RESEARCHREPORT

PORK SAFETY

Title: Effect of Lairage on Prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) in market swine - **NPB#11-131**

Investigator: James D. McKean

Institution: Iowa State University

Date Submitted: December 26, 2013

Industry Summary

The bacterium *Staphylococcus aureus* is a commonly found on the skin and mucous membranes of a wide range of mammals; and usually exists in a non-clinical state. One subgroup of *Staphylococcus aureus* that has been particularly important clinically in healthcare and community settings is Methicillin-Resistant *Staphylococcus aureus* (MRSA). This bio-type has received significant attention from the medical community; and currently is a high priority for reduction in hospital environments by the Centers for Disease Control and Prevention (CDC website, 2013). Dutch researchers first identified MRSA ST398 as a clonal type in animals. This finding has raised the issue of MRSA ST398 as a potential human food safety issue in meats. One frequently raised assertion is: The presence of on-farm carriage of ST398 results in ST398 contamination of retail pork. Between the farm and retail sectors are a series of harvest and processing steps which may impact that connection. This study was designed to examine the impact of harvest and initial carcass handling on the presence of MRSA. It serves as an introductory foray into the potential impacts on MRSA carriage through lairage and initial carcass processing. In addition the relationship between skin swabs and nasal swabs on individual swine was studied to demonstrate correlative values for these different sampling strategies.

From the 15 observation dates, 58 lots complete sample sets representing four (4) production steps – receiving (skin swab), post lairage (pre-gondola skin swab), post-stun (shackle nasal swab), and pre-chill (carcass swab), were analyzed for the presence of MRSA using traditional bacteriologic methods. The first and most striking observation was the drop in carrier isolations pre-chill when compared to receiving, and post-lairage samples (1.2%, 29.3% and 65.5%, respectively). Such an observation is consistent with current plant-based HACCP procedures substantially reducing MRSA carriage from the live animal to pre-chill carcass stage. Because the lots were selected from direct site to abattoir loads, the entry rate may be used as an estimate for on-farm carriage rates. The post-lairage isolation rate was greater than at entry (29.3% vs. 65.5%), which supports the proposition that during the lairage period additional MRSA transmission between animals occurs due to skin contact and environmental contamination. Within this general rise in MRSA isolation, the results varied widely by date. Second, MRSA typing from a randomized draw of post-lairage and post-stun results demonstrated the presence of 21 ST398 and 12 ST5 patterns across all sampling dates. All but one ST398 isolate (20) were found

in the March 27 cohort, while the ST5 isolates were scattered throughout the 15 sampling dates. Within the March 27th cohort all four lots had post-lairage positive ST398 isolates, but no carcass (pre-chill) swabs were ST398 positive, nor were any other MRSA bio-types identified pre-chill on that date. Conversely, the seven ST5 isolates on pre-chill carcasses were derived from four lots on three different days. Only one of the four receiving lots contained an MRSA positive sample. These observations lend credence to the lack of direct MRSA transfer from live swine to pre-chill carcasses. A probable source for the observed contamination at the pre-chill level could not found because of the project objectives limitations.

At the animal level individual post lairage and post-stun samples were collected to answer whether skin swabs taken post-lairage or nasal swabs taken post stun of individual animals would result in isolation compatibility. Skin or nasal swabs were obtained from 619 post lairage and 615 post-stun animals. A total of 589 post-lairage skin and post stun nasal swab pairs were available for analysis; with 351 pairs both negative and 72 both positive. The remaining 166 pairs were either post-lairage negative and nasal swab positive (88) or reversed designation (78). There was no statistically significant difference in the number of positive isolates each method detected within lots, but they did not statistically predict the same pairs as positive. McNemar test result (p value of 0.48) suggests no reason to suspect that the proportion of samples positive differed between the two locations.

In summary, this data supports observations reported elsewhere: 1) that a potential direct link between live swine MRSA and pre-chill contamination appears to be very low 2) that current HACCP-based interventions in processing provide significant protections from MRSA transfer at the pre-chill carcass level and 3) that skin swabs and nasal swab isolations do not detect the same individual animal carriage, but may be used interchangeably to determine group contamination rates. These data should be interpreted within the context that additional characterization of MRSA ecology in the peri-harvest environment is needed. However, in the interim, routine plant HACCP-based interventions appear capable of greatly reducing the transfer of *Staphylococcus aureus* from production sites to pre-chill carcass.

Key words: pork, swine, MRSA, peri-harvest, *Staphylococcus aureus*, lairage

Contact information: Dr. James McKean, DVM, JD; 2225 Lloyd Veterinary Medical Center, Iowa State University, Ames, Iowa, 50011-1250. Telephone: 515-294-8792; Email: x2mckean@iastate.edu.

Scientific abstract

The bacterium *Staphylococcus aureus* is a commonly found on the skin and mucous membranes of a wide range of mammals; and usually exists in a non-clinical state. Dutch researchers were the first to identify MRSA ST398 as a new clonal type. Subsequent research demonstrated presence in various species and with world-wide distribution. The popular and scientific press has highlighted MRSA398 presence in retail pork as a food safety concern. Little MRSA ecologic data is available for live pigs during normal harvest and processing steps, particularly lairage. An observational study to examine MRSA presence at multiple harvest points from abattoir entry to pre-chill carcass was carried out to gather such information.

Sample procurement occurred from November 2011 to April 2012. Four direct delivery production site-to-abattoir lots were selected by the plant on each of 15 dates. Lairage and harvest impacts on MRSA isolations were measured by rubbing a moistened sponge across the shoulder area (approximately a 10cm X 10cm area) of 10 pigs per lot upon entry (receiving), after several hours in lairage (post-lairage) and at the post-evisceration (pre-chill). Equivalency of skin and nasal swab sampling was tested by collecting individually identified pigs by post-lairage shoulder swab and post-stun nasal swab. All sponges were hydrated and maintained in sterile and uniquely identified whirl-paks overnight. Each sponge was returned to the original bag after use. Nasal swabs, uniquely identified to match post-lairage code, were inserted for each identified pig. Swabs and sponges were chilled after use and delivered to the ISU Veterinary Diagnostic Laboratory within 6-8 hours of collection for *Staphylococcus aureus* culture. Clonal characterization was a PCR for 16s, *mecA* and PVL genes and by PFGE. This project was designed to determine the impact of normal lairage and pre-chill interventions on MRSA carriage under commercial conditions. Receiving, post-lairage, post-stun and pre-chill isolation rates were 29.3%, 65.5%, 84.4% and 6.9%, respectively. The receiving rate represents on-delivery carriage. Post-lairage isolation rate was greater than at receiving (29.3% vs. 65.5%), which supports MRSA transmission from direct contact and environmental contamination in lairage. With a general rise in MRSA isolation, individual sample date results varied widely. March 27th is instructive: receiving lots with 9/40 positive isolates from 3 of 4 lots: post-lairage with 27/40 samples and 26/38 post-stun swabs across all lots, and no MRSA isolates at pre-chill. Of those isolates further characterized, all 16 from the post lairage (8) and nasal swabs (8) were ST398. Alternatively the four lots found on other dates with positive pre-chill carcasses were all ST5 with minimal (1/40) receiving lot positives.

The third objective was to determine comparability for MRSA isolation rates from shoulder swabs and nasal swabs from individual pigs. Results demonstrate no direct animal correlation with these sampling strategies. However, both sampling sites produced comparable results at the lot level. Therefore collection of skin swabs before stunning or nasal swabs may be useful to measuring MRSA carriage. As described above, the seven ST5 isolates found on the pre-chill carcasses (7/580) did not provide a path of carriage from the live animals to carcass. Neither MRSA presence/absence on nasal swabs nor any antemortem skin locations was a good predictor of carcass status at pre-chill.

In summary, this data supports:1) that potential direct linkage between live swine MRSA and pre-chill contamination is low 2) that current plant interventions provide significant protections from MRSA contamination to pre-chill MRSA carcass, and 3) that skin swabs and nasal swab isolations do not detect the same individual animal carriage, but may be used interchangeably to determine group contamination.

Introduction and project rationale:

The bacterium *Staphylococcus aureus* is a commonly found on the skin and mucous membranes of a wide range of mammals; and usually exists in a non-clinical state. However, these bacteria can invade the body and cause mild to serious clinical manifestations, primarily recognized in humans. One subgroup of *Staphylococcus aureus* that has been particularly important clinically in healthcare and community settings is Methicillin-Resistant *Staphylococcus aureus* (MRSA). This bio-type has received significant press and attention from the medical community; and currently is a high priority for reduction in hospital environments by the Centers for Disease Control and Prevention (CDC website, 2013). Human exposures were generally considered to result from nosocomial contamination by other humans. Dutch researchers were the first to identify MRSA ST398 as a new clonal type in animals. Carriage of ST398 has subsequently been detected in poultry, horses, and ruminants. ST398 has a world-wide distribution and has been found in Canada and in the USA associated with pigs, pork, swine producers and swine veterinarians (Weese et al., 2010, 2011). Finding a distinctive clonal type in food animals has raised the issue of MRSA ST398 as a potential human food safety issue in meats.

Davies (2009) found swine associated spa types in 4.9% (7/143) retail pork, but isolated *Staphylococcus aureus* from 80% of all samples examined. An oft-proposed retail pork MRSA contamination scenario is routing from on-farm carriage passed through processing to retail cuts. This pathway and its relative efficacy for meat contamination have not been well demonstrated under USA commercial abattoir conditions. Little is known about the ecology of MRSA in live pigs at harvest and subsequent contamination of pork products in the peri-harvest environment. Since *Staphylococcus aureus* contamination occurs commonly on the skin and mucous membranes a high carriage rate can be expected in sampled mammals. Unlike Europe where ST398 is the predominant clonal type reported from livestock, Canadian and US studies demonstrated a wider array of clonal types in swine (Weese et al.; 2010; Khannat et al., 2008; Molla et. al, 2012; Frana et. al., 2013). In several North American studies a range from 25-50% of sampled pigs have been identified with ST398.

Methicillin resistant *Staphylococcus aureus* (MRSA) has recently gained increased attention as a marker for antimicrobial resistance. The popular and scientific press has highlighted MRSA presence in retail pork as a food safety concern related to antimicrobial resistance transfer. Given the wide-spread occurrence of *Staphylococcus aureus* in human and animal populations, introduction of MRSA onto retail foods may occur at many points in the farm to retail continuum. Statements that pork is a primary source of MRSA in retail meats have appeared; coupled with a proposed narrative which ties on-farm MRSA carriage transfer directly through processing to retail pork. Cross-contamination with MRSA during lairage has been posited as a possible source for variations in group prevalence seen within different abattoir populations, and to question a direct linkage from farm to retail. Experiences with *Salmonella enterica* transfer and carriage amplification within lairage might provide guidance about possible MRSA transfer in lairage. Additionally, in previous lairage work our observations were that in spite of a high carriage rate for *Salmonella* in pigs leaving lairage, normal processing and sanitation steps required by in plant HACCP plans greatly reduced carcass contamination prevalence prior to chill (Hurd et al., 2001) This effect may be relevant with MRSA transfer, however, it is not known that such prevalence amplification or diminution in lairage and processing steps, respectively, occurs with MRSA in commercial abattoir environments. This study will serve as an introductory foray into the potential peri-harvest impacts on MRSA carriage through lairage and initial processing of carcasses.

Objectives of Research Project

Objective 1: Determine the impact of lairage on the prevalence of MRSA carriage in swine
Objective 2: Determine the impact of normal pre-chill harvest procedures on MRSA prevalence Objective 3: Compare the relative agreement of nasal vs. skin swabs on MRSA detection

Materials and Methods:

Study design: The study was an observational study with data collected from pigs at multiple times points along the harvest process from entry to the abattoir to pre-chill carcass.

Study setting:
Sample procurement occurred at a high-volume commercial Midwestern (Iowa) abattoir from November 2011 to April 2012. Pigs marketed where chosen by the owner. Upon arrival each lot of pigs was tattooed by plant personnel with a unique lot identifier per company procedures. Four direct delivery production site-to-abattoir lots were selected by plant personnel for each of 15 periods. From these lots 10 individual pigs were selected to

represent the group status. Individual pig selection was based on convenience within test groups as no unique individual ID or sampling frame was available. The same pigs were not sampled at each point for Objectives 1 and 2. Identified test lots were sampled with skin swabs of 10 individual animals where all pigs in the lot were eligible. Skin samples were taken by swabbing the moistened sponge (Whirlpak, Nasco, Fort Atkinson, WI) across the shoulder area (approximately a 10cm X 10cm area) of 10 pigs per lot upon entry (receiving), at the gondola-loading area after several hours in lairage (post-lairage) and at the post-evisceration (pre-chill) area to fulfill Objectives 1 and 2.

For Objective 3, skin samples were collected from individually identified pigs at the gondola-loading area (post-lairage) and those pigs were resampled after stun (shackle) using nasal swabs (post-stun). All sponges were hydrated with 15 ml. of phosphate buffered saline (PBS) and maintained in individual, sterile and uniquely identified whirl-paks overnight. Nasal swabs were prepared with transport media and each tube was uniquely identified to enable individual sample capture and match to post-lairage samples previously collected. Each sponge was returned to the original bag after skin swabbing was completed. After post-lairage sampling the individual animal was identified with a unique code by spray marking the back. At post-stun, the individual animals to be sampled were identified by that spray code and the appropriately numbered nasal swab applied. Nasal swabs were inserted deeply into the nostrils for each sampled pig and the swab was returned to the identified tube. All swabs and sponges were chilled and delivered to the Iowa State University Veterinary Diagnostic Laboratory for processing within 6-8 hours of collection for processing.

Bacterial Isolation and identification of MRSA isolates: Upon receipt at the Iowa State University Veterinary Diagnostic Laboratory individual samples were assigned a VDL number per normal procedures to maintain information integrity through the laboratory processes. Each sponge or swab was inoculated into enrichment broth containing 10g tryptone/L, 75g NaCl/L, 10g mannitol/L and 2.5g yeast extract/L and incubated for 24h at 35°C, then a loopful of the broth was inoculated onto selective MRSA agar plates (Bio-Rad Laboratories, Redmond, WA). Plates were incubated for 18-24 hours at 35ºC and examined *Staphylococcus* colonies. (Weese, et al., 2010; Weese, et al., 2011). Up to 3 suspect colonies from a sample were further identified by hemolytic pattern, gram stain, catalase test, and conventional biochemical tests as described in Carter and Smith (1957) or by matrix-assisted laser desorption ionization time-of-flight (MALDI TOF) mass spectrometry (Bruker, Billerica, MA). All *Staphylococcus aureus* isolates were screened for methicillin resistance on Mueller Hinton agar with 4% NaCL and 6 μg/ml Oxacillin. Confirmation of MRSA status was initially conducted using the MRSA latex agglutination test (Oxoid Ltd., Hants, UK). Isolates were stored at -80°C and transferred to the laboratory of Dr Catherine Logue for molecular characterization.

Molecular testing of MRSA isolates: A subset of isolates collected were tested for the presence of 16s, *mecA* and PVL genes by PCR (Buyukcangaz et. al., 2013. *Multiplex PCR*

All *S. aureus* strains were recovered from frozen stock to TSA plates and incubated at 37°C for 18-24 hours. DNA extraction was carried out by suspending one colony in 50 µL of DNase/RNase-free distilled water, heating the suspension (99°C, 10 min) and then centrifugation (30,000 x *g*, 1 min) to remove cellular debris. The remaining DNA was transferred to a new tube and stored frozen at -20°C. Multiplex PCR assay for detection of 16S rRNA, *mecA* and PVL genes included 2 µL of the DNA template (described above) added to a 50 µL final reaction mixture: 1X Go Taq® Reaction Buffer (Promega, Madison, WI, USA), 0.025 U/µL of Go Taq® DNA polymerase (Promega), 200 µM dNTP (Promega) and 1 µM of primers (16S rRNA, *mecA*, LukS/F-PV) (Integrated DNA Technologies, Inc., Coralville, IA, USA) (McClure *et al*., 2006). Multiplex PCR settings was carried out according to Makgotlho *et al*. (2009), using a thermocycler (Eppendorf, Hamburg, Germany). Ten microliters of the PCR amplicons were loaded into a 1.5% (wt/vol) agarose gel (Agarose I^{TM}, Amresco, Solon, OH, USA) in 1X TAE buffer and loading dye, and run at 100 v in 1X TAE buffer for 1 h. A molecular weight marker 100-bp ladder (Promega) and a positive control (ATCC 35591) were included on each gel. Bands were visualized using an Alpha Innotech UV imager (FluorChem^{TM}).

Pulse-field gel electrophoresis (PFGE)

The PulseNet protocol with slight modifications was used (McDougal *et al*., 2003). Briefly, frozen isolates were struck in TSA plates and incubated at 37°C for 18-24 h. A single colony was inoculated into a second TSA plate and incubated at 37°C for 18-24 h. Colonies were transferred to 5 mL polystyrene round-bottom tubes containing 2 mL of cell suspension buffer (CSB) (100 mM Tris HCl [pH 8.0], Invitrogen; and 100mM EDTA [pH 8.0], Gibco), adjusting the concentrations to an absorbance of 0.9 to 1.1 in a spectrophotometer (Smart Spec^{TM} plus, Bio-Rad Laboratories, USA) at 610 nm. After that, the preparation, lysis and washes of plugs, and then the *SmaI* enzyme restriction digestion were performed according to the PulseNet protocol. *Salmonella* Branderup H9812 was used as a DNA marker (Ribot *et al*., 2006). The electrophoresis was carried out in a Chef Mapper (Bio-Rad Laboratories) PFGE rig, with an initial switch time of 5s, a final switch time of 40s, and a total running time of 17 h 45 min.

After staining the gels with ethidium bromide (1.5 µg/mL), they were visualized using a UVP imager (UVP, Upland, CA). Macrorestriction patterns were compared using the BioNumerics Fingerprinting software (Ver 6.5 Applied Math, Austin, TX). The similarity index was calculated using the Dice coefficient, a band position tolerance of 1% and an optimization of 0.5%. The unweighted-pair group method with arithmethic averages (UPGMA) was used to construct a dendrogram and clusters were selected using a cutoff at 80%.

Multilocus sequence typing (MLST)

Briefly, *S. aureus* isolates were struck to TSA plates and incubated at 37°C for 18-24 h. Colonies were picked to 40 µL of single cell lysing buffer (50 µg/mL of Proteinase K, Amresco; in TE buffer [pH=8]), and then lysed by heating to 80°C for 10 minutes followed by 55°C for 10 minutes in a thermocycler. The final suspension was diluted 1:2 in sterile water, centrifuged to remove cellular debris and transferred to a sterile tube (Marmur, 1961). The housekeeping genes: *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*, were amplified (Table 1) (Enright *et al*., 2000). All PCR reactions were carried out in 50 µL volumes: 1 µL of DNA template, *Taq* DNA polymerase (Promega) (1.25 U), 1X PCR buffer (Promega), primers (0.1 µM) (Integrated DNA Tecnologies, Inc.), and dNTPs (200 µM) (Promega). PCR settings were adjusted according to Enright *et al*. (2000) using a thermocycler (Eppendorf). Ten microliters of the PCR products were loaded into 1% agarose gels in 1X TAE with EzVision One loading dye, and run at 100 V in 1X TAE for 1 h. Images were captured using an Alpha Innotech imager.

After PCR, each amplicon was purified of amplification primer using the QIAquick® PCR Purification Kit (Qiagen, Valencia, CA) as per manufacturer's instructions. Purified DNA was sequenced at Iowa State University's DNA Facility (Ames, IA, USA) using an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequence data was imported into DNAStar (Lasergene, Madison, WI), trimmed and aligned to control sequences (from MLST site) and interrogated against the MLST database (http://saureus.mlst.net/). Sequence types were added to the strain information for analysis in BioNumerics identification and analysis program.

Statistical Analysis

Objectives 1 and 2: We calculated the proportion of positive lots and the 95% confidence interval for the proportion using Fishers exact methods (Using OPEN EPI). The unit of concern will be the lot and outcome of interest was prevalence of MRSA positive lots pre-lairage and post-lairage using McNemars paired test of proportions.

Objective 3: The unit of concern was the individual pig and outcome was MRSA status. The analysis assessed the agreement between nasal and skin samples collected from the same pigs before and after the gondola. The null hypothesis tested with a Kappa test was that status agreement between nasal and skin swabs be > 90%.

Results

From the 15 trips, 58 lots had a complete set of samples (10 at each point) collected representing four (4) production steps – receiving (skin swab), post lairage (pre-gondola skin swab), post-stun (shackle nasal swab), and pre-chill (carcass swab). Summary data are presented in Table 3, and are the subject of this analysis. Cumulative results were as follows:

		Number of positive lots – at least one positive isolate/lot	
Receiving (skin swab).		17/58	29.3% (18.09-42.73)
	1 positive/lot	8	
	2 - 4 positive/lots	6	
	5 positive/lot	2	
	> 5/lot	1	
Post-lairage (pre-gondala skin swab)		38/58	65.5% (51.88-77.51)
	1 positive/lot	13	
	2 – 4 positives/lot	14	
	5 positives/lot	4	
	> 5positive/lot	7	
Post-stun (shackle nasal swab)		49/58	84.4% (75.58-92.65)
	1 positive/lot	16	
	2 – 4 positives/lot	19	
	5 positive/lot	7	
	> 5 positives/lot	7	
Pre-chill lots (carcass swab)		4	6.9% (2.23-15.8)
	1 positive/lot	2	
	2 – 4 positives/lot	2	

The first and most striking observation is the drop in carrier isolations pre-chill when compared to receiving, post-lairage and to post-stun samples. In only one of the four lots with pre-chill positive isolates were any positive samples obtained at receiving. Second, MRSA typing from a randomized draw of post-lairage and post-stun results demonstrated the presence of 21 ST398 and 12 ST5 patterns across all sampling dates. All but one ST398 isolate (n = 20) were found in the March 27 cohort, while the ST5 isolates were scattered throughout the 15 sampling dates. The March 27th groups yielded 9/40 MRSA positive entry samples and a total of 43/78 positive isolates when combining post-lairage and post-stun. No MRSA ST398 carcass positive isolations (0/40) or other ST types were found on this date. Within the March 27th cohort all four lots had post-lairage positive ST398 isolates. One lot had 3/10 post lairage and 1/10 post-stun positives for ST398 with one of 10 individual matches for post-lairage and post-stun. Another contained 4/10 post lairage and 4/10 post-stun positives with ST398 originating from the same four animals. The third lot had ST398 in1/10 post lairage and 1/10 post-stun from the same animal. The final lot had no post lairage positives, but 2/9 post-stun positive ST398 isolates.

Table 1: Lot level data comparing pre-lairage and post-lairage samples testing for the presence of MRSA in swine

		Pre-lairage skin samples		
		Positive	Negative	
Post-lairage nasal samples	Positive	14	26	40
	Negative	3	15	18
		17	41	58

A positive lot is defined as a lot with at least 1 positive sample, the unit of concern is the lot

The McNemars test was significant (p value of <0.001), suggesting a substantial increase in the proportion of positive lots during the lairage period based on the definition of 1 positive sample being a positive lot (McNemar chi-square tests statistic =16.69, p value=0.00004402).

No ST398 isolates were found on pre-chill carcasses over the 15 sample dates and 58 lots tested. All (7) positive carcass (pre-chill) isolates were ST5, and were found in 3 different date cohorts. On two dates when carcass isolates (ST5) were found all receiving samples were negative (0/40) for any MRSA isolate. On the third date the originating lot containing the positive carcass isolate was negative (0/10), but the other three lots produced 9/30 MRSA isolates on delivery. The initially negative lot presented 1/10 and 2/10 for post-lairage and post-stun isolates, respectively. The other three receiving lots presented 1/10, 4/10 and 5/10 isolates; post-lairage lots presented 3/10, 9/10, and 7/10; and the post-stun presented 4/10, 6/10, and 10/10 isolates respectively, but all carcasses were negative (0/30). In contrast on the March 20th sampling when 3/40 MRSA positive carcasses were found, the post-lairage isolates were 0/40 and post stun positives were 2/39. Receiving samples from all four lots were negative (0/40). Source(s) of the isolates of ST5 were not determinable from this dataset.

At the animal level individual post lairage and post-stun samples were collected to determine compatibility of results to answer Objective 3. The question was posited as to whether skin swabs taken post-lairage or nasal swabs taken post stun of individual animals would result in isolation compatibility. Skin or nasal swabs were obtained from 619 post lairage and 615 post-stun animals. There were 160 post-lairage positive and 150 nasal swab isolate positive results. A total of 589 matched post-lairage skin and post stun nasal swab pairs were available for analysis; with 351 pairs both negative and 72 both positive. The remaining 166 pairs were either post-lairage negative and nasal swab positive (88) or reversed designation (78).

Table 2: Individual level results for the comparison of post-lairage and post-stun samples testing for the presence of MRSA in swine.

		Post-lairage skin samples		
		Positive	Negative	
Post-stun nasal samples	Positive	72	88	160
	Negative	78	351	429
		150	439	589

There was no statistically significant difference in the number of positive isolates each method detected within lots, but they did not statistically predict the same pairs as positive. McNemar test (p value of 0.48) suggests no reason to suspect that the proportion of samples positive differed between the two locations. This is clear from the table above. Although the agreement between post-lairage and post stun samples was statistical significant we considered this too low to be of practical significance (Kappa-0.27, SEM Kappa=0.041, 95% CI= 0.19-0.35, Z test-statistic =6.64, p value=1.515654e-11).

Discussion

This project was designed to determine the impact of normal lairage and pre-chill HACCP-based interventions applied during abattoir processing on the presence of MRSA carriage in multiple lots of market swine held under commercial conditions. Each lot of market swine were selected by plant personnel from regular direct farm to abattoir deliveries on each sampling date. The presence of MRSA carriage within each lot was estimated at four points in the harvest process to assess changes in group carriage rates. In this study the MRSA individual lot carriage rate at entry was estimated to be 29.3%. Because the lots were selected from direct site to abattoir loads, this rate may be used as an estimate for expected on-farm carriage rates. The post-lairage isolation rate was greater than at entry (29.3% vs. 65.5%), which supports the proposition that during the lairage period additional MRSA transmission between animals is occurring due to skin contact and environmental contamination. Within this general rise in MRSA isolation, the results varied widely by sample day or lot. The seven ST5 isolates on pre-chill carcasses were derived from four lots on three different days. Only one of the four receiving lots contained an MRSA positive isolate. The March 27th date is instructive in the inverse as three of four entry lots contained 9/40 positive isolates. After several hours in lairage 27/40 samples and 26/38 post-stun swabs were positive for MRSA and were spread across all 4 lots. Of those isolates further characterized, 16 from the post lairage (8) and nasal swabs (8) were ST398. No carcass (pre-chill) swabs were ST398 positive, nor were any MRSA bio-types identified pre-chill on that date. Such observations are consistent with current HACCP-based procedures substantially reducing MRSA carriage from the live animal to pre-chill carcass stage.

The third objective for this study was to determine whether MRSA isolations resulting from swabbing the skin of pigs was comparable to nasal swab results in individual animal pairs. Based on the individual pairs of samples available there is not a direct animal correlation for these sampling strategies. However, both sampling sites produced comparable results in quantity and ST-type distribution when evaluated at the lot level. Therefore collection of skin swabs before stunning or nasal swabs after stunning may provide useful information about group MRSA carriage. Importantly, neither nasal swabs nor antemortem skin sampling were a good predictor of carcass skin contamination at the pre-chill level. As described above, the seven (7/580) ST5 isolates found on the pre-chill carcasses did not present a clear path of carriage from the live animals to carcass as they originated from lots with minimal antemortem isolate recovery from the three sample sets before processing. This observation could also be made about those lots where substantial MRSA contamination found prior to processing (post lairage and post stun). In none of the 58 lots sampled at each of the four locations (receiving, post-lairage, post-stun, and pre-chill) were the same isolates able to be detected moving through these progressive steps.

In summary, this data supports observations reported elsewhere: 1) that a potential direct link between live swine MRSA and pre-chill contamination appears to be very low (thereby reducing risk for transfer to retail pork from the live animal); 2) that current HACCP-based interventions in processing provide significant protections from MRSA transfer at the pre-chill carcass level; and 3) that skin swabs and nasal swab isolations do not detect the same individual animal carriage, but may be used interchangeably to determine group contamination rates. These data should be interpreted within the context that additional characterization of MRSA ecology in the peri-harvest environment is needed. However, in the interim, routine plant HACCP-based interventions associated with pre-cooler actions appear capable of greatly reducing the transfer of *Staphylococcus aureus* from production sites to pre-chill carcass.. This data should be interpreted within the context that additional characterization of MRSA ecology in the peri-harvest environment is needed to confirm and amplify these observations.

References

Aires-de-Sousa, M., et al., (2006) High interlaboratory reproducibility of DNA sequence-based typingof bacteria in a multicenter study. J Clin Microbiol, **44**(2): p. 619-21.

Buyukcangaz, E., V. Velasco, et. al. (2013) Molecular Typing of *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* (MRSA) Isolated from Animals and Retail Meat in North Dakota, United States. Foodborne Pathogens and Disease. 10(7): 608-617.

Carter, C. F., A.L. Smith (1957). Principles of Microbiology. St. Louis, Mosby.

Davies, P. (2009). Methicillin Resistant Staphylococcus aureus in pigs, pork products and Swine Veterinarians (Published on Pork.org NPB #07-196)

Frana TS, AR Beahm et. al. , (2013) Isolation and characterization of methicillin-resistant Staphylococcus aureus from pork farms and visiting veterinary students.PLoS One. 2013;8(1):e53738. doi: 10.1371/journal.pone.0053738. Epub 2013 Jan 3.

Hurd, H.S., J.D. McKean, et al. (2001) The Effect of Lairage on Salmonella Isolation from Market Swine. J. Food Protection, 64(7), 939-46.

Khannat., Friendship R., Dewey C., Weese J.S.s.,(2008), Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers. Vet Microbiol. 128:298-303.

Lina, G., et al., (1999) Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis,. **29**(5): p. 1128-32.

Molla B, M. Byrne et. al., (2012) Epidemiology and genotypic characteristics of methicillin-resistant Staphylococcus aureus strains of porcine origin. J Clin Microbiol. 50 (11):3687-93.

Murakami, K., et al.,(1991) Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J Clin Microbiol,. **29**(10): p. 2240-4.

Shopsin, B., et al., (1999) Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. J Clin Microbiol, **37**(11): p. 3556-63.

http://online.liebertpub.com/doi/pdfplus/10.1089/fpd.2012.1427

Weese, J. S., B. P. Avery, et al. (2010). "Detection and quantification of methicillin-resistant Staphylococcus aureus (MRSA) clones in retail meat products." Lett Appl Microbiol 51(3): 338-42.

Weese, J. S., A. Zwambag, et al. (2011). "Longitudinal Investigation of Methicillin-Resistant Staphylococcus aureus in Piglets." Zoonoses Public Health 58(4): 238-43. Principles of Microbiology. St. Louis, Mosby.

Table 3: Summary of all lots in test groups. (Positive isolates/samples)

Date	Lot	Data	Receive Sponge	Post-lairage Sponge	Post-stun Swab	Prechill Sponge
11/1/2011	2458	Positive Samples/lot	0	6	0	0
		Sum of Total Samples	10	10	0	10
	3457	Positive Samples/lot	0	8	6	0
		Sum of Total Samples	10	11	20	10
	3914	Positive Samples/lot	0	5	7	0
		Sum of Total Samples	10	11	22	10
	3915	Positive Samples/lot	0	1	0	0
		Sum of Total Samples	10	2	0	10
11/8/2011	3555	Positive Samples/lot	4	9	7	0
		Sum of Total Samples	10	10	10	10
	3556	Positive Samples/lot	0	1	3	0
		Sum of Total Samples	10	10	10	10
	3814	Positive Samples/lot	0	6	0	0
		Sum of Total Samples	10	10	10	10

	3815	Positive Samples/lot	3	6	5	0
		Sum of Total Samples	10	10	10	10
11/15/2011	3631	Positive Samples/lot	0	1	2	1
		Sum of Total Samples	10	10	10	10
	3632	Positive Samples/lot	1	7	10	2
		Sum of Total Samples	10	10	10	10
	3633	Positive Samples/lot	0	3	8	0
		Sum of Total Samples	10	10	10	10
	3929	Positive Samples/lot	0	2	2	0
		Sum of Total Samples	10	10	10	0
11/29/2011	3321	Positive Samples/lot	0	0	1	0
		Sum of Total Samples	10	10	10	10
	3921	Positive Samples/lot	1	1	3	0
		Sum of Total Samples	10	10	10	10
	3922	Positive Samples/lot	0	0	3	0
		Sum of Total Samples	5	10	10	10
	3923	Positive Samples/lot	0	1	6	0
		Sum of Total Samples	10	10	10	10
12/6/2011	3447	Positive Samples/lot	0	2	1	0
		Sum of Total Samples	10	10	10	10
	3855	Positive Samples/lot	0	2	0	0
		Sum of Total Samples	9	10	10	10
	3856	Positive Samples/lot	1	3	1	0
		Sum of Total Samples	10	10	10	10
	3857	Positive Samples/lot	0	2	1	0
		Sum of Total Samples	10	10	9	10
12/13/2011	3578	Positive Samples/lot	0	3	0	0
		Sum of Total Samples	10	10	10	10
	3579	Positive Samples/lot	2	2	2	0
		Sum of Total Samples	10	10	10	10
	3983	Positive Samples/lot	0	6	0	0
		Sum of Total Samples	10	10	10	10
	3985	Positive Samples/lot	0	5	1	0
		Sum of Total Samples	10	10	10	10
1/10/2012	3332	Positive Samples/lot	1	3	4	0
		Sum of Total Samples	10	10	10	10
	3333	Positive Samples/lot	0	1	2	1
		Sum of Total Samples	10	10	10	10
	3972	Positive Samples/lot	5	9	12	0
		Sum of Total Samples	10	10	20	10
	3973	Positive Samples/lot	4	7	10	0
		Sum of Total Samples	10	10	10	10
1/24/2012	3424	Positive Samples/lot	0	2	2	0
		Sum of Total Samples	10	10	7	10
	3427	Positive Samples/lot	0	1	0	0
		Sum of Total Samples	10	10	10	10
	3838	Positive Samples/lot	0	1	1	0
		Sum of Total Samples	10	10	11	10
	3839	Positive Samples/lot	1	0	0	0

		Sum of Total Samples	10	8	10	10
1/31/2012	3446	Positive Samples/lot	0	0	2	0
		Sum of Total Samples	10	10	10	10
	3447	Positive Samples/lot	0	1	1	0
		Sum of Total Samples	10	10	10	10
	3839	Positive Samples/lot	0	0	1	0
		Sum of Total Samples	10	10	10	10
	3842	Positive Samples/lot	0	2	2	0
		Sum of Total Samples	10	10	9	10
2/7/2012	3445	Positive Samples/lot	0	0	1	0
		Sum of Total Samples	10	10	8	10
	3446	Positive Samples/lot	0	5	1	0
		Sum of Total Samples	10	8	10	10
	3894	Positive Samples/lot	0	0	0	0
		Sum of Total Samples	10	10	10	10
	3897	Positive Samples/lot	0	2	1	0
		Sum of Total Samples	10	10	9	10
2/14/2012	3496	Positive Samples/lot	0	1	2	0
		Sum of Total Samples	10	10	9	10
	3499	Positive Samples/lot	0	0	1	0
		Sum of Total Samples	10	10	9	10
	3838	Positive Samples/lot	0	0	1	0
		Sum of Total Samples	10	10	9	10
	3841	Positive Samples/lot	1	7	7	0
		Sum of Total Samples	10	10	10	10
3/6/2012	3527	Positive Samples/lot	1	1	0	0
		Sum of Total Samples	10	10	10	10
	3891	Positive Samples/lot	1	0	0	0
		Sum of Total Samples	10	10	10	9
	3893	Positive Samples/lot	0	0	1	0
		Sum of Total Samples	10	10	10	10
	3894	Positive Samples/lot	6	0	1	0
		Sum of Total Samples	10	10	9	10
3/20/2012	3614	Positive Samples/lot	0	0	1	0
		Sum of Total Samples	10	9	10	10
	3615	Positive Samples/lot	0	0	0	3
		Sum of Total Samples	10	10	9	10
	3909	Positive Samples/lot	0	0	1	0
		Sum of Total Samples	10	10	10	10
	3914	Positive Samples/lot	0	0	0	0
		Sum of Total Samples	10	10	10	10
3/27/2012	3643	Positive Samples/lot	1	4	7	0
		Sum of Total Samples	10	10	9	10
	3645	Positive Samples/lot	0	4	5	0
		Sum of Total Samples	10	10	9	10
	3929	Positive Samples/lot	5	9	6	0
		Sum of Total Samples	10	10	10	10
	3931	Positive Samples/lot	3	10	8	0

		Sum of Total Samples	10	10	9	10
4/3/2012	3673	Positive Samples/lot	0	0	3	0
		Sum of Total Samples	10	10	10	10
	3981	Positive Samples/lot	0	0	3	0
		Sum of Total Samples	10	10	10	10
	3983	Positive Samples/lot	0	1	2	0
		Sum of Total Samples	10	10	9	10
4/10/2012	3697	Positive Samples/lot	0	5	3	0
		Sum of Total Samples	10	10	10	10
	3699	Positive Samples/lot	0	1	4	0
		Sum of Total Samples	10	10	10	10
	3701	Positive Samples/lot	0	1	3	N/A
		Sum of Total Samples	10	10	10	N/A
	3805	Positive Samples/lot	0	0	4	0
		Sum of Total Samples	10	10	9	10