# PREVALENCE AND CHARACTERIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* DURING COMMERCIAL PORK PROCESSING

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*Staphylococcus aureus* although largely considered a human commensal and opportunistic pathogen, has been isolated from numerous vertebrate species, indicating it's capacity to survive in multiple host environments *(1, 3, 6, 12)*. In the past several years, a new reservoir of methicillin resistant *Staphylococcus aureus* (MRSA) has been identified in the food animals and people in contact with these animals. This involves a specific clone of the multilocus sequence type 398 (ST398), which has spread extensively among animals in recent years *(1)*. MRSA in pigs was first reported in France and subsequently it was found that it belonged to the ST398 genotype *(2)*. Later on ST398 MRSA was discovered to be widespread in pig farms in the Netherlands *(26)* and has been identified in several other countries in Europe, North America and Asia *(7, 11, 25)*. The ST398 strain has also been found in retail meat samples in surveys from different parts of the world and appears to be a predominant lineage that poses a potential threat to human health *(17)*. Livestock-Associated MRSA (LA-MRSA) belonging to the ST398 lineage, common among pigs and other animals, emerged in central and northern Europe is now becoming a new risk factor among farm workers *(15, 21)*. Indeed, the typical multi-resistant phenotype of MRSA ST398 and its ability to easily acquire genetic elements suggests that MRSA ST398 strains with an increased virulence potential may emerge, for which few therapeutic options would be available. This highlighted the need to implement intervention strategies to control the presence and spread of MRSA clone ST398 among pigs.

The transmission dynamics and risk factors for introduction and persistence of MRSA in farms and pork processing plants, workers handling pork and contamination of pork products is not fully understood and deserves investigation. The shift in the epidemiology of MRSA, possibly involving the food chain, may pose risks to human health. To address these challenges we studied the epidemiology of MRSA during commercial pork processing and on retail pork produced at these plants. This information allowed us to better understand the transmission dynamics of MRSA in pork processing plants should help in assessing the risks of spread of MRSA infections to humans.

**Material and Methods**

Sampling was performed during multiple visits to three commercial pork processing plants in Alberta, Canada (September 2010 to August 2011). Samples were collected from four points during pig slaughter and pork processing. Two of the pork plants designated as A and B performed skinning of the carcasses after bleeding. The third plant designated as plant C performed scalding after bleeding and was the only plant that pasteurized carcasses with hot water (80°C). During each visit a total of 40-44 samples comprising about 10 samples from each of the following four sampling points were obtained: nasal swab sample after bleeding (NSAB); nasal swab sample after scalding or skinning depending on the operation (NSASc or NSASk); carcass samples after pasteurization or washing (CSAP or CSAW) depending on the operation; and retail pork products (RP).

Samples were transported to the AAFC laboratory in liquid Stuart's medium stored at 4 °C. Initial enrichment of samples was carried out in 2 mL of enrichment broth containing 10 g tryptone/L, 75 g sodium chloride/L, 10 g mannitol/L and 2.5 g of yeast extract/L. Selective MRSA chromogenic agar plates (BBL CHROMagar MRSA, Becton, Dickinson and Company, *Spa*rks, Maryland, USA) were used for MRSA isolation. MRSA colonies based on appearance (mauve-colored, round colonies) were selected for further analysis. Presumptive MRSA colonies were tested and confirmed as *S. aureus* by various

biochemical and molecular methods. The *spa* typing of MRSA isolates was performed using a modified method originally described by Shopsin et al. (1999) *(24)* and modified by Khanna et al. (2008). *(16)*.

## Results

*MRSA prevalence in pork processing plants*

All confirmed MRSA isolates carried the *mec*A gene and all of the isolates were negative for Panton Valentine Leucocidin (cytotoxin) gene (PVL). Overall 24.8% (655/2641) of the samples were found positive for MRSA. The overall MRSA prevalence by sample area was as follows: 61.9% (410/662) of NSAB samples, 28.4% (187/659) of NSASs/Sk samples; 7.6% (50/660) of CSAP/W and only 1.2% (8/660) of retail pork product samples (Table 1).

**Table 1**: Prevalence of MRSA in pigs nasal swab after bleeding (NSAB), nasal swab after scalding (NSASc: plant C) or skinning (NSASk; plants A, B), carcass swab after pasteurization (CSAP; plant C) or washing (CSAW; plants A, B) and in retail pork (RP) from three commercial pork processing plants in Alberta, Canada

| Plant | NSAB | NSASc/Sk | CSAP/W | Retail Pork | *P* value |
|---|---|---|---|---|---|
| A | 77.3 % (170/220) | 48.9% (107/219)$^{Sk}$ | 20.9% (46/220)$^{W}$ | 3.2% (7/220) | <0.0001 |
| B | 34.7% (77/222) | 14.1% (31/220)$^{Sk}$ | 3.57% (4/220)$^{W}$ | 0% (0/220) | <0.0001 |
| C | 74.1 (163/220) | 22.3% (49/220)$^{Sc}$ | 0.0% (0/220) $^{P}$ | 0.5% (1/220) | <0.0001 |

*Sk= after skinning, Sc= after scalding, P= after pasteurization, W= after washing*

The prevalence of MRSA was significantly different among various processing points and among the three participating plants (Table 1). For retail pork products plant A showed the highest prevalence (3.2%), followed by plant C (0.5%) (Table 1). The prevalence of MRSA significantly decreased (P <0.0001) along the various pork processing steps in all the participating pork processing plants. Eleven different *spa*-types were found among 655 MRSA isolates that were subjected to *spa* typing. Diverse *spa*-type patterns were observed in the three pork processing plants and along the various processing steps. The pig associated *spa*-types t034, t002 and t111 were the most common *spa*-types found in the three plants (Figure 1).

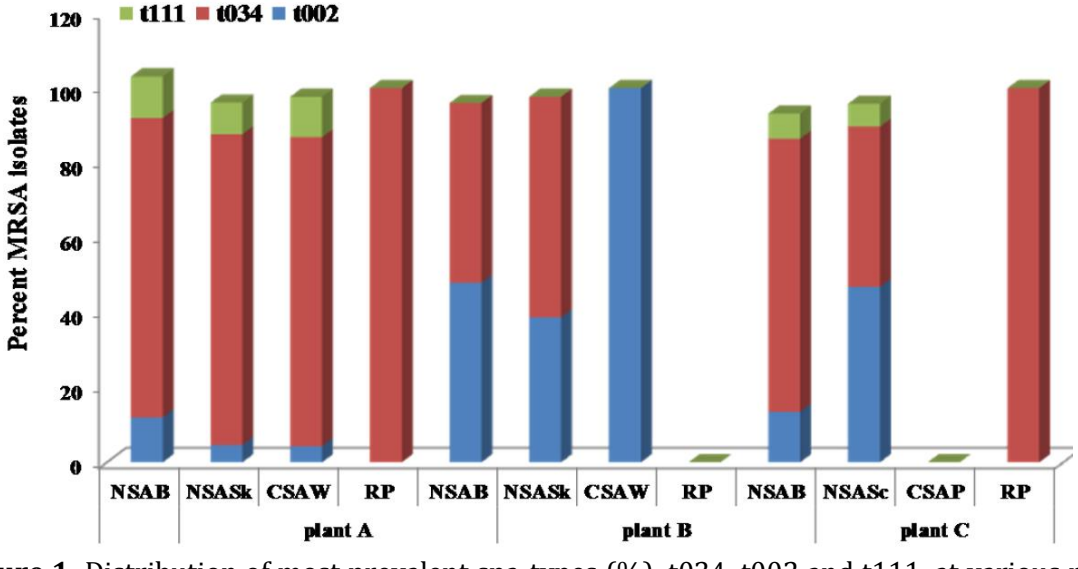


**Figure 1**: Distribution of most prevalent spa-types (%), t034, t002 and t111, at various processing steps in commercial pork plants in Alberta, Canada. NSAB= nasal swab after bleeding, NSASc= nasal swab after scalding (plant C), NSASk= nasal swab after skinning (plants A, B), CSAP= carcass swab after pasteurization (plant C), CSAW= carcass swab after washing (plants A, B), RP= retail pork.

Out of 330 MRSA isolates from plant A, *spa*-type t034 was found in 82% of isolates followed by *spa*types t002 (10% of isolates) and t111 (5.7% of isolates). In NSAB samples about 80% of isolates belonged to *spa*-type t034 and *spa*-types t111 and t002 were found in 11% and 7% of MRSA isolates respectively (Table 3; Figure 1). The *spa*-type t034 remains the most prevalent *spa*-type in NSASk (83% of isolates), CSAW (82.6% of isolates) and in RP samples (100% of isolates).

From plant B the pig associated *spa*-types t034 and t002 were also the most prevalent *spa*-types found (49.1% of isolates and 47.3% of isolates, respectively). In NSAB and NSASk, about 96% of isolates belonged to both *spa*-types t034 and t002 and all four isolates from CSAW belonged to *spa*-type t002 (Table 3; Figure 1).

Overall 66% of MRSA isolates from plant C were belonged to pig associated *spa*-type t034 followed by *spa*-type t002 (21.1% isolates) and *spa*-type t111 (6.5% of isolates; Table 3 and Figure 1). Diverse *spa*types were more commonly recovered from plant C. About 3% of MRSA isolates belonged to *spa*-type t1094 whereas *spa*-types t2971, t4030, t6408, t777, t808, t067 and t1184 were found in < 1% of MRSA isolates. In NSAB and NSASc samples the majority of isolates belonged to *spa*-type t034 (73%, 42.8% respectively) and *spa*-type t002 was found in 13.5% and 46.9% of MRSA isolates (Table 3; Figure 1). One isolate from an RP sample from plant C belonged to the pig associated *spa*-type t034.

**Table 3:** Distribution of spa-types (%) at various processing steps in three commercial pork plants in Alberta, Canada.

| *spa types* | Plant (A) | | | | Plant (B) | | | | Plant (C) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NSAB | NSASk | CSAW | RP | NSAB | NSASk | CSAW | RP | NSAB | NSASc | CSAP | RP |
| t002 | 12 | 4.6 | 4.3 | 0 | 48 | 38.7 | 100 | 0 | 13.5 | 46.9 | 0 | 0 |
| t034 | 80 | 83.1 | 82.6 | 100 | 48 | 59 | 0 | 0 | 73 | 42.8 | 0 | 100.0 |
| t1094 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.1 | 1 | 0 | 0 |
| t111 | 11.1 | 8.4 | 10.8 | 0 | 0 | 0 | 0 | 0 | 6.7 | 6.1 | 0 | 0 |
| t2971 | 0 | 0.9 | 0.0 | 0 | 0 | 0 | 0 | 0 | 1.2 | 0.0 | 0 | 0 |
| t4030 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.6 | 0.0 | 0 | 0 |
| t6408 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.6 | 0.0 | 0 | 0 |
| t777 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.6 | 0.0 | 0 | 0 |
| t808 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.6 | 0.0 | 0 | 0 |
| t067 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| t1184 | 0 | 0 | 0 | 0 | 1.29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unknown | 0.5 | 2.8 | 2.7 | 0 | 2.59 | 3.2 | 0 | 0 | 0 | 0 | 0 | 0 |

*NSAB= nasal swab after bleeding, NSASc= nasal swab after scalding (plant C), NSASk= nasal swab after skinning (plants A, B), CSAP= carcass swab after pasteurization (plant C), CSAW= carcass swab after washing (plants A, B), RP= retail pork.*

## Discussion

Previous studies in Canada have been focused on either prevalence of MRSA in pigs at the farm or in retail pork *(27-29)*. This research was focused on the prevalence of MRSA in three pork processing plants in Alberta and looked into how various processing steps may have an impact on the prevalence of MRSA. Compared with recent studies reporting MRSA prevalence (4.6% 21/460) in Canadian pig farms *(28)*, our results from three processing plants showed that a higher than expected percentage of incoming pigs carried MRSA in their nasal cavity (61.9%), suggesting a potential risk of contamination of pork during the slaughter process. The higher prevalence of MRSA in Alberta pigs found in this study may be in part due to increased nasal shedding because of stress of shipping, transmission among pigs during transportation and in the holding pen area, when compared to the aforementioned study that sampled pigs on the farms. Furthermore the ability to get better samples from carcasses

compared to live slaughter age pigs may have also contributed to the higher MRSA prevalence in this study.

Despite a higher MRSA prevalence in the nasal cavity of incoming pigs, only 1.2% retail pork products were contaminated with MRSA. Previously, a report from Germany analyzing MRSA in the fresh pork production chain and slaughter environment found that 2.8% of the final pork products were contaminated with MRSA *(3)*. A number of previous studies reported a prevalence of MRSA on retail pork products that ranged from as low as 2% to 45% *(3, 5, 13, 19)* based on these studies the prevalence found in this study can be considered low.

MRSA prevalence reports in retail pork varied greatly from various regions. In Canada, a MRSA prevalence of 5.8% in retail pork was reported by Weese et al. 2010. A study from United States showed a MRSA prevalence of 6.6% in retail pork products *(22)*. A higher prevalence (21.9% and 45.6%) of MRSA has been reported in retail pork samples elsewhere *(4, 23)*. These estimates of MRSA prevalence in retail pork are higher than reported in our study and suggest that hygienic and sanitation procedures applied at Alberta commercial pork processing plants are effectively reducing MRSA contamination. These differences in the prevalence among various geographical locations may be due to sampling locations, number of isolates analyzed, MRSA isolation procedures used, food safety regulations and hygienic practices used at the pork processing facilities from where samples were obtained.

A previous study estimated the prevalence of MRSA in slaughterhouses and workers found that the status of MRSA in the environmental samples such as holding pens and slaughter areas of pigs correlated well with the MRSA status of humans working in the slaughterhouse *(26)*. An MRSA prevalence of 12% has been reported in environmental samples collected from a large pig slaughterhouse with an integrated pork-processing unit *(3)*. In this study although a higher prevalence (61.9%) of MRSA in the nasal swab samples after bleeding was found, it reduced significantly along the various processing steps. Our results are consistent with a previously published research reporting that about 65% of samples taken after bleeding of pigs were found to be positive for MRSA *(3)* but contamination of retail products was very low. It appears that the pasteurization of carcasses at plant C and skinning and washing of carcasses at plant A and B might have helped reducing the retail pork contamination in our study. At plant B, MRSA was not found in the pork products whereas a very low MRSA prevalence (0.5%; 1/220) was observed in the retail pork obtained from plant C. Although plant C slaughtered about 8000 pigs per day, despite the higher volume of animal passing through the process, it appears that carcass pasteurization used as intervention step might have helped to reduce the MRSA contamination. On the other hand plant B skinned and washed carcasses during the processing steps and slaughter only about 500 animals per day. It is possible that sanitation practices at this plant are working well and the process was under control.

No significant difference in the MRSA prevalence was observed in plant A compared to plant C. However, at plant B, an overall reduction of MRSA prevalence was observed at all four processing steps. These differences in MRSA prevalence among processing plants suggests that hygienic practices applied at individual plant might have played a role in reducing the MRSA contamination at processing steps. Similarly, a low prevalence of MRSA at early slaughter and processing steps at plant B was translated into 0% prevalence on the retail pork products. On the other hand although plant A and plant C had a higher initial MRSA load at the early slaughter and processing steps, MRSA contamination of retail products was reduced significantly at both plants suggesting that food safety and hygienic procedures applied at the plants are effective. *Spa*-typing results showed that mostly pig associated *spa*-types are present during commercial pork processing in these Alberta plants. A total of 11 different *spa*-types were found in our study with *spa*types t034, t002 and t111 being the major ones. These *spa*-types belong to ST398 which can colonize humans and cause infection, mainly in areas

with high livestock-farming *(21)*. It appears that these *spa*types originated from the incoming pigs and a possibility exists that these *spa*-types can colonize plant workers either at the time of slaughter or during pork processing. *Spa*-type t002 has been associated with human infections and is considered as a livestock indicator strain for MRSA infections in the community *(18)*. *Spa*-type t008 was found in this study, its prevalence was very low, but it has been reported as a pig associated MRSA type and its presence on contaminated retail pork has contributed to community associated MRSA infections *(14)*.

All retail pork MRSA in our study belonged to *spa*-type t034 which have the potential to disseminate in the community *(6, 10)*. *Spa*-type t034 is a sequence type 398 which is LA-MRSA *(20)*. In a study from Belgium performed on pig farms, farm workers and personnel associated with pig farming, t034 represented 16% of the isolates and these strains closely matched those recovered from people showing clinical infection *(10)*. *Spa*-types obtained in a mixed farming environment were reported to be t011, t034, t567, t571, t1451, t2974, t3423 and t5943 and are ST398. Although, the clinical importance of ST398/*spa*-type t034 is still not fully understood, reports suggested that it can cause infections in the human population *(8, 9)*.

To better understand the dynamics of MRSA in pork processing plants, the processes need to be evaluated on a regular basis in order to identify critical control points where MRSA contamination may occur. By optimizing processes for carcass decontamination and avoiding recontamination by implementation of effective cleaning and personal hygiene practices, MRSA cross contamination during slaughter can be minimized. A higher MRSA prevalence during pig production makes it easy for the colonized animals to enter the slaughter plants. MRSA can colonize pigs without any clinical symptoms, therefore, without microbiological screening it is not possible to distinguish between MRSApositive or negative herds entering the slaughter plants. MRSA prevalence data collected prior to slaughter process may limit our ability to exactly pinpoint MRSA prevalence at later stages of pork processing because cross-contamination during transport or in the holding pens area can impact MRSA prevalence. The higher prevalence of MRSA in the incoming pigs to slaughter plants raises questions about how and to what extent MRSA can disseminate along the pork processing steps. Our research has established that a notable reduction of MRSA along the pork processing chain occurs in these plants. The observed differences along the processing steps suggest that the sanitation practices applied at the individual plants may have more impact on the MRSA prevalence on the final product than the initial MRSA carriage rate in the animals.

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