Survival of Methicillin-Resistant Staphylococcus aureus During Thermal Processing of Frankfurters, Summer Sausage, and Ham

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Abstract
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Keywords
Food Science & Human Nutrition

Disciplines
Agriculture | Food Chemistry | Food Processing | Meat Science | Parasitology

Comments
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Survival of Methicillin-Resistant *Staphylococcus aureus* During Thermal Processing of Frankfurters, Summer Sausage, and Ham

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**Abstract**

Infections from antibiotic-resistant bacteria are a major concern for human health professionals around the world. Methicillin-resistant *Staphylococcus aureus* (MRSA) is just one of the resistant organisms of concern. MRSA prevalence has also been recently reported in retail meat products at rates higher than originally thought. Although the risk of contracting an infection from handling contaminated meat products is thought to be low, very little is known about this organism from a food safety perspective. The objective of this study was to determine the survival of MRSA during thermal processing of frankfurters, summer sausage, and boneless ham. Frankfurters, summer sausage, and boneless ham were manufactured using formulations and processing procedures developed at the Iowa State University meat laboratory. Thermal processing resulted in a significant log reduction (\( p < 0.05 \)) for boneless ham, summer sausage, and frankfurters when compared to uncooked, positive controls for each of the three processed meat products. All products were thermally processed to an internal temperature of 70°C and promptly cooled to 7.2°C. Boneless ham showed the highest log reduction (7.28 logs) from cooking, followed by summer sausage (6.75 logs) and frankfurters (5.53 logs). The results of this study indicate that thermal processing of ham, summer sausage, and frankfurters to 70°C is sufficient to reduce the risk of MRSA as a potential food safety hazard.

**Introduction**

* Methicillin-resistant *Staphylococcus aureus* (MRSA) is a variant strain of *S. aureus*, a known food pathogen that commonly inhabits the nasal passage and skin of humans. MRSA was first discovered in the United States in the 1960s at Boston City Hospital (Barrett et al., 1968), and since its discovery more than 40 years ago, MRSA has emerged as one of the leading and most severe infectious microorganisms acquired from exposure in health care settings (CDC NNIS, 2003). According to an 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). MRSA infections have evolved from being solely acquired in a health care setting to that of a community-acquired exposure (Zetola et al., 2005; Maree et al., 2007; Witte et al., 2007). Wenzel and Perl (1995) estimated that up to 10%–15% of adults are continually colonized with the organism.

MRSA has also been isolated from companion animals and livestock, especially swine (APHIS, 2007). Lewis et al. (2008) discussed the link to pig farming and the increased risk for MRSA exposure and subsequent infections 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. With the increase of MRSA colonization in livestock and invasive infections in humans outside of a nosocomial setting, reasons for the increased incidences come into question. Opponents of animal agriculture offer their opinion on the link to the increased MRSA colonization and community-acquired infections by describing the “insane overuse of antibiotics in livestock feeds” (Kristof, 2009). Price et al. (2012) refute popular views on antibiotic overuse in livestock production and explain the potential for a “bidirectional zoonotic exchange” between humans and livestock. This means that humans can transfer potential resistant microorganisms to livestock and vice versa.

Prevalence of MRSA has also been documented for numerous meat products, especially pork. 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). Recent research evaluated...
various pork products around the world to determine the prevalence of MRSA at the retail meat counter. Pu et al. (2009) reported that 5.6% of the 90 pork products samples in Louisiana retail food stores were positive for MRSA. A similar study in The Netherlands revealed that MRSA was present in 25% of 64 pork products sampled (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 majority of the literature suggests that MRSA contamination of meat products is very low (<100 colony-forming units (CFU)/g detected).

Although these studies in the United States and Europe indicate the presence of MRSA on fresh 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 (Kluymans et al., 1997). This was the first report of its kind until Jones et al. (2002) linked a community-acquired foodborne illness from MRSA to shredded pork barbecue in Tennessee. The report from Tennessee is the first to note that MRSA is also capable of producing food poisoning from enterotoxin produced in food.

Results of increased MRSA incidence from past research on pork products brings into question the safety of meat products at the retail level. Is it possible that MRSA, with its genetic ability to be resistant to antimicrobials, is also resistant to methods used to thermally process meat products? To answer this question, the following study was designed to determine the survival of MRSA after thermal processing of various processed meat products.

Materials and Methods

Bacteriological cultures

MRSA cultures used for the experiment were obtained from Tara C. Smith at the University of Iowa College of Veterinary Medicine, Ames, IA. The specific strains used during testing were ST398(HU010111N) from human origin, 1337(MN55) from an adult swine, ST398(R35) from retail ground pork, and ATCC strain BA1-44(R31) as a reference organism. Cultures of each MRSA strain were grown at 35°C in trypticase soy broth (TSB; Difco®, Becton, Dickinson & Co., Franklin Lakes, NJ) for 24 h. The four strains were then combined and vortexed to create the mixed culture.

Frankfurter manufacture

A blend of pork and beef frankfurters were made using the following formulation: 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), 0.23% 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). The pork and beef trim were ground through a 12.7-mm plate using a Biro model 7552SS (Biro Mfg. Co., Marblehead, OH) grinder. The emulsion was produced using a vacuum-bowl chopper (model VSM65, Krämer & Grebe GmbH & Co. KG, Biendenkopf-Wallau, Germany) with six knives at varying speeds. The emulsion was then vacuum packaged, frozen at -28°C, and stored for approximately 2 weeks until used for testing. Prior to inoculation, the vacuum pouches were slowly thawed in a 0°C walk-in cooler.

Summer sausage manufacture

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) summer sausage seasoning (0.47% wt/wt), Legg’s cure (A.C. Legg) 6.25% nitrite curing salt (0.23% wt/wt), SAGA® 200 (Pediococcus acidilactici) (Kerry Seasoning, Beloit, WI) lactic acid starter culture (0.2% wt/wt). The pork and beef trim were ground through a 9.5-mm plate using a Biro model 7552SS grinder (Biro Mfg. Co.). The ground trim was then transferred to a Hollymatic® model 175 mixer grinder (Hollymatic Corporation, Countryside, IL) and mixed for 2 min along with the salt, water, and curing salt. The dextrose, seasoning blend, and starter culture dissolved into 300 mL of distilled water was then added to the mixer grinder and mixed for an additional 2 min. The product was then re-ground through a 3.2-mm plate, placed into vacuum pouches, and stored as previously described.

Boneless ham manufacture

Pork inside and outside ham muscles obtained from swine harvested and fabricated at the Iowa State University meat laboratory were trimmed to remove surface connective tissue. The ham muscles were injected to 25% over raw weight using the following brine ingredients: water (80.7% wt/wt), salt (11% wt/wt), sugar (6.6% wt/wt), Brifisol® 450 Super (BK Giuliani Corp., Simi Valley, CA) sodium tri-poly 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 a Günther® PI 21 model injector (Günter Maschinenbau GmbH, Dieburg, Germany) and then macerated using a model PMT-41 Stork-Protecon™ macerator (Oss, Holland, The Netherlands). The ham macerate was stored in vacuum pouches as previously described.

Inoculation and microbial sampling

A 2-h culture of each MRSA strain was grown as previously described and vortexed together in a single test tube to create a four-strain mixed culture. For frankfurters, 4 mL of the mixed culture was mixed with 36 g of the emulsion batter. The inoculated batter was stuffed into a plastic 50-mL Corning® centrifuge tube (Corning Inc., Corning, NY) using a sterile plastic syringe. The cap was secured onto the tube, placed into a three-tube centrifuge cassette, and the cassette was submerged into a Thermo/NESLAB® model RTE-211 water bath/circulator (Thermo Scientific, Portsmouth, NH) at 79.5°C. Tubes similar in diameter to retail frankfurters were used in place of frankfurter casing to keep the emulsion fully
submersed in the water bath during the thermal process to an internal temperature of 70°C. The cooked sausages were immediately transferred to a slush ice bath and cooled to 7.2°C prior to microbial analysis. For all meat product types, an uncooked, positive control of each meat product type was used to determine the inoculum level (target 6 log CFU/g). A cooked, negative control, inoculated with sterile TSB, was also created to monitor the internal temperatures during thermal processing and chilling, and to determine the presence of any naturally occurring microflora. Ten grams of each frankfurter (including positive and negative controls) were aseptically transferred to a Whirl-Pak® filter stomacher bag (Nasco, Ft. Atkinson, WI) and homogenized with 90 mL of 0.1% buffered peptone water (Difco™, Becton, Dickinson & Co.) for 120 s in a stomacher (model 400 lab blender Seward Medical, London, UK).

For summer sausage, 20 mL of the mixed culture was blended with 180 g of the sausage batter. The inoculate was stuffed into a 250-mL glass Pyrex® beaker (Corning Inc., Corning, NY), covered with “Parafilm M” laboratory film (Bemis Flexible Packaging, Neenah, WI), and placed into a 45°C fermentation chamber for 12 h. The pH of the fermented sausages was determined using a calibrated Accumet® pH meter (Thermo Fisher Scientific, Inc., Pittsburgh, PA). The fermented sausages were placed in a water bath at 79.5°C and cooked and chilled prior to microbial analysis as previously described for frankfurters. Glass beakers, approximately the same diameter as typical retail summer sausage, were used in place of fibrous casing to prevent contamination of the sample during thermal processing in the water bath. Twenty-five grams of each fermented and cooked summer sausage was blended along with 225 mL of 0.1% buffered peptone water into a homogenate, as previously described.

For boneless hams, 14 mL of the mixed culture was mixed with 1386 g of the ham macerate. The inoculated ham was stuffed into a T8 · 30-inch pre-tied fibrous casing (Kalle UK Ltd., Witham, Essex, UK) using a Biro DFS 30 (Biro MFG Co., Marblehead, OH) piston stuffer. The fibrous ham casing was clipped using a Poly-Clip® System SCH 6210 model clipper (Poly-Clip System GmbH & Co. KG, Frankfurt, Germany). The hams were cooked in an Alkar® model 700 HP single-truck processing oven (Alkar-RapidPak, Inc., Lodi, WI) to an internal temperature of 70°C according to the schedule outlined in Table 1. The cooked hams were transferred to a 4°C walk-in cooler and chilled to 7.2°C in accordance with option 3 outlined in Appendix B, Compliance Guidelines for Cooling Heat-Treated Meat & Poultry Products (USDA-FSIS, 1999). Fifty grams of each cooked and chilled boneless ham were homogenized as described for frankfurters along with 450 mL of 0.1% buffered peptone water.

The homogenized slurry for each meat product type was serially diluted, and surface plated (including the slurry contents) in duplicate on Baird-Parker agar with egg yolk Tellurite enrichment (BPA-EY; Difco™, Becton, Dickinson & Co.). Preliminary results (data not shown) from heat shocking of the mixed culture at various time intervals indicated BPA-EY enrichment plating to be a more inclusive method to enumerate surviving MRSA cells when compared to trypticase soy agar and BPA without enrichment. The BPA-EY plates for all meat products sampled for surviving MRSA were incubated at 35°C for 48 h, and colonies were enumerated and the results recorded. Three independent replications of the experiment were performed. Confirmation of MRSA colonies for all products was performed using a blue chromogenic agar (Brilliance™ MRSA agar, Oxoid Ltd., Basingstokes, Hantz, UK).

Data analysis

Log10 transformations for surviving MRSA colonies were calculated in duplicate for each of three independent replications for frankfurter, summer sausage, and boneless ham experiments. Values below the detection limit of the assay (<50 CFU/g) were recorded as the minimum detection limit for frankfurters, summer sausage and boneless ham. The frankfurter, summer sausage, and boneless ham experiments were arranged in a completely randomized design. Least significant differences for the results were calculated using the general linear model procedure (PROC GLM) and the mixed-effects model procedure (PROC MIXED) of the Statistical Analysis Software (SAS Institute Inc., Cary, NC) at a significance level of p < 0.05.

Results

Table 2 shows the mean log10 count (CFU/g) and standard deviation for the negative control (addition of sterile TSB), positive control (inoculated, uncooked), fermented (summer sausage) and cooked processed meat products sampled for the experiment. Table 3 shows the average cook and chill times for frankfurters, summer sausage, and boneless ham.

Frankfurters

All three treatments were statistically different (p < 0.001) from one another. There was an overall 5.5 log10 reduction in the cooked, inoculated samples when compared to the

| Table 1. Boneless Ham Thermal Processing Schedule |
|-------|-----------|-----------|-----------|----------|-----------|
| Step | Time | DB | WB | Humidity | IT (°F) | Dampers |
| Cook | 0:40 | 165 | 0 | 0% | Auto |
| Cook | 0:30 | 170 | 0 | 0% | Auto |
| Cook | 0:45 | 175 | 0 | 0% | Closed |
| Cook | 1:00 | 175 | 161 | 71% | 126 | Closed |
| Cook | 0:01 | 180 | 160 | 62% | 140 | Auto |
| Steam cook | 0:01 | 185 | 185 | 100% | 158 | Closed |
| Cold shower | 0:10 | 50 | 50 | 0% | Auto |

DB, dry bulb temperature; WB, wet bulb temperature; IT, internal temperature.

| Table 2. Mean and Standard Error of Mean for Survival of Methicillin-Resistant Staphylococcus aureus in Frankfurters, Summer Sausage, and Boneless Ham |
|-----------------|-----------------|-----------------|
| Treatment | Frankfurters | Summer sausage | Boneless ham |
| Negative | 1.70 ± 0.10a | 1.70 ± 0.10a | <1.70a |
| Positive | 7.76 ± 0.10b | 7.75 ± 0.09b | 7.73 ± 0.24b |
| Fermented (45°C) | 3.75 ± 0.32c | 3.75 ± 0.32c | |
| Cooked (79.5°C) | 2.23 ± 1.06c | 0.93 ± 0.69a,d | 0.45 ± 0.73c |

Means in a column with different superscripts are statistically different (p < 0.05).

CFU, colony-forming units.
uncooked, positive control. As expected, populations of MRSA were not detected in the negative control. Cook and chill time for the experiment was not significant for the results of the main treatment effects.

**Summer sausage**

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 uncooked, positive controls. Growth was not detected \((<50\,\text{CFU/g})\) for the negative control group. The average starting \(\text{pH}\) of the raw sausages prior to fermentation was 6.02, with a range of 0.2. The average 12-h \(\text{pH}\) after fermentation was calculated to be 4.32 with a range of 0.05. Cook/chill times and \(\text{pH}\) were not significantly different between replications and did not impact the fixed main treatment effects.

**Boneless ham**

The results of the boneless ham experiment are shown in Table 2. There was a significant effect \((p<0.001)\) of the thermal treatment when compared to the uncooked, positive control, and demonstrated an average \(\log_{10}\) reduction of 7.28. The results of the cooked, inoculated 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 were well within the 15-h time limit for option 3 in FSIS–Appendix B (USDA-FSIS, 1999). Cook/chill times were not a significant effect in the model.

**Discussion**

**Frankfurters**

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 from the experiment described above. This is probably attributed to the fact that the Heiszler and Palumbo studies more closely mimicked large-scale commercial manufacturing of frankfurters (30 min versus 95 min). Heiszler et al. (1972) showed the greatest reduction of surviving microorganisms at an internal temperature of 60\(^\circ\)C, but the microorganisms continued to decrease with increasing temperatures at the end of processing. 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 the compared 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 was not different from \(S.\) aureus and should not be of great concern to consumers in these types of processed meats.

**Summer sausage**

Since many strains of enterotoxin producing \(S.\) aureus have the ability to survive at varying salt concentrations, \(\text{pH}\), and water activities, these intrinsic and extrinsic factors are manipulated and monitored by meat processors during the production and storage of dry and semidry sausages. Results from Ingham et al. (2005) indicate that fermented, commercial summer sausages range in \(\text{pH}\) from 4.4 to 4.9, which was slightly higher than the \(\text{pH}\) measured in the current study. The numbers of viable cells is not nearly as important as enterotoxin production in fermented sausages. Extent and rate of \(\text{pH}\) decline in fermented dry and semidry sausages appear to be the main factors in controlling toxin production (Genngeorgis et al., 1969). Also, \(S.\) aureus does not compete well with other bacterial populations (McCoy, 1965), which suggests that dry and semidry 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**

Although thermal processing of large-diameter meat products such as 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. (2005) 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. (2005) should be considered for processed meats thermally processed using slow-cooking procedures.

**Conclusions**

The results of this study indicate that thermal processing and subsequent chilling of frankfurters, summer sausage, and boneless ham allowed for at least a 5.5 \(\log_{10}\) reduction of MRSA when cooked to an internal temperature of 70\(^\circ\)C. This is important when considering the risk of foodborne illness from MRSA in processed meats, since the literature indicates that MRSA contamination of fresh pork is very low \((<100\,\text{cells per gram of meat})\). Reduced \(\text{pH}\) products and larger-diameter processed meats showed an increased safety level with regard to survival of MRSA colonies. It is important to consider good manufacturing practices for fermentation degree-hours in reduced \(\text{pH}\) products fermented with starter cultures. Although acid production and subsequent thermal processing drastically reduce survival of viable organisms, improper fermentation procedures or failed fermentations could allow for ideal conditions for enterotoxin production by MRSA. Adequate temperature control, proper sanitation, and prevention of cross-contamination by food handlers are still

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### Table 3. Average Cook and Chill Times for Frankfurters, Summer Sausage, and Boneless Ham

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Frankfurters</th>
<th>Summer sausage</th>
<th>Boneless ham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to 70°C</td>
<td>26.18±0.25</td>
<td>39.95±7.97</td>
<td>330±10</td>
</tr>
<tr>
<td>Time to 7.2°C</td>
<td>29.56±6.56</td>
<td>52.35±12.30</td>
<td>439±21</td>
</tr>
</tbody>
</table>

Values are mean±standard deviation.
the main components for reduced risk of foodborne illness by MRSA.

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Disclosure Statement

No competing financial interests exist.

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