RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: MRSA resistance to thermal processes commonly used with pork and pork products. - **NPB #09-176** **revised**

Investigator: James S Dickson

Institution: Iowa State University

Date Submitted: April 1, 2012

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 would survive many of the common processes used with pork products, including cooked emulsion products (hot dogs), ham, bacon, and summer sausage. The processes used for the fully cooked products resulted in dramatic reductions in the inoculated populations of MRSA. The mild treatment used in the production of slab bacon resulted in a smaller reduction, although it is expected that bacon will be cooked further by the consumer before consumption. The results of these experiments show that the commonly used industry processes are more than adequate to control MRSA at populations that would be expected to occur in these products.

Keywords: methicillin resistant Staphylococcus aureus, MRSA, consumer, thermal process, ham, bacon, summer sausage, hot dog

Scientific Abstract: Methicillin-resistant *Staphylococcus aureus*, also known as MRSA, is a variant strain of *S. aureus*, a gram positive cocci that is commonly found in the nasal passage, mucous membranes and on the skin of humans. MRSA is resistant to antibiotic therapies, which can include methicillin (no longer used in human medicine), penicillin, tetracycline and amoxicillin, commonly administered to treat *S. aureus* infections. MRSA strains that are resistant to three or more antibiotics are said to be multi-drug resistant and tend to be more virulent in nature than their methicillin sensitive (MSSA) relative.

Introduction: MRSA was first discovered in the United States in the 1960's at Boston City Hospital (Barrett *et al.*, 1968). Since its discovery more than 50 years ago, MRSA has emerged as one of the leading and most severe infectious microorganisms acquired from exposure in a health care setting (CDC NNIS system, 2003). MRSA colonization and infections have recently evolved from being solely

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

For more information contact:
National Pork Board • PO Box 9114 • Des Moines, IA 50306 USA • 800-456-7675 • Fax: 515-223-2646 • pork.org

acquired in a health care setting to that of a community acquired exposure (Zetola *et al.,* 2005; Maree *et al.* 2007 and Witte *et al.,* 2007). This means that transmission of MRSA from person-to-person outside of a medical facility is common. According to a review by Kluytmans *et al.* (1997), the nasal passage is a key harborage area for colonization of the bacteria, and it was estimated that 10-15% of adults are continually colonized (Wenzel and Perl, 1995). Community acquired MRSA cases tend to be less severe and more responsive to drug therapy than nosocomial MRSA infections (APHIS, 2007). MRSA infections are usually acquired through a wound, exposed skin or soft tissue area, and clinical signs include furunculosis, multiple infected boils, and necrosis of the skin (Zetola *et al.*, 2005). According to a recent article in the Journal of the American Medical Association, the incidence of invasive MRSA infections in the United States is low (<1%) and prevalence varies by location (Klevens *et al.,* 2007). Voss[a] *et al.* (1994) reported that MRSA prevalence was highest in southern European countries (Spain, Italy and France) when compared to northern European countries (Denmark, Sweden and The Netherlands), which also suggests that geographic location may play a role in the incidence of MRSA cases. Although infection rates remain low, prevalence (colonization) and isolation of MRSA strains in the United States, especially community acquired cases, have increased dramatically since its discovery in the 1960's.

Although originally thought to be rare, MRSA has also been isolated from companion animals and livestock, especially swine (APHIS 2007). The recent increase in community and livestock acquired MRSA cases throughout the world has caused epidemiologists to suggest a possible link to zoonotic transmission from animals to humans. Voss[b] *et al.* (2005) showed a link between pigs at one location in The Netherlands and the family who lived and worked at the farm. A second MRSA case in The Netherlands was also examined by Huijsdens *et al.* (2006) in which a mother with mastitis and no known risk factors for MRSA carriage was linked to a swine operation. In the study, 80% of the pigs sampled, three employees and all of the family members were found to have genetically identical strains of MRSA. Lewis *et al.* (2008) discussed the link to pig farming and the increased risk for MRSA exposure and subsequent infection in humans. More recently, Smith et al. (2009) reported a 49% MRSA prevalence in swine confinement operations in eastern Iowa and Illinois with a 45% carriage rate in farm employees. It is interesting to note from this pilot study that of the two production systems sampled, only one of the confinement systems was positive for MRSA in pigs and human subjects.

With all of this evidence of increased MRSA prevalence and new emerging strains of this "super bug", as described by New York Times columnist Nicholas Kristof, the question of how this is occurring is

still of concern to scientists and the general public alike. Kristof offered his opinion on the link to the increased MRSA colonization in pigs and people by describing the “insane overuse of antibiotics in livestock feeds” (Kristof[a], 2009). Price *et al.* (2012) described an alternative theory to livestock associated MRSA in humans. This complicated genomic study showed that a MRSA strain known to be associated with both swine and humans probably originated in humans. The study also refutes popular views on antibiotic overuse in livestock and explains the potential for a “bidirectional zoonotic exchange” between humans and livestock.

More alarming than a bacterium becoming part of a pig’s common nasal microflora is its effect on the world’s most popular meat commodity. Pork is the number one meat product consumed in the world (USDA-FSIS, 2008), and U.S. pork consumption is around 51 pounds per person per year (USDA-ERS, 2005). Methicillin-sensitive *Staphylococcus aureus* (MSSA), a known food pathogen to humans, has been researched extensively in food for the intoxication it causes. However, very little is known about its “super” clone counterpart with respect to meat products. Recent research conducted has evaluated various meat products around the world to determine the prevalence of MRSA at the retail meat counter. Pu *et al.* (2008) found MRSA in 5.6% out of 90 pork products sampled in Louisiana retail food stores. A total of 5 isolates were identified in retail pork, of which all were from either USA 100 (a hospital acquired form of MRSA) or USA 300 (a community acquired form of MRSA). Of particular interest was that 19 of the 22 total MRSA isolates in the study came from a single store, which may suggest a carrier in the meat department at that store. A similar study in The Netherlands revealed MRSA present in 2.5% out of 64 pork samples tested (van Loo *et al.*, 2007). More recently, O’Brien *et al.* (2012) reported that 6.6% of 395 pork products purchased from retail outlets in Iowa, Minnesota and New Jersey were positive for MRSA. The study recovered a total of 26 MRSA isolates from 394 samples of retail pork. 7 of the 26 isolates were considered “livestock associated” MRSA, while 12 of the remaining isolates were either USA 100 (a hospital acquired form of MRSA) or USA 300 (a community acquired form of MRSA). The remaining 7 isolates belonged to various other MRSA types. This study also concluded no significant difference between MRSA or MSSA prevalence in conventional and alternatively raised (“raised without antibiotics”) pork.

Although these studies in the United States and Europe indicate the presence of MRSA on pork products in retail meat outlets, transmission of MRSA as a food contaminant to humans is thought to be rare. The European Food Safety Authority’s Panel on Biological Hazards states that “there is no current evidence that eating or handling food” contaminated with MRSA will result in an infection (Byrne, 2009). Epidemiologists at The University Hospital Rotterdam, Dijkzigt, The Netherlands,

however believe a MRSA outbreak was initiated by a dietary worker who transferred the bacterium through food to patients (Kluytmans[b] *et al.*, 1995). This was the first report of its kind until Jones *et al.* (2002) linked a community acquired foodborne illness from MRSA to coleslaw in Tennessee. However, the actual reported illness was classical Staphylococcal food poisoning, and not an actual infection with MRSA. It was only after the illnesses had resolved that it was determined that the causative agent was in fact MRSA, and not MSSA. In addition, a nasal isolate from one of the food preparers matched the isolate in the slaw, suggesting possible contamination from the preparer during the preparation of the slaw. The report from Tennessee is the first to note that MRSA is also capable of causing intoxication from enterotoxin produced in food.

These findings make for media opinionated opportunities like a recent New York Times article entitled "Pathogens in Our Pork" (Kristof[b], 2009). Although Nicholas Kristof may not fully understand the science behind his article, one main question arises from the opinion editorial. Does normal cooking or heating methods "kill" the bacteria?

Objectives: The purpose of the designed study was to test the following objectives: **1)** Determine the survival of methicillin-resistant *Staphylococcus aureus* during thermal lethality processes used by commercial industry to prepare pork and pork products and **2)** Determine the survival of methicillin-resistant *Staphylococcus aureus* during thermal lethality processes comparable to those used at the consumer level to prepare pork and pork products.

Materials & Methods: Objective 1 – Survival of MRSA during commercial thermal processes

Bacteriological Cultures – Cultures of methicillin-resistant *Staphylococcus aureus* used for the designed experiment were obtained from Tara C. Smith at the Center for Emerging Infectious Diseases, University of Iowa College of Public Health, Iowa City, IA and from Catherine M. Logue at the department of Veterinary Microbiology and Preventative Medicine, Iowa State University College of Veterinary Medicine, Ames, IA. The specific strains used during testing were ST398(HU010111N) from a 40 year old adult human male, t337(MN55) from an adult swine, ST398(R35) from retail ground pork and ATCC strain BAA-44(R31) as a reference organism.

Pork Products – The pork products used to test objective 1 were selected to cover a variety of processed meat products commercially produced at varying end point thermal processes. The

following is a list of the pork products used in the experiment to test objective 1: Cooked and emulsified sausage (hot dogs), fermented and cooked sausage (summer sausage), boneless ham and slab bacon.

Cooked and emulsified sausages (hot dogs) – A blend of pork and beef frankfurter style cooked sausages were made using formulations developed at the Iowa State University Meat Laboratory. The formula included the following ingredients: 90% lean beef trim (36.9% wt/wt), 50% lean pork trim (36.9% wt/wt), water (22.15% wt/wt), salt (1.5% wt/wt), 6.25% sodium nitrite curing salt (0.15% wt/wt) and spice blend (2.4% wt/wt). The frankfurter spice blend used was blend EJ-93-150-001 from A.C. Legg Packing Company (Calera, AL, USA). The pork and beef trim was ground through a 12.7mm plate using a Biro model 7552SS (Biro MFG Co., Marblehead, OH, USA) grinder. The emulsion was manufactured using a Krämer-Grebe model VSM65 (Krämer & Grebe GmbH & Co. KG., Biendenkopf-Wallau, Germany) vacuum bowl chopper. The emulsion was then vacuum packaged and frozen at -28°C and stored until used for testing.

A twenty-four hour culture of each MRSA strain was grown at 35°C in Trypticase Soy Broth (TSB; Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA). A four strain cocktail was then prepared by transferring 1mL of each strain into a test tube. The cocktail was vortexed and mixed with 36g of the emulsion batter. The inoculated batter was aseptically stuffed into a 50mL Corning® centrifuge tube (Corning Inc., Corning, NY, USA) and placed into a 79.5°C water bath. The sausages were cooked to an internal temperature of 70°C and immediately transferred to a slush ice bath. The emulsified cooked sausages were cooled to 7.2°C prior to microbial analysis. An uncooked, positive control was used to determine the inoculum level. A negative control, inoculated with 4mL of sterile TSB, was also created to monitor internal temperatures of the sausage and to determine the presence of any naturally occurring microflora.

Ten grams of each sausage (including positive and negative controls) were aseptically transferred to a Whirl-Pak® filter stomacher bag (Nasco, Ft. Atkinson, WI, USA) and filled with 90mL of 0.1% buffered peptone water (Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA). The contents were mixed for 120 seconds using a Seward model 400 lab blender stomacher (Seward Medical, London SE1 1PP, UK) and the slurry was serially diluted to 10^{-6}. Each dilution (including the slurry contents) was plated in duplicate on Baird-Parker Agar with egg yolk Tellurite enrichment (BPA-EY; Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA) and incubated at 35°C for 48hrs. Plates

were counted for growth at 24 and 48 hours and the results were recorded. Three replications of this experiment were performed.

Fermented and cooked sausage (summer sausage) – A blend of pork and beef summer sausage was manufactured at the Iowa State University Meat Laboratory using the following formulation: 80% lean beef trim (47.17% wt/wt), 80% lean pork trim (47.17% wt/wt), salt (1.89% wt/wt), water (1.42% wt/wt), dextrose (1.42% wt/wt), Newly Weds (Newly Weds Foods®, Inc., Chicago, IL, USA) summer sausage seasoning (0.47% wt/wt), 6.25% curing salt (0.23% wt/wt), lactic acid starter culture (0.23% wt/wt). The pork and beef trim were ground through a 9.5mm plate using a Biro model 7552SS (Biro MFG Co., Marblehead, OH, USA) grinder. The ground trim was then transferred to a Hollymatic® (Hollymatic Corporation, Countryside, IL, USA) model 175 mixer grinder and mixed for 2 minutes along with the salt, water and curing salt. The dextrose, seasoning blend and starter culture dissolved into 300mL of distilled water was then added and mixed for an additional 2 minutes. The product was then ground through a 3.2mm plate and placed into vacuum pouches (Cryovac *bag style*, Cryovac-Sealed Air Corp., Duncan, SC, USA). The vacuum pouches were sealed and stored at -28°C until used for testing.

A twenty-four hour culture of each MRSA strain was grown at 35°C in Trypticase Soy Broth (TSB; Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA). A four strain cocktail was then prepared by transferring 5mL of each strain into a test tube. The cocktail was vortexed and mixed with 180g of the sausage batter. The inoculated batter was aseptically stuffed into a 250mL Pyrex® beaker (Corning Inc., Corning, NY, USA), covered with Parafilm M laboratory film (Bemis Flexible Packaging, Neenah, WI, USA) and placed into a 45°C fermentation chamber for 12 hours. The fermented beakers were removed and analyzed for pH using a Accumet® model 15 pH meter (Thermo Fisher Scientific, Inc., Pittsburg, PA, USA) and a calibrated (pH 4.0 and 7.0 calibrating buffers) Accumet® liquid filled electrode (Thermo Fisher Scientific, Inc., Pittsburg, PA, USA). The sausages were cooked in a water bath at 79.5°C and finished to an internal temperature of 70°C. The beakers were immediately transferred to a slush ice bath and cooled to 7.2°C prior to microbial analysis. A fermented, uncooked, positive control was used to determine the inoculum level. A negative control, inoculated with 20mL of sterile TSB, was also created to monitor internal temperatures of the sausage and to determine the presence of any naturally occurring microflora.

Twenty five grams of each sausage after fermentation and thermal processing (including positive and negative controls) were aseptically transferred to a Whirl-Pak® filter stomacher bag (Nasco, Ft.

Atkinson, WI, USA) and filled with 225mL of 0.1% buffered peptone water (Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA). The contents were mixed for 120 seconds using a Seward model 400 lab blender stomacher (Seward Medical, London SE1 1PP, UK) and the slurry was serially diluted to 10^{-6}. Each dilution (including the slurry contents) was plated in duplicate on Baird-Parker Agar with egg yolk Tellurite enrichment (BPA-EY; Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA) and incubated at 35°C for 48hrs. Plates were counted for growth at 24 and 48 hours and the results were recorded. Three replications of this experiment were performed.

Boneless Ham – Pork inside and outside ham muscles were obtained from the Iowa State University Meat Laboratory. The ham muscles were injected to 25% over green weight using the following brine ingredients: water (80.7%wt/wt), salt (11%wt/wt), sugar (6.6%wt/wt), phosphate (1.4%wt/wt), sodium erythorbate (0.22%wt/wt) and sodium nitrite (0.08%wt/wt). The pork insides and outsides were injected using Günther® PI 21 model injector (Günther Maschinenbau GmbH, Dieburg, DE) and then macerated using a model PMT-41 Stork-Protecon™ macerator (Oss, Holland, The Netherlands). The ham macerate was vacuum sealed and stored at -28°C until used for testing.

A twenty-four hour culture of each MRSA strain was grown at 35°C in Trypticase Soy Broth (TSB; Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA). A four strain cocktail was then prepared by transferring 3.5mL of each strain into a test tube (14mL total volume). The cocktail was vortexed and mixed with 1396g of the ham macerate. The inoculated ham was stuffed using a Biro DFS 30 (Biro MFG Co., Marblehead, OH, USA) piston stuffer. The ham was stuffed into a T8 x 30 inch pre-tied fibrous casing (Kalle UK Ltd., Witham, Essex, UK) and clipped using a Poly-Clip® System SCH 6210 model clipper (Poly-clip System GmbH & Co. KG, Frankfurt, Germany, DE). The hams were cooked in an Alkar® model 700 HP single truck processing oven (ALKAR-RapidPak, Inc., Lodi, WI, USA) to an internal temperature of 70°C according to the schedule below (See Table 1.).

The cooked hams were immediately transferred to a 4°C walk-in cooler and chilled to 7.2°C in accordance with appendix B (USDA-FSIS, 1999) prior to microbial analysis. An uncooked, positive control was used to determine the inoculum level. A negative control, inoculated with 14mL of sterile TSB, was also created to monitor internal temperatures of the hams and to determine the presence of any naturally occurring microflora.

Fifty grams of each ham after thermal processing and chilling (including positive and negative controls) were aseptically transferred to a Whirl-Pak® filter stomacher bag (Nasco, Ft. Atkinson, WI, USA) and filled with 450mL of 0.1% buffered peptone water (Difco™, Becton, Dickson & Co., Franklin

Lakes, NJ, USA). The contents were mixed for 120 seconds using a Seward model 400 lab blender stomacher (Seward Medical, London SE1 1PP, UK) and the slurry was serially diluted to 10^{-6}. Each dilution (including the slurry contents) was plated in duplicate on Baird-Parker Agar with egg yolk Tellurite enrichment (BPA-EY; Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA) and incubated at 35°C for 48hrs. Plates were counted for growth at 24 and 48 hours and the results were recorded. Three replications of this experiment were performed.

Slab Bacon – Fresh pork bellies ranging in thickness from 2.38 – 2.71 cm were obtained from the Iowa State University Meat Laboratory. The bellies were injected to 12% over green weight using the following brine ingredients: water (79.5%wt/wt), salt (12.75%wt/wt), sugar (4.25%wt/wt), phosphate (2.94%wt/wt), sodium erythorbate (0.458%wt/wt) and sodium nitrite (0.10%wt/wt). The pork bellies were injected using a Günther® PI 21 model injector (Günther Maschinenbau GmbH, Dieburg, Germany, DE). The injected bellies were vacuum sealed and stored at -28°C until used for testing.
A twenty-four hour culture of each MRSA strain was grown at 35°C in Trypticase Soy Broth (TSB; Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA). A four strain cocktail was then prepared by transferring 1mL of each strain into a test tube and vortexing. Each belly slab was scored with six – 5cm x 5cm squares. Three of the $25cm^2$ sections were scored on the lateral (skin) side and three $25cm^2$ sections were scored on the medial (rib) side. 100µL of the four strain cocktail was spread over four of the $25cm^2$ scored belly surfaces (two lateral and two medial) with a sterilized glass "hockey stick" spreader. The inoculated bellies were allowed to dry for 30 minutes at 22°C (method modified from Burnham *et al.* JFP 69(3):602-608) prior to being cooked in an Alkar® model 700 HP single truck processing oven (ALKAR-RapidPak, Inc., Lodi, WI, USA) to an internal temperature of 52°C according to the schedule below (See Table 2.). The slab bacon was stabilized in a 4°C walk-in cooler to and internal temperature of 7.2°C according to Appendix B (USDA-FSIS, 1999).
Each of the six $25cm^2$ sections were excised and aseptically transferred to a Whirl-Pak® filter stomacher bag (Nasco, Ft. Atkinson, WI, USA) and filled with 99mL of 0.1% buffered peptone water (Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA) (method modified from Burnham *et al.*, 2006). One $25cm^2$ section from each side (medial and lateral) was inoculated and excised prior to thermal processing and chilling to evaluate the initial inoculum level (positive control). One $25cm^2$ section from each side (medial and lateral) was inoculated with sterile TSB to serve as a negative control and to detect the presence of any naturally occurring microflora. Figure 1 depicts the location of the six 25cm2 sections on the belly surfaces.

The contents of the filter bag were mixed for 120 seconds using a Seward model 400 lab blender stomacher (Seward Medical, London SE1 1PP, UK) and the slurry was serially diluted to 10^{-6}. Each dilution (including the slurry contents) was plated in duplicate on Baird-Parker Agar with egg yolk Tellurite enrichment (BPA-EY; Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA) and incubated at 35°C for 48hrs. Plates were counted for growth at 24 and 48 hours and the results were recorded. Three replications of this experiment were performed.

Objective 2 – Survival of MRSA during thermal processes comparable to those used at the consumer level

Bacteriological Cultures – Cultures of methicillin-resistant *Staphylococcus aureus* used for the designed experiment were obtained from Tara C. Smith at the Center for Emerging Infectious Diseases, University of Iowa College of Public Health, Iowa City, IA and from Catherine M. Logue at the department of Veterinary Microbiology and Preventative Medicine, Iowa State University College of Veterinary Medicine, Ames, IA. The specific strains used during testing were ST398 (HU010111N) from a 40 year old adult human male, t337(MN55) from an adult swine, ST398(R35) from retail ground pork and ATCC strain BAA-44(R31) as a reference organism.

Pork Products – Sliced bacon was used to test objective 2.

Sliced Bacon – Three brands of commercially available sliced bacon, selected on the basis of similar "use by" dates, were purchased at retail outlets in Ames, IA and brought back to the Iowa State University Food Safety Research Laboratory for testing and evaluation.
A twenty-four hour culture of each MRSA strain was grown at 35°C in Trypticase Soy Broth (TSB; Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA). A four strain cocktail was then prepared by transferring 2.5mL of each strain into a test tube (10mL total volume) and vortexing. Two 25g slices of bacon from each brand were inoculated on both sides with 0.5mL of the cocktail. Two degrees of doneness, "very crispy" and "less crispy" were used to test Objective 2. "Very crispy" slices were grilled on a 16-inch, covered Presto® electric skillet (National Presto Industries, Inc., Eau Claire, WI) at 177°C (350°F) for 5 minutes on each side. "Less crispy" bacon slices were grilled on a 16-inch, covered Presto® electric skillet (National Presto Industries, Inc., Eau Claire, WI) for 2 minutes on each side. Slices were removed from the skillet and 10g of each brand of cooked bacon

for the two doneness variables were aseptically transferred to a Whirl-Pak® filter stomacher bag (Nasco, Ft. Atkinson, WI, USA) and filled with 90mL of 0.1% buffered peptone water (Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA) The contents were mixed for 120 seconds using a Seward model 400 lab blender stomacher (Seward Medical, London SE1 1PP, UK) and the slurry was serially diluted to 10^{-4}. Each dilution (including the stomacher slurry) was plated in duplicate on Baird-Parker Agar with egg yolk Tellurite enrichment (BPA-EY; Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA) and incubated at 35°C for 24 hours. Plates were counted for growth and recorded. The remaining portion of cooked bacon slices from each brand for the two doneness variables were placed into a test tube of Brain Heart Infusion broth (BHI, Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA) enrichment and incubated at 35°C for 24 hours. The incubated BHI was then streaked onto BPA-EY plates to test for presence or absence of severely heat injured MRSA requiring time and enrichment to recover.

Results and Discussion:

Frankfurters. Figure 2 graphically depicts the survival of MRSA in cooked, emulsified sausages. Table 3 shows the mean $\log_{10}$ count (cfu/g) and standard deviation for the negative control, positive control and cooked, inoculated treatments for the experiment. All three treatments were statistically different ($P<0.001$) from one another. There was an overall 5.5 $\log_{10}$ reduction in the cooked, inoculated samples when compared to the uncooked, positive control. As expected, populations of MRSA were not detected in the negative control. The average time for the frankfurters to reach an internal temperature of 70°C was 26.18 minutes with a range of 0.25 minutes. The average chill time in slush ice to an internal temperature of 7.2°C was 29.56 minutes with a range 6.56 minutes. Cook and chill time for the experiment was not significant for the results of the main treatment effects. The results of this study on survival of MRSA during thermal processing do not differ from the results of Heiszler *et al.* (1972) and Palumbo *et al.* (1977) on thermal inactivation of strains of *S. aureus*. These two studies differed in cook time to the described experiment above however, because they more closely mimicked large scale commercial manufacturing of frankfurters (30min vs. 95min). Heiszler *et al.* (1972) showed the greatest reduction of surviving microorganisms at an internal temperature of 60°C, but continued to decrease with increasing finished temperatures. It is interesting to note that *S. aureus* was only detected in 1.67% of the 120 frankfurters exposed to various time/temperature combinations. Although some of the frankfurters in these studies were exposed to higher ambient temperatures, addition of smoke and longer cook times than the water bath utilized in

the current study, survival of MRSA is not different from *S. aureus* and should not be of great concern to consumers in these types of processed meats.

Fermented and cooked sausage (summer sausage). Figure 3 illustrates the survival of MRSA in fermented and cooked sausages. Table 4 shows the mean $\log_{10}$ count (cfu/g) and standard error for the negative control, positive control, fermented and cooked treatments for the experiment. All four treatments were statistically different ($P<0.001$) from one another. There was an overall 6.75 $\log_{10}$ reduction in the cooked, inoculated samples when compared to the positive controls. As illustrated in the figure 3 and listed in table 4, growth did not occur in the negative control group. The average starting pH of the raw sausages prior to fermentation was 6.02 with a range of 0.2. The average 12-hour pH after fermentation was calculated to be 4.32 with a range of 0.05. The average time for the summer sausage to reach an internal temperature of 70°C was 39.95 minutes with a range of 7.97 minutes. The average chill time in slush ice to an internal temperature of 7.2°C was 52.35 minutes with a range of 12.3 minutes. Cook / Chill times and pH were not significantly different between replications and did not impact the fixed main treatment effects.
Since many strains of enterotoxin producing *S. aureus* have the ability to survive at varying salt concentrations, pH and water activities, these intrinsic and extrinsic factors are manipulated and monitored by meat processors during the production and storage of dry and semi-dry sausages. Results from Ingham *et al.* (2005) indicate that fermented, commercial summer sausages range in pH from 4.4 – 4.9 which was slightly higher than pH measured in the current study. The survival of cells is not nearly as important as enterotoxin production in fermented sausages. Extent and rate of pH decline in fermented dry and semi-dry sausages appears to be main factors in controlling toxin production (Genigeorgis *et al.* 1969). Also *S. aureus* does not compete well with other bacterial populations (McCoy 1965) which suggests that dry and semi-dry sausages fermented with commercial starter cultures have a reduced risk of *S. aureus* growth and enterotoxin production. Appropriate fermentation procedures, as outlined by the American Meat Institute (AMI 1997), are vital for ensuring the safety of these processed meats.

Boneless Ham. The results of the boneless ham experiment are shown in Figure 4. Table 5 shows the mean $\log_{10}$ count (cfu/g) and standard error for the negative control, positive control, and cooked treatments for the experiment. There was a significant effect ($P<0.001$) of the thermal treatment when compared to the uncooked, positive control. The total cooking process took an average of 5.5 hours

and demonstrated an average Log_{10} reduction of 7.28. The cooked treatment means were not different (P=0.26) when compared to the negative control (ham with sterile TSB added). Day of replication was not a significant effect in the model (P=0.28). Chilling (stabilization) times of the hams to an internal temperature of 7.2°C ranged from 6.8 hours to 7.5 hours with an average time to stabilization of 7.15 hours. These times are well within the 15 hour time limit for option 3 in FSIS-Appendix B (USDA-FSIS, 1999). Cook / Chill times were not a significant effect in the model. Although thermal processing of large diameter meat products like ham can serve as potential growth reservoirs for *S. aureus* due to the slow come-up times of the product during cooking, the boneless ham results in this study showed the least survival for MRSA. Ingham *et al.* (2004) reported that although slow cooking procedures were adequate in controlling pathogen survival, control of *S. aureus* toxin production was paramount. Since some strains of MRSA are capable of producing enterotoxin, critical limits for time and temperature combinations like those validated by Ingham *et al.* (2004) should be considered for processed meats thermally processed using slow-cooking procedures.

Slab Bacon. The results of the slab bacon study are illustrated in Figure 5, as well as the means summarized in Table 6. The results of the experiment show average log_{10} reduction of 1.89 when comparing the fixed main treatment effects of heat treatment to the uncooked, positive control. These results are similar to a study by Taormina and Bartholomew (12) who reported a 1.26 log_{10} reduction after smoking and subsequent chilling of whole belly pieces. It should be noted that whole belly pieces in this similar report were heat treated to a lower temperature (48.9°C) which could explain the smaller reduction in population. Although not examined in the current study, the addition of smoke to whole cured pork bellies inhibits both *S. aureus* growth and enterotoxin production. (12)
Although not significantly different (P=0.21) from the medial side negative control group (MS neg con), there was observed growth on the lateral side negative control group (SS neg con). Day of replication was a significant factor in the model (P=0.01), so the mixed effect day within treatment interaction was added to the statistical model. This term in the model may explain the observed growth for SS neg con, which means contamination of the sample could have occurred for that particular group. The block of location on the belly was not significantly different (P>0.05) within the main treatment effects and did not significantly impact the fixed main treatment effect of cooking. The average time for heat treatment to an internal temperature of 52°C was 5.9 hours ±0.7 hours. Average chill time for stabilization was 8.4 hours. Cook / Chill times were not a significant factor to

explain the data. Cook and chill times differ from commercial processes reported in the literature (12). The time difference is mainly due to the blast chill devices utilized in commercial bacon operations to rapidly chill and crust whole, cured and smoked bellies prior to slicing.

Sliced Bacon. The results for the survival of MRSA on sliced bacon cooked at a temperature of 177°C (350°F) are shown in Figure 6. The means of observed growth for the experiment are listed in Table 7. There was not a significant difference ($P>0.05$) between brands or for the brand within treatment effect (cook time) in the model. Day of replication also did not impact the fixed main treatment effect of time of cooking (0, 2 or 5 minutes @ 177°C). Overall, the three independent replications showed a Log_{10} reduction of > 6.5. There was not a significant difference ($P=0.91$) in the treatment effect between cooking at 2 minutes per side or 5 minutes per side, but were different ($P<0.001$) when compared to the uncooked, positive control. Although not measured in the current study, the literature suggests that water activity is a better measure of safety of cooked sliced bacon with respect to *S. aureus* growth and toxin production (6).

Figure 7 shows the results of the percent positive MRSA samples observed from a Brain Heart Infusion (BHI) enrichment of cooked pieces. The percent positive results were pooled by treatment only since there was not a significant difference between brands in the model. The pooled Least Square Means (LSMeans) ± standard errors of the means for the percent positive results for the BHI enrichment are listed in Table 8. Although not statistically different, recovery of MRSA from BHI enrichment was not observed in Brand A.

These results indicate that some samples were potentially positive for viable MRSA cells below the detection limit of the assay (50 cells/g). The survival of MRSA from heat injured cells could also explain the results from the enrichment experiment. This could be a result of the bacteria being exposed to a high temperature for shorter periods of time. It is obvious; however that sliced bacon grilled at high temperatures is sufficient to greatly reduce the risk of MRSA survival.

Figure 1. Schematic of bacon slab

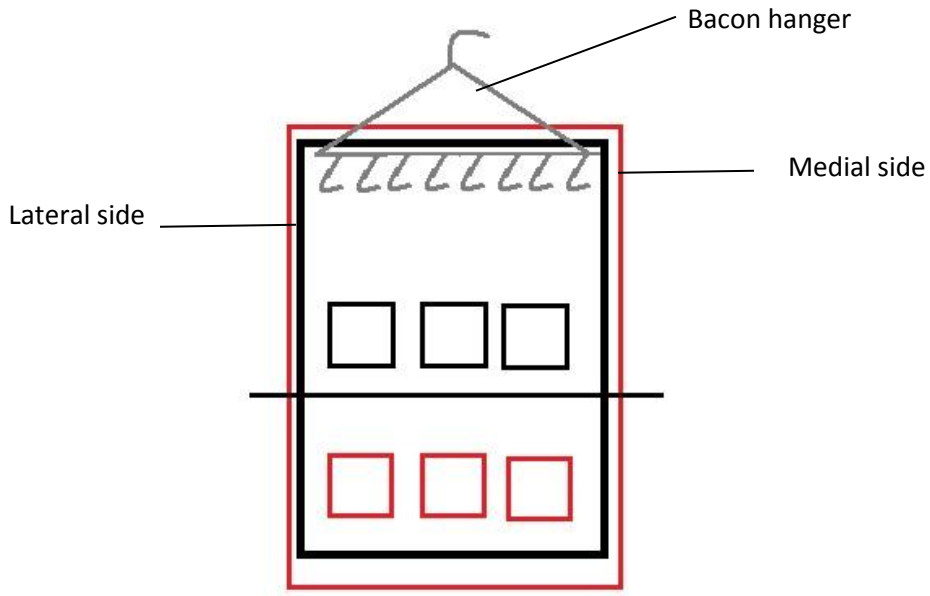

Figure 2.

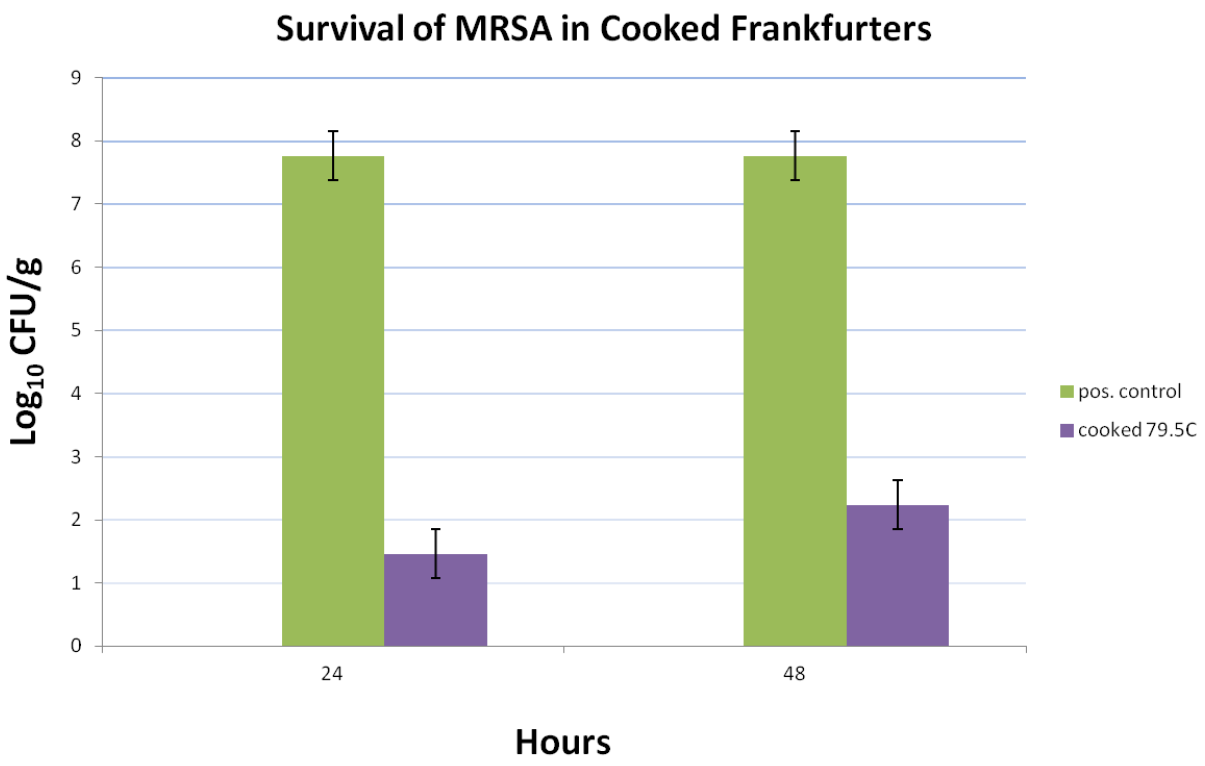

Figure 3.

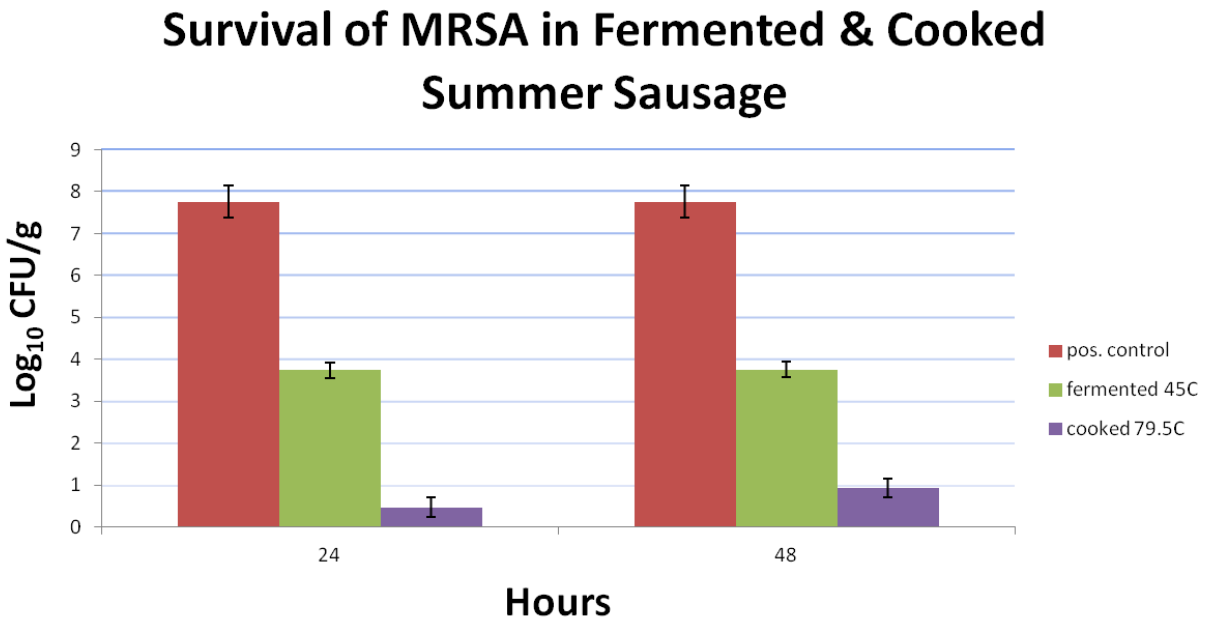

Figure 4.

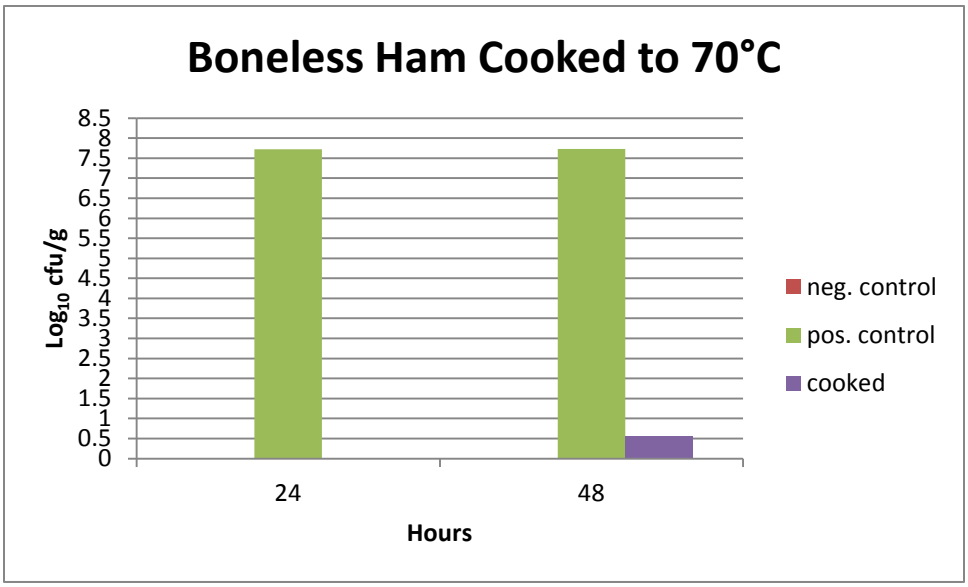
Boneless Ham Cooked to 70°C
Log10 cfu/g
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
24
48
Hours
neg. control
pos. control
cooked

Figure 5.

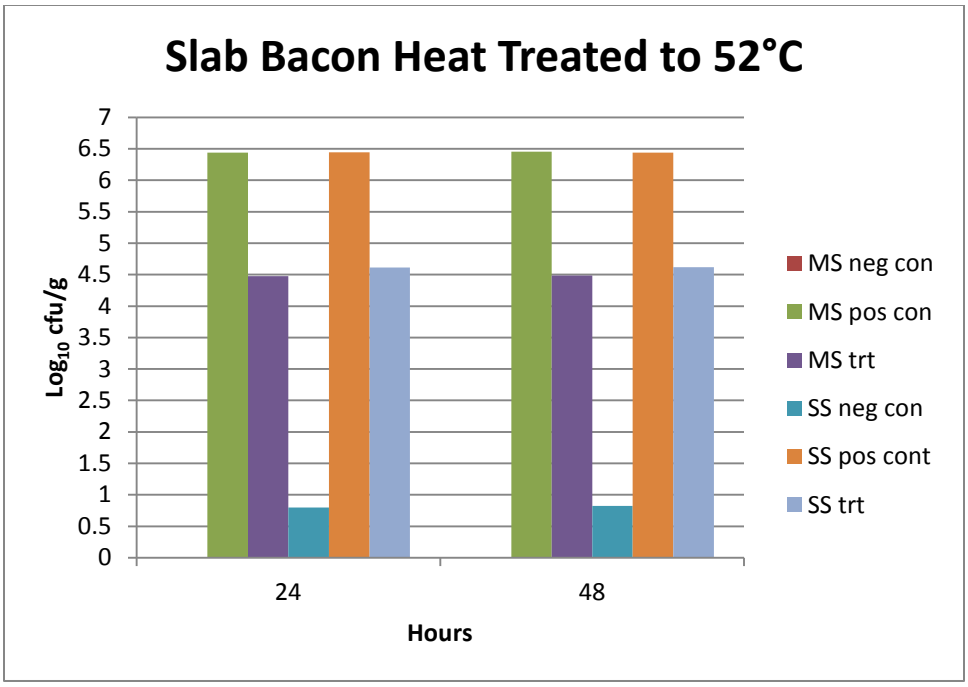

Figure 6. Survival of MRSA in sliced bacon cooked at 177°C (350°F)

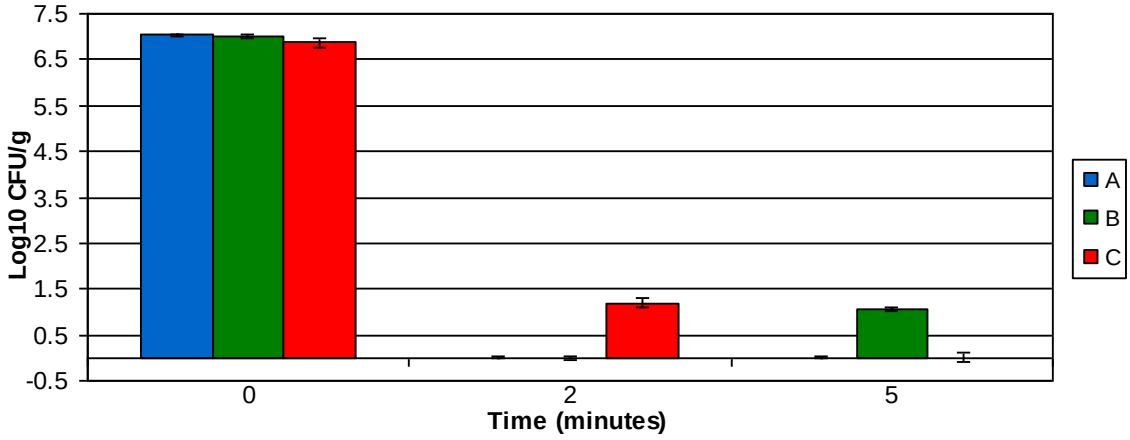

Figure 7.

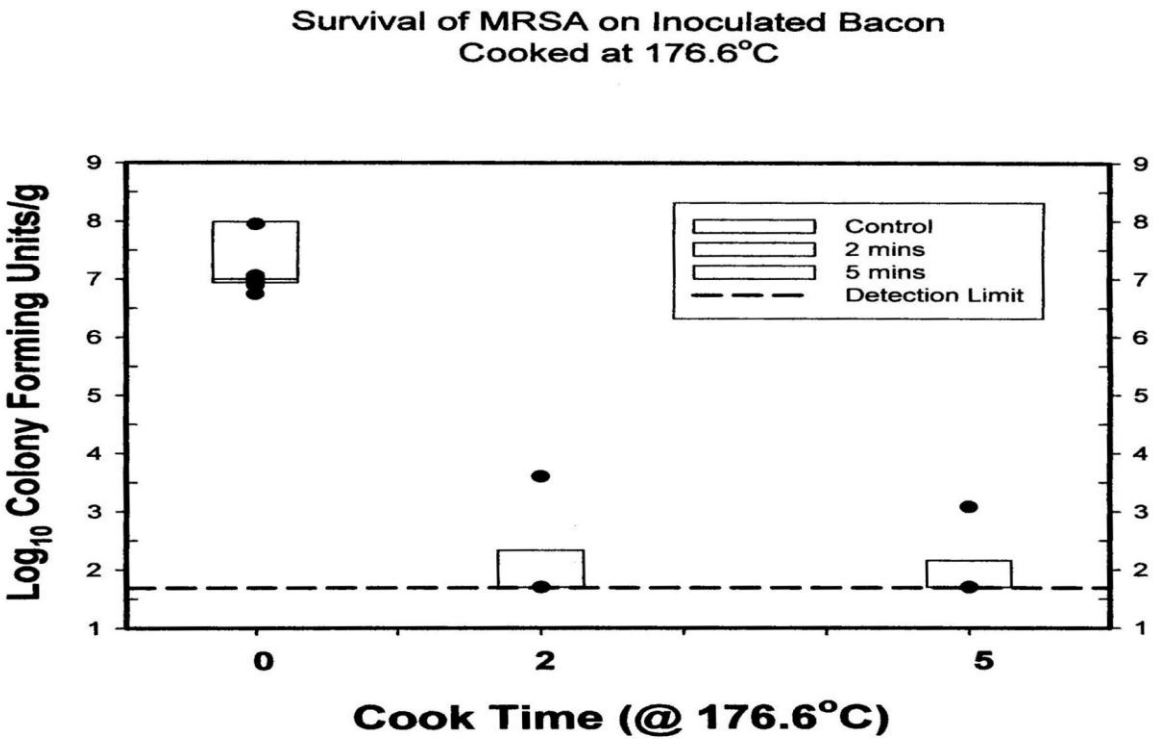
Survival of MRSA on Inoculated Bacon
Cooked at 176.6°C
Log10 Colony Forming Units/g
Control
2 mins
5 mins
Detection Limit
9
8
7
6
5
4
3
2
1
0
2
5
Cook Time (@ 176.6°C)

Table 1. Boneless Ham Thermal Processing Schedule

Step	Step Time	D.B.	W.B.	rH	Blower	IT (°F)	Dampers
Cook	0:40	165	0	0%	8		Auto
Cook	0:30	170	0	0%	8		Auto
Cook	0:45	175	0	0%	6		Closed
Cook	1:00	175	161	71%	8	126	Closed
Cook	0:01	180	160	62%	10	140	Auto
Steam Cook	0:01	185	185	100%	10	158	Closed
Cold Shower	0:10	50	50	0%	0		Auto

Table 2. Slab Bacon Thermal Processing Schedule

Step	Step Time	D.B.	W.B.	rH	Blower	IT (°F)	Dampers
Cook	3:00	104	86	48%	8		Auto
Cook	1:10	125	100	42%	8		Closed
Cook	2:00	125	0	0%	6		Auto
Cook	0:01	135	100	30%	7	126	Auto

Table 3. Mean and standard error of mean for survival of MRSA in frankfurters

Treatment	Means (Log_{10} CFU/g)
Negative	0^{a}
Positive	7.76 ± 0.10^{b}
Cooked (79.5°C)	2.23 ± 1.06^{c}

[a-c]Means in a column with different superscripts are statistically different (P<0.05)

Table 4. Mean and standard error for survival of MRSA in summer sausage

Treatment	Means (Log_{10} CFU/g)
Negative	0^{a}
Positive	7.75 ± 0.09^{b}
Fermented (45°C)	$3.75 \pm 0.32_{c}$
Cooked (79.5°C)	0.93 ± 0.69^{d}

[a-d]Means in a column with different superscripts are statistically different (P<0.05)

Table 5. Mean and standard error of mean for survival of MRSA in boneless ham

Treatment	Means (Log_{10} CFU/g)
Negative	0^{a}
Positive	7.73 ± 0.24^{b}
Cooked (79.5°C)	0.45 ± 0.73^{c}

[a-c]Means in a column with different superscripts are statistically different (P<0.05)

Table 6. Mean and standard error of mean for survival of MRSA in slab bacon

Treatment	Means (Log_{10} CFU/g)
MS Negative	0^{a}
SS Negative	0.81 ± 1.15^{a}
MS Positive	6.45 ± 0.49^{b}
SS Positive	6.44 ± 0.46^{b}
MS Treatment	4.48 ± 1.01^{c}
SS Treatment	4.61 ± 1.07^{c}

[a-c]Means in a column with different superscripts are statistically different (P<0.05)

MS = medial side of belly

SS = lateral (skin) side

Table 7. Mean and standard error of mean for survival of MRSA in sliced bacon

	Time (minutes)		
Brand	**0**	**2**	**5**
A	7.05 ± 0.02^{d}	0^{e}	0^{e}
B	7.02 ± 0.05^{d}	0^{e}	1.05 ± 1.82^{e}
C	6.86 ± 0.11^{d}	1.20 ± 2.08^{e}	0^{e}

[d-e]Means in a column with different superscripts are statistically different (P<0.05)

Table 8. LSMean and standard error of mean for pooled data of percentage of MRSA present in sliced bacon after BHI enrichment

Time (minutes)	LSMEAN ± Standard Error Mean (n=27)
0	**100 ± 0.13[a]**
2	**44.44 ± 0.13[b]**
5	**22.22 ± 0.13[b]**

[a-b]Means in a column with different superscripts are statistically different (P<0.05)

References

APHIS Veterinary Services Centers for Epidemiology and Animal Health. Methicillin-resistant *Staphylococcus aureus* A growing Concern for Animal and Human Health. *CEI info sheet* December 2007; http://www.aphis.usda.gov/vs/ceah/cei.

Barrett FF, M[c]Gehee RF Jr and Finland M. Methicillin-resistant *Staphylococcus aureus* at Boston City Hospital: bacteriologic and epidemiologic observations. *New Eng J Med* 1968; 279: 441-448.

Burnham, GM, Fanslau, MA, Ingham, SC. Evaluating Microbial Safety of Slow Partial-Cooking Processes for Bacon: Use of a Predictive Tool Based on Small-Scale Isothermal Meat Inoculation Studies. *Jour Food Protect* 2006; 69(3):602-608.

Byrne, J. Low Risk of MRSA contamination from food, says EFSA. *Meat Process.com* 27 March 2009. http://www.meatprocess.com/Safety-Legislation/Low-risk-of-MRSA-contamination-from-food-says-EFSA. Retrieved April 10, 2009.

Centers for Disease Control and Prevention. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2003, issued August 2003. *Am Jour Infect Control* 2003; 31: 481 – 498.

Huijsdens XW, van Dijke BJ, Spalburg E, van Santen-Verheuvel MG et al. Community-acquired MRSA and pig-farming. *Annal Clin Micro AntiMicro* 2006; 5: 26 – 28.

Jones TF, Kellum ME, Porter SS, Bell M and Schaffner W. An Outbreak of Community-Acquired Foodborne Illness Caused by Methicillin-Resistant *Staphylococcus aureus*. *Emerg Infect Dis* 2002; 8(1): 82 – 84.

Khanna T, Friendship R, Dewey C and Weese JS. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. *Vet Micro* 2007; 128: 298 – 303.

Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K *et al.* Invasive Methicillin-Resistant *Staphylococcus aureus* Infections in the United States. *JAMA* 2007; 298(15): 1763 – 1771.

Kluytmans[a] J, van Leeuwen W, Goessens W, Hollis R, Messer S *et al.* Food-Initiated Outbreak of Methicillin-Resistant *Staphylococcus aureus* Analyzed by Pheno- and Genotyping. *Jou Clin Micro* 1995; 33(5): 1121 – 1128.

Kluytmans[b] J, van Belkum A and Verbrugh H. Nasal Carriage of *staphylococcus aureus*: Epidemiology, Underlying Mechanisms, and Associated Risks. *Clin Micro Rev* 1997; 10(3): 505 – 520.

Kristof[a] ND. Our Pigs, Our Food, Our Health. *New York Times* 11 March 2009. http://www.nytimes.com/2009/03/12/opinion/12kristof.html

Kristof[b] ND. Pathogens in Our Pork. *New York Times* 15 March 2009. http://www.nytimes.com/2009/03/15/opinion/15kristof.html

Lewis HC, Mølbak K, Reese C, Aarestrup FM, Selchau M, Sørum M and Skov RL. Pigs as Source of Methicillin-Resistant *Staphylococcus aureus* CC398 Infections in Humans, Denmark

Maree CL, Daum RS, Boyle-Vavra S, Matayoshi K and Miller LG. Community-associated Methicillin-resistant *Staphylococcus aureus* Isolates Causing Healthcare-associated Infections. *Emerg Infect Dis* 2007; 13(2): 236 – 242.

O'Brien AM, Hanson BM, Farina SA, Wu JY, Simmering JE, Wardyn SE, Forshey BM, Kulick ME, Wallinga DB & Smith, TC. MRSA in Conventional and Alternative Retail Pork Products. PLoS ONE 2012; 7(1):e30092.

Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, *et al. Staphylococcus aureus CC398: Host Adaptation and Emergence of Methicillin Resistance in Livestock*. mBio 3(1):1-6 e00305-11

Pu S, Han F and Ge B. Isolation and Characterization of Methicillin-Resistant *Staphylococcus aureus* Strains from Louisiana Retail Meats. *App Env Micro* 2009; 75(1): 265 – 267.

Smith TC, Male MJ, Harper AL, Kroegar JS et al. Methicillin-Resistant *staphylococcus aureus* (MRSA) Strain ST398 Is Present in Midwestern US Swine and Swine Workers. *PLoS ONE* 2009; 4(1):e4248.

United States Department of Agriculture – Economic Research Service. 2005. Factors Affecting U.S. Pork Consumption. http://transcoder.usablenet.com/tt/http://www.ers.usda.gov/Publications/LDP/May05/LDPM13001/ Retrieved 22 April 2009.

United States Department of Agriculture – Food Safety and Inspection Service. 1999. *Appendix B – Compliance Guidelines for Cooling Heat Treated Meat and Poultry Products (Stabilization)*. http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/95-033F/95-033F_Appendix_B.htm. Retrieved 14 January 2010.

United States Department of Agriculture – Food Safety and Inspection Service. 2008. Fact Sheet: Safety of Fresh Pork . . . From Farm to Table. http://www.fsis.usda.gov/Fact_Sheets/Pork_from_Farm_to_Table/index.asp. Retrieved 22 April 2009.

Van Loo IHM, Diederen BMW, Savelkoul PHM, Woudenberg JHC *et al.* Methicillin-Resistant *Staphylococcus aureus* in Meat Products, The Netherlands. *Emerg Infect Dis* 2007; 13(1): 1753 – 1755.

Voss A, Milatovic D, Wallrauch-Schwarz C, Rosdahl VT and J Verhoef. Methicillin *resistant Staphylococcus* aureus in Europe. *Eur Jou Clin Micro Infect Dis* 1994; 13: 50 – 55.

Voss A, Loeffen F, Bakker J, Klaassen C and M. Wulf. Methicillin-resistant *Staphylococcus aureus* in Pig Farming. *Emerg Infect Dis* 2005; 11(12): 1965 – 1966.

Wenzel RP and TM Perl. The significance of nasal carriage of *staphylococcus* aureus and the incidence of postoperative wound infection. Jou *Hosp Infect* 1995; 31: 13 – 24.

Witte W, Strommenger B, Stanek C and C Cuny. Methicillin-resistant *Staphylococcus aureus* ST 398 in Humans and Animals, Central Europe. *Emerg Infect Dis* 2007; 13(2): 255 – 258.

Zetola N, Francis JS, Nuermberger EL and WR Bishai. Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging threat. *Lancet Infect Dis* 2005; 5: 275 - 286.