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The fates of microbial populations on pig carcasses during slaughtering process, on retail cuts after slaughter, and intervention efficiency of lactic acid spraying

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ABSTRACT

This study was conducted to detect and identify microbial populations on pig carcasses at different slaughtering stages and on retail pork cuts at 24 h after slaughter as well as to evaluate the intervention efficiency of sprays containing different concentrations (2% and 4%) of lactic acid. The sprays were applied to the carcass surfaces at the end of the slaughter line. Microbial samples were collected from carcass surfaces after bleeding and after eviscerating, and from retail cuts at 24 h after chilling/spraying. The detected microorganisms were identified through using a Microflex identification instrument and 16S rRNA gene sequencing. The diversity of the bacterial genera; *Staphylococcus*, *Salmonella*, *Shigella*, *Enterococci*, *Escherichia*, *Acinetobacter* and *Corynebacterium* spp. showed counts ranging from 2.70 to 4.91 $\log_{10}$ cfu/100 cm^2 on the carcasses during slaughter. Most of these genera were also detected on the carcasses after 24 h of chilling. Three species (*Staphylococcus hyicus*, *Acinetobacter albensis*, and *Corynebacterium xerosis*) were also found on the retail cuts of non-sprayed carcasses but not on those of the sprayed groups. Significantly greater reductions in all bacterial species were observed on the carcasses and retail cuts that were sprayed with lactic acid, particularly at the 4% level. Thus, spraying with 4% lactic acid may be an effective intervention for controlling bacterial contamination on pig carcasses to improve the microbiological safety of pork meat.

1. Introduction

Food safety is an important concern in society, particularly for food processors and producers. Pork and pork-based products are the most widely consumed protein sources worldwide, but also are important sources of foodborne pathogens (Baer et al., 2013). Although meat from healthy animals is generally considered as safe, there is still an implicit risk of foodborne bacterial contamination or cross-contamination from faeces as well as equipment, utensils, and other materials used during slaughter (Buncic et al., 2014; Loretz et al., 2011). Recent surveillance studies revealed large numbers of foodborne disease outbreaks associated with the ingestion of contaminated meat and meat products. According to a report by the World Health Organization (WHO, 2015), 420,000 deaths occurred worldwide after consuming contaminated foods in 2015. The European Food Safety Authority (EFSA, 2016) reported 4362 foodborne disease outbreaks resulting in 45,874 cases of illness and 3892 hospitalizations among European member states. The number of foodborne diseases outbreaks has increased (approximately 4786 outbreaks) among European member states as reported by the EFSA (2017). In the United States, the Centers for Disease Control and Prevention (2017) estimated that each year, approximately 48 million people become sick from foodborne diseases, resulting in 128,000 hospitalizations and 3000 deaths.

Meat-producing animals, raw meats, and their processed products are the main sources of foodborne disease outbreaks and death annually (EFSA, 2017). Studies have shown that *Campylobacter*, *Salmonella*, Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, and staphylococci, among others, are the main foodborne bacteria (Baer et al., 2013; EFSA, 2017). Pork has been estimated as the second most important source of foodborne diseases, as pigs are reservoirs of pathogens

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that contaminate/cross-transmit to the carcasses or muscle tissues during slaughtering and processing (EFSA, 2016, 2017). Schmidt et al. (2012) reported a relatively high prevalence (91%) of pre-scald carcasses positive for *Salmonella* spp. These authors also reported that approximately 3.7% of carcasses were positive for *Salmonella* after the final washing and chilling. Similarly, Duggan et al. (2010) found that 69% of *Salmonella* contamination on carcasses was due to contamination in the slaughter environment. Although broiler meat is considered as the main source of *Campylobacter* contamination (EFSA, 2017), *Campylobacter* together with *Salmonella enterica* and *Yersinia enterocolitica* are the three most frequently reported hazards in human clinical cases related to pork consumption (Fosse et al., 2008). The most commonly detected *Campylobacter* species in the pig digestive carriage and pig carcasses are *Campylobacter coli* followed by *Campylobacter jejuni* and *Campylobacter lari* (Fosse et al., 2009). Similarly, *Listeria monocytogenes* was found to be the main pathogenic agent causing human listeriosis, with approximately 1500 cases of foodborne listeriosis per year reported in the United States (Scallan et al., 2011). Approximately 9% of raw pork samples were found to be positive for *L. monocytogenes* (Mataragas et al., 2009) because of contamination of live pigs or the slaughter environment (Hellstrom et al., 2010). Additionally, *Staphylococcus aureus* is a well-known pathogenic bacteria causing foodborne diseases in humans. Live pigs (e.g., sow, piglet, and finishing pig) are the major sources of staphylococci (Baer et al., 2013). A high prevalence of *S. aureus* on nasal, faecal, pork carcass, and raw pork samples has been reported in many countries (Baer et al., 2013; Beneke et al., 2011).

In Korea, according to a surveillance study by Kim (2010), 9472 salmonellosis outbreaks were caused by ingestion of contaminated foods in 1998–2007. Additionally, Lee et al. (2009) showed that fresh pork was the most important source (14.9%) of pathogenic *E. coli*, followed by poultry meat (4.6%) and beef (4.1%).

Many of these pathogenic foodborne bacteria are carried primarily in the pig's faeces, respiratory and gastrointestinal tracts, and scalding at 60 °C or a singeing process during commercial slaughter does not eliminate these species (Baer et al., 2013). As a result, head removal and evisceration are the main risk factors of cross-contaminations/transmissions from pigs to their carcasses which subsequently contaminate pork and pork products (Baer et al., 2013; Beneke et al., 2011; Dickson et al., 2002). In this context, an effective intervention of pork carcasses after head removal and the evisceration process is needed to reduce bacterial contamination on the muscle tissues. Previous studies have focused on intervention treatments of beef carcasses and beef muscle tissues by spraying with organic acids such as lactic acid and acetic acid (Ba et al., 2018; Buncic et al., 2014). To the best of our knowledge, however, limited information regarding intervention treatments on pork carcasses during slaughter under commercial conditions is available (Loretz et al., 2011). Since lactic acid is considered as a safe food additive and is commonly used in the meat industry because of its high microbial decontamination efficiency on naturally-contaminated beef carcasses (Ba et al., 2018). Thus, the present study was conducted to: (i) identify contamination by microbial populations and their fates of on pig carcasses during different slaughtering process and their muscle tissues after slaughter and (ii) investigate the antimicrobial efficiency of spraying with different concentrations of lactic acid on the microbial reduction on pig carcasses and retail pork cuts.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and media

Lactic acid and other chemicals used for analyses were purchased from Sigma-Aldrich (St. Louis, MO, USA). Media for bacterial culture and enumeration were as follows: Baird-Parker agar (supplemented with egg-yolk emulsion) was purchased from Oxoid, Ltd. (Hampshire, UK). Xylose lysine deoxycholate (XLD) agar, tryptic soy agar, Enterococcus selective agar, violet red bile agar, and Listeria selective agar were purchased from Sigma-Aldrich; 3M™ Petrifilm™ *E. coli*/coliform count plates and Petrifilm aerobic plate counts were purchased from 3 M Health Care (St. Paul, MN, USA). Sterile gauze sponges were purchased from McKesson Corp. (Richmond, VA, USA).

2.1.2. Animals

Pigs with average live weights of approximately 80 kg obtained from different local farms in Korea were used in this study. All animals were raised under the same conditions.

2.2. Experimental designs and preparations

2.2.1. Gauze sponge and lactic acid solution preparation

Sterile gauze sponges (3 × 3 cm) wrapped in aluminium foil were autoclaved for 15 min at 121 °C, and then each sponge was aseptically placed in an individual sampling bag containing 10 mL of peptone water before sealing.

Lactic acid at 2 different concentrations (2% and 4% v/v, pH 2.22 and 2.05, respectively) was used as the bacterial decontamination agent to compare their decontamination efficiencies.

2.2.2. Animals slaughter and decontamination spraying

The experiments were carried out at the pig slaughterhouse of the National Institute of Animal Science (Jeonju, South Korea). Animals were slaughtered using a commercial process as follows: stunning, bleeding, scalding (in 60 °C water for 5 min), dehairing, singeing, head removal and evisceration, splitting, final washing, and chilling (Fig. 1).

Spraying treatment of the skin surfaces of carcasses was conducted immediately after final washing (before entering the chilling room) as shown in Fig. 1. For each spraying group, the pig carcasses ($n = 12$ each) were sprayed with either 2% or 4% lactic acid. Each carcass side was sprayed with 100 mL of 2% or 4% lactic acid using a manual sprayer. Following spraying, all carcasses were immediately transferred into a chilling room (∼2 °C) and left for 24 h before microbiological samples were collected. Untreated pig carcasses ($n = 12$) which received no spraying treatment were served as controls.

2.2.3. Microbiological sampling

To determine the fates of microbial populations on pig carcasses during slaughter, microbiological samples were collected from the (i) surfaces of the carcasses at different slaughter stages: after bleeding and evisceration (before spraying treatment was applied); (ii) surfaces of carcass sides at 24 h after chilling/spraying; and (iii) retail cuts in the non-sprayed and sprayed carcass groups, as follows:

(i) Sampling of carcasses at different slaughter stages: The microbiological samples were collected from 6 representative locations (3 locations per side × 2 sides): Shoulder, hip (cranial-caudal midline), and ham of each carcass immediately after bleeding and after evisceration.
(ii) Sampling of carcass sides at 24 h after chilling/spraying: After 24 h spraying and chilling (before carcass grading), the same numbers of samples were collected from the same locations (as described above) of the non-sprayed control and sprayed carcass sides.
(iii) Sampling of retail cuts: On the same day (24 h *post-mortem*), three representative primal cuts: butt (neck), loin, and trimmed ham (butt end of ham) from each of the non-sprayed and sprayed carcasses were collected for microbiological analysis.

For all sampling stages, samples were collected by firmly rubbing the pre-moistened sterile sponge over a 100-cm^2 surface area (10 × 10 cm) of each selected region of the carcass or retail cut using sterile forceps. All sponge samples were then placed back into the bags, sealed, and transported on ice to the Laboratory of Microbiology for

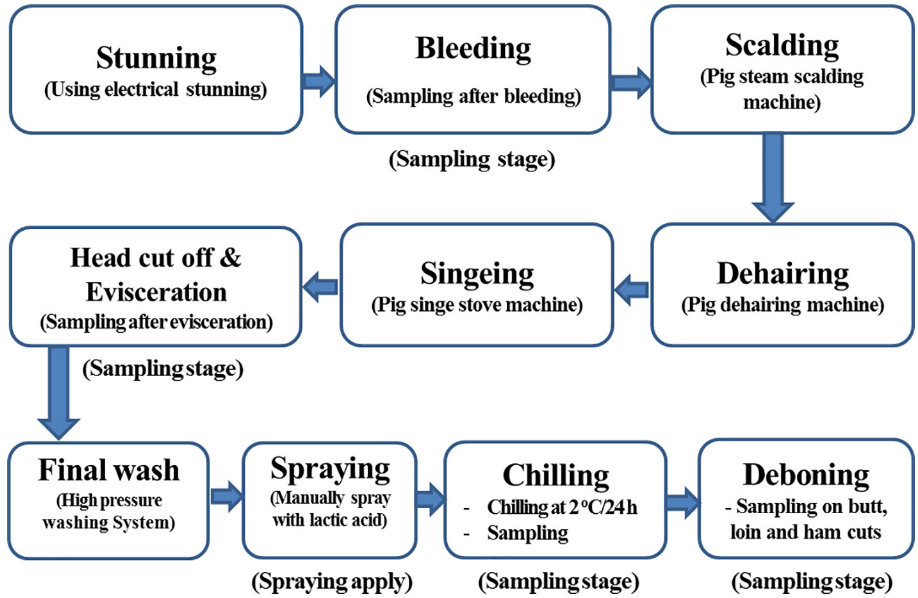

Fig. 1. The process of commercial pig slaughter and experimental steps (e.g., sampling and spraying) used in the study.

bacterial analyses.

2.2.4. Microbiological analysis

At approximately 2 h after sampling, microbiological analyses were performed. The bags containing sponge samples were hand-massaged inside the plastic bag for 1 min, and serial dilutions were prepared using 0.85% sodium chloride solution for each sample.

Aerobic plate counts (*APC*): APC were enumerated by plating 1 mL of a diluted sample solution onto the Petrifilm Aerobic Plate Counts. After spreading, the Petrifilms were incubated at 37 °C for 48 h in an incubator.

E. coli and *total coliform*: For ach sample, 1 mL of the diluted sample solutions was plated onto the 3M™ Petrifilm™ *E. coli*/coliform count plates, which were then incubated at 37 °C for 48 h in an incubator.

Staphylococcus spp.: Approximately 0.1 mL of the diluted sample solutions was plated on Baird-Parker agar supplemented with egg-yolk emulsion.

Enterococcus spp.: These species were determined by plating 0.1 mL of a diluted sample on Enterococcus selective agar.

Pantoea, *Acinetobacter*, and *Corynebacterium* spp.: Bacteria were evaluated by plating 0.1 mL of diluted sample solution on tryptic soy agar and violet red bile agar.

Shigella, *Salmonella*, and *Escherichia*: These bacteria were evaluated by plating 0.1 mL of diluted sample solution on xylose lysine deoxycholate agar plates.

All bacteria were cultured under aseptic conditions on a clean bench and grown in an incubator at 37 °C for 24–48 h as described in our previous study (Ba et al., 2018).

2.2.5. Methods for identifying bacteria

The bacterial species/genus were identified as described below:

(i) Typical biochemical properties (e.g., morphology and colour of colonies): Only APC, *E. coli*, and total coliforms were identified using this method according to the manufacturer's instructions, with all red colonies appearing on the Petrifilm aerobic plate counts considered as APC. The colonies with entrapped gas and red or blue colour were counted as coliforms. Colonies with entrapped gas and showing a blue to red-blue colour were counted as *E. coli*.

(ii) Using the highly reliable Microflex identification instruments (Brucker Daltonik GmbH: Bremen, Germany). Except for APC, *E. coli*, and total coliform, all other remaining bacterial species were identified with this instrument. Briefly, each typically single colony on each kind of agar medium (at initial culture) was taken and sub-cultured on the same type of agar medium. Following sub-culturing the cells for 24 h at 37 °C, 5 individual colonies from each type of agar medium were picked up and then analysed with the Microflex system. The identification procedure of bacterial species/genus was performed according to the manufacturer's instructions as described by Porte et al. (2017).

(iii) Using 16S rRNA gene sequencing: Following identification with the Microflex system, 3 individual sub-cultured colonies from each type of agar medium were collected and analysed by 16S rRNA sequencing as described in our previous study (Ba et al., 2018).

2.3. Statistical analysis

The Statistic Analysis System (SAS) package (SAS Institute, Cary, NC, USA, 2007) was used for data analysis. Each microbial sample was analysed in duplicate and bacterial species were calculated and converted to $\log_{10}$ of colony forming units/100 cm^2 (cfu/100 cm^2) of the carcass surface or retail cut surface. The means of the $\log_{10}$ cfu/100 cm^2 of all bacterial species obtained during the slaughter process (after bleeding and evisceration) were averaged from all pig carcasses including the non-spayed control, 2% lactic acid, and 4% lactic acid groups. Differences between the $\log_{10}$ cfu/100 cm^2 of the carcass or retail cut surfaces from the non-sprayed control and those treated with 2% or 4% lactic acid were calculated as the log reduction. The means of the bacterial populations for samples collected during the slaughter process or carcass surfaces after chilling/spraying and retail cut surfaces as well as the log reduction were statistically analysed by using the General Linear Model procedure of SAS. Differences between means were compared by using Duncan's multiple range test, and significance was defined at $P < 0.05$.

3. Results and discussion

According to the Korean Food Standard Codex (No. 53-2018) of Ministry of Food and Drug Safety (2018), organic acids such as lactic acid can be legally applied as chemical decontamination treatments of animal carcasses. In the present study, natural contamination of pig carcasses with microbial populations and bacterial fates at different

Table 1
Number ($\log_{10}$ cfu/100 cm^2) of aerobic plate count (APC), *E. coli* and total coliform on carcass's surfaces at different slaughter stages and decontamination efficiency of spraying with different levels of lactic acid (LA).

	Bleeding[1]	Evisceration[2]	Carcass surfaces			Log reduction on carcass[3]		IM[4]
			Non-spray	2% LA spray	4% LA spray	2% LA spray	4% LA spray	
APC	7.03 ± 0.31a	6.32 ± 0.14b	3.27 ± 0.16a	2.78 ± 0.02b	2.22 ± 0.02c	0.49 ± 0.01b	1.05 ± 0.01a	BP
E. coli	3.63 ± 0.02a	3.43 ± 0.04b	2.70 ± 0.05a	1.97 ± 0.01b	1.32 ± 0.01c	0.73 ± 0.01b	1.38 ± 0.03a	BP
Total coliform	3.25 ± 0.18a	3.29 ± 0.38a	2.57 ± 0.03a	2.31 ± 0.04b	1.54 ± 0.01c	0.26 ± 0.01b	1.03 ± 0.01a	BP

Values with different letters (a–c) within each parameter in the same row differ significantly ($P < 0.05$).
[1] Sampling was done on the surfaces of all carcasses in all groups following the bleeding process.
[2] Sampling was done on the surfaces of all carcasses in all groups following the evisceration process.
[3] $\log_{10}$ reduction = ($\log_{10}$ cfu/100 cm^2 carcass surfaces of non-sprayed control) − ($\log_{10}$ cfu/100 cm^2 surfaces of sprayed carcass).
[4] Identification method: the identification was based on their biochemical properties (BP) such as; morphology and colour etc. of colonies on their cultured media according to the recommendation by manufacturer.

slaughter stages were studied. Furthermore, the results of our previous study of decontamination efficiency of lactic acid spray on beef carcass (Ba et al., 2018) were used as a basis for developing an effective method for controlling cross-contaminations on pig carcasses during slaughter. By using the Microflex identification instrument and 16S rRNA gene sequencing methods, various bacterial species/genus were detected on the carcass surfaces and retail cuts, whereas no *Listeria* spp. or *Campylobacter* ss. was detected on the samples in the present study.

3.1. APC, E. coli, and total coliform on carcass surfaces during slaughter and the spray effect

The numbers of APC, *E. coli*, and total coliforms on the carcass surfaces at different slaughter stages (after bleeding and evisceration) and 24 h following spraying are shown in Table 1. The APC, *E. coli*, and total coliforms are considered as good indicators of the contamination level or hygienic situation of foods and food products (Bredie and de Boer, 1992). During slaughter, the APC, *E. coli*, and total coliform were higher (7.03, 3.36, and 3.25 $\log_{10}$/100 cm^2, respectively) on the carcass surfaces after the bleeding process, while their numbers were significantly decreased following the evisceration process (6.32, 3.43, and 3.29 $\log_{10}$/100 cm^2, respectively), except for the total coliform which remained unchanged. The reductions in the numbers of APC and *E. coli* may be related to the synergic effects of scalding with hot water and singeing during the slaughter process. Our results agree with those of Pearce et al. (2004) and Spescha et al. (2006), who reported that scalding (59–62 °C) pig carcasses resulted in reduction of aerobic bacteria.

After 24 h of chilling, the non-sprayed carcasses contained 3.27, 2.70, and 2.57 $\log_{10}$/100 cm^2 of APC, *E. coli*, and total coliform, respectively, while the carcasses sprayed with 2% or 4% lactic acid showed significantly lower numbers of APC (2.22 $\log_{10}$/100 cm^2), *E. coli* (1.32 $\log_{10}$/100 cm^2), and total coliform (1.54 $\log_{10}$/100 cm^2) ($P < 0.05$). Statistical analysis also revealed that the concentration of lactic acid used significantly affected the decontamination efficiency. For instance, spraying with 4% lactic acid yielded significantly ($P < 0.05$) higher reductions in APC (by 1.05 log), *E. coli* (by 1.38 log), and total coliform (by 1.03 log) compared to spraying with 2% lactic acid (e.g., APC, *E. coli*, and total coliform were reduced by 0.49, 0.73, and 0.26 $\log_{10}$/100 cm^2, respectively). Similar to our findings, previous studies reported that spraying pig carcasses with 1–2% lactic acid at the end of slaughter reduced aerobic bacteria by 0.2–1.0 log $\log_{10}/cm^2$ (Fu et al., 1994; Prasai et al., 1992). Compared to the numbers of APC, *E. coli*, and total coliform found on samples in the present study, Spescha et al. (2006) reported much higher numbers of aerobic bacteria (2.77–3.07 log per cm^2) on pig carcass surfaces after the chilling process. These contrasting results may be related to differences in hygienic situations or initial bacterial loads on pig carcasses between studies. Furthermore, it has been reported that psychotropic and psychrophilic microorganisms may contribute to the spoilage of chilled meat (Gill and Newton, 1978). In fact, low temperatures are typically used to store carcasses after slaughter (Ercolini et al., 2009); however, the psychotropic and psychrophilic bacteria can grow at cold temperatures, which may result in increased aerobic counts on the carcasses after chilling.

3.2. Staphylococcus species on carcass surfaces during slaughter and spray effect

The numbers ($\log_{10}$ cfu/100 cm^2) of *Staphylococcus* species detected on carcass surfaces during slaughter, 24 h after chilling/spraying, and lactic acid spraying are presented in Table 2. By plating the samples on agar medium specific for staphylococci and using highly reliable identification methods, we detected three strains, *S. hyicus*, *S. saprophyticus*, and *S. epidermidis*, on the carcass surfaces. During slaughter, after the bleeding step, the numbers of *S. hyicus*, *S. saprophyticus*, and *S. epidermidis* were 4.70, 4.44, and 2.94 $\log_{10}$ cfu/100 cm^2, respectively. After evisceration, these numbers were significantly reduced to 3.29, 3.14, and 2.12 $\log_{10}$ cfu/100 cm^2 for *S. hyicus*, *S. saprophyticus*, and *S. epidermidis*, respectively. These reductions may be related to the effects of the scalding and singeing process during slaughter. After the final wash and chilling for 24 h, the non-sprayed carcasses were contaminated with 2 of these 3 species [*S. hyicus* (2.58 $\log_{10}$ cfu/100 cm^2) and *S. saprophyticus* (2.10 $\log_{10}$ cfu/100 cm^2)]. Thus, the final washing and chilling process did not eliminate these bacteria from the carcasses. Regarding the spray effect, it was interesting to note that all carcasses were free from *S. saprophyticus* and *S. epidermidis* when sprayed with 2% or 4% lactic acid. Only *S. hyicus* was detected on all sprayed carcasses, but its count was significantly lower on the carcasses sprayed with 4% lactic acid (0.85 $\log_{10}$ cfu/100 cm^2) than on those sprayed with 2% lactic acid (1.64 $\log_{10}$ cfu/100 cm^2). The application of 2% and 4% lactic acid spray reduced *S. hyicus* by 0.94 and 1.73 $\log_{10}$ cfu/100 cm^2, respectively.

Staphylococci are commonly found in various environments (e.g., skin and digestive tracts of humans and animals). Among the 3 detected species, *S. hyicus* is a commensal flora in various animals and has been reported as the main pathogenic bacteria causing exudative epidermitis in pigs (Andresen, 1998); the major symptoms of infection with these bacteria are injured skin and histopathological alterations in pigs (Tanabe et al., 1996). *S. hyicus* is also known as the causative agent of sepsis in humans and is often misidentified as *S. aureus* (Casanova et al., 2011). Additionally, *S. saprophyticus* is frequently detected in foods (e.g., meat and meat products) and the gastrointestinal tracts of humans and animals (Hedman et al., 1990; Leroy et al., 2010). This strain has been recognized as the major pathogen causing urinary tract injection in humans (Raz et al., 2005). In humans, infection with *S. saprophyticus* typically results from eating contaminated foods (Hedman et al., 1990). Similarly, *S. epidermidis* has been isolated from the skin and mucous membranes of animals including pigs (Argudin et al., 2015b).

Table 2
Number ($\log_{10}$ cfu/100 cm^2) of Staphylococcus species on the carcass's surfaces at different slaughter stages and decontamination efficiency of spraying with different levels of lactic acid (LA).

	Bleeding[1]	Evisceration[2]	Carcass surfaces			Log reduction on carcass[3]		IM[4]
			Non-spray	2% LA spray	4% LA spray	2% LA spray	4% LA spray	
Staphylococcus hyicus	4.70 ± 0.25a	3.29 ± 0.16b	2.58 ± 0.06a	1.64 ± 0.01b	0.85 ± 0.01c	0.94 ± 0.01b	1.73 ± 0.05a	MI + 16S rRNA gene sequencing
Staphylococcus saprophyticus	4.44 ± 0.17a	3.14 ± 0.15b	2.10 ± 0.01	nd	nd	–	–	MI + 16S rRNA gene sequencing
Staphylococcus epidermidis	2.94 ± 0.05a	2.12 ± 0.29b	nd	nd	nd	–	–	MI + 16S rRNA gene sequencing

Values with different letters (a–c) within each parameter in the same row differ significantly ($P < 0.05$).
[1] Sampling was done on the surfaces of all carcasses in all groups following the bleeding process.
[2] Sampling was done on the surfaces of all carcasses in all groups following the evisceration process.
[3] Log_{10} reduction = (Log_{10} cfu/100 cm^2 carcass surfaces of non-sprayed control) − (Log_{10} cfu/100 cm^2 surfaces of sprayed carcass).
[4] Identification method: MI: Microflex identification instrument.

Staphylococcus epidermidis is an opportunistic pathogen causing mastitis in humans, and high transmission of this strain occurs between humans and animals (Argudin et al., 2015a). Thus, lactic acid spraying considerably reduced contamination by *S. hyicus* and eliminated *S. saprophyticus*, therefore improving the microbiological safety of pig carcasses.

3.3. Salmonella, Shigella, and Enterococcus species on carcass surfaces during slaughter and effects of spraying

The numbers of *Salmonella enterica*, *Shigella flexneri*, *Enterococcus faecium*, and *Enterococcus durans* detected on the pig carcass surfaces during slaughter and at 24 h after chilling/spraying are shown in Table 3. After bleeding, all carcasses were found to be contaminated with *S. enterica*, *S. flexneri*, *E. faecium*, and *E. durans* with maximum numbers of 4.50, 3.56, 3.83, and 3.46 $\log_{10}$ cfu/100 cm^2, respectively. Following evisceration, approximately 1.0 log reduction was observed on the carcasses for *S. enterica*, *S. flexneri*, *E. faecium*, and *E. durans* (3.59, 2.65, 2.78, and 2.26 $\log_{10}$ cfu/100 cm^2, respectively). After 24 h of chilling, all carcasses were positive for these 4 species; however, their counts differed depending on the control and spraying groups. Particularly, non-spayed carcasses showed the highest counts of *S. enterica* (1.79 $\log_{10}$ cfu/100 cm^2), *S. flexneri* (2.04 $\log_{10}$ cfu/100 cm^2), *E. faecium* (1.35 $\log_{10}$ cfu/100 cm^2), and *E. durans* (1.26 $\log_{10}$ cfu/100 cm^2), followed by those sprayed with 2% lactic acid, with the lowest counts found in the group sprayed with 4% lactic acid. The results also revealed that significantly ($P < 0.05$) higher reductions (by 0.89–1.12 log $\log_{10}$ cfu/100 cm^2) in all species were obtained on carcasses sprayed with 4% lactic acid compared to those sprayed with 2% lactic acid (by 0.26–0.48 log $\log_{10}$ cfu/100 cm^2). Lactic acid is well-known as an antimicrobial agent; the results of this study support those of Epling et al. (1993), who observed reduced *Salmonella* counts on pig carcasses after spraying with 2% lactic acid.

Among the detected species (Table 3), *S. enterica*, belonging to Enterobacteriaceae, is a major cause of foodborne disease, and results in diarrhoea, vomiting, pain, and fever symptoms (Kim, 2010). *Salmonella* infections in humans are associated with ingestion of contaminated foods of animal origin (Fardsanei et al., 2017; Kim, 2010). *Salmonella* is an important concern in the pork industry, as these bacteria are typically present in the intestinal tract of infected pigs, and contamination of pork carcasses with bacteria often occurs during slaughter because of cross-contamination from the contaminated intestinal tract which has been lacerated (Rostagno and Callaway, 2012). Because the washing process may result in the widespread distribution of bacterial contamination over carcasses, treating pig carcasses (e.g., by spraying with organic acid) after evisceration appears to be an effective and safe method for reducing the contamination risk of pig carcasses. Additionally, *S. flexneri* is the major cause of human shigellosis in many countries worldwide, resulting in typical symptoms such as diarrhoea (Shen et al., 2017). *Shigella flexneri* has also been isolated from meat and meat products (Mokhtari et al., 2012). *Shigella* is often transmitted through a faecal-oral route, and thus foods that directly contact animal waste can transmit these bacteria. Recently, studies showed that shigellosis outbreaks are often caused by contact with or ingestion of contaminated foods (Shen et al., 2017). Regarding *Enterococcus* species, both of *E. faecium* and *E. durans* are commensal bacteria in the gastrointestinal tract of animals and humans. These strains are frequently detected in pork, beef, chicken, and fermented sausages (Cho et al., 2013). Researchers have found that although *E. faecium* of animal origin does not constitute a health hazard to humans, they can act as donors of antimicrobial resistance genes for other pathogenic enterococci (Hammerum, 2012).

Table 3

Number ($\log_{10}$ cfu/100 cm^2) of Salmonella, Shigella and Enterococcus species on the carcass's surfaces at different slaughter stages and decontamination efficiency of spraying with different levels of lactic acid (LA).

	Bleeding[1]	Evisceration[2]	Carcass surfaces			Log reduction on carcass[3]		IM[4]
			Non-spray	2% LA spray	4% LA spray	2% LA spray	4% LA spray	
Salmonella enterica	4.50 ± 0.38a	3.59 ± 0.30b	1.79 ± 0.11a	1.31 ± 0.02b	0.69 ± 0.01c	0.48 ± 0.01b	1.10 ± 0.04a	MI + 16S rRNA gene sequencing
Shigella flexneri	3.56 ± 0.21a	2.65 ± 0.21b	2.04 ± 0.01a	1.63 ± 0.01b	0.92 ± 0.01c	0.41 ± 0.01b	1.12 ± 0.02a	MI + 16S rRNA gene sequencing
Enterococcus faecium	3.83 ± 0.29a	2.78 ± 0.22b	1.35 ± 0.07a	1.03 ± 0.01b	0.43 ± 0.01c	0.32 ± 0.01b	0.92 ± 0.02a	MI + 16S rRNA gene sequencing
Enterococcus durans	3.46 ± 0.34a	2.26 ± 0.23b	1.26 ± 0.01a	1.00 ± 0.00b	0.37 ± 0.01c	0.26 ± 0.01b	0.89 ± 0.02a	MI + 16S rRNA gene sequencing

Values with different letters (a–c) within each parameter in the same row differ significantly ($P < 0.05$). nd: Not detected; (–): insufficient data for calculation.

[1] Sampling was done on the surfaces of all carcasses in all groups following the bleeding process.

[2] Sampling was done on the surfaces of all carcasses in all groups following the evisceration process.

[3] Log_{10} reduction = (Log_{10} cfu/100 cm^2 carcass surfaces of non-sprayed control) − (Log_{10} cfu/100 cm^2 surfaces of sprayed carcass).

[4] Identification method: MI: Microflex identification instrument.

3.4. *Pantoea dispersa, Escherichia fergusonii, Acinetobacter albensis, and Corynebacterium xerosis on carcass surfaces during slaughter and effects of spraying*

The numbers of *Pantoea dispersa*, *Escherichia fergusonii*, *Acinetobacter albensis*, and *Corynebacterium xerosis* on carcass surfaces at different slaughtering stages and 24 h after chilling/spraying are shown in Table 4. The results showed that *P. dispersa* counts remained unchanged during slaughter, ranging from 2.70 to 2.52 $\log_{10}$ cfu/100 cm^2 on carcass surfaces after bleeding and evisceration, respectively. *Escherichia fergusonii* was only found on the carcasses at 2.93 $\log_{10}$ cfu/100 cm^2 after bleeding and was not found after evisceration. The counts of *A. albensis* and *C. xerosis* were typically reduced after evisceration compared to those after bleeding. After 24 h chilling/spraying, these bacterial species were detected on the surfaces of non-sprayed and sprayed carcasses, except for *E. fergusonii*. In general, the non-sprayed carcasses showed significantly ($P < 0.05$) higher counts of these bacterial species, followed by carcasses sprayed with 2% and 4% lactic acid. Spraying with 4% lactic acid caused significantly larger reductions in *P. dispersa* (by 0.41 log), *A. albensis (*by 1.16 log), and *C. xerosis* (by 1.11 log) compared to 2% lactic acid spray. Similar to our results, Fabrizio and Cutter (2004) reported that spraying inoculated pig carcasses with 2% lactic acid reduced *E. coli* and other pathogenic bacteria by 1.1–1.8 log cfu/cm^2.

In the present study, the prevalence of *P. dispersa*, *A. albensis*, and *C. xerosis* on pig carcasses during slaughter and at 24 h after chilling was determined. Among these species, *P. dispersa* belonging to the Enterbacteriaceae family is frequently found in the gastrointestinal tract of animals and humans and in foodstuffs; some genera of *Pantoea* have been identified as human pathogens causing blood poisoning (Liberto et al., 2009). *Escherichia fergusonii* has frequently been found in the faeces of warm-blooded animals (Hariharan et al., 2007) and in animal meats (Fegan et al., 2006). *Escherichia fergusonii* is a pathogenic bacterium causing diarrhoea symptoms in humans (Gaastra et al., 2014). Additionally, *Acinetobacter* species are commonly found in nature, such as on vegetables, animals, and humans (Hamouda et al., 2011), and some *Acinetobacter* species such as *A. septicus* rather than *A. albensis* were shown to cause human infections (Sung et al., 2014). Additionally, *C. xerosis* is widely distributed in nature and the microbiota of animals. In industry, some *Corynebacterium* species (e.g., *C. glutamicum*) have been used for amino acid production, whereas other species (e.g., *C. diphtheria* and *C. xerosis*) are known to cause infections in humans and animals (Vela et al., 2006).

3.5. *Microbial populations on retail cuts and effects of spraying*

After 24 h of chilling and spraying, 3 representative retail cuts from each carcass side were sampled and subjected to microbiological analysis; the results are presented in Table 5. Our results revealed that all cut samples were contaminated with APC and total coliform, in which the samples from the non-sprayed carcasses showed significantly higher counts (2.64 and 1.97 $\log_{10}$ cfu/100 cm^2 for APC and total coliform, respectively) than those from sprayed carcasses. Additionally, greater reductions in APC (by 1.07 $\log_{10}$ cfu/100 cm^2) and total coliform (0.60 $\log_{10}$ cfu/100 cm^2) were obtained on cuts from carcasses sprayed with 4% lactic acid compared to the cuts from carcasses sprayed with 2% lactic acid. Notably, cut samples from non-sprayed carcasses were positive for *S. hyicus* (0.77 $\log_{10}$ cfu/100 cm^2), *A. albensis* (0.71 $\log_{10}$ cfu/100 cm^2), and *C. xerosis* (0.67 $\log_{10}$ cfu/100 cm^2). Interestingly, all cut samples from the sprayed carcasses were negative for all these bacterial species. Fu et al. (1994) sprayed pig carcasses with 1.5% lactic acid and found values of 3–4 $\log_{10}$ cfu/g of APC for pork loin at 24 h *post-mortem*. In general, the numbers of APC and coliform detected on retail cuts after 24 h chilling/spraying for all groups were lower than those (4 log cfu/g of APC) previously reported for pork samples (Li et al., 2014).

Table 4

Number ($\log_{10}$ cfu/100 cm^2) of *Pantoea dispersa*, *Escherichia fergusonii*, *Acinetobacter albensis* and *Corynebacterium xerosis* on carcass's surfaces at different slaughter stages and decontamination efficiency of spraying with different levels of lactic acid (LA).

	Bleeding[1]	Evisceration[2]	Carcass surfaces			Log Reduction on carcass[3]		IM[4]
			Non-spray	2% LA spray	4% LA spray	2% LA spray	4% LA spray	
Pantoea dispersa	2.70 ± 0.20a	2.52 ± 0.23a	1.71 ± 0.13a	1.43 ± 0.13b	1.30 ± 0.01c	0.30 ± 0.01b	0.41 ± 0.01a	MI + 16S rRNA gene sequencing
Escherichia fergusonii	2.93 ± 0.26	nd	nd	nd	nd	–	–	MI + 16S rRNA gene sequencing
Acinetobacter albensis	3.41 ± 0.32a	2.62 ± 0.07b	2.06 ± 0.11a	1.64 ± 0.01b	0.90 ± 0.01c	0.42 ± 0.01b	1.16 ± 0.03a	MI + 16S rRNA gene sequencing
Corynebacterium xerosis	4.91 ± 0.19a	3.36 ± 0.02b	2.52 ± 0.08a	1.89 ± 0.01b	1.42 ± 0.02c	0.63 ± 0.01b	1.11 ± 0.04a	MI + 16S rRNA gene sequencing

Values with different letters (a–c) within each parameter in the same row differ significantly ($P < 0.05$). nd: Not detected; (–): insufficient data for calculation.

[1] Sampling was done on the surfaces of all carcasses in all groups following the bleeding process.

[2] Sampling was done on the surfaces of all carcasses in all groups following the evisceration process.

[3] Log_{10} reduction = (Log_{10} cfu/100 cm^2 carcass surfaces of non-sprayed control) − (Log_{10} cfu/100 cm^2 surfaces of sprayed carcass).

[4] Identification method: MI: Microflex identification instrument.

Table 5
Number ($\log_{10}$ cfu/100 cm^2) of microbial populations on retail cut's surfaces and decontamination efficiency of spraying with lactic acid (LA).

	Retail cut			Log Reduction on retail cut[1]		IM[2]
	Non-spray	2% LA spray	4% LA spray	2% LA spray	4% LA spray	
APC	2.64 ± 0.11a	2.04 ± 0.02b	1.57 ± 0.01c	0.60 ± 0.01b	1.07 ± 0.02a	BP
E. coli	nd	nd	nd	–	–	BP
Total coliform	1.97 ± 0.02a	1.78 ± 0.01b	1.24 ± 0.01c	0.19 ± 0.01b	0.74 ± 0.01a	BP
Staphylococcus hyicus	0.77 ± 0.01	nd	nd	–	–	MI + 16S rRNA gene sequencing
Staphylococcus saprophyticus	nd	nd	nd	–	–	MI + 16S rRNA gene sequencing
Staphylococcus epidermidis	nd	nd	nd	–	–	MI + 16S rRNA gene sequencing
Salmonella enterica	nd	nd	nd	–	–	MI + 16S rRNA gene sequencing
Shigella flexneri	nd	nd	nd	–	–	MI + 16S rRNA gene sequencing
Enterococcus faecium	nd	nd	nd	–	–	MI + 16S rRNA gene sequencing
Enterococcus durans	nd	nd	nd	–	–	MI + 16S rRNA gene sequencing
Pantoea dispersa	nd	nd	nd	–	–	MI + 16S rRNA gene sequencing
Escherichia fergusonii	nd	nd	nd	–	–	MI + 16S rRNA gene sequencing
Acinetobacter albensis	0.71 ± 0.01	nd	nd	–	–	MI + 16S rRNA gene sequencing
Corynebacterium xerosis	0.67 ± 0.01	nd	nd	–	–	MI + 16S rRNA gene sequencing

Values with different letters (a–c) within each parameter in the same row differ significantly ($P < 0.05$). nd: Not detected; (–): insufficient data for calculation.
[1] Log_{10} reduction = (Log_{10} cfu/100 cm^2 cut surfaces of non-sprayed control) − (Log_{10} cfu/100 cm^2 cut surfaces of spray).
[2] Identification method: the identification was based on their biochemical properties (BP), MI: Microflex identification instrument.

Taken together, the prevalence of *staphylococci*, *Salmonella*, *Shigella*, *Enterococci*, *Escherichia*, *Acinetobacter*, and *Corynebacterium* on the surfaces of pig carcasses during slaughter indicates that pigs are sources of many bacterial species. Furthermore, their frequent detections on carcass surfaces after 24 h of chilling indicate that during normal commercial slaughtering processes, most bacterial species cannot be eliminated entirely, whereas there natural and cross-contamination occurs during the slaughter process. Among these detected bacteria, most are known to reside in the gastrointestinal tracts of animals in general and pigs in particular, thus, the scalding and singeing process do not affect these bacteria. In contrast, washing distributes the bacterial contamination over the carcasses (Spescha et al., 2006). Finally, the presence of some bacterial species such as *S. hyicus*, *A. albensis*, and *Corynebacterium xerosis* on the pork cuts may indicate the natural contamination and transmission from the pigs to their carcasses and muscle tissues during slaughter and processing. Based on our results, efficient intervention methods such as organic acid spraying of pig carcasses at the end of the slaughter line are needed to increase the microbiological safety of pork meat.

4. Conclusion

As a part of the project on 2nd national establishment of procedures for pig slaughter hygiene and meat quality control, the present work was conducted to develop an intervention for improving the microbiological safety of pig carcasses and retail cuts. The present study revealed diverse bacterial genera such as *staphylococci*, *Salmonella*, *Shigella*, *Enterococci*, *Escherichia*, *Acinetobacter*, and *Corynebacterium* on pig carcasses at different stages of slaughter. Most of these bacterial genera were also detected on the carcass surfaces after 24 h of chilling, and some were found on retail cuts. Spraying pig carcasses with 2% or 4% lactic acid at the end of the slaughter line significantly reduced all bacterial species, with a greater reduction efficiency obtained when a higher concentration of lactic acid (4%) was used. These findings are valuable for pork producers, processors, and traders because we provide an overview of the prevalence of bacterial populations on pigs, natural contamination, and fates of the bacterial species on carcasses during slaughter under the commercial slaughtering process. Our results emphasize the suitability of 4% lactic acid spraying as an effective intervention for controlling bacterial contamination/transmission risks on pig carcasses, thus ensuring the microbiological safety of pork meat.

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