



PUBLIC HEALTH/WORKER SAFETY

Title: The potential for human contamination with Methicillin Resistant Staphylococcus aureus

from handling contaminated pork products. NPB #09-179

revised

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Industry Summary: Methicillin resistant Staphylococcus aureus (MRSA) has been linked to livestock production (referred to as Livestock Associated or LA-MRSA). The bacterium has been found in some swine herds as well as in some of the livestock handlers associated with swine. MRSA has also been found in retail meats. The purpose of this study was to determine if MRSA could be transferred from pork to common consumer surfaces (knives, cutting boards) and to human skin. A pork skin model was used as a model system for human skin. MRSA was found to transfer between surfaces, and the amount of transfer was not affected by the initial population. MRSA was also found to transfer from surfaces to the pork skin model.

Keywords: Methicillin resistant Staphylococcus aureus, MRSA, skin transfer, consumer, cutting board, knife

Scientific Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen that has developed resistance to beta-lactam antibiotics. MRSA was initially associated with hospital acquired infections but emerged in association with community and livestock acquired infections. Recently MRSA has been isolated at low levels in retail meat products in the United States and other countries. Pork loins, bacon and pork sausage were inoculated with four strains of MRSA cocktail, swabbed for initial bacterial populations, vacuum packaged and stored for two weeks at 4°C to simulate normal packaging and distribution. Polyethylene cutting boards, knives and pork skin were contaminated with the inoculated product laving on the surface for 5 minutes. Polyethylene cutting boards and knives

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were also contaminated by placing a 500g lead donut on the product while it is dragged across the transfer surface. 5cmx5cm² areas were swabbed and bacterial populations of the inoculated pork products and contact surfaces were enumerated on Baird-Parker Agar and reported as Log10 cfu/cm². Percent transfer from the inoculated products to the cutting board ranged from 76% to 88% across all 5 cell concentrations. Percent transfer from inoculated products to the knife ranged from 53% to 87% across all 5 cell concentrations. Percent transfer from the inoculated products to the pork skin ranged from 71% to 91% across all 5 cell concentrations. Statistical analysis performed by SAS showed no significant differences in amounts of transfer between transfer surfaces and across cell concentrations. This research illustrates the potential for MRSA transfer to food contact surfaces and skin even at lower initial cell concentrations.

Introduction: Methicillin-resistant *Staphylococcus aureus* is a strain of *Staphylococcus aureus* that has acquired resistance to beta-lactam antibiotics. It first gained notice as the agent responsible for nosocomial infections referred to as hospital acquired (HA MRSA) and it has since been increasingly isolated from infections in the community (CA MRSA) among those with no history of long term hospitalization or surgical procedures (28). Within the last decade livestock acquired (LA MRSA) also known as ST398 has been isolated from healthy livestock, primarily swine. This strain has also been isolated from those who work in close contact with swine and some human cases have also resulted in infections (6,9). There have also been cases in those with no previous animal contact (2). Recently concerns have been raised from a food safety standpoint as ST398 and other MRSA strains have been isolated from raw retail meats (4, 16, 52). Though there have not been any documented cases of human infection with ST398 due to raw meats (40) the fact remains that MRSA strains including ST398 are present in retail raw meat. Therefore they serve as a possible source of bacterial infection

that could enter open wounds on hands of food preparers in the food industry (5) and hands of the consumer in the home.

Contamination of retail meats with MRSA can occur in a number of ways. It may commonly be found on animals at harvest and the animals themselves may be the source. Research has shown MRSA to be present on carcasses and to carry through to the final products (11). In fact ST398 has been isolated in several studies as the source of contamination raw meat (16, 4, 35). Other findings point to humans as the source. On occasion, the MRSA contamination was found to be USA 100 or USA 300, common human strains (52). Current research supports the possibility of more CA-MRSA infections due to contaminated retail products.

MRSA has demonstrated the ability to survive a range of pH, temperature, salt levels and to survive on raw meat that could reach the consumer. These attributes make MRSA a potential source of contamination and infection when found on meat prepared in the home. Just as MRSA is able to be carried through the stages of a production facility (Beneke) it may also be carried around the kitchen. Several studies have reported that knives and cutting boards are major sources of cross contamination with bacteria during food preparation and that bacteria on these surfaces from raw meat may in turn contaminate hands(29, 39,48). Research suggests that bacteria will transfer readily to food contact surfaces and to skin (32, 7). Despite these findings, in the case of MRSA, a dose-response level is still not known so it is difficult to make a quantitative risk assessment regarding consumer (3).

Objectives:

1. Determine the potential for transfer of Methicillin Resistant Staphylococcus aureus from pork and pork products to food contact surfaces at the consumer level.

2. Estimate the probability of transfer of Methicillin Resistant Staphylococcus aureus from pork and pork products at the consumer level.

Materials & Methods:

Experimental Design. Two exposure methods, three types of retail pork products, and three different transfer surfaces were used for these experiments. Exposures were light and heavy using inoculated fresh boneless pork loins, bacon and raw pork sausage. Light exposure involved laying the inoculated product on the transfer surface for 5 minutes. Heavy exposure involved placing a 500g lead donut on top of the inoculated product and sliding the product back and forth on a 10cm path on the transfer surface for 20 complete transits. One transit consisted of one back and forth movement along the 10cm path across the transfer surface. Pork loins, bacon and raw pork sausage were inoculated with a 4 strain MRSA cocktail, vacuum packaged and stored at 4°C for two weeks to simulate typical distribution and storage conditions before the product arrives at the consumer level. The transfer surfaces were polyethylene cutting boards, knives and the pork skin model to simulate human skin (55). All surfaces were at ambient temperature during the experiments. The surfaces were chosen because they are commonly used in the preparation of raw meat products by the consumer and are potential sources of cross-contamination.

Bacterial Strains. Four isolates of methicillin-resistant *Stapylococcus aureus* were used in this experiment. They are as follows: ST398 isolate from ground pork, ST398 isolate from pork chops (both provided by Dr. Catherine Logue, Iowa State University College of Veterinary Medicine; Ames, Iowa), an ST398 isolate from a 24 week old hog (Dr. Tara Smith, University of Iowa, College of Public Health Department of Epidemiology, Center for Emerging Infectious Diseases; Iowa City, Iowa), and ATCC strain BAA-44 (Dr. Brehm-Stecher, Iowa State University Department of Food Science and Human Nutrition; Ames, Iowa) was used as a reference organism. Dr. Logue's laboratory performed

the MLST (multi-locus sequence typing) on the ground pork and pork chop isolates to confirm they were MRSA ST398. Dr. Smith's laboratory performed PFGE and MLST on the ST398 from the 24 week old hog.

Inoculum Preparation. Prior to inoculation, isolates were streaked onto Baird-Parker Agar (Difco, Becton-Dickinson) and Spectra MRSA Agar (Remel, Lenexa, KS) and incubated at 37°C for 24 hours. Black colonies on Baird-Parker was indicative of *Stapylococcus aureus* and denim colored colonies on Spectra MRSA was indicative of methicillin-resistant *Stapylococcus aureus*. All 4 isolates were black on Baird-Parker Agar and denim blue on Spectra MRSA Agar. Isolated colonies were also tested on the Staphylase Test (Oxoid Ltd., Basingstoke, Hants, UK) and the PBP2' Latex Agglutination Test (Oxoid Ltd., Basingstoke, Hants, UK) and were MRSA positive. On the day of inoculation, the four cultures were combined to form a cocktail with a cell concentration of 10⁹ cfu/ml.

Bacterial Growth Conditions. All strains of methicillin-resistant *Stapylococcus aureus* were maintained in Tryptic Soy Broth (TSB; Difco, Becton-Dickinson, Sparks, MD). The day before inoculations, the cultures were transferred to TSB and grown aerobically at 37°C for 24 hours. 50 ml of Egg yolk tellurite enrichment (Difco, Becton-Dickinson) was aseptically added to 950 ml of sterilized Baird-Parker agar (BPA) base(Difco, Becton-Dickinson) that was cooled in a water bath to 49°C. This media was chosen because it is commonly used for isolation and enumeration of *Stapylococcus aureus*. (Drosinos) Since the identity of methicillin-resistant *Stapylococcus aureus* in this experiment had been determined with ancillary tests, BPA was used for these experiments. Spectra MRSA Agar (Remel, Lenexa,KS) was also used initially to confirm the identity of the four isolates as MRSA. This was based on colony color, MRSA colonies will appear denim blue on Spectra MRSA Agar.

Inoculation Procedure. Fresh boneless pork loins were purchased from a local supplier and held at 4°C until used for the experiments. Each of the 3 light and 3 heavy replications involved 6 pork loins, 5 loins were separately inoculated with 10ml of cell concentrations of 10, 100, 1,000, 10,000 and 100,000 cfu/ml diluted from the initial MRSA cocktail. One loin was used as a negative control and swabbed with a speci-sponge (Nasco Whirl-Pak "Speci-Sponge" Bags, Fort Atkinson, WI) hydrated with 10ml Buffered Peptone Water, 0.01%(BPW, Difco, Becton-Dickinson) before and after the 2 week storage period to determine the presence of Staphylococcus aureus and/or MRSA, if any, prior to my inoculation of the products. One tenth of a ml was plated onto Baird-Parker in duplicate and 0.1ml was plated onto Spectra MRSA in duplicate. No growth was observed after 24 hours incubation at 37°C. All 5 inoculated loins were put in the cooler for 30 minutes to allow attachment of the bacteria. Next, a 5cm x 5cm area of each loin was swabbed with a speci-sponge (Nasco Whirl-Pak "Speci-Sponge" Bags, Fort Atkinson, WI) hydrated with 10ml Buffered Peptone Water, 0.01%(BPW, Difco, Becton-Dickinson). The loins were then each vacuum packaged with a MultiVac vacuum packaging machine (Figure 1) and stored for 2 weeks at 4°C to simulate typical packaging and distribution. Swabs were taken to determine bacterial counts after inoculation but before storage. All 6 swab bags were hand stomached for one minute, serially diluted in 9.0ml BPW tubes, plated on Baird-Parker Agar in duplicate (Difco, Becton-Dickinson) and incubated at 37°C for 24 hours.

Bacon was purchased from a local supplier and held at 4°C until used for the experiments. Each of the 3 light and 3 heavy replications involved 4 strips of bacon, 5 groups of 4 strips were separately inoculated with 10ml of cell concentrations of 10, 100, 1,000, 10,000 and 100,000 cfu/ml diluted from the initial MRSA cocktail. One group of 4 strips was the negative control. All inoculated bacon was put in the cooler for 30 minutes to allow attachment of the bacteria. Next, a 5cm x 5cm area of each of

the groups of 4 strips was swabbed with a speci-sponge (Nasco Whirl-Pak "Speci-Sponge" Bags) hydrated with 10ml Buffered Peptone Water, 0.01%(BPW, Difco, Becton-Dickinson). The bacon was then vacuum packaged (the name of the vac. pak machine and the type of vac pak bags used) and stored for 2 weeks at 4°C to simulate typical packaging and distribution. Swabs were taken to determine bacterial counts after inoculation but before storage. All 6 swabs were hand stomached for one minute, serially diluted in 9.0ml BPW tubes, plated on Baird-Parker Agar (Difco, Becton-Dickinson) and incubated at 37°C for 24 hours.

Raw pork sausage was purchased from a local supplier and held at 4°C until used for the experiments. Each of the 3 light and 3 heavy replications involved 30g of sausage inoculated with 10ml of cell concentrations of 10, 100, 1,000, 10,000 and 100,000 cfu/ml diluted from the initial MRSA cocktail. The 30g was then divided up into 3 10g portions and formed into 2cm x 10cm x 5mm thick strips. One additional strip was formed to serve as the negative control. All inoculated sausage strips were put in the cooler for 30 minutes to allow attachment of the bacteria. Next, the 2cm x 10cm surface was swabbed with a speci-sponge (Nasco Whirl-Pak "Speci-Sponge" Bags) hydrated with 10ml Buffered Peptone Water, 0.01%(BPW, Difco, Becton-Dickinson). The sausage strips were then vacuum packaged and stored for 2 weeks at 4°C to simulate typical packaging and distribution. Swabs were taken to determine bacterial counts after inoculation but before storage. All 6 swabs were hand stomached for one minute, serially diluted in 9.0ml BPW tubes, plated on Baird-Parker Agar (Difco, Becton-Dickinson) and incubated at 37°C for 24 hours.

Sampling for Light Exposure. After the two week storage period, the vacuum packaged loins were taken out of the cooler. The transfer surfaces: 5 cutting boards, 5 knives and 5 portions of pork skin were set up to correspond to the 5 cell concentrations the loins had been inoculated with. The pork

skin was sanitized underneath a UV light in a biosafety cabinet for 15 minutes and the cutting boards and knives were autoclaved at 121°C for 15 minutes. All transfer surfaces were at ambient temperature during the experiments. Each of the 5 loins was divided up into 3 parts, a part for each transfer surface. The loins were removed from the vacuum package with a sterile scalpel blade and laid on the transfer surfaces for 5 minutes without any movement or additional pressure. After 5 minutes, the loins were moved aside and a 5cm x 5cm² area of each pork loin was swabbed with a speci-sponge (Nasco Whirl-Pak "Speci-Sponge" Bags, Fort Atkinson, WI) rehydrated with 10ml Buffered Peptone Water, 0.01% (BPW, Difco, Becton-Dickinson). A 5cm x 5cm² area of each cutting board and each pork skin were also swabbed. The entire surface of the knife was swabbed, for knives the blade measured 14cm x 1.5cm. All swabs were hand stomached for one minute, serially diluted in 9.0ml BPW tubes to extinction, plated on Baird-Parker Agar (Difco, Becton-Dickinson) and incubated at 37°C for 24 hours. After transfer surfaces were swabbed, a 5cm x 5cm² x 5mm thick area of each loin was excised (samples weighed 15g +/- 0.5g) and placed in a Whirl Pak filter stomacher bag (Nasco Whirl-Pak Bags, Fort Atkinson, WI) and diluted to a 10% dilution using a dilutor pump with Buffered Peptone Water, 0.01% (BPW, Difco, Becton-Dickinson). All excised loins were stomached for a minute in a stomacher (Seward Laboratory Blender 400, Tekmar Co., Cincinnati, Ohio) and serially diluted to extinction in 9.0ml BPW test tubes, plated on Baird-Parker Agar (Difco, Becton-Dickinson) and incubated at 37°C for 24 hours.

The procedure for the pork loins was also followed for the bacon and raw pork sausage including plating and incubation times. For the bacon, the following changes were observed: 4 slices of bacon were used to achieve a surface area of 5cm x 5cm² x 5mm thick that could be swabbed and excised for enumeration. Excised portions of bacon weighed 25g +/-0.5g depending on the thickness and fat content of the individual pieces of bacon. For the raw pork sausage, the following changes were

observed: a strip of sausage 2cm x 10cm x 5mm thick was formed by using a mold made by the Department of Engineering, Iowa State University, Ames, Iowa. The sausage was removed from the mold and the weight of each strip was determined to be 10g +/- 0.5g.

Sampling for Heavy Exposure. After the two week storage period, the vacuum packaged loins were taken out of the cooler and the transfer surfaces were set up as they were for the light exposure. The loins were removed from the vacuum package with a sterile scalpel blade and placed on the transfer surface. The 500g lead donut was then placed on the loin and the loin with weight was moved back and forth on a 10cm path on the transfer surface for 20 complete transits. One transit consisted of one back and forth movement along a 10cm path. After the surfaces were exposed to the inoculated loins they sat at room temperature for five minutes. Next, the pork skin was applied to the surface with the 500g lead donut on top and the skin was passed along the same 10cm path for 20 back and forth transits. The surfaces and the pork skin were then swabbed with a speci-sponge (Nasco Whirl-Pak "Speci-Sponge" Bags,Fort Atkinson, WI) rehydrated with 10ml Buffered Peptone Water, 0.01%(BPW, Difco, Becton-Dickinson) and the pork skin was placed in a Whirl-Pak bag (Nasco Whirl-Pak Bags) and diluted to a 1:10 dilution with Buffered Peptone Water, 0.01%(BPW, Difco, Becton-Dickinson). All swabs were plated in duplicate on Baird-Parker Agar (Difco, Becton-Dickinson) as were the pork skin samples. Plates were incubated at 37°C for 24 hours.

Quantitative Methods. The direct plate counts of MRSA were transformed to Log CFU in a Microsoft Excel Spreadsheet. For swabs the counts are reported as Log CFU/cm² of the surface area swabbed. For all of the meat samples and pork skin samples the counts are reported as Log CFU/ml. All retail meat samples were tested in triplicate at each of the 5 cell concentrations and on each transfer surface. Percent transfer rates of MRSA from the pork products to the surfaces was determined by

dividing the final Log CFU on the surface swab by the initial Log CFU present on the product after inoculation. (Perez-Rodriguez) Average percent transfer was obtained by adding the percentages together and dividing by the total number of observations.

Data Analysis. Three replicate experiments were performed for each of the retail products: pork loins, bacon and raw pork sausage. MRSA populations were determined by calculating the Log values (Microsoft Excel) of bacterial counts on duplicate plates for each sample. The bacterial counts were an indicator of bacterial transfer to surfaces so the Log values were converted into percentages. The data were analyzed by SAS (Statistical Analysis System version 9.2, SAS Institute, Inc., Cary, N.C.) with a general linear model using least square means and Q-Q plots (which indicated that the data were normally distributed.) A least square means test was performed to show that there was no significant difference in the ability of the bacteria to transfer among the surfaces: the polyethylene cutting board, the knife and the pork skin.

Results: The first part of this study, the light exposure, was designed to estimate the percentage of MRSA transfer from inoculated retail pork products to other surfaces across a broad range of initial concentrations. The second part, the heavy exposure, looks at the ability of MRSA to transfer from a contaminated surface to skin using the pork skin model (55) across a broad range of initial concentrations. All samples were vacuum packaged after inoculation and stored at 4°C to simulate normal packaging and distribution to suppliers. Before inoculation, all pork products used were tested and no *Staphylococcus aureus* was detected.

Light Exposure

For light exposure, the initial counts of MRSA, an average of all 3 replications, on the 5 pork loins after inoculation were 4.8, 5.2, 6.5, 7.7 and 8.6 Log CFU/cm² for inoculum concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml respectively. The area of loin sampled was 5cm x 5cm². At the end of two weeks the vacuum packaged loins were taken from the cooler and aseptically removed from their packages and exposed to the 3 transfer surfaces for 5 minutes. For the 10 CFU/ml loins, transfer occurred on all 3 surfaces, the cutting board, knife and pork skin at rates of 76%, 53%, and 80% respectively. However, for 2 of the 3 replications no growth nor transfer was observed at 10 CFU/ml. This may have been due to the initial cell concentration being lower than expected, the cells in the inoculum may have been non-viable due to some stress not accounted for during incubation. For the remaining concentrations of 100, 1,000, 10,000 and 100,000 CFU/ml, average transfer rates are displayed in Table 1.

The bacon light exposure initial counts of MRSA, an average of all 3 replications, after inoculation were 5.3, 6.0, 7.3, 7.9 and 9.1 Log CFU/cm² for inoculum concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml respectively. The area of 4 inoculated bacon slices sampled was 5cm x 5cm². At the end of two weeks the vacuum packaged bacon was taken from the cooler and aseptically removed from the packages and exposed to the 3 transfer surfaces. The average percent transfer from the bacon to each of the 3 transfer surfaces is summarized in Table 2.

The raw pork sausage light exposure initial counts of MRSA, an average of all 3 replications, after inoculation were 5.6, 6.9, 8.0, 9.0 and 10.0 Log CFU/cm² for inoculum concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml respectively. For the sausage, molds (Figure 2) were made in order to form the sausage into standard portions that were 2cm wide, 5mm thick and 10cm in length and this is the area that was swabbed after inoculation. At the end of two weeks the vacuum

packaged sausage strips were taken from the cooler and aseptically removed from their packages and exposed to the 3 transfer surfaces. The average percent transfer from the sausage to each of the 3 transfer surfaces is summarized in Table 3.

The average percent recovery of MRSA from the products after two weeks of vacuum packaged storage at 4°C is summarized in Tables 4, 5 and 6 for pork loins, bacon and raw pork sausage respectively. The surfaces are listed to correlate the percent transfer from that product to the surface and the amount of bacteria remaining on the surface of the product at the time of sampling/transfer. Although there was no significant difference among transfer to the surfaces across the cell concentrations, the cutting board and the skin generally seemed to have more cells transferred but this may have been due to the total surface area differences in the knife swabs and the 5cm x 5cm area of the cutting board and pork skin swabs.

Heavy Exposure

The initial counts of MRSA on the 5 pork loins, an average of all 3 replications, after inoculation were 5.1, 5.7, 7.1, 7.9 and 9.0 Log CFU/cm² for inoculum concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml respectively. The area of loin sampled was 5cm x 5cm². For the bacon, counts were as follows, 5.2, 5.7, 7.0, 7.8 and 8.9 Log CFU/cm² for inoculum concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml respectively. The initial sausage counts were 5.4, 6.7, 8.3, 8.4 and 9.5 Log CFU/cm² for inoculum concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml respectively. At the end of two weeks the vacuum packaged loins were taken from the cooler and aseptically removed from their packages and exposed to the 3 transfer surfaces following the procedures previously described for heavy exposure. The results of the percent transfer to the pork skin from the cutting board and knife after those surfaces were exposed to the inoculated pork loin

are summarized in Table 7. The results for the bacon and raw pork sausage are in tables 8 and 9 respectively. The surface least square means test (SAS version 9.2, SAS Institute, Inc., Cary, N.C.) showed that among the 3 products, there was not a significant difference in the ability of the inoculated product to transfer bacteria nor was there a significant difference among the surfaces in the ability to transfer bacteria to the skin.

IX. **Discussion:** The first part of this study employing the light exposure technique shows that bacteria, in this case MRSA, is able to survive refrigeration temperatures inside vacuum packages for at least two weeks, a standard shipping and distribution time. This implies that retail pork products contaminated with MRSA could reach the consumer and present a source of possible contamination or infection. Viable cells remain on the retail pork products and are capable of contaminating food contact surfaces as has been previously elucidated 26, 29, 32). This study did not find a significant difference in the ability of cells to adhere to the 3 different surfaces which is consistent with a finding in earlier research (32). However, other research has shown that levels of bacterial transfer are dependent on the texture of the surface (48). The cutting boards used in these experiments were new, smooth polyethylene cutting boards that had not been previously used and so they had no cut marks or grooves on the surface. Regarding the lack of growth and transfer of cells at 10 CFU/ml for the pork loins, this may have been due in part to the way the inoculum was prepared. In this paper, inoculum was prepared by growing up the 4 MRSA cultures in TSB for 24 hours and then combining them together on the day of inoculation. For each cell concentration (10, 100, 1,000, 10,000 and 100,000) the stock was plated to extinction to determine the original cell Log CFU/ml. A method that might have been more reliable would have been to centrifuge my cultures and harvest the cells and resuspend them followed by testing the concentration using a refractometer.

Findings in this study also show that the amount transferred from the product to the surface during light exposure increased as the initial inoculum amount on the products increased. While this was expected, other studies have described an inverse relationship in which a high initial bacterial load leads to a lower total amount transferred and lower initial bacterial counts will lead to a higher percent transfer due to bacterial interactions on the meat surface (26,29). However, one of these studies used raw poultry that was naturally contaminated (29) while this research used inoculated products. Also, pork product surfaces vary among type (loin, bacon, sausage) and may be quite different from the surfaces of raw products such as chicken and beef. Thus, bacterial interactions on inoculated pork and naturally contaminated poultry cannot be readily compared due to the differences in their surface type.

For the heavy exposure experiments, tables 7, 8 and 9 show that among the products and across the cell concentrations there was not a significant difference in transfer to the pork skin from the contact surfaces.

In the heavy exposure experiments, it was expected that the amounts of cell transfer would be higher if pressure, like the 500g lead donut used here, was applied to the product while it was being moved across a surface. One study reported that applied pressure would facilitate bacterial removal from one surface to another resulting in higher transfer rates (26). It was also suspected that subsequent weighted skin contact on that surface would result in a high transfer. These percentages are lower than those from the light exposure when the contaminated product was simply laid on the pork skin for 5 minutes. However, a direct comparison cannot be made because heavy exposure was to determine if a contaminated surface could transfer bacteria to skin whereas the light exposure showed that direct skin contact on the contaminated product allowed bacterial transfer. Still, the

results show that transfer occurs across all levels of initial contamination which indicates that risk of consumer contact and possible colonization or infection exist.

It is possible that some of the Log CFU/ml counts were lower than they actually may have been in the heavy exposure due to the movement of the product on the surface spreading out the cells away from the area swabbed. Additionally, whole pork loins were inoculated with 10ml of inoculum and this may have added a dilution effect to the final cell counts and percentages as well. Contact times may have also been a factor in the final transfer percentages. Light exposure consisted of 5 minutes while the transits of the heavy exposure were not timed.

In order for a pathogen to present a risk to the consumer it must be able to survive on meat surfaces and on surfaces used in home food preparation such as cutting boards and knives (8). These experiments quantified percent transfer rates of retail pork products contaminated with 4 strains of MRSA to food contact surfaces at the consumer level and estimated the percent risk of the consumer being exposed to MRSA via contaminated surfaces in the home. Since raw meat is frequently handled by the consumer in the kitchen, it is important to understand what risks exist. As the dose-response of MRSA is still not known, these data may allow the construction of a model to determine what MRSA bacterial cell range places the consumer at the most risk of becoming colonized with MRSA or developing a MRSA infection due to the preparation of a contaminated retail product in the home kitchen.

References

- Aarestrup, F.M., H.C. Lewis, K. Molbak, C. Reese, M. Selchau, M. Sorum and R.L. Skov. 2008. Pigs as Source of Methicillin-Resistant Staphylococcus aureus CC398 Infection in Humans, Denmark. Emerg Infect Dis. 14:1383-1389.
- 2. Ahren, C., C. Welinder-Olsson, K. Floren-Johansson, L. Larsson, S. Oberg and L. Karlsson. 2008. Infection with Panton-Valentine Leukocidin-Positive Methicillin-Resistant *Staphylococcus aureus* t034. *Emerg Infect Dis.* 14:1271-1272.
- 3. Alt, K., A. Fetsch, B. Kraushaar, G. Krause, B. Guerra-Roman, J.A. Hammerl, A. Kasbohrer, J. Braeunig, B. Appel and B.A. Tenhagen. 2011. Methicillin susceptible and resistant *Staphylococcus aureus* from farm to fork impact on food safety. 2011. *Tehnologija Mesa*. 52:60-65.
- 4. Avery B., J.S. Weese and R. Reid-Smith. 2010. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) clones in retail meat products. *Letters Applied Microbiol*. 51:338-342.
- 5. Avery, B., J.S. Weese, R. Reid-Smith and J. Rousseau. 2010. Methicillin-resistant *Staphylococcus aureus* contamination of retail pork. Canadian Vet Journal 51:749-752.
- 6. Bakker, J, A. Voss, F. Loeffen, C. Klaassen and M. Wulf. 2005. Methicillin-resistant *Staphylococcus aureus* in pig farming. *Emerg Infect Dis.* 11:1965-1966.
- 7. Bartleson, C.A., E.C.D. Todd, J.D. Grieg, D. Smith, J. Holah and B.S. Michaels. 2010. Outbreaks Where Food Workers Have Been Implicated in the Spread of Foodborne Disease. Part 7. Barriers to Reduce Contamination of Food by Workers. *J. Food Protect.* 73:1552-1565.
- 8. Bartleson, C.A., E.C.D. Todd, J.D. Grieg and B.S. Michaels. 2009. Outbreaks Where Food Workers Have Been Implicated in the Spread of Foodborne Disease. Part 6.Transmission and Survival of Pathogens in the Food Processing and Preparation Environment. *J. Food Protect.* 72:202-219.
- 9. Beaujean, D., I. van Loo, X. Huijsdens, E. Tiemersma, A. de Neeling, N. van de Sande-Bruinsma, A. Voss and J. Kluytmans. 2007. Emergence of Methicillin-Resistant *Staphylococcus aureus* of Animal Origin in Humans. *Emerg Infect Dis.* 13: 1834–1839.

Bartolomeoli, I., M. Marino, F. Frigo and M. Maifreni. 2010. Safety-related properties of staphylococci isolated form food and food environments *J. Applied Microbiol.* 110:550-561.

Beneke, B., S. Klees, B. Stuhrenberg, A. Fetsch, B. Kraushaar and B.A. Tenhagen. 2011. Prevalence of Methicillin-Resistant *Staphylococcus aureus* in a Fresh Meat Pork Production Chain. *J. Food Prot.* 74:126-129.

Beumer, R.R., H.D. Kusumaningrum, E.D. van Asselt and M.H. Zwietering. 2004. A Quantitative Analysis of Cross-Contamination of *Salmonella* and *Campylobacter spp*. Via Domestic Kitchen Surfaces. *J. Food Prot.* 67:1892-1903.

- Bhargava, K., X. Wang, S. Donabedien, M. Zervos, L. de Rocha and Y. Zhang. 2011. Methicillin-Resistant *Staphylococcus aureus* in Retail Meat, Detroit, Michigan, USA [letter]. *Emerg Infect Dis* [serial on the internet]. http://dx.doi.org/10.3201/eid1706.101095.
- Bhat, M., C. Dumortier, B.S. Taylor, M. Miller, G. Vasquez, J. Yunen, K. Brudney, J. Sanchez, C. Rodriguez-Taveras, R. Rojas, P. Leon and F.D. Lowy. 2009. *Staphylococcus aureus* ST398, New York City and Dominican. Republic. *Emerg Infect Dis.* 15:285-287.
- Borch, E., T. Nesbakken and H. Christensen. 1996. Hazard Identification in swine slaughter with respect to foodborne bacteria. *Int J Food Micro*. 30:9-25.
- Bosch, T., E. de Boer, J.T.M. Zwartkruis-Nahuis, B. Wit, X.W. Huijsdens, A.J. de Neeling, R.A.A. van Oosterom, A. Vila and A.E. Heuvelink. 2009. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *Int J Food Micro*. 134:52-56.
- Boshuizen, H.C., A.J. de Neeling, M.J.M. van den Broek, E.C. Spalburg, M.G. van Santen-Verheuvel, W.D.C. Dam-Deisz, A.W. van de Giessen, E. van Duijkeren and X.W. Huijsdens. 2007. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Vet Microbiol*. 122:366-372.
- Bremer, P.J., S.H. Flint and J.D. Brooks. 2000. Properties of the stainless steel substrate, influencing the adhesion of thermoresistant streptococci. *J. Food Engineering*. 43:235-242.
- Broens, E.M., B.A.G.L. Van Cleef, A. Voss, W. Huijsdens, L. Zuchner, B.H.B. Van Benthem, J.A.J.W. Kluytmans, M.N. Mulders and A.W. Van de Geissen. 2010. High prevalence of nasal MRSA carriage in slaughterhouse workers in contact with live pigs in The Netherlands. *Epidemiol Infect.* 138:756-763.
- Brown, T., A. Kelman, Y. Soong, N. Dupuy, D. Shafer, W. Richbourg, K. Johnson, E. Kestler, Y. Li, J. Zheng, P. McDermott and J. Meng. 2011. Antimicrobial Susceptibility of *Staphylococcus aureus* from Retail Ground Meats. *J. Food Prot.* 74:1625-1629.
- Brudvig, J., H.S. Hurd, J.S. Dickson, J. Mirceta, M. Polovinski, N. Matthews and R. Griffith. 2008. Swine Health Impact on Carcass Contamination and Human Foodborne Risk. *Public Health Reports*. 123:343-351.
- Bryden, L., G.R. Golding, P.N. Levett, R.R. McDonald, A. Wong, J. Wylie, M.R. Graham, S. Tyler, G.van Domselaar, A.E. Simor, D. Gravel and M.R. Mulvey. 2010. Livestock-Associated Methicillin Resistant *Staphylococcus aureus* Sequence Type 398 in Humans, Canada. *Emerg Infect Dis.* 16:587-594.
- Busscher, H. J., B. Knobben, H. van der Mei and J. van Horn. 2007. Transfer of bacteria between biomaterials surfaces in the operating room—An experimental study. *J Biomed Mat Research*. 80A:790-799.
- Butaye, P., W. Vanderhaeghen, K. Hermans and F. Haesebrouck. 2009. Methicillin-resistant *Staphylococcus aureus* (MRSA) in food production animals. *Epidemiol Infect.* 138:606-625.

Capuano, A.W., T.C. Smith, M.J. Male, A.L. Harper, J.S. Kroeger, G.P. Tinkler, E.D.Moritz, L.A. Herwaldt and D.J. Diekema. 2009. Methicillin-Resistant *Staphylococcus aureus* (MRSA) Strain ST398 Present in Midwestern U.S. Swine and Swine Workers. PLoS ONE. 4:e4258. 10.1371/journal.pone.0004258.

Carrasco, E., F. Rodriquez-Perez, A. Valero, R.M. Garcia and G. Zurera. 2008. Understanding and modelling bacterial transfer to foods: a review. *Trends in Food Sci Tech.* 19:131-144.

Catry, B., E. Van Duijkeren, M. C. Pomba, C. Greko, M. A. Moreno, S. Pyorala, M. Ruzauskas, P. Sanders, E. J. Threlfall, F. Ungemach, K. Torneke, C. Munoz-Madero and J. Torren-Edo. 2010. Reflection paper on MRSA in food-producing and companion animals: epidemiology and control options for human and animal health. *Epidemiol Infect*. 138:626-644.

Center for Food Safety and Public Health, Iowa State University. Methicillin-resistant *Staphylococcus aureus*. CFSPH; 2011 January. Available at: www.cfsph.iastate.edu/Factsheets/pdfs/mrsa.pdf. Accessed 19 January 2012.

Chemaly, M, P. Fravalo, M.J. Laisney, M.O. Gillard and G. Salvat. 2009. *Campylobacter* Transfer from Naturally Contaminated Chicken Thighs to Cutting Boards is Inversely Related to Initial Load. *J. Food Prot.* 72:1836-1840.

Comodo, N., G. Pesavento, B. Ducci and A. Lo Nostro. 2007. Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: A research for methicillin-resistant *Staphylococcus aureus* (MRSA). *Food Control*. 18:196-200.

Cox Jr., L.A., R.S. Singer, J.S. Dickson, H.S. Hurd, I. Phillips and G.Y. Miller. 2007. Modeling the relationship between food animal health and human foodborne illness. *Preventive Vet Med*. 79:186-203.

Da Silva Malheiros, P. C. Tavares dos Passos, L. S. Casarin, L. Serraglio and E.C. Tondo. 2010. Evaluation of growth and transfer of *Staphylococcus aureus* from poultry meat to surfaces of stainless steel and polyethylene and their disinfection. *Food Control.* 21:298-301.

Denis, O., A. Deplano, M. Nemati, K. Hermans, U. Lipinska, L.A. Devriese, F. Pasmans and F. Haesebrouck. 2008. Antimicrobial resistance of old and recent *Staphylococcus aureus* isolates from poultry: first detection of livestock-associated methicillin-resistant strain ST398. *Antimicrob Agents Chemother*. 52:3817-3819.

Desmarchelier, P.M., P.B. Vanderlinde, N. Feganand L. Mills. 1999. Use of pulse field gel electrophoresis for the epidemiological characterization of coagulase positive *Staphylococcus* isolated from meat workers and beef carcasses. *Int J Food Microbiol* 48:81-85.

Dressler, A.E., B.M. Hanson, A.L. Harper, R.P. Scheibel, S.E. Wardyn, L.K. Roberts, LJ.S. Kroeger and T.C. Smith. 2011. Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*(MRSA) on retail meat in Iowa. *J Infet Public Health*. 4:169-174.

Drosinos, E.H. and M. Mataragas. 2010. Microbial Foodborne Pathogens. p. 22-40. *In* L.M.L. Nollet and F. Toldra. Safety Analysis of Foods of Animal Origin. CRC Press Boca Raton, Florida.

Duijkeren, E, A. van Belkum, D.C. Melles, J.K Peeters and W.B. van Leeuwen. 2008. Methicillin-Resistant and -Susceptible *Staphylococcus aureus* Sequence Type 398 in Pigs and Humans. *Emerg Infect. Dis.* 14:479-483.

EFSA, 2009. Assessment of the Public Health significance of methicillin resistant *Staphylococcus aureus* (MRSA) in animals and foods. *The EFSA Journal*. 993:1-73.

Fanning, S., J. Kennedy, A. Nolan, S. Gibney, S. O'Brien, M.A.S. McMahon, K. McKenzie, B. Healy and P.G. Wall. 2011. Determinants of cross-contamination during home food preparation. *British Food Journal*. 113:80-297.

Farina, S.A., A.M. O'Brien, B.M. Hanson, J.Y. Wu, J.E. Simmering, S.E. Wardyn, B.M. Forshey, M.E. Kulick, D.B. Wallinga and T.C. Smith. 2011. MRSA in Conventional and Alternative Retail Pork Products. *PLoS ONE*. 7:e30092. doi:10.1371/journal.pone.0030092.

Filipe, S.R., M.G. Pinho, H. de Lencastre and A. Tomasz. 2001. Complementation of the essential peptidoglycan transpeptidase function of penicillin-binding protein 2 (PBP2) by the drug resistance protein PBP2A in *Staphylococcus aureus*. *J. Bacteriol*. 183:6525-6531.

Fischer, A.R.H., A.E.I. de Jong, E.D. van Asselt, R. de Jonge, L.J. Frewer and M.J. Nauta. 2007. Food Safety in the Domestic Environment: An Interdisciplinary Investigation of Microbial Hazards During Food Preparation. *Risk Analysis*. 27:1065-1082.

Fluit, A.C., E. van Duijkeren, M. Moleman, M.M. Sloet van Oldruitenborgh-Oosterbaan, J. Multem, A. Troelstra, W.J.B. van Wamel, D.J. Houwers, A.J. de Neeling and J.A. Wagenaar. 2010. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: An investigation of several outbreaks. *Vet Microbiol*. 141:96-102.

French, G.L. and J. A. Otter. 2010. Molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus* in Europe. *The Lancet*. 10:227-239.

Gahrn-Hansen, B., G.N. Hartmeyer, R.L. Skov and H.J. Kolmos. 2010. Pig-associated methicillin-resistant Staphylococcus aureus: Family transmission and severe pneumonia in a newborn. *Scandinavian J Infect Dis.* 4:318-320.

Graham III, P.L., S.X. Lin and E. Larson. 2006. A U.S. Population-Based Survey of Staphylococcus aureus Colonization. *Ann Intern* Med. 144:318-325.

Griffith, C.J. and E.C. Redmond. 2003. Consumer Food Handling in the Home: A Review of Food Safety Studies. *J. Food Protect.* 66:130-161.

Ghazali, F.M., J.Y.H. Tang, M. Nishibuchi and Y. Nakaguchi. 2010. Transfer of *Campylobacter jejuni* from raw to cooked chicken via wood and plastic cutting boards. *Letters Applied Microbiol*ogy. 52:581-588.

Gillespie, B.E., A.A. Sawant and S.P. Oliver. 2009. Antimicrobial susceptibility of coagulase-negative *Staphylococcus* species isolated from bovine milk. *Vet Microbiol*. 134:73-81.

Goering, R.V. and F.C.Tenover. 2009. Methicillin-resistant *Staphylococcus aureus* strain USA300: origin and epidemiology. *J. Antimicrob. Chemother.* (2009) 64(3): 441-446.

Guardabassi, L., M. Stegger and R. Skov. 2007. Retrospective detection of methicillin resistant and susceptible *Staphylococcus aureus* ST398 in Danish slaughter pigs. *Vet Microbiol*. 122:384-386.

Han, F., S. Pu and B. Ge. 2009. Isolation and Characterization of Methicillin-Resistant *Staphylococcus aureus* Strains from Louisiana Retail Meats. *Applied Environ Microbiol.* 75:265-267.

Huijsdens, X.W., B.J. van Dijke, E. Spalburg, M.G. van Santen-Verheuvel, M. Heck, G.N. Pluister, A. Voss, W. Wannet and A. de Neeling. 2006. Community-acquired MRSA and pig-farming. *Clin Microbiol Antimicrob*. 5:26.

Jimenez, S.M., M.C. Tiburzi, M.S. Salsi, M.A. Moguilevsky, and M.E. Pirovani. 2009. Survival of *Salmonella* on refrigerated chicken carcasses and subsequent transfer to cutting board. *Letters in Applied Microbiol.* 48:687-691.

Klein, D.A. 2008. Microbiological Testing of Disinfectants and Decontaminants for Critical Surfaces. p. 215-227. *In* G. Manivannan. Disinfection and decontamination: principles, applications and related issues. CRC Press Boca Raton, Florida.

Kluytmans, J.A.J.W. 2009. Methicillin-resistant *Staphylococcus aureus* in food products: cause for concern or case for complacency? *Clin Microbiol Infect Dis.*16:11-15.

Lee, J.H. 2003. Methicillin (Oxacillin)-Resistant *Staphylococcus aureus* Strains Isolated from Major Food Animals and Their Potential Transmission to Humans. *Applied Environ Microbiol.* 69:6489-6494.

Melles, D.C., H.F.L. Wertheim, MC. Vos, W. van Leuwen, A. van Belkum, H.A. Verbrugh and J.L. Nouwen. 2005. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet*. 5:751-762.

Pearson, N. and T.C. Smith. 2011. The Emergence of *Staphylococcus aureus* ST398. *Vector-Borne Zoo Dis.* 11:327-339.

Ryser, E., K. Vorst and E.C.D. Todd. 2006. Transfer of *Listeria monocytogenes* during Mechanical Slicing of Turkey Breast, Bologna, and Salami. *J. Food Protect.* 69:619-626.

Voss, A., M. Wulf. 2008. MRSA in livestock animals—an epidemic waiting to happen? *Clin Microbiol Infect.* 14:519-521.

igure 1. Vacuum Packaging Machine



Figure 2a. Sausage Mold



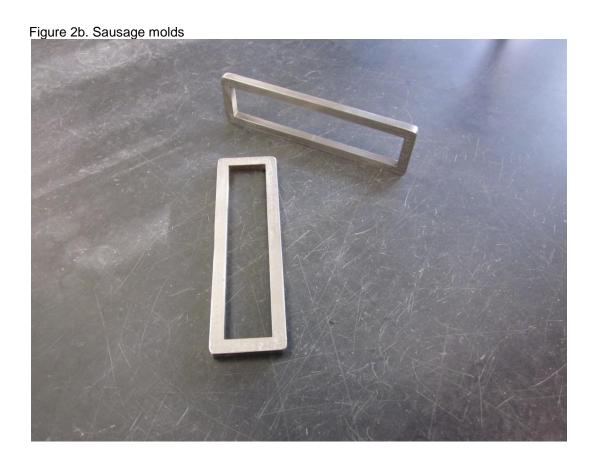


Figure 3. Vacuum packaged products from left: bacon, sausage. Foreground: loin



Figure 4. Pork skin to serve as the human skin model



Pork Loin CFU/ml	Cutting board	Knife	Pork skin
100,000	88	80	89
10,000	78	78	77
1,000	83	80	84
100	79	79	91

Table 1. Average Percent Transfer for 3 replications at each cell concentration for each surface.

Bacon CFU/ml	Cutting Board	Knife	Pork Skin
100,000	88	83	86
10,000	88	87	85
1,000	80	74	83
100	80	81	81
10	72	69	71

Table 2. Average Percent Transfer for 3 replications at each cell concentration for each surface.

Sausage CFU/ml	Cutting Board	Knife	Pork Skin
100,000	88	87	86
10,000	87	86	89
1,000	81	87	84
100	76	82	78
10	82	75	81

Table 3. Average Percent Transfer for 3 replications at each cell concentration for each surface.

Pork Loin CFU/ml	Cutting board	Knife	Pork Skin
100,000	86	87	88
10,000	88	85	85
1,000	84	86	84
100	85	82	82
10	86	74	84

Table 4.Average percent recovery of MRSA after two weeks of vacuum packaged storage at 4°C.

Bacon	Cutting board	Knife	Pork skin
100,000	91	88	93
10,000	94	93	94
1,000	84	89	89
100	88	91	92
10	87	85	76

Table 5. Average percent recovery of MRSA after two weeks of vacuum packaged storage at 4°C.

Sausage CFU/ml	Cutting board	Knife	Pork skin
100,000	87	85	85
10,000	87	89	87
1,000	86	86	84
100	80	85	82
10	90	87	79

Table 6. Average percent recovery of MRSA after two weeks of vacuum packaged storage at 4°C.

Pork Loin Heavy	knife to pork skin	cutting board to pork skin
10	59	57
100	63	58
1,000	64	68
10,000	68	71
100,000	73	74

Table 7. Summary of average percent transfer from surface to skin with a 500g lead donut applied as weighted exposure.

Bacon Heavy	Knife to skin	Cutting board to skin
10	53	59
100	58	63
1,000	61	61
10,000	76	75
100,000	76	75

Table 8. Summary of average percent transfer from surface to skin with a 500g lead donut applied as weighted exposure.

Sausage Heavy	Knife to skin	Cutting Board to skin
10	55	66
100	54	58
1,000	67	66
10,000	74	70
100,000	74	76

Table 9. Summary of average percent transfer from surface to skin with a 500g lead donut applied as weighted exposure.