)^{A}	3 (1.5)^{B,C}
4.01–5.00	16 (8.0)^{B}	0 (0)^{C}
5.01–6.00	17 (8.5)^{B}	0 (0)^{C}
6.01–7.00	14 (7.0)^{B}	0 (0)^{C}
≥ 7.01	6 (3.0)^{B,C}	0 (0)^{C}

Values with different superscript letters represent significant differences between columns ($P < 0.05$).

TABLE 3 Prevalence of foodborne pathogens in pig slaughterhouses and carcasses.

Pathogen	No. positive samples (%)	
	Slaughterhouses ($n = 20$)	Carcasses ($n = 200$)
Campylobacter coli	2 (10.0)^{A,B}	8 (4.0)^{A,B,C}
Campylobacter jejuni	0 (0)^{B}	0 (0)^{C}
Clostridium perfringens	5 (25.0)^{A,B}	8 (4.0)^{A,B,C}
Listeria monocytogenes	3 (15.0)^{A,B}	3 (1.5)^{B,C}
Listeria monocytogenes 1/2a	1 (5.0)	1 (0.5)
Listeria monocytogenes 1/2b	1 (5.0)	1 (0.5)
Listeria monocytogenes 1/2c	1 (5.0)	1 (0.5)
Salmonella Agona	1 (5.0)^{A,B}	1 (0.5)^{C}
Staphylococcus aureus	8 (40.0)^{A}	23 (11.5)^{A}
Shiga toxin-producing *Escherichia coli*	0 (0)^{B}	0 (0)^{C}
Yersinia enterocolitica	6 (30.0)^{A,B}	14 (7.0)^{A,B}
Yersinia enterocolitica O:3	1 (5.0)	1 (0.5)
Yersinia enterocolitica O:5	5 (25.0)	10 (5.0)
Yersinia enterocolitica O:untypable	2 (10.0)	3 (1.5)

Values with different superscript letters represent significant differences between columns ($P < 0.05$).

3. Results

3.1. Distribution of levels of aerobic bacteria and *E. coli*

The distribution of aerobic bacteria and *E. coli* counts in pig carcasses is presented in Table 2. Distribution of microbial counts were significantly higher for aerobic bacteria at 3.01–4.00 log_{10} CFU/cm^2 (42.0%) and 2.01–3.00 log_{10} CFU/cm^2 (28.5%), whereas most of *E. coli* showed the counts under 1.00 log_{10} CFU/cm^2 (87.0%) ($P < 0.05$).

3.2. Prevalence of foodborne pathogens

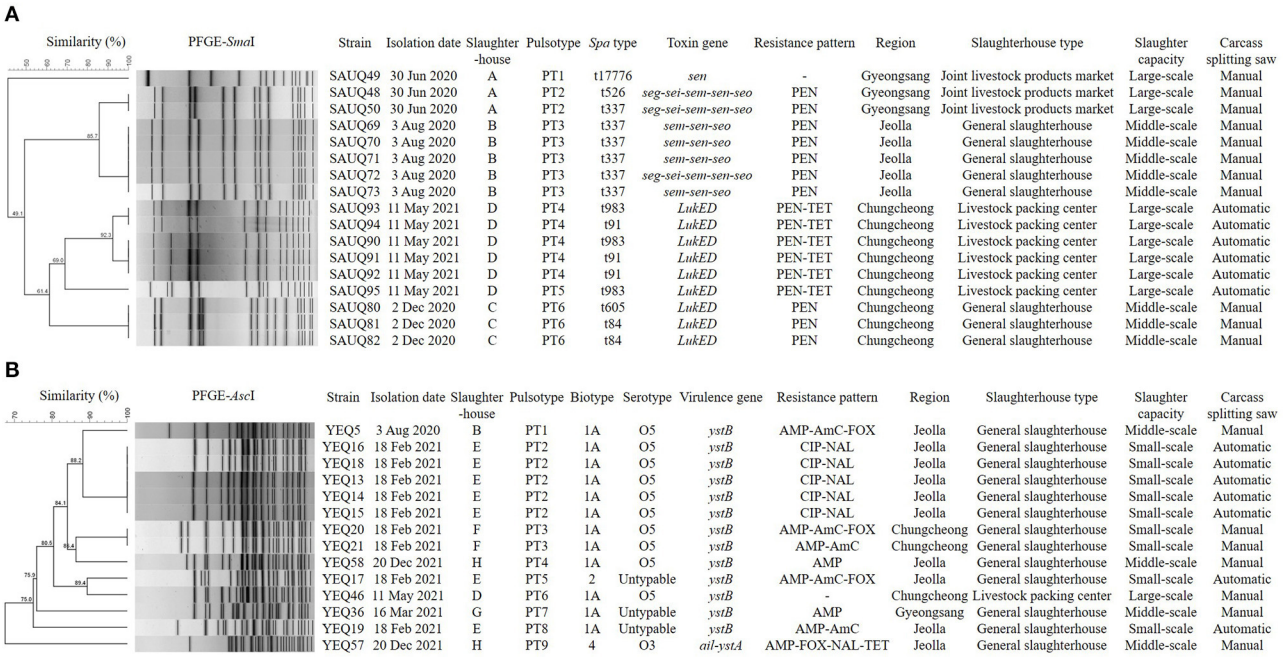
The prevalence of foodborne pathogens in slaughterhouses and pig carcasses is presented in Table 3. The most common pathogen isolated from 200 carcasses was *S. aureus* (11.5%), followed by *Y. enterocolitica* (7.0%), *C. perfringens* (4.0%), and *C. coli* (4.0%), and the most prevalent pathogens in 20 slaughterhouses were *S. aureus* (40.0%), *Y. enterocolitica* (30.0%), and *C. perfringens* (25.0%) ($P < 0.05$). In particular, *Y. enterocolitica* was divided into three serotypes, and *Y. enterocolitica* O:5 showed the highest prevalence in slaughterhouses (25.0%) and carcasses (5.0%). Moreover, two *C. coli* and one *S.* Agona isolates were obtained from two (10.0%) and one (5.0%) slaughterhouses, respectively, and three *L. monocytogenes* isolates obtained from three slaughterhouses were divided into three serotypes: 1/2a, 1/2b, and 1/2c.

3.3. Characteristics of *S. aureus* and *Y. enterocolitica*

Characteristics of phylogenetic, antibiotic resistance, and biotypic profiles of two major pathogens, *S. aureus* and *Y. enterocolitica* are presented in Figure 1. In total, 17 *S. aureus* isolates from four slaughterhouses were divided into six pulsotypes and seven *spa* types, and showed the same or different types depending on the slaughterhouses. Specifically, five isolates from slaughterhouse B showed the same pulsotype and *spa* type, but those from slaughterhouse D could be divided into two pulsotypes and two *spa* types. Furthermore, isolates from slaughterhouses A and C divided into two and one pulsotypes, and three and two *spa* types, respectively. Interestingly, isolates from slaughterhouses C and D only carried *LukED* associated with the promotion of bacterial virulence, whereas those from slaughterhouses A and B carried one or more toxin genes associated with enterotoxins, including *sen*. In total, 14 *Y. enterocolitica* isolates from six slaughterhouses were divided into nine pulsotypes, but isolates showed three biotypes and two serotypes, excluding three O:untypable isolates. Moreover, 13 isolates belonging to biotype 1A or 2 carried only *ystB* encoding an enterotoxin, and one isolate belonging to bio-serotype 4/O:3 carried both *ail* and *ystA*, which encode an attachment invasion locus and enterotoxin, respectively.

4. Discussion

Microbial contamination of meat is unavoidable because microorganisms are present on animals and in their environments. Thus, the initial level of bacteria in carcasses is important because as it directly affects spoilage and the shelf life (5). According to the Livestock Products Sanitary Control Act and Modernization of Swine Slaughter Inspection (10, 26), the hygienic quality of pig carcass is considered satisfactory when aerobic bacteria and *E. coli* counts are < 5.00 log_{10} CFU/cm^2 and < 4.00 log_{10} CFU/cm^2, respectively. In this study, although 163 (81.5%) among 200 carcasses met this criterion for aerobic bacterial counts, 37 (18.5%) carcasses showed aerobic bacterial counts exceeding 5.00 log_{10} CFU/cm^2. Lebret and Candek-Potokar (27) reported that microbial growth in pork carcasses depends on the environmental



A

Strain	Isolation date	Slaughter-house	Pulsotype	*Spa* type	Toxin gene	Resistance pattern	Region	Slaughterhouse type	Slaughter capacity	Carcass splitting saw
SAUQ49	30 Jun 2020	A	PT1	t17776	*sen*	-	Gyeongsang	Joint livestock products market	Large-scale	Manual
SAUQ48	30 Jun 2020	A	PT2	t526	*seg-sei-sem-sen-seo*	PEN	Gyeongsang	Joint livestock products market	Large-scale	Manual
SAUQ50	30 Jun 2020	A	PT2	t337	*seg-sei-sem-sen-seo*	PEN	Gyeongsang	Joint livestock products market	Large-scale	Manual
SAUQ69	3 Aug 2020	B	PT3	t337	*sem-sen-seo*	PEN	Jeolla	General slaughterhouse	Middle-scale	Manual
SAUQ70	3 Aug 2020	B	PT3	t337	*sem-sen-seo*	PEN	Jeolla	General slaughterhouse	Middle-scale	Manual
SAUQ71	3 Aug 2020	B	PT3	t337	*sem-sen-seo*	PEN	Jeolla	General slaughterhouse	Middle-scale	Manual
SAUQ72	3 Aug 2020	B	PT3	t337	*seg-sei-sem-sen-seo*	PEN	Jeolla	General slaughterhouse	Middle-scale	Manual
SAUQ73	3 Aug 2020	B	PT3	t337	*sem-sen-seo*	PEN	Jeolla	General slaughterhouse	Middle-scale	Manual
SAUQ93	11 May 2021	D	PT4	t983	*LukED*	PEN-TET	Chungcheong	Livestock packing center	Large-scale	Automatic
SAUQ94	11 May 2021	D	PT4	t91	*LukED*	PEN-TET	Chungcheong	Livestock packing center	Large-scale	Automatic
SAUQ90	11 May 2021	D	PT4	t983	*LukED*	PEN-TET	Chungcheong	Livestock packing center	Large-scale	Automatic
SAUQ91	11 May 2021	D	PT4	t91	*LukED*	PEN-TET	Chungcheong	Livestock packing center	Large-scale	Automatic
SAUQ92	11 May 2021	D	PT4	t91	*LukED*	PEN-TET	Chungcheong	Livestock packing center	Large-scale	Automatic
SAUQ95	11 May 2021	D	PT5	t983	*LukED*	PEN-TET	Chungcheong	Livestock packing center	Large-scale	Automatic
SAUQ80	2 Dec 2020	C	PT6	t605	*LukED*	PEN	Chungcheong	General slaughterhouse	Middle-scale	Manual
SAUQ81	2 Dec 2020	C	PT6	t84	*LukED*	PEN	Chungcheong	General slaughterhouse	Middle-scale	Manual
SAUQ82	2 Dec 2020	C	PT6	t84	*LukED*	PEN	Chungcheong	General slaughterhouse	Middle-scale	Manual

B

Strain	Isolation date	Slaughter-house	Pulsotype	Biotype	Serotype	Virulence gene	Resistance pattern	Region	Slaughterhouse type	Slaughter capacity	Carcass splitting saw
YEQ5	3 Aug 2020	B	PT1	1A	O5	*ystB*	AMP-AmC-FOX	Jeolla	General slaughterhouse	Middle-scale	Manual
YEQ16	18 Feb 2021	E	PT2	1A	O5	*ystB*	CIP-NAL	Jeolla	General slaughterhouse	Small-scale	Automatic
YEQ18	18 Feb 2021	E	PT2	1A	O5	*ystB*	CIP-NAL	Jeolla	General slaughterhouse	Small-scale	Automatic
YEQ13	18 Feb 2021	E	PT2	1A	O5	*ystB*	CIP-NAL	Jeolla	General slaughterhouse	Small-scale	Automatic
YEQ14	18 Feb 2021	E	PT2	1A	O5	*ystB*	CIP-NAL	Jeolla	General slaughterhouse	Small-scale	Automatic
YEQ15	18 Feb 2021	E	PT2	1A	O5	*ystB*	CIP-NAL	Jeolla	General slaughterhouse	Small-scale	Automatic
YEQ20	18 Feb 2021	F	PT3	1A	O5	*ystB*	AMP-AmC-FOX	Chungcheong	General slaughterhouse	Small-scale	Manual
YEQ21	18 Feb 2021	F	PT3	1A	O5	*ystB*	AMP-AmC	Chungcheong	General slaughterhouse	Small-scale	Manual
YEQ58	20 Dec 2021	H	PT4	1A	O5	*ystB*	AMP	Jeolla	General slaughterhouse	Middle-scale	Manual
YEQ17	18 Feb 2021	E	PT5	2	Untypable	*ystB*	AMP-AmC-FOX	Jeolla	General slaughterhouse	Small-scale	Automatic
YEQ46	11 May 2021	D	PT6	1A	O5	*ystB*	-	Chungcheong	Livestock packing center	Large-scale	Manual
YEQ36	16 Mar 2021	G	PT7	1A	Untypable	*ystB*	AMP	Gyeongsang	General slaughterhouse	Middle-scale	Manual
YEQ19	18 Feb 2021	E	PT8	1A	Untypable	*ystB*	AMP-AmC	Jeolla	General slaughterhouse	Small-scale	Automatic
YEQ57	20 Dec 2021	H	PT9	4	O3	*ail-ystA*	AMP-FOX-NAL-TET	Jeolla	General slaughterhouse	Middle-scale	Manual

FIGURE 1
Dendrogram showing genetic relationships among the strains characterized by PFGE profiles **(A)** *Staphylococcus aureus*, **(B)** *Yersinia enterocolitica*. Showing similarities of < 90% in PFGE were considered to be unrelated. The types of slaughterhouses are divided into Livestock packing center (slaughterhouse for slaughter, processing, and sale), Joint livestock products market (slaughterhouse for slaughter and sale), and General slaughterhouse (slaughterhouse for slaughter). Slaughter capacities (pigs/day) are divided into small-scale (≤ 900), middle-scale (901–1,500), and large-scale (≥ 1,501). PEN, Penicillin; TET, Tetracycline; AMP, Ampicillin; AmC, Amoxicillin/ Clavulanic acid; FOX, Cefoxitin; CIP, Ciprofloxacin; NAL, Nalidixic acid.

conditions during the aging process, and microbes ultimately affect pork spoilage and quality deterioration. Therefore, it is important to control microbial growth during storage by enhancing hygiene. In contrast, all carcasses had *E. coli* counts of < 4.00 $\log_{10}$ CFU/cm^2, with 87.0% of carcasses showing counts of ≤ 1.00 $\log_{10}$ CFU/cm^2. Van Ba et al. (28) previously reported that the average counts of aerobic bacteria and *E. coli* in pig carcasses in Korea were satisfactory, and Lindblad et al. (29) and Bohaychuk et al. (30) also reported that pig carcasses from Sweden and Canada met the criteria for aerobic bacteria and *E. coli* counts, respectively. In developed countries, risk factors in slaughterhouses are strictly managed because a high initial microbial load, poor hygiene practices, or high temperatures (>15°C) in the slaughtering lines can affect the distribution of microorganisms (31), and the quality of carcasses can be improved through food safety/HACCP implementation, non-conformities control, site hygiene, and pest control (32).

Several studies have reported that the most common foodborne pathogens associated with pigs are *Campylobacter* spp., *Salmonella* spp., *S. aureus*, *L. monocytogenes*, and *Y. enterocolitica* (33–35). In this study, the prevalence of seven major foodborne pathogens was investigated, and five pathogens were isolated from carcasses with *S. aureus* being the most prevalent (40.0% of slaughterhouses and 11.5% of carcasses). The MFDS (36) reported 21 cases of staphylococcal food poisoning involving 378 patients in Korea from 2018 to 2022. *S. aureus* is one of the most common causes of food poisoning, and is commonly found on the skin and in the mucous membranes of human beings and animals, and is particularly dangerous at slaughterhouses because of its potential for transmission from animals to slaughter operators and *vice-versa* (37). In Germany, Greece, and South Africa, the prevalence of *S. aureus* in pig carcasses were reported to be 6.0%, 15.5%, and 32.5%, respectively (38–40). The high prevalence of *S. aureus* in pig carcasses may be related to a lack of skinning of pigs during slaughter; therefore, it is important to minimize skin contamination during slaughter.

In this study, the second most frequently isolated pathogen was *Y. enterocolitica* (30.0% of slaughterhouses and 7.0% of carcasses). In the United States, *Y. enterocolitica* is estimated to cause ∼117,000 cases of illnesses, 640 hospitalizations, and 35 deaths each year (41). In Europe, human yersiniosis is the third most common foodborne zoonotic disease after campylobacteriosis and salmonellosis (42). Pigs are considered the primary reservoirs of human yersiniosis globally because pigs are the only animal species from which pathogenic strains have frequently been isolated so far (43). Moreover, pigs infected with *Y. enterocolitica* shed the organism in feces on farms for prolonged periods, and *Y. enterocolitica* has been frequently isolated from the tonsils of pigs at slaughter (44). Therefore, pig carcasses can be contaminated based on their infected tissues and intestinal contents in slaughterhouses (45).

C. perfringens is one of the bacterial hazards identified in the Guides to Good Hygiene Practices, and of application of HACCP principles in the slaughtering because *C. perfringens* frequently colonizers of the intestinal tracts of various food animals (46). In this study, the prevalence of *C. perfringens* was only 4.0% in

carcasses, but it was detected in as high as 25.0% of slaughterhouses. Therefore, for hygienic carcass production, it is necessary to fast animals on the farm before slaughter and reduce the contents of the gastrointestinal during slaughter (47).

Campylobacter jejuni was not found in this study, but *C. coli* was identified in 4.0% of carcasses. Several researchers have reported that the predominant species of *Campylobacter* in pigs is *C. coli*, whereas that in poultry and cattle is *C. jejuni* (48, 49). *Campylobacter* spp. do not usually cause clinical signs in animals, but reducing the abundance of *Campylobacter* in carcasses at slaughter can be an important step in the farm-to-table continuum through which *Campylobacter* enters the food chain.

Mechesso et al. (50) reported that the prevalence of *S. agona* was 10.8% in domestic pig carcasses from 2016 to 2018 in Korea, but in this study, this serovar was only isolated from one carcass. *S.* Agona is an important cause of food poisoning, and infection in humans usually occurs *via* the consumption of contaminated meat and eggs (51). Recently, Trinetta et al. (52) reported that the prevalence of *S.* Agona was 24.5% at 11 feed mills in eight states representative of the main pig production areas within the United States. Although the risk of feed-borne salmonellosis is difficult to quantify, this report indicated that *Salmonella* can be transmitted *via* contaminated feed through the food chain to pig farms, slaughterhouses, and ultimately to humans. Therefore, risk assessment studies for *Salmonella*-contaminated feed should be continuously performed to identify potential hazards.

In this study, the genetic and phenotypic characteristics of *S. aureus* and *Y. enterocolitica*, the main pathogens isolated from pig carcasses, were investigated. The prevalence of *S. aureus* and *Y. enterocolitica* had no relationship with the season, region, slaughterhouse type, slaughter capacity, and type of carcass splitting even though carcasses were collected from slaughterhouses across Korea (data not shown). Moreover, some isolates from the same slaughterhouse clustered in the same pulsotype, but 17 *S. aureus* isolates were ultimately divided into six pulsotypes. For identifying the epidemiological relevance of food poisoning bacteria, PFGE is generally preferred over other typing methods such as multilocus sequence typing or *spa* typing because standardization of the PFGE protocol has established a nomenclature for local pulsotypes in many countries (53), and because it is a highly discriminatory and valuable technique for the typing of classification within species (54).

The *S. aureus* isolates were divided into seven *spa* types, showing more diverse *spa* types than pulsotypes within the same slaughterhouse. Shopsin et al. (23) reported that *spa* typing provides clonal groupings that PFGE techniques cannot identify individually; thus, analyzing *spa* types together provides high differentiation in describing PFGE subtyping. In particular, among the *spa* types, t34 and t337 have been reported to be predominant in pigs worldwide (55), and t337 was also frequently confirmed in this study, consistent with previous reports (56).

In this study, 14 *Y. enterocolitica* isolates were divided into nine pulsotypes, whereas only three biotypes (1A, 2, and 5) and two serotypes (O3 and O5) were identified, excluding the untypable serotype. In general, *Y. enterocolitica* is divided into the non-pathogenic biotype 1A, weakly pathogenic biotypes 2–5, and highly pathogenic biotype 1B (57). Fortunately, biotype 1B was not identified in this study. *Y. enterocolitica* O:5, which was the most common serogroup in this study, has been reported to be the most prevalent serogroup worldwide, and it is mainly associated with non-pathogenicity type (16, 44). However, *Y. enterocolitica* bio-serotype 4/O:3, which was isolated from only one carcass in this study, is known to be pathogenic to humans (58).

In this study, all *S. aureus* isolates carried staphylococcal enterotoxin genes that induce food poisoning or leukotoxin genes that promote virulence (59). These two genes are located on mobile elements in bacterial genomes such as plasmids or pathogenic islands; thus they can easily be transferred horizontally between strains (60, 61).

In *Y. enterocolitica*, pathogenicity is associated with the presence of plasmids and chromosomal virulence genes, but the most commonly encoded virulence determinants are *ail* and the enterotoxin-encoding gene *yst*, which are chromosomal virulence markers (62). In particular, *Y. enterocolitica* biotype 1A, which is regarded as a non-pathogenic environmental strain, lacks the pYV plasmid (plasmid of *Yersinia* virulence) and most chromosomal virulence markers (63). In this study, all 12 *Y. enterocolitica* biotype 1A isolates harbored only *ystB*, which is recovered from wild animals and the environments, as previously described (64). However the *ail*, which usually accompanies *ystA* in pathogenic *Y. enterocolitica*, was identified in one *Y. enterocolitica* bio-serotype 4/O:3 isolate.

In this study, all *S. aureus* except one isolate showed the resistance to penicillin, and isolates from slaughterhouse D showed the resistance to both penicillin and tetracycline. Moreover, *Y. enterocolitica* showed the resistance to various antimicrobial subclasses, although there were similarities in antimicrobial resistant classes by slaughterhouse. The difference in the resistance of isolates by slaughterhouse is presumed to be attributable to differences in the antimicrobial classes mainly used in farms by region, because pigs are mainly slaughtered at slaughterhouses located in the region in which they are raised.

This is the first study to investigate microbial quality and the prevalence of foodborne pathogens in carcasses from slaughterhouses nationally, and the findings support the need for ongoing slaughterhouse monitoring to improve the microbiological safety of pig carcasses.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical review and approval was not required for the study on animals in accordance with the local legislation and institutional requirements. Written informed consent from the owners for the participation of their animals in this study was not required in accordance with the national legislation and the institutional requirements.

Author contributions

SH, J-SM, S-SY, H-YK, and YL conceived and designed all the experiments. SH, HK, H-YL, H-RJ, and H-YK participated in collecting the data and performing the tests. SH, H-YK, and YL analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food and Rural affairs, and Republic of Korea (grant number B-1543081-2022-23-02).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. World Health Organization (WHO). *World Health Organization Estimates of the Global Burden of Foodborne Disease: Foodborne Diseases Burden Epidemiology Reference Group 2007–2015*. Geneva, Switzerland (2015). doi: 10.1007/978-3-662-43978-4_3884

2. Abebe E, Gugsa G, Ahmed M. Review on major food-borne zoonotic bacterial pathogens. *J Trop Med.* (2020) 2020:1–19. doi: 10.1155/2020/4674235

3. Imre K, Herman V, Morar A. Scientific achievements in the study of the occurrence and antimicrobial susceptibility profile of major foodborne pathogenic bacteria in foods and food processing environments in romania: review of the last decade. *Biomed Res Int.* (2020) 2020:1–9. doi: 10.1155/2020/5134764

4. World Health Organization (WHO). *Food Safety*. (2022). Availableonline at: https://www.who.int/news-room/fact-sheets/detail/food-safety (accessed May 19, 2022).

5. Das AK, Nanda PK, Das A, Biswas S. Hazards and safety issues of meat and meat products. In: *Food Safety and Human Health*. Amsterdam: Elsevier (2019). p. 145–168 doi: 10.1016/B978-0-12-816333-7.00006-0

6. Biasino W, De Zutter L, Mattheus W, Bertrand S, Uyttendaele M, Van Damme I. Correlation between slaughter practices and the distribution of *Salmonella* and hygiene indicator bacteria on pig carcasses during slaughter. *Food Microbiol.* (2018) 70:192–9. doi: 10.1016/j.fm.2017.10.003

7. Swart AN, Evers EG, Simons RLL, Swanenburg M. Modeling of *salmonella* contamination in the pig slaughterhouse. *Risk Anal.* (2016) 36:498–515. doi: 10.1111/risa.12514

8. Bae D, Macoy DM, Ahmad W, Peseth S, Kim B, Chon J, et al. Distribution and characterization of antimicrobial resistant pathogens in a pig farm, slaughterhouse, meat processing plant, and in retail stores. *Microorganisms.* (2022) 10:2252. doi: 10.3390/microorganisms10112252

9. Im MC, Seo KW, Bae DH, Lee YJ. Bacterial quality and prevalence of foodborne pathogens in edible offal from slaughterhouses in Korea. *J Food Prot.* (2016) 79:163–8. doi: 10.4315/0362-028X.JFP-15-251

10. Food Safety and Inspection Service (FSIS). *Modernization of Swine Slaughter Inspection; Final Rule.* (2019). Available online at: https://www.fsis.usda.gov/federal-register/rules/modernization-swine-slaughter-inspection (accessed January 18, 2023).

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

12. Jung B, Lim H, Jung S. Development of differential media and multiplex PCR assays for the rapid detection of *Listeria monocytogenes*. *Korean J Vet Res.* (2003) 43:231–237.

13. Arnold T, Scholz HC, Marg H, Rösler U, Hensel A. 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. *J Vet Med B.* (2004) 51:459–63. doi: 10.1111/j.1439-0450.2004.00808.x

14. Franck SM, Bosworth BT, Moon HW. Multiplex PCR for enterotoxigenic, attaching and effacing, and Shiga toxin-producing *Escherichia coli* strains from calves. *J Clin Microbiol.* (1998) 36:1795–7. doi: 10.1128/JCM.36.6.1795-1797.1998

15. Mason WJ, Blevins JS, Beenken K, Wibowo N, Ojha N, Smeltzer MS. Multiplex PCR protocol for the diagnosis of staphylococcal infection. *J Clin Microbiol.* (2001) 39:3332–8. doi: 10.1128/JCM.39.9.3332-3338.2001

16. Wannet WJ, Reessink M, Brunings HA, Maas HM. Detection of pathogenic *Yersinia enterocolitica* by a rapid and sensitive duplex PCR assay. *J Clin Microbiol.* (2001) 39:4483–6. doi: 10.1128/JCM.39.12.4483-4486.2001

17. Grimont P, Weill F-X. *Antigenic Formulae of the Salmonella Servovars: WHO Collaborating Centre for Reference and Research on Salmonella*. Paris, France (2007). p. 1–166. Available online at: https://www.pasteur.fr/sites/default/files/veng_0.pdf (accessed May 20, 2023).

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

19. On SLW, Jordan PJ. Evaluation of 11 PCR assays for species-level identification of *Campylobacter jejuni* and *Campylobacter coli*. *J Clin Microbiol.* (2003) 41:330–6. doi: 10.1128/JCM.41.1.330-336.2003

20. Yoo HS, Lee SU, Park KY, Park YH. Molecular typing and epidemiological survey of prevalence of *Clostridium perfringens* types by multiplex PCR. *J Clin Microbiol.* (1997) 35:228–32. doi: 10.1128/jcm.35.1.228-232.1997

21. Van Duijkeren E, Ikawaty R, Broekhuizen-Stins MJ, Jansen MD, Spalburg EC, de Neeling AJ, et al. Transmission of methicillin-resistant *Staphylococcus aureus* strains between different kinds of pig farms. *Vet Microbiol.* (2008) 126:383–9. doi: 10.1016/j.vetmic.2007.07.021

22. Platt-Samoraj A, Ugorski M, Szweda W, Szczerba-Turek A, Wojciech K, Procajło Z. Analysis of the presence of *ail, ystA* and *ystB* genes in *Yersinia enterocolitica* Strains isolated from aborting sows and aborted fetuses. *J Vet Med Ser B.* (2006) 53:341–6. doi: 10.1111/j.1439-0450.2006.00969.x

23. Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol.* (1999) 37:3556–63. doi: 10.1128/JCM.37.11.3556-3563.1999

24. Weagant, S. D. Feng, P. Stanfield JT. *BAM Chapter 8, Yersinia enterocolitica. Bacteriological Analytical Manual.* (2017). Availale online at: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-8-yersinia-enterocolitica (accessed December 20, 2022).

25. Centers for Disease Control and Prevention (CDC). *Pulsed-Field Gel Electrophoresis (PFGE)*. (2016). Available online at: https://www.cdc.gov/pulsenet/pathogens/pfge.html (accessed January 18, 2023).

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

27. Lebret B, Candek-Potokar M. Review: pork quality attributes from farm to fork. Part I Carcass and fresh meat. *Animal.* (2022) 16:100402. doi: 10.1016/j.animal.2021.100402

28. Van Ba H, Seo H-W, Seong P-N, Kang S-M, Cho S-H, Kim Y-S, et al. The fates of microbial populations on pig carcasses during slaughtering process, on retail cuts after slaughter, and intervention efficiency of lactic acid spraying. *Int J Food Microbiol.* (2019) 294:10–7. doi: 10.1016/j.ijfoodmicro.2019.01.015

29. Lindblad M, Lindmark H, Lambertz ST, Lindqvist R. Microbiological baseline study of swine carcasses at swedish slaughterhouses. *J Food Prot.* (2007) 70:1790–7. doi: 10.4315/0362-028X-70.8.1790

30. Bohaychuk VM, Gensler GE, Barrios PR. Microbiological baseline study of beef and pork carcasses from provincially inspected abattoirs in Alberta, Canada. *Can Vet J.* (2011) 52:1095–100.

31. Manios SG, Grivokostopoulos NC, Bikouli VC, Doultsos DA, Zilelidou EA, Gialitaki MA, et al. 3-year hygiene and safety monitoring of a meat processing plant which uses raw materials of global origin. *Int J Food Microbiol.* (2015) 209:60–9. doi: 10.1016/j.ijfoodmicro.2014.12.028

32. Jakubowska-Gawlik K, Kolanowski W, Murali AP, Trafialek J. A comparison of food safety conformity between cattle and pig slaughterhouses. *Food Control.* (2022) 140:109143. doi: 10.1016/j.foodcont.2022.109143

33. Drummond N, Murphy BP, Ringwood T, Prentice MB, Buckley JF, Fanning S. *Yersinia enterocolitica*: a brief review of the issues relating to the zoonotic pathogen, public health challenges, and the pork production chain. *Foodborne Pathog Dis.* (2012) 9:179–89. doi: 10.1089/fpd.2011.0938

34. Bennett SD, Walsh KA, Gould LH. Foodborne disease outbreaks caused by *Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus*—United States, 1998–2008. *Clin Infect Dis.* (2013) 57:425–33. doi: 10.1093/cid/cit244

35. Heredia N, García S. Animals as sources of food-borne pathogens: a review. *Anim Nutr.* (2018) 4:250–5. doi: 10.1016/j.aninu.2018.04.006

36. Ministry of Food and Drug Safety (MFDS). *Food Poisoning Statistics.* (2023). Available online at: https://www.foodsafetykorea.go.kr/portal/healthyfoodlife/foodPoisoningStat.do?menu_no=4425&menu_grp=MENU_NEW02 (accessed January 20, 2023).

37. Peton V, Le Loir Y. *Staphylococcus aureus* in veterinary medicine. *Infect Genet Evol.* (2014) 21:602–15. doi: 10.1016/j.meegid.2013.08.011

38. Beneke B, Klees S, Stührenberg B, Fetsch A, Kraushaar B, Tenhagen B-A. Prevalence of methicillin-resistant *Staphylococcus aureus* in a fresh meat pork production chain. *J Food Prot.* (2011) 74:126–9. doi: 10.4315/0362-028X.JFP-10-250

39. Komodromos D, Kotzamanidis C, Giantzi V, Angelidis AS, Zdragas A, Sergelidis D. Prevalence and biofilm-formation ability of *Staphylococcus aureus* isolated from livestock, carcasses, the environment, and workers of three abattoirs in Greece. *J Hell Vet Med Soc.* (2022) 73:4097–104. doi: 10.12681/jhvms.26469

40. Tanih NF, Sekwadi E, Ndip RN, Bessong PO. Detection of pathogenic *Escherichia coli* and *Staphylococcus aureus* from cattle and pigs slaughtered in abattoirs in Vhembe District, South Africa. *Sci World J.* (2015) 2015:1–8. doi: 10.1155/2015/195972

41. Centers for Disease Control and Prevention (CDC). *Yersinia enterocolitica* (Yersiniosis) (2016). Available online at: https://www.cdc.gov/yersinia/index.html (accessed January 18, 2023).

42. European Food Safety Authority (EFSA). *The European Union One Health 2019 Zoonoses Report. EFSA J.* (2021) 19:6406. doi: 10.2903/j.efsa.2021.6406

43. Bari ML, Hossain MA, Isshiki K, Ukuku D. Behavior of *Yersinia enterocolitica* in foods. *J Pathog.* (2011) 2011:1–13. doi: 10.4061/2011/420732

44. Fredriksson-Ahomaa M, Stolle A, Stephan R. Prevalence of pathogenic *Yersinia enterocolitica* in pigs slaughtered at a Swiss abattoir. *Int J Food Microbiol.* (2007) 119:207–12. doi: 10.1016/j.ijfoodmicro.2007.07.050

45. Liang J, Wang X, Xiao Y, Cui Z, Xia S, Hao Q, et al. Prevalence of *Yersinia enterocolitica* in pigs slaughtered in chinese abattoirs. *Appl Environ Microbiol.* (2012) 78:2949–56. doi: 10.1128/AEM.07893-11

46. Songer JG, Uzal FA. Clostridial enteric infections in pigs. *J Vet Diagnostic Investig.* (2005) 17:528–36. doi: 10.1177/104063870501700602

47. Driessen B, Freson L, Buyse J. Fasting finisher pigs before slaughter influences pork safety, pork quality and animal welfare. *Animals.* (2020) 10:2206. doi: 10.3390/ani10122206

48. Whyte P, McGill K, Cowley D, Madden R, Moran L, Scates P, et al. Occurrence of *Campylobacter* in retail foods in Ireland. *Int J Food Microbiol.* (2004) 95:111–8. doi: 10.1016/j.ijfoodmicro.2003.10.018

49. Wieczorek K, Osek J. Characteristics and antimicrobial resistance of *Campylobacter* isolated from pig and cattle carcasses in Poland. *Pol J Vet Sci.* (2013) 16:501–8. doi: 10.2478/pjvs-2013-0070

50. Mechesso AF, Moon DC, Kim S-J, Song H-J, Kang HY, Na SH, et al. Nationwide surveillance on serotype distribution and antimicrobial resistance profiles of non-typhoidal *Salmonella* serovars isolated from food-producing animals in South Korea. *Int J Food Microbiol.* (2020) 335:108893. doi: 10.1016/j.ijfoodmicro.2020.108893

51. Foley SL, Lynne AM, Nayak R. *Salmonella* challenges: prevalence in swine and poultry and potential pathogenicity of such isolates1,2. *J Anim Sci.* (2008) 86:E149–62. doi: 10.2527/jas.2007-0464

52. Trinetta V, Magossi G, Allard MW, Tallent SM, Brown EW, Lomonaco S. Characterization of *Salmonella enterica* isolates from selected us swine feed mills by whole-genome sequencing. *Foodborne Pathog Dis.* (2020) 17:126–36. doi: 10.1089/fpd.2019.2701

53. Neoh H, Tan X-E, Sapri HF, Tan TL. Pulsed-field gel electrophoresis (PFGE): a review of the "gold standard" for bacteria typing and current alternatives. *Infect Genet Evol.* (2019) 74:103935. doi: 10.1016/j.meegid.2019.103935

54. Cookson BD, Robinson DA, Monk AB, Murchan S, Deplano A, de Ryck R, et al. Evaluation of molecular typing methods in characterizing a european collection of epidemic methicillin-resistant *Staphylococcus aureus* strains: the HARMONY collection. *J Clin Microbiol.* (2007) 45:1830–7. doi: 10.1128/JCM.02402-06

55. Sun J, Yang M, Sreevatsan S, Davies PR. Prevalence and characterization of *Staphylococcus aureus* in growing pigs in the USA. *PLoS one.* (2015) 10:e0143670. doi: 10.1371/journal.pone.0143670

56. Kang HY, Moon DC, Mechesso AF, Choi J-H, Kim S-J, Song H-J, et al. Emergence of CFR-mediated linezolid resistance in *Staphylococcus aureus* isolated from pig carcasses. *Antibiotics.* (2020) 9:769. doi: 10.3390/antibiotics9110769

57. Bancerz-Kisiel A, Pieczywek M, Łada P, Szweda W. The most important virulence markers of *Yersinia enterocolitica* and their role during infection. *Genes (Basel).* (2018) 9:235. doi: 10.3390/genes9050235

58. Laukkanen R, Martínez PO, Siekkinen K-M, Ranta J, Maijala R, Korkeala H. Contamination of carcasses with human pathogenic *Yersinia enterocolitica* 4/O:3 originates from pigs infected on farms. *Foodborne Pathog Dis.* (2009) 6:681–8. doi: 10.1089/fpd.2009.0265

59. Vasquez MT, Lubkin A, Reyes-Robles T, Day CJ, Lacey KA, Jennings MP, et al. Identification of a domain critical for *Staphylococcus aureus* LukED receptor targeting and lysis of erythrocytes. *J Biol Chem.* (2020) 295:17241–50. doi: 10.1074/jbc.RA120.015757

60. Varshney AK, Mediavilla JR, Robiou N, Guh A, Wang X, Gialanella P, et al. Diverse enterotoxin gene profiles among clonal complexes of *Staphylococcus aureus* isolates from the Bronx, New York. *Appl Environ Microbiol.* (2009) 75:6839–49. doi: 10.1128/AEM.00272-09

61. Moon BY, Park JY, Hwang SY, Robinson DA, Thomas JC, Fitzgerald JR, et al. Phage-mediated horizontal transfer of a *Staphylococcus aureus* virulence-associated genomic island. *Sci Rep.* (2015) 5:9784. doi: 10.1038/srep09784

62. Fois F, Piras F, Torpdahl M, Mazza R, Ladu D, Consolati SG, et al. Prevalence, bioserotyping and antibiotic resistance of pathogenic *Yersinia enterocolitica* detected in pigs at slaughter in Sardinia. *Int J Food Microbiol.* (2018) 283:1–6. doi: 10.1016/j.ijfoodmicro.2018.06.010

63. Bonardi S, Bassi L, Brindani F, D'Incau M, Barco L, Carra E, et al. Prevalence, characterization and antimicrobial susceptibility of *Salmonella enterica* and *Yersinia enterocolitica* in pigs at slaughter in Italy. *Int J Food Microbiol.* (2013) 163:248–57. doi: 10.1016/j.ijfoodmicro.2013.02.012

64. Platt-Samoraj A, Syczyło K, Szczerba-Turek A, Bancerz-Kisiel A, Jabłoński A, Łabuć S, et al. Presence of *ail* and *ystB* genes in *Yersinia enterocolitica* biotype 1A isolates from game animals in Poland. *Vet J.* (2017) 221:11–3. doi: 10.1016/j.tvjl.2017.01.013