Effect of lairage on prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in market swine

McKean, J.D.; Frana, T.1; Logue, C.M.1; O’Connor, A.M.1

**Introduction**

This project had three objectives:

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.

Here we present the interim summary of the project.

**Materials and Methods:**

**Design and setting:** The study was an observational study with data collected from pigs at multiple time points along the harvest process from entry to the abattoir to pre-chill carcass. 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. Pigs 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 across the shoulder area (approximately a 10cm X 10cm area) of 10 pigs per lot upon entry (receiving), at the gondola-loading area (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 (post-lairage) area and those pigs were resampled after stun (shackle) using nasal swabs (nasal swabs).

**Bacterial Isolation and identification of MRSA isolates:** 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 18-24hours at 35°C and examined for *Staphylococcus* species. (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). 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 For multilocus sequence typing (MLST)*) the housekeeping genes were: *arcC, aroE, glpF, gmk, pta, tpi,* and *vlp*., were amplified (Table 1) (Enright et al., 2000). Sequence data was imported into DNAStar (Lasergene, Madison, WI), trimmed and aligned to the control sequences (from the MLST site) and interrogated against the MLST database (http://saureus.mlst.net/).
Results

These results are interim. From the 15 trips data from 63 lots were collected. 58 lots had a complete set of samples (10 at each point) were 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). The patterns of recovery for Staphylococcus aureus at the four locations are provided in Figure 1. The change in prevalence of Staphylococcus aureus at the three locations (pre-lairage, post lairage and chill) that used skin swabs is also shown in Figure 2, differentiated by the lots arrival status i.e., comparing lots that arrived with at least one positive sample compared to those that did not have at least one positive sample. The first and most striking observation is the drop in carrier isolations pre-chill when compared to receiving-and post-lairage (pre-gondola) lots and to Post-stun lots. This result was consistent regardless of the status of the pigs upon arrival i.e. positive and negative lots both had increases while in the abattoir but where negative at the end of processing.

MRSA typing from a random sampling of post-lairage and post-stun Staphylococcus aureus demonstrated the presence of 21 ST398 and 12 ST5 patterns across all sampling dates. All but one ST398 isolate was found in the March 27 cohort, while the ST5 isolates were scattered throughout the 15 sampling dates. No MRSA ST398 carcass positive isolates (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 nasal swab positives for ST398 with one of 10 individual matches for post-lairage and nasal swabs. Another contained 4/10 post lairage and 4/10 nasal swab positives with ST398 originating from the same animals. The third lot had 1/10 post lairage isolates and 1/10 nasal swabs with ST398 with one match. The final lot had no post lairage positives, but 2/9 nasal swab positive ST398 isolates. The McNemars test was significant with a p value of <0.001, suggesting here was 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.

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

<table>
<thead>
<tr>
<th>Pre-lairage skin samples</th>
<th>Post-lairage nasal samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>14</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
</tr>
</tbody>
</table>

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

At the animal level individual post lairage and post-stun samples were collected to determine compatibility of results and to answer the Objective 3. The question was posited as whether skin swabs taken post-lairage or nasal swabs take 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. 589 matched post-lairage skin swabs and post stun nasal swab pairs were available for analysis; with 351 pairs both negative and 72 pairs both positive. The remaining 166 pairs were 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.

<table>
<thead>
<tr>
<th>Post-stun samples</th>
<th>Post-lairage skin samples</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
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<td>78</td>
</tr>
</tbody>
</table>

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 had a p value of 0. 48 suggesting no reason to suspect that the proportion of samples positive differed between the two locations. 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). (statistics code is provided at the end).

Discussion

In this study the MRSA individual lot carriage rate at entry was estimated to be 28.8%. 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 (28.8% vs. 64.4%), which supports the proposition that during the lairage period additional MRSA transmission between animals is probably occurring due to skin contact and contamination. Within this general rise in MRSA isolation, the results varied widely. No carcass (pre-chill) swabs were ST398 positive, nor were any MRSA bio-types identified pre-chill on that date. Such an observation is 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.

Conclusion

In summary, these data support observations elsewhere: 1) that the potential risk of MRSA ST398 from animal products causing human disease appears to be very low and 2) that current HACCP-based intervention in processing provide significant protections by reducing the risk of MRSA ST398 at the pre-chill carcass level. Worth noting is that the ST5 type has been previously implicated in human illness as a potential source for community acquired strains. These data identified only ST5 on pre-chill carcasses.

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**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%.

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Epidemiology and control of hazards in pork production chain – SAFEPORK
One health approach under a concept of farm to fork

Control strategies to mitigate foodborne pathogens

References

Listeria control programs in the production environment
Fernàndez, E.C.

Listeria is a universal problem for food manufacturers and even when all reasonable hygiene measures have been put in place, problems can still arise. The crux of the Listeria monocytogenes challenge is this bacteria’s natural resistance to many of the standard food preservation methods. The factory sources of Listeria are many and varied and include handling, processing and maintenance equipment, packaging systems, drains, surfaces, as well as refrigeration and chilling units.

Sealed Air has defined practical hygiene solutions for the effective management of the Listeria threat. Sealed Air’s preventative approach targets all potential sources of the bacteria’s spread in the form of a comprehensive hygiene programme. It addresses three main risk types – those deriving from infrastructure, processes and personal hygiene. From our expertise in implementing hygiene practices in several types of food industries, we claim that a previous assessment needs to be performed before providing the solution that better fits the requirements and specific conditions of the producer. The first step is a full and objective audit of the factory facilities, including the identification of any potential Listeria “hot spots”. Our product range facilitates hygiene excellence in all processing equipment and food contact surfaces. Proven solutions also incorporate training programmes for personal hygiene and working practices, measures for the prevention of cross-contamination and water control. Last development has been a specific assessment about Listeria based on our know-how, which provides specific (and some generic) solutions to control the impact of this bacteria on final product.

Finally, we have to highlight that no magical solution exists to fight Listeria: control requires good hygienic practices to be followed and continuous monitoring, but no hygienic practices alone will be able to eliminate Listeria from the production environment. Implementation & management of correct hygiene tasks can minimize the cross-contamination of the finished product and support the manufacture of microbiologically-safe food. Additionally other aspects need to be included as well such as a good HACCP program as well as Good Manufacturing Practices.

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Figure 1: The pattern of recovery of Staphylococcus aureus from the lots of pigs each date. For each lot, 4 locations where sampled: receiving (pr.rs), post lairage (pr.pls), post-stun (pr.ss), and pre-chill (pr.ps).

Figure 2: Pattern of Staphylococcus aureus recovery from lots of swine, at three locations: at arrival (recived sponge), after lairage (post-lairage sponge) and at chill (prechill sponge)