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Enumeration and identification of microorganisms present in bioaerosols in a swine harvesting facility

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The objectives of this study were to isolate and enumerate airborne bacteria, and yeast and mold before and during pork harvesting in a USDA-inspected processing facility, and determine the impact of bioaerosols on the harvesting and chill room environments. Air samples were collected before and during three separate pork harvesting processes at the bleeding, dehairing, and back splitting areas and in the holding cooler (0°C), using an Andersen N6 single stage impactor. Carcass surface bacteria swabs were collected from the chilled carcasses after being held in the cooler for 24 h. Total airborne bacteria, Staphylococcus spp., and Enterobacteriaceae counts increased ($P < 0.05$) during harvesting when compared to counts prior to harvest. Predominant Gram negative airborne bacteria isolated during harvesting included *Enterobacteriaceae* and *Pseudomonadaceae* families, and predominant Gram positives were *Staphylococcus*, *Microbacterium*, *Bacillus* and *Micrococcus* species. Potentially pathogenic *Staphylococcus aureus* and *Salmonella* spp. were isolated from bioaerosols generated during harvesting and from the pork carcasses. Neither *Listeria* spp. nor *Escherichia coli* O157:H7 were isolated from the bioaerosol samples or carcasses. The isolation of *Staphylococcus* and *Salmonella* from air samples and carcass swabs revealed that bioaerosols during harvesting may transport bacteria and contribute significantly to contamination of pork carcasses, and the resulting finished products.

Keywords: Bioaerosols, microbiology, airborne bacteria, swine processing, *Salmonella spp. Escherichia coli*

INTRODUCTION

Bioaerosols, or biological aerosols, are tiny airborne particles varying in size from 0.01 to 100 microns that come from a living organism (such as dander from indoor pets or pollen from trees) or are living organisms (such as bacteria and viruses) (Lennox International Inc., 2019). Bioaerosols or organic dust may occur as single unattached organisms or as aggregates of organisms. They may consist of pathogenic or non-pathogenic live or dead bacteria and fungi, viruses, high molecular weight allergens, bacterial endotoxins, mycotoxins, peptidoglycans, ß(1→3)-glucans, pollen or plant fibers (Douwes *et al., 2003).* Bioaerosols are of major concern to the health of humans, livestock, and workers in plants (Anderson *et al.*, 2017), swine confinement units (Cambra-Lopez *et al.*, 2010), individuals living in close proximity to pig farms (Kristiansen *et al.,* 2012) and contamination of the food supply with pathogenic bacteria (Anderson *et al.*, 2017; Ren and Frank, 1992). In processing plants, microorganisms grow in a liquid medium, such as wastewater, rinse water or spilled product that may become aerosolized due to sources or actions in the facility (Ren and Frank, 1992). They are also generated in the processing facility by ventilation

systems, worker activity, equipment operation, sink and floor drains, and high pressure spraying (Hedrick and Heldman, 1969). Most of the processes during pork harvesting are associated with the creation of bioaerosols. Bacteria on the surface of animals may become airborne during initial spraying and exsanguination, and during the hair removal process. Rahkio and Korkeala, (1997) reported higher bacterial counts during harvest in the back splitting area, which they attributed to the blade movement and water flowing from the cutting saw.

Bioaerosols have the potential to cause human illness, whether by respiratory inhalation or by the consumption of food contaminated with airborne microorganisms. Saide-Albornoz *et al.* (1995) determined that increased human handling in a pork processing plant was the main contributor to the linear increase of *S. aureus* from slaughter to fabrication. Butera *et al.* (1991) reported that the microflora isolated in a hog growing facility was composed of 72% Gram positive cocci (*Staphylococcus*, *Micrococcus*, *Leuconostoc*, *Streptococcus*, and *Aerococcus*), 7.2% Gram positive rods (*Bacillus*) and 20.8% Gram negative rods (*Enterobacteriaceae*). It is essential to determine the microbial content of bioaerosols and their impact on the safety of pork and related food animal species during harvesting and further processing. This can be accomplished by identifying the points/locations in the processing plant that have the potential to contribute to microbial contamination of the pork carcasses. The objectives of this study were to isolate and enumerate airborne bacteria, and yeast and mold before and during pork harvesting, and determine the impact of bioaerosols on the harvesting and the chill room environments.

MATERIALS AND METHODS

Air samples were collected at the University of Florida USDA inspected harvest facility in four areas before and during harvesting (slaughter), and coded: Before harvest-bleeding pit (A), hide removal/dehairing area (B), back splitting area (C) and holding cooler for carcasses (D); During harvest - bleeding pit (E), hide removal/dehairing area (F), back splitting area (G) and holding cooler (H, after 24 h of chilling carcasses). “Before harvest” refers to sampling of the clean and sanitized harvesting area and cooler 24 h prior to harvesting. “During harvest” refers to sampling during the actual time of harvesting in the designated areas. Duplicate air samples were collected from each area during three separate harvesting days using the N6 single stage impactor (Thermo Andersen Corp., Smyrna, Ga). Prior to sample collection, the impactor was shipped to the manufacturer for calibration. Each harvest consisted of 20 to 25 pigs. The impactor was operated at a flow rate of 28.3 liters/min (1 cubic foot/ minute) with a cut-off diameter of 0.65 µm as recommended by the manufacturer. Prior to sampling, the impactor was washed at the beginning of each harvest with soapy water and disinfected with 100°C distilled water containing a chlorine concentration of 100 parts per million (7.1 ml per 3.8 liters of water). The jet stage was checked for clogged holes and the impactor was air dried. At the beginning of each harvest the inlet cone, jet stage, and base plate were disinfected with alcohol swabs. The impactor was also disinfected with alcohol swabs between sampling points. The sampler was turned on for two minutes prior to sampling to allow the alcohol to evaporate and not affect the number of bacteria recovered. Air samples were taken at a height of 1.5 meters from the floor and within one meter from the carcass. It was determined during preliminary evaluations that the most effective sampling conditions were one minute with a flow rate of 28.3 ± 0.1 liters/minute in order to achieve countable bacterial growth on petri plates (25 to 250 CFU).

Air sampling analysis

Direct plating and pre-enrichment methods were employed. The air was sampled for total aerobic bacteria, yeast and mold, *Escherichia coli* O157:H7, *Listeria* spp., *Salmonella* spp., and *Staphylococcus* spp. using duplicate sterile prepoured 15 mm by 95 mm petri dishes containing tryptic soy agar (TSA), potato dextrose agar (PDA), violet red bile agar (VRBA), modified oxford agar (MOX), xylose-lysine-tergitol 4 agar (XLT4) and mannitol salt agar (MSA), respectively (USDA FSIS, 2019; USFDA, 2019). Each plate was inserted, without lid, into the disinfected N6 single stage impactor and the vacuum was turned on for one minute at a flow rate of 28.3 ± 0.1 liters/minute. After one minute, the sampler was turned off and the Petri dish was removed and inverted into its cover. The plates were incubated at 25^{o}C for 3 days (PDA only) and 35°C for 24 h (all other media) and counted at the end of the incubation period. The counts were recorded and expressed as log colony forming units per m^3 of air (log cfu/m^3 of air).

Identification of microorganisms

Air samples were enriched by flooding TSA plates with 10 mL of 0.1% peptone water and allowing the plates to set at room temperature for approximately five minutes for bacterial colonies to soften and separate from the media. 1 mL of the bacteria slurry was then transferred to duplicate test tubes containing 9 mL of either Modified EC plus Novobiacin (mEC) for *Escherichia coli* O157: H7, University of Vermont Broth (UVM) and Fraser broth (FB) for *Listeria* spp., Lactose broth (LB) and Rappaport Vassiladis broth (RV) for *Salmonella* spp., or Brain heart infusion broth for *Staphylococcus* spp., and incubated at

35°C for 24 h, 30°C for 24 h, 35°C for 24 to 48 h, 37°C for 18 h, 37°C for 18 h and at 35°C for 24 h, respectively (USDA FSIS, 2019). Following incubation, test tubes were vortexed and a loop full of broth was streaked onto duplicate selective media plates containing VRBA, MOX, XLT4 and MSA for isolation of Escherichia *coli* O157:H7, *Listeria* spp., *Salmonella* spp., and *Staphylococcus* spp., respectively. All plates were incubated at the appropriate temperature and time combinations as previously described. Typical 24 h colonies were selected and streaked onto TSA agar and subjected to identification assays.

Carcass sample collection and analysis

Carcasses were swabbed 24 h after being in the holding cooler at 0°C using swine carcass sampling kits (Solar Biologicals Inc., Ogdensburg, NY). Each individual sterile kit consisted of a pre-moistened sponge, 15 ml Butterfield's phosphate buffer, disposable template, sample bags and gloves. Carcass swab samples were collected as instructed by USDA FSIS (1999). In general, a 100 cm^2 area was marked using the template, and swabbed using the sterile pre-moistened sponge 10 times horizontally and 10 times vertically. The wiping force was sufficient to remove dried blood. The belly and jowl areas were swabbed first with one side of the sponge, and the other side of the sponge was used to swab the ham 10 times horizontally and 10 times vertically. Individual sponges were placed into sample bags along with 15 mL of Butterfield's phosphate buffer. The bags were closed, kneaded several times, and stored in a walk-in cooler (5 ± 1°C) for approximately one hour maximum prior to analysis. Analyses included presence of Escherichia coli O157: H7, Listeria spp., Salmonella spp., and Staphylococcus spp. One milliliter was aseptically transferred from the bags to test tubes containing 9 ml of the enrichment broths: Modified EC plus Novobiacin (mEC), Fraser broth (FB), Rappaport Vassiladis broth (RV), and Brain Heart Infusion broth (BHI). The resulting isolated colonies were analyzed to determine identification, and some were suspended in 2 mL Cryovials containing 40% glycerol peptone water solutions and frozen at -80°C for future analyses.

Bacterial identification of air and carcass samples

Frozen bacterial colonies were thawed under running cold tap water. 1 mL was transferred from individual Cryovials to test tubes containing 9 ml of the appropriate enrichment broths as previously described. The tubes were incubated under the same conditions as discussed earlier. A loop full of the cell suspension was transferred onto duplicate selective media plates, streaked and incubated as previously described. Isolated colonies were observed for color, size, texture and color changes in the media. Gram stains were conducted, and colony morphology was determined in order to select the appropriate colonies. The Analytical Profile Index identification test strip system (API, bioMérieux Inc., 2002) and the microbial Identification System (MIDI Inc., Newark, DE) were employed to identify the isolated colonies. The API method is for manual identification of Gram positive and Gram negative bacteria and yeast based on their unique biochemical properties. API 20E Microbial Identification Kit was used to identify species/subspecies of Enterobacteriaceae (Escherichia coli and Salmonella spp.) and/or non-fastidious Gram-negative rods. API Staph was used for 24 h identification of Staphylococci spp., Micrococci spp., and Kocuria spp. API Listeria was used to identify Listeria species and was specific for Listeria species only. The Microbial Identification System identifies bacterial cells by gas chromatographic analysis of cellular fatty acids. The system works by comparing the composition of the bacterial sample fatty acid methyl esters to a stored database of bacteria using pattern recognition software (Sasser, 2001). Samples positively identified as Escherichia coli by API 20E and/or MIDI microbial identification system were further analyzed using the confirmation methods Bacto® E. coli O antiserum O157 and Bacto® E. coli H antiserum H7 (Fisher Scientific., Detroit, MI), and the Reveal antigen-antibody detection system for Escherichia coli O157:H7 (Neogen Corp., Lansing, MI).

Statistical analyses

The experimental design utilized in this study was a randomized complete factorial with sampling points (8 levels), harvests (3 levels), species (1 level), samples analyzed (2 per location), and media (6). Each harvest consisted of 20 to 25 pigs. The dependent variables for each of the factors tested were types of media (6). Each air sample collected in this study consisted of two subsamples. All data collected in this study were analyzed using SAS for Windows analysis of variance method for General Linear Model Procedures (Proc GLM) (SAS, version 9, SAS Institute, Cary, NC). Any significant differences were analyzed by the multiple comparisons procedure of LSD (least significant difference), using a level of significance of alpha = 0.05.

RESULTS AND DISCUSSION

Total aerobic bacteria in bioaerosols

Total aerobic bacteria (TAB) counts were similar ($P > 0.05$) among the three harvests. TAB counts for bleeding pit, scalding tank and back splitting areas increased ($P < 0.05$) during harvesting when compared to the same areas before harvesting. Except for the cooler after 24 h,

TAB counts were similar among all areas before harvesting and among all areas during harvesting. After 24 h of chilling the carcasses, the cooler had lower TAB counts ($P< 0.05$) than all other areas before (including the cooler) and during harvesting. There was a significant decrease in TAB for the cooler after 24 h when compared to TAB counts before harvesting.

Rahkio and Korkeala (1997) reported higher values of 3.13 to 4.07 log CFU/m^3 of air counts in the back splitting area and 2.13 log CFU/m^3 of air counts inside the holding cooler in a pork harvesting facility. The higher values might have been directly related to the stocking density in the cooler. Data in this study revealed that as the carcasses moved closer to the cooler, Salmonella spp. counts and prevalence decreased. Storage in the cooler (0ºC) resulted in decrease in prevalence of Salmonella. O'Connor et al. (2012) reviewed 17 publications, describing 44 studies conducted in the European Union States where the presence of Salmonella was evaluated at 14 points during harvesting, that included stun, bleed, kill, scald, dehair, singe, polish, bung removal, evisceration, split, stamp, final wash, immediately after chill, and 18–48 h after chilling. The review revealed that the processing procedures currently in use in swine harvesting facilities generally result in decreased prevalence of Salmonella spp. as the carcasses move toward the cooler. The predominant mesophilic microflora on the carcasses is very sensitive to the cooler temperature of 0°C because mesophiles grow best at 30 to 40°C.

Total airborne yeast and mold

The data revealed no significant differences ($P > 0.05$) in yeast and mold (YAM) counts among the three harvests. The cooler, before and during harvest, had lower YAM counts ($P < 0.05$) than all other locations (Table 1). The bleeding area during harvest had higher counts ($P< 0.05$) than all other areas before and during harvesting except the back splitting area. In general YAM counts ranged from 1.73 to 3.01 log cfu per m^3 of air which were similar to counts reported by Kotula and Emswiler-Rose (1988) during pork harvest.

Mannitol salt agar

Bacterial counts on Mannitol Salt agar (MSA) were similar ($P > 0.05$) among harvests. Except for the holding cooler, all areas sampled had higher MSA counts during harvest when compared to MSA counts before harvest (Table 2). All MSA counts were similar ($P > 0.05$) among areas sampled before harvest. Except for the cooler, MSA counts were also similar ($P > 0.05$) among areas sampled during harvest. MSA counts were less than 1 log cfu per m^3 of air in all areas sampled before harvest and increased to approximately 3 log cfu per m^3 of air during harvest. Identification of the bacteria growing on the Mannitol Salt agar plates revealed 89.0% Staphylococcus spp., and 11% other Gram positives (Bacillus megaterium (5.5%) and Kocuria kristinae (5.5%)) (Table 3). Sixteen species of Staphylococci were identified with 5.55% representing Staphylococcus aureus. The rarely pathogenic Staphylococcus epidermidis is the most ubiquitous of the various Staphylococcus species found on the skin of humans and animals. Staphylococcus aureus is a potential food safety hazard since many of its strains produce enterotoxins which cause Staphylococcal food poisoning when ingested. Staphylococcus aureus contamination of pork carcasses does not only come from human contact but also from animal hides, skin, lesions and bruised tissue (Salfinger and Tortorello, 2015). Staphylococcus hyicus has also been shown to produce enterotoxins in food. The presence of Staphylococcus, Micrococcus and Bacillus species in bioaerosols collected during three pork harvests could be attributed to their association with mammalian skin, soil and animal feces.

Modified oxford agar

Airborne bacteria collected on Modified Oxford Agar (MOX) resulted in no significant differences ($P > 0.05$) among harvests. Although colonies grew on the MOX agar plates, identification of the bacteria revealed no growth of Listeria spp. Except for the holding cooler, all areas sampled had higher ($P < 0.05$) counts during harvest when compared to counts before harvest (Table 2). Except for the back splitting area, counts were similar ($P > 0.05$) among all areas sampled before harvest. The back splitting area before harvest had lower ($P < 0.05$) counts than the cooler and similar counts ($P > 0.05$) as the bleeding pit and scalding area. Counts were similar ($P > 0.05$) among all areas except the cooler during harvesting, and lower ($P < 0.05$) for the cooler area than all other areas during harvesting. The data for the cooler demonstrated that after 24 h the counts had decreased from 1.13 log cfu/m^3 of air to 0.31 log cfu/m^3 of air. Growth on MOX plates included 80% Gram positive bacteria and 20% Gram negative bacteria (Table 3). The genus Listeria belongs to the bacilli class along with Bacillus, Paenibacillus, Staphylococcus, Streptococcus, Lactobacillus, and Brochothrix. The presence of Bacillus, Brevibacterium, Brochothrix, Cellulomonas, Microbacterium and Micrococcus in bioaerosols collected during three pork harvests could be attributed to their predominance in soil, mammalian skin and animal feces. These bacteria could have entered the harvesting facility through contaminated hides of animals, feces, soil on workers' clothing and shoes, equipment transport and human carriers. Ren and Frank, (1992) isolated Listeria spp. from specific environmental surfaces of dairy plants

Table 1. Mean total aerobic bacteria and yeast and mold counts for bioaerosols collected before and during three harvest periods.

Total counts (log CFU/m^3 of air)[c]				
	Total aerobic count		Yeast and mold	
Sampling Area[a]	Harvest	SEM[b]	Harvest	SEM[b]
A	2.21B	0.08	2.90B	0.05
B	1.82B	0.68	2.85B	0.06
C	2.27B	0.12	2.83B	0.01
D	2.12B	0.06	2.39C	0.12
E	3.47A	0.20	3.32A	0.03
F	3.61A	0.07	3.00B	0.17
G	3.43A	0.01	3.01AB	0.02
H	0.26C	0.44	1.73D	0.14

a Before harvest: A = bleeding pit; B = scalding tank; C = back splitting; D = cooler;
During harvest: E = bleeding pit; F = Scalding tank; G = back splitting; H = cooler after 24 h,
b Standard error of the mean.
c Means within a column followed by different letters (A, B, C, D, E) are significantly different ($P < 0.05$).

Table 2. Mean airborne bacteria collected on Mannitol Salt Agar and Modified Oxford Agar in eight areas during three pork harvests.

	Total counts (log CFU/m^3 of air)[c]			
	Mannitol Agar		Modified Oxford Agar	
Sampling Area[a]	Average	SEM[b]	Average	SEM[b]
A	0.52B	0.01	0.62BC	0.76
B	0.26B	0.45	0.57BC	0.70
C	0.26B	0.45	0.26C	0.45
D	0.52B	0.01	1.13B	0.17
E	3.05A	0.12	2.75A	0.06
F	3.12A	0.56	2.85A	0.08
G	2.87A	0.01	2.33A	0.27
H	0.01B	0.00	0.31C	0.53

[a] Before harvest: A = bleeding pit; B = scalding tank; C = back splitting; D = cooler;
During harvest: E = bleeding pit; F = Scalding tank; G = back splitting; H = cooler after 24 h,
[b] Standard error of the mean
[c] Means within a column followed by different letters (A, B, C, D, E) are significantly different ($P < 0.05$).

Table 3. Bioaerosol bacteria isolated on Mannitol Agar and Modified Oxford agar during three pork harvests.

Mannitol Agar		
Staphylococcus spp. (89.0%)		**Others (11.0%)**
Staphylococcus arlettae	*Staphylococcus hominis*	*Bacillus megaterium*
Staphylococcus aureus	*Staphylococcus hyicus*	*Kocuria kristinae*
Staphylococcus auricularis	*Staphylococcus kloosii*	
Staphylococcus capitis	*Staphylococcus lugdunensis*	
Staphylococcus chromogenes	*Staphylococcus saprophyticus*	
Staphylococcus cohnii	*Staphylococcus sciuri*	
Staphylococcus epidermidis	*Staphylococcus warneri*	
Staphylococcus gallinarum	*Staphylococcus xylosus*	
Modified Oxford Agar		
Gram Positive (80.0%)		**Gram Negative (20%)**
Bacillus pumilus	*Microbacterium barkeri*	*Escherichia coli*
Brevibacterium casei	*Microbacterium saperdae*	*Salmonella bongori*
Brevibacterium epidermidis	*Nesterenkonia halobia*	*Shigella boydii*
Brochothrix campestris	*Staphylococcus aureus*	
Cellulomonas fimi	*Staphylococcus epidermidis*	
Kocuria kristinae	*Staphylococcus haemolyticus*	

Table 4. Mean airborne bacteria collected on Violet Red Bile Agar and Xylose-Lysine-Tergitol 4 Agar in eight areas during three pork harvests.

	Airborne bacteria (log CFU/m^3 of air)[c]			
	Violet red bile agar		Xylose-Lysine-Tergitol 4 Agar	
Sampling Area[a]	Mean	SEM[b]	Mean	SEM[b]
A	NG	-	NG	-
B	NG	-	NG	-
C	NG	-	NG	-
D	NG	-	NG	-
E	0.94A	0.46	NG	-
F	0.92A	0.53	0.60A	0.73
G	0.85A	0.58	0.01B	0.01
H	0.01B	0.01	0.01B	0.01

[a] Before harvest: A = bleeding pit; B = scalding tank; C = back splitting; D = cooler; During harvest: E = bleeding pit; F = Scalding tank; G = back splitting; H = cooler after 24 h, [b] Standard error of the mean,[c] Means within a column followed by different letters (A, B, C, D, E) are significantly different ($P < 0.05$). NG = No growth, - denotes no SEM could be calculated because no bacterial counts were detected/grew on the plates.

but could not recover it from air samples collected close to these surfaces. It is hypothesized that Listeria species suspended in air may lose their colony forming capability even though they retain overall viability. Waldron (2017) determined that isolation of Listeria innocua was dependent on relative humidity and time of sampling air. The researcher reported that mean cell recovery (2.2 CFU/L) from air samples was significantly higher ($P < 0.05$) when collected 5 or 10 min after nebulization of 5 log CFU/mL into a 154 L biosafety chamber at 83% RH, compared to collection after 20 or 40 min, or compared to all times (5 to 40 min intervals) under 65% humidity (0.4 CFU/L).

Escherichia coli

No Escherichia coli 0157:H7 was isolated in the areas sampled before harvest. During harvest, generic E. coli was isolated in the bleeding pit, back splitting and scalding tank areas, which indicated that the presence of the animals and actions occurring in the harvesting area contributed to the presence of microorganisms in the facility (Table 4). However, the E. coli counts were less than 1 log cfu per m^3 of air in all areas sampled. None of the E. coli isolated was E. coli O157:H7 as was determined by E. coli O157:H7 agglutination test. Approximately 90% of the microflora isolated was Gram negative, with 3.33% being generic E. coli, and 10% of the microflora was Gram positive bacteria (Table 5). The Gram negative bacteria were largely from the Enterobacteriaceae family. Enterobacteriaceae have a worldwide distribution and are found in soil, water, fruits, vegetables, plants, trees and animals, and inhabit a wide variety of niches which include human and animal gastrointestinal tracts and various environmental sites (Salfinger and Tortorello, 2015; Forbes et al., 2002). Other Gram negative bacteria present such as Pseudomonas spp., Chryseobacterium indologenes, Chryseomonas luteola, Flavimonas oryzihabitans, and Stenotrophomonas maltophilia are all members of the Pseudomonadaceae family. These species are normally associated with soil and fecal matter and therefore, are found on the hides of animals. Gram positive bacteria isolated from VRBA plates were Kocuria kristinae, Microbacterium barkeri and Bacillus pumilus which are frequently isolated from meat and dairy products (Salfinger and Tortorello, 2015). The presence of Enterobacteriaceae species in bioaerosols collected during the three pork harvests could be attributed to their association with mammalian skin, soil and animal feces. These bacteria could have entered the harvesting facility on the hides of animals, feces and by the soil on workers' shoes.

Salmonella species

The Data revealed no significant differences ($P > 0.05$) among harvests. Except for the scalding tank area during harvest, Salmonella spp. was not detected before nor during harvest (Table 4). Salmonella counts during harvest were less than 1.0 log cfu/m^3 of air. Approximately 85.7% of the total isolates were Gram negative with 5.0% representing Salmonella, and 14.3 were Gram positive Staphylococcus bacteria (Table 5).

Carcass swabs collected during pork harvests

The predominant bacteria isolated from pork carcass swabs were Gram negative (Table 6). Gram Negative and Gram Positive bacteria represented approximately 68.2%, and 31.8%, respectively, of the total microflora on the pork carcasses. The Gram negative microflora represented approximately 54.5% Enterobacteriaceae,

Table 5. Bioaerosol bacteria isolated on Violet Red Bile Agar and Xylose-Lysine Tergitol 4 Agar during three pork harvests.

Violet Red Bile agar		
Gram Negative (90%)		**Gram Positive (10%)**
Cedecea neteri	*Pantoea spp.*	*Bacillus pumilus*
Chryseobacterium indologenes	*Pseudomonas aeruginosa*	*Kocuria kristinae*
Chryseomonas luteola	*Pseudomonas fluorescens*	*Microbacterium barkeri*
Enterobacter aerogenes	*Pseudomonas putida*	
Enterobacter cloacae	*Salmonella bongori*	
Escherichia coli	*Salmonella choleraesuis s*	
Escherichia fergusonii	*Salmonella typhi*	
Flavimonas oryzihabitans	*Serratia liquefaciens*	
Klebsiella pneumoniae ozaenae	*Serratia rubidaea*	
Klebsiella pneumoniae	*Shigella boydii*	
Klebsiella terrigena	*Shigella flexneri*	
Kluyvera spp.	*Shigella sonnei*	
Leclercia adecarboxylata	*Stenotrophomonas maltophilia*	
Xylose-Lysine-Tergitol 4 agar		
Gram Negative (87.5%)		**Gram Positive (14.3%)**
Enterobacter aerogenes	*Morganella morganii*	*Bacillus sphaericus*
Enterobacter asburiae	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Enterobacter cancerogenus	*Pseudomonas fluorescens*	*Staphylococcus epidermidis*
Enterobacter cloacae	*Pseudomonas putida*	
Escherichia coli	*Salmonella choleraesuis*	
Escherichia fergusonii	*Salmonella typhimurium*	
Klebsiella ornithinolytica	*Serratia liquefaciens*	
Klebsiella pneumoniae	*Serratia marcescens*	
	Shigella flexneri	
	Shigella sonnei	

Table 6. Bacteria isolated during three swine harvests on pork carcasses held at 0°C for 24 h.

Gram negative (68.2%)	Gram positive (31.8%)
Chryseobacterium indologenes	*Staphylococcus aureus*
Chryseomonas luteola	*Staphylococcus chromogenes*
Enterobacter asburiae	*Staphylococcus epidermidis*
Enterobacter cancerogenus	*Staphylococcus hominis*
Enterobacter cloacae	*Staphylococcus hyicus*
Escherichia coli	*Staphylococcus warneri*
Escherichia fergusonii	*Staphylococcus xylosus*
Klebsiella pneumoniae	
Leclercia adecarboxylata	
Morganella morganii	
Pantoea spp.	
Pseudomonas aeruginosa	
Salmonella choleraesuis	
Shigella flexneri	
Shigella sonnei	

and 13.6% Pseudomonas and other non-Enterobacteriaceae bacteria. All Gram Positive bacteria were identified as Staphylococcus with S. aureus representing 4.5%. Generic Escherichia coli and Salmonella species were identified as 14.3% and 4.8%, respectively, of Gram negative bacteria isolated from pork carcass swabs. Listeria species and Escherichia coli O157:H7 were not isolated from any of the pork carcass swabs. As a proactive antimicrobial intervention procedure in our meat processing facility, all carcasses (pork, beef, lamb and goat) are treated with carcass washing, followed by a 2.0% lactic acid spray solution prior to chilling (0°C). The combination of carcass wash, lactic acid treatment, followed by low temperature chilling

Table 7. Bacteria isolated before and during three pork harvests.

Organism (Genus Species)		
Before Harvest	**During Harvest**	
Gram negative	**Gram negative**	**Gram positive**
Escherichia coli	*Cedecea neteri*	*Bacillus megaterium*
Flavimonas oryzihabitans	*Chryseobacterium indologenes*	*Bacillus pumilus*
Salmonella bongori	*Chryseomonas luteola*	*Brevibacterium casei*
Shigella boydii	*Enterobacter aerogenes*	*Brevibacterium epidermidis*
	Enterobacter asburiae	*Brochothrix campestris*
	Enterobacter cancerogenus	*Cellulomonas fimi*
	Enterobacter cloacae	*Kocuria kristinae*
Gram positive	*Escherichia coli*	*Microbacterium barkeri*
Bacillus megaterium	*Escherichia fergusonii*	*Microbacterium saperdae*
Kocuria kristinae	*Flavimonas oryzihabitans*	*Nesterenkonia halobia*
Staphylococcus auricularis	*Klebsiella ornithinolytica*	*Staphylococcus arlettae*
Staphylococcus capitis	*Klebsiella pneumoniae ozaenae*	*Staphylococcus aureus*
Staphylococcus chromogenes	*Klebsiella pneumoniae*	*Staphylococcus auricularis*
Staphylococcus cohnii	*Klebsiella terrigena*	*Staphylococcus capitis*
Staphylococcus epidermidis	*Kluyvera* spp.	*Staphylococcus chromogenes*
Staphylococcus hominis	*Leclercia adecarboxylata*	*Staphylococcus cohnii*
Staphylococcus lugdunensis	*Morganella morganii*	*Staphylococcus epidermidis*
Staphylococcus sciuri	*Pantoea spp.*	*Staphylococcus gallinarum*
Staphylococcus warneri	*Pseudomonas aeruginosa*	*Staphylococcus haemolyticus*
	Pseudomonas fluorescens	*Staphylococcus hominis*
	Pseudomonas putida	*Staphylococcus hyicus*
	Salmonella bongori	*Staphylococcus kloosii*
	Salmonella choleraesuis	*Staphylococcus lugdunensis*
	Salmonella typhi	*Staphylococcus saprophyticus*
	Serratia liquefaciens	*Staphylococcus sciuri*
	Serratia marcescens	*Staphylococcus warneri*
	Serratia rubidaea	*Staphylococcus xylosus*
	Shigella boydii	
	Shigella flexneri	
	Shigella sonnei	
	Stenotrophomonas maltophilia	

ensure that pathogens and general microflora are eliminated or maintained at a minimal level.

Comparison of bioaerosol and carcass microflora

Approximately 38.6% of the bacteria isolated on the pork carcass (Table 6) were also isolated in the bioaerosol during harvesting (Table 7). In addition, approximately 22.7 % of the bacteria isolated on the pork carcasses were also isolated in the bioaerosol before harvesting. None of the airborne bacteria isolated from the empty cooler (D) and the cooler containing carcasses (H) were isolated from the pork carcasses. The isolation of Staphylococcus, Escherichia, and Salmonella species from air samples taken during harvesting and carcass swabs supported the theory that bioaerosols may transport bacteria and contribute to the contamination of pork carcasses. In this study a total of 171 bacterial colonies were isolated from various selective media used to collect air samples, and carcass swab samples during three pork harvests. Of the bacteria isolated, a total of 115 were from air samples, and 56 were from pork carcasses. Staphylococcus species were identified from 46.7% of the bacterial colonies isolated from air samples during three pork harvests, with 15% of the colonies representing Staphylococcus aureus. Similar findings were reported by Syne et al. (2013) for processing plants where ready-to-eat meat products were produced. The researchers reported that pre- and post- cooking air environment and surfaces had relatively high levels of aerobic bacteria, Staphylococcus aureus and coliforms. Higher S. aureus, coliforms and total bacteria were reported in the precooking raw meat areas. Although, not currently available for pork harvesting and processing facilities, Vyscosil et al. (2019) evaluated the effectiveness of a percolating biofilter developed by the Research and Development Institute for the Agri-environment (IRDA) to capture airborne contaminants, such as bacteria and viruses emitted from a swine finishing room over a 10-month period. The data revealed a decrease in total bacteria (qPCR) (75.0%) and other qPCR targeted organisms that included archaea (42.1%), coliphages (25.6%), Enterococcus (76.1%), and Escherichia coli (40.9%). The researchers concluded that the 10-month study revealed evidence for bioaerosols

reduction using the percolating biofilter system.

Conclusion

Microbial counts (total airborne bacteria, Staphylococcus spp., and Enterobacteriaceae) increased when swine were brought into the processing plant and harvested, when compared to counts in the absence of animals in the facility.

Predominant Gram negative airborne bacteria isolated during harvesting were from the Enterobacteriaceae and Pseudomonadaceae families, and predominant Gram positives were Staphylococcus, Microbacterium, Bacillus and Micrococcus species. Potentially pathogenic Staphylococcus aureus and Salmonella spp. were isolated from bioaerosols generated during harvesting and from the pork carcasses. Neither Listeria spp. nor Escherichia coli O157:H7 were isolated from the air samples or carcasses.

The isolation of Staphylococcus and Salmonella from air samples and carcass swabs revealed that bioaerosols during harvesting may transport bacteria and contribute significantly to contamination of pork carcasses, and the resulting finished products.

Recommendations for improving the health of consumers and staff that work in swine and related harvesting facilities should include comprehensive food safety, employee education and training programs. In addition, the installation of innovative filtering systems will function significantly to control foodborne illnesses, and respiratory complications that might be experienced by workers. For example, the filtering system evaluated by Vyskosil et al. (2019) may have future applications in red meat, poultry and related food processing facilities to control the concentration of bioaerosols in production and harvesting facilities.

Proactive food safety programs to reduce the levels of pathogenic bacteria on the live animal prior to entering the harvesting facility must continue to be a top priority. Finally, employers must ensure that the proper respiratory protection equipment and, showering and other facilities are made available to workers to ensure minimal contact with swine plant bioaerosols.

Authors' declaration

We declare that this study is original research by our research team.

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