Graduate Theses and Dissertations

Iowa State University Capstones, Theses and Dissertations

2012

Survival of methicillin-resistant Staphylococcus aureus during various thermal lethality processes used to manufacture pork products

Jonathan Alexander Campbell
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/etd

Part of the Agriculture Commons, Animal Sciences Commons, and the Microbiology Commons

**Survival of methicillin-resistant *Staphylococcus aureus* during various
thermal lethality processes used to manufacture pork products**

by

Jonathan Alexander Campbell

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Meat Science

Program of Study Committee:
Joseph Cordray, Major Professor
James Dickson
Aubrey Mendonca
Dennis Olson
Kenneth Prusa

Iowa State University

Ames, Iowa

2012

DEDICATION

This work is dedicated to my fiancée, Andrea Beisser, whose love and support helped me to complete this project. Her encouragement and presence in my life is appreciated and will be cherished. I look forward to our life together. A special "thank you" is also due to my mom and dad. I love you both.

TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION

Human health professionals have long been concerned with microorganism gaining resistance to antimicrobials and antibiotics commonly used to treat and prevent infections. Antibiotic resistant bacteria have a genetic make-up different than antibiotic susceptible organisms, which allows them to survive antibiotic treatments and other potential stressors in their environment that would have otherwise been lethal. Methicillin-resistant *Staphylococcus* aureus, or MRSA, is just one of many species of bacteria that have adapted to a specific class of antibiotics. MRSA has gained notoriety over other antibiotic resistant species, because it seems to be so ubiquitous in our environment.

Bacterial resistance not only affects human health, but also domesticated pets and livestock. Antibiotics used to treat disease in pets and livestock are similar, and in some cases exactly the same, as those antibiotics prescribed by physicians in human health. This practice is not alarming, since the common mircoflora of both humans and animals can be identical. If a drug works well in one animal for a specific organism of interest causing an infection, it makes sense that the same antibiotic or antimicrobial will be as effective for the same bacteria in a different host species.

The main problem with antibiotic resistance in livestock species is the fact that they are utilized for meat and milk. The issue of antibiotic resistance in livestock and poultry health is very complex when compared to human health, because of the fact that they provide essential nutrition for humans. Livestock

and poultry species have sustained human life for generations, and ensuring the safety of these commodities is paramount to support life for future generations.

Production practices for livestock have also changed since the first meat animal species were domesticated. Livestock and poultry production practices are managed more intensely today. Production operations, along with genetic selection, allow for increased growth and efficiency of the species being produced for meat or milk. Modern animal production farms are very large in scale and productivity when compared to the smaller, but more numerous, family farms in this country at the turn of the 20th century. Opponents of modern animal agriculture would argue that it is these production practices that now threaten human life instead of preserving it.

Meat and poultry, especially pork, have been found to be positive for MRSA, and prevalence of MRSA in meat at the retail level is much higher than originally thought. This can be quite alarming since the mechanism of how this is occurring is not well understood. *Staphylococcal aureus* intoxication has long been associated with foodborne illness around the world, however, very little is known about it's antibiotic resistant relative with respect to food safety. Although earlier studies sought to discover the presence of MRSA in meat, the survival of MRSA during thermal processing is not well understood. Therefore, the overall objective of this research was to determine the potential for MRSA survival during thermal lethality processes common to produce pork. The first objective investigated the survival of MRSA during commercial thermal processing. The

second objective evaluated MRSA survival during common consumer cooking methods for pork.

Dissertation Organization

This dissertation is organized into five chapters. Chapter 1 is a general introduction. Chapter 2 is a review of literature pertinent to the objectives of this research project. Chapter 3 is a manuscript to be submitted to *Foodborne Pathogens and Disease* entitled "Survival of methicillin-resistant *Staphylococcus aureus* during commercial thermal processes for frankfurters, summer sausage and ham". Chapter 4 is a manuscript to be submitted to *Journal of Food Protection* entitled "Survival of methicillin-resistant *Staphylococcus aureus* during commercial heat treatment of slab bacon and consumer frying of sliced bacon". References, figures and tables referred to in the literature review and manuscripts will be at the end of each chapter. Chapter 5 provides a general summary of the research project and final conclusions for the obtained results.

CHAPTER 2. LITERATURE REVIEW

Staphylococcus

Genus

The genus *Staphylococcus* is a group of microorganisms that test positive for gram staining and appear, under the microscope, blue to purple in color from the absorption of the stain by the thick peptidoglycan layer component of the bacterial cell wall (Reaveley and Burge 1972, Beveridge 2000). *Staphylococci* are small cocci or round cells that are usually arranged in pairs and small irregular clusters of cells (3 to 4). The word *Staphylococcus* stems from the Greek word "σταφύλια" (*staphylia),* which means "bunch of grapes" (Stavropoulos and Hornby 2008). This is why some texts and documents refer to these organisms as being arranged in "grape-like clusters" of cells (ICMSF 1996, FDA 2012[a]). *Staphylococci* are facultative anaerobes, which mean these organisms can utilize both aerobic and anaerobic metabolic pathways, depending on the presence or absence of oxygen in their environment. Organisms from this genus are catalase positive, or have the ability to change hydrogen peroxide ($2\ H_2O_2$) into water ($2\ H_2O$) and oxygen (O_2). Staphylococci have a low percentage (30% – 39%) of guanine plus cytosine (G+C) in their genetic make-up when compared to other gram positive cocci (Prescott *et al.* 1996).

Taxonomy

Some people still recognize bacteria as either 'good' or 'bad', but modern classification of microorganisms didn't really begin until the American

society for microbiology published the first edition of Bergey's Manual in 1923 (Bergey 1923). That first edition of the manual listed only six species of the genus *Staphylococcus*. Today, almost forty species (depending on the source) are assigned to the genus by well-defined genotypic and phenotypic criteria (De Vos *et al*. 2009). Genotypic evaluation of all species can be performed using ribosomal RNA. The ribosome is the site of protein synthesis in a cell. Literature refers to the use of the 16s subunit of rRNA (Takahashi *et al.* 1999) to be able to sequence and classify members of the *Staphylococcus* genus. The 16s genetic code is unique for each identified species and is compared to a known database during evaluation and naming. Even though numerous species, sub-species and strains have been identified since Bergey's systematic approach began, only two species (*S. aureus* and *S. epidermis*) are really known to be associated with human diseases.

Staphylococcus spp. causing human disease

Staphylococcus epidermis

One of the two *Staphylococcal* species associated with human disease and infection is *S. epidermis. S. epidermis* is a lesser known pathogen recognized by the general public, and as its name suggests, is commonly found on the skin of humans. *S. epidermis* has been associated with infections of the heart valves, skin lesions and other minor infections (Heilmann and Peters 2000). *S. epidermis* does not possess the ability to clot rabbit plasma and is classified in a *Staphylococcal* group as coagulase negative. The coagulase test is a phenotypic method used to classify *Staphylococcal* species and will be explained

in more detail in a following section. According to a review by Kloos and Bannerman (1994), most infections caused by coagulase negative *Staphylococci* (mainly *S. epidermis*) are acquired in a nosocomial setting.

Staphylococcus aureus

The second and more severe pathogenic *Staphylococcal* species associated with human disease is *S. aureus.* According to Prescott *et al.* (1996), "*S. aureus* is the most important human *Staphylococcal* pathogen". *S. aureus* is resistant to high salt concentrations (10-15%) and temperatures of 50°C or more (Smith and Marmer 1991). This ubiquitous microorganism is commonly found in the nasal passage, mucous membranes and on the skin of humans (Williams 1963). *S. aureus is* non-motile, non-spore forming and infections tend to be pyogenic or pus forming in nature. Unlike *S. epidermis, S. aureus* is coagulase positive. Human *S. aureus* infections included the following: impetigo, folliculitis, endocarditis, pneumonia and various bone and joint infections.

The Coagulase test

The coagulase test tends to be one of the main determinants for classifying unknown species of *Staphylococci*. The ability to clot rabbit plasma in a test tube or on a microscope slide is a widely accepted method to phenotypically identify unknown, gram positive cocci as *S. aureus*. Clotting factors of the enzyme's activity react with plasma components fibrinogen and/or thrombin. Agglutination or clumping factor is a visible reaction between the bound coagulase (intracellular) and fibrinogen component of the plasma. These two components cross link to form fibrin on the cell wall of the bacterial cell and

immediately allow cocci to bind together or "clump" and separate from the remaining plasma fraction.

Extracellular coagulase reacts similarly with thrombin in test tube plasma. The combined thrombin allows reaction with fibrinogen to again cross link and form fibrin. The clumping or grouping of cocci in a tube is similar to results seen on a slide (Arvidson 2000).

Prevalence and carriage of *S. aureus*

S. aureus is very prevalent in our society, especially in health care facilities, and carriage rates of human populations have been reported to range between 29% – 51% (Williams 1963, Wenzel and Perl 1995). High risk groups, like health care workers and hospital employees, have been documented to have carriage rates as high as 90% (Tenover and Gaynes 2000). Results between *S. aureus* carriage studies of healthy human populations can vary depending on how swab samples were taken and the anatomical location of the sample. Prevalence of *S. aureus* carriage seems to be affected by age and health of normal populations, but does not appear to have seasonality trends (Noble *et al. 1967)*. Infections caused by *S. aureus* have "higher morbidity and mortality" when compared to other bacteremia rates (Naber 2009).

<p align="center">***S. aureus* Infections in Humans**</p>

*S. aureus i*nfections in humans are thought to be initiated by invasion of the bacteria through a wound, open skin or soft tissue. This pathogen is also associated with infections from medical procedures, such as surgical incisions and catheterizations. Infection appears to be linked to carriage of the bacteria.

Impetigo

Impetigo is a highly contagious skin infection commonly seen in infants and young children. This type of infection can be caused by *S. aureus* and may result from skin associated trauma, for example an abrasion from falling, an insect or animal bite and other disruptions of the outer dermal layer, allowing opportunistic bacteria to invade. Symptoms of the infection appear as large, pus filled blisters or bullae (Vorvick 2010) usually on the face and around the mouth. Once breakage of these bullae occurs, the remaining infected area appears as a red, sometimes itchy rash that can easily be propagated by scratching. Other symptoms include swelling of the lymph nodes and skin lesions. Antibacterial creams are prescribed as treatment, and in more severe infections, antibiotics will be administered orally (WebMD 2010).

Folliculitis

Folliculitis is a dermatological infection of the skin and surrounding hair follicle. Causes of this infection include cuts from shaving and severe friction from clothing rubbing against the skin causing potential irritation and blockages of the hair follicle. Symptoms include small pustules around an infected hair follicle or a red, itchy rash on the skin surrounding the follicle. Treatment of these minor skin infections is usually non-invasive, since the infected skin area is usually localized. Antibiotic ointments, topical antifungal creams and hot compresses are common treatments with symptoms subsiding in two to three days (Vorvick and Zieve 2010).

Bacterial Endocarditis

Endocarditis is a severe and invasive infection of the innermost layer of the heart and heart valves caused by *S. aureus*. This severe illness usually occurs from bacterial carriage of *S. aureus* entering the blood stream and attaching to the surface of a congenital malformation of the heart muscle or defective valve. Routine oral prophylaxis is a major source of *S. aureus* entering the circulatory system. Once infection is diagnosed, severe damage to the heart and heart valves can occur from the propagation of *S. aureus* in the affected area. Symptoms of bacterial endocarditis usually begin with high, persistent fever and also include loss of appetite, joint pain and general lethargy. According to Brookfield (1994), bacterial endocarditis may result from any number of heart related malformations, "but is most common in aortic valve lesions." An antibiotic administered orally is generally the prescribed method for prevention prior to dental visits for children and young adults with known congenital heart defects. Treatment of this infection is usually fairly invasive and requires intravenous antibiotics to be administered by a health professional (Brookfield 1994, Fox 2010).

Bone and Joint Infections

Osteomyelitis and septic arthritis are two of the most common bone and joint afflictions caused by an *S. aureus* infection. In many cases, osteomyelitis occurs as a result of a secondary infection from *S. aureus* in the vascular system. According to a recent article in *Best Practice and Research Clinical Rheumatology*, the majority of clinical cases occur in the feet, vertebral column

and long bones of the human body (Sia and Berbari 2006). Individuals with increased risk factors for osteomyelitis include patients recovering from surgery, those with diabetes, the immunocompromised and habitual intravenous drug users (Chihara and Segreti 2010). Early onset symptoms are similar to the flu and include joint pain, high fever, chills and general lethargy. Although infection rates are low due to the protective properties of bone, morbidity of the disease can be significant. In many cases, the infection is not localized but spreads to infect other soft tissue areas surrounding the initial bone site. Diagnosis is usually confirmed via blood test for suspecting microorganisms, bone biopsy and the use of magnetic resonance imaging. The infection is usually treated with intravenous antibiotics for four to six weeks. Recovery is slow and relapse rates of infection are estimated to be around 20% and depends on the risk factors of the individual afflicted (Sia and Berbari 2006, Chihara and   Segreti 2010).

Septic arthritis or infectious arthritis is an infection and inflammation of the joint caused by *S. aureus*. Infection usually occurs as a result of joint surgery or can be spread directly to the joint by the vascular system. According to Dugdale *et al.* (2011), "the most common sites for … infection are the knee and hip." Prompt diagnosis and subsequent antibiotic therapy is the key for a good prognosis of the patient (Dugdale *et al.* 2011).

Pneumonia

Bacterial pneumonia, an infection of the lungs, is predominantly caused by *S. aureus*. Those individuals most at risk for contracting bacterial pneumonia are the elderly (> 65 years of age) and infants under the age of two. Pneumonia is

typically grouped into categories by origination of the infection. Kollef and Micek (2005), give examples of these groups by describing pneumonia contracted in nosocomial settings, health care facilities or at home. According to CDC's National Center for Health Statistics (2012[b]), greater than 1 million patients are hospitalized for pneumonia annually and the nosocomial mortality rate is around 3.4%. Diagnosis occurs through the use of x-ray technology, sputum culture results and blood test confirmation. As with most bacterial infections, antibiotic therapy is the preferred treatment (Kollef and Micek 2005).

S. aureus has been also been researched for disease caused by the toxin some strains produced. Several toxins are produced by *S. aureus* and cause a wide variety of illnesses.

S. aureus toxin production

S. aureus produces a number of toxins both inside and outside of the cell that cause human illness. Some of the toxins that are produced include alpha, beta, gamma, delta and leucocidin. Most of these cell toxins cause damage to the plasma membrane of healthy cells, which ultimately leads to damage of healthy tissues and cell death (Fox 2010).

Alpha toxin

Alpha (α) toxin, a short chain protein secreted by most *S. aureus* strains, was the first pore forming toxin discovered in the bacterial species. A-toxin interacts directly with healthy cell membranes by integrating itself into the plasma membrane and creating a channel or "pore" for free ion exchange into and out of the cell, typically human epithelial cells. This channel creates a severe osmotic

imbalance and exchange of ions and ultimately results in lysis of the cell. (Bhakdii and Tranum-Jensen 1991, Bohach and Foster 2000, Fox 2010).

Beta toxin

Beta (β) toxin is another virulence factor located on the cell surface and secreted outside of the bacterial cell by some strains of *S. aureus*. This small protein (~34KDa) is recognized by researchers as the "hot-cold toxin" (Huseby *et al.* 2007), because of the protein's unique ability to bind with sheep blood cells at 37°C, but not lyse them until the erythrocytes are maintained at 4°C. B–toxin is also known for its neutral sphingomyelinase activity, which attaches to and damages lipids in the plasma membrane of cells. The enzyme activity is enhanced in the presence of certain cations, for example Ca^{2+} (Bohach and Foster 2000, Huseby *et al.* 2007, Fox 2010).

Gamma toxin

Gamma (Γ) toxin, like a-toxin, is a pore forming protein made by most strains of *S. aureus* that damages the plasma membrane of healthy neutrophils and macrophages (Fox 2010). Γ-toxin differs from a-toxin (single chain) in that it is made up of two polypeptide chains (S and F). These protein side chains are encoded for genetically by the *hlg* loci (HlgA = S chain and HlgB = F chain) that is carried by most *S. aureus* strains. These two proteins are categorized by ion exchange to be either fast (F) or slow (S). These two proteins are hemolytic in nature and work synergistically to disrupt cell homeostasis. Γ-toxin has also been found to interact with other *Staph.* toxins that cause specific human illnesses (Nilsson *et al.* 1999, Bohach and Foster 2000, Fox 2010).

Delta toxin

Delta (Δ) toxins are small proteins produced by most strains of *S. aureus* that damage the lipid bi-layer of cell membranes. Δ-toxin acts like a detergent when interacting with cell membranes and causes "cytoplasmic leakage and lysis of cells" (Bohach and Foster 2000). Similar to Γ-toxin, Δ-toxin enhances other factors related to human disease (Schmitz *et al.* 1997, Bohach and Foster 2000, Fox 2010).

Leukocidins

Leukocidins are very similar in structure, function and genetic encoding as Γ-toxin. Instead of the *hlg* loci, leukocidins are encoded for by the *luk* gene, a virulence factor not common to most strains of *S. aureus*. Unlike Γ-toxin, however, leukocidin does not posses hemolytic capability (Bohach and Foster 2000, Fox 2010).

S. aureus toxin mediated disease

Many human illnesses are initiated by *S. aureus* infections, but some of the most feared are those diseases initiated by toxins that the microorganism produces. *S. aureus* has been extensively researched for both exotoxin and enterotoxin production with regard to human diseases mediated by these toxic proteins.

Toxic Shock Syndrome

Toxic Shock Syndrome (TSS) is a human disease initiated by the superantigen TSST-1. Toxic Shock Syndrome Toxin is historically associated with tampon use by females during menstruation, but TSS can also be

enterotoxicosis is contracted by consuming food that is contaminated by SEs secreted into the food by various toxin producing strains of *S. aureus*. According to Cassman (1965), SEA is the most common toxin related to SE food poisoning, accounting for almost 78% of all cases investigated. Table 1 lists the types of SEs and the gene that encodes for these single chain polypeptide superantigens.

Table 1. List of SE superantigens and encoding genes

Staphylococcal Enterotoxin	# of Amino Acids	Gene
SE-A	*233*	*entA*
SE-B	*240*	*entB*
SE-C Subtypes: SE-C1, SE-C2 & SE-C3	*C1-230* *C2-239* *C3-240*	*entC1, entC2 & entC3*
SE-D	*228*	*entD*
SE-E	—	*entE*
SE-G	*233*	*entG*
SE-H	—	*entH*
SE-I	*218*	*entI*
SE-J	*248*	*entJ (same plasmid as SE-D)*

Source: Balaban, N and Rasooley, A. 2000. Intl J Food Micro 61(1)1-10.

Symptoms of Enterotoxicosis

According to Bennett and Hait (2011), clinical symptoms of the intoxication occur within two to six hours after ingesting the toxin. It is unknown what the

thresholds for each toxin are, but it was estimated that patients who became ill ingested at least 100ng of the virulent protein (Evenson *et al.* 1988). In addition to surviving very high temperatures (boiling for 30 min), enterotoxins are resistant to normal digestive proteases (e.g. pepsin and trypsin) found in the gastrointestinal tract to help digest proteins. Signs of the intoxication include nausea, vomiting, diarrhea and gastroenteritis, however, no fever is observed. Since enterotoxicosis does not involve viable pathogens, antibiotics are not necessary to treat symptoms. Fluid therapy to prevent dehydration is generally the means of treatment (Fox 2010).

Enterocolitis

Enterocolitis is a condition similar to *Staphylococcal* food poisoning with one major difference, the infection of viable *S. aureus* cells in the gut. Symptoms of this infection are similar to that of enterotoxicosis, but include fever. Since this illness is associated with live bacteria, antibiotic therapy is the preferred course of treatment after positive identification of the microorganism has been confirmed from the patient's stool sample (Fox 2010).

Foodborne Illness in the United States

Foodborne illness in the United States is a major concern for public health experts. Although regulations for food processors are constantly increasing and more restrictive legislation passed to secure the safety of the food supply, millions of people report illness from food consumption annually. It has been estimated that 75 – 80 million people become ill each year from consuming food products (Mead *et al.* 1999, Balaban and Rasooley 2000). According to CDC

(2011) however, it was estimated that human illness in 2011 from foodborne agents would be around 48 million cases. Foodborne illnesses result in 5,000 – 9,000 deaths annually with an economic impact estimated to be greater than 6 billion dollars to the industry (Altekruse 1997, Buzby and Roberts 1997, Mead *et al.* 1999). Unfortunately, underreporting of illness makes these estimates and projected economic figures inaccurate (Mead *et al.* 1999). There are many reasons why a consumer would choose not to report an illness contracted from a food product, but this major flaw still lies in the heart of how foodborne illness data are gathered, investigated and reported.

Burden of Illness pyramid

The burden of illness pyramid is a model to outline how foodborne illnesses are reported in the United States (Figure 1). Centers for disease control actively monitor foodborne illness cases through the active surveillance network called FoodNet (CDC 2012[a]). Unfortunately, active surveillance alone represents only a small portion of a specific population that became ill. An "outbreak" is defined as two or more people presenting to a medical professional with common symptoms and food commonalities (CDC 2012[a]).

The pyramid model was designed to encompass a more accurate estimate of the population by providing data from the general population, physician questionnaires and laboratory surveys as foodborne illness data moves up the pyramid (FSIS 1998).

One of the main flaws with any model is estimating for unknowns. In the burden of illness pyramid model, FoodNet attempts to estimate for

underreporting by consumers and illnesses contracted from unknown agents. Underreporting can occur at any step in the pyramid model, but would generally begin at step three of the pyramid (FSIS 1998, CDC 2012[a]). Step three, "seeking medical care", may be avoided by consumers for many reasons. Using *S. aureus* as an example, the quick onset and duration of an intoxication episode could lead to an individual enduring the symptoms without seeking medical assistance. This type of underreporting of illness decreases the accuracy of actual incidence of foodborne illness in a population (Mead *et al.* 1999) and could be much higher than believed for an organism like *S. aureus*.

Figure 1. Burden of Illness Pyramid
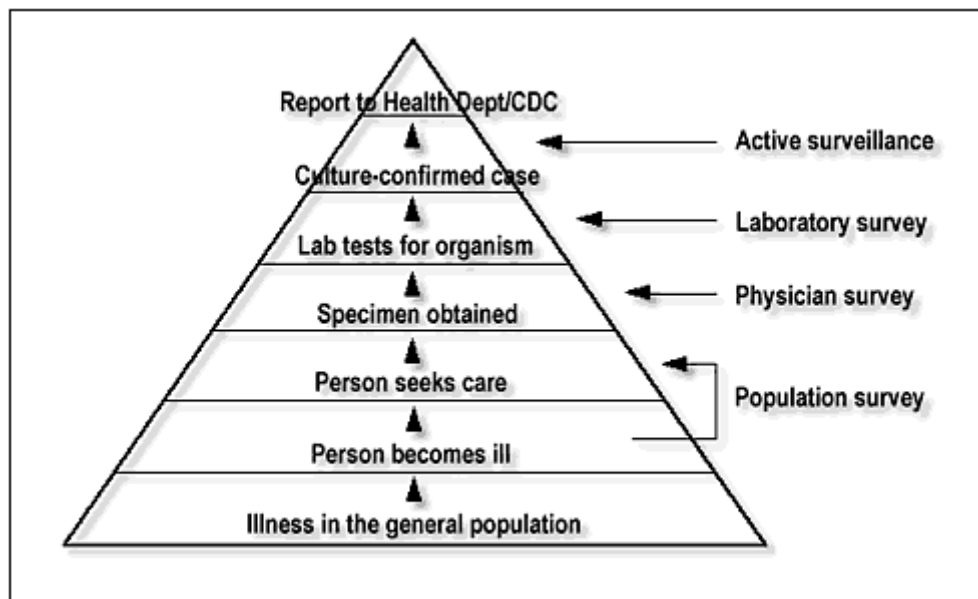
Source: USDA Food Safety & Inspection Service 2008
http://www.fsis.usda.gov/ophs/rpcong98/rpcong98.htm

Incidence of *S. aureus* Foodborne Illness

According to a review by Balaban and Rasooley (2000), *S. aureus* and SE production in food was one of the most common (2nd) causes of foodborne illness in the United States. This trend in disease from consumption of contaminated food products is fairly static from the years 1983 through the mid 1990s and then rates appear to decrease in the number of cases reported (Bean *et al.* 1990, 1996). Table 2 shows CDC estimates for foodborne illness in the U.S. for 2011.

Table 2. Estimated annual number of domestically acquired, foodborne illnesses, hospitalizations, and deaths due to 31 pathogens and unspecified agents transmitted through food, United States.

Foodborne Agents	Estimated annual number of illnesses (90% credible interval)	%	Estimated annual number of hospitalizations (90% credible interval)	%	Estimated annual number of deaths (90% credible interval)	%
31 known pathogens	9.4 million (6.6–12.7 million)	20	55,961 (39,534–75,741)	44	1,351 (712–2,268)	44
Unspecified agents	38.4 million (19.8–61.2 million)	80	71,878 (9,924–157,340)	56	1,686 (369–3,338)	56
Total	47.8 million (28.7–71.1 million)	100	127,839 (62,529–215,562)	100		100

Source: CDC 2011. http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html.

More recent trends for foodborne illness in the U.S. indicate that *S. aureus* has dropped from near the top of causal food agents to the fifth most common pathogen known to be attributed to foodborne illness. Table 3 outlines the current top five pathogens attributed to foodborne illness in the United States (CDC 2011).

Although these estimates seem to show that *S. aureus* mediated illness via food consumption has decreased over the past few decades, actual percent of reported cases has remained fairly steady at around 2.5 to 3% (Bean *et al.*

Table 3. Top five pathogens contributing to domestically acquired foodborne illnesses

Pathogen	Estimated number of illnesses	%
Norovirus	5,461,731	58
Salmonella, nontyphoidal	1,027,561	11
Clostridium perfringens	965,958	10
Campylobacter spp.	845,024	9
Staphylococcus aureus	241,148	3
Subtotal	8,541,422	91

Source: CDC 2011. http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html.

1990, 1996, CDC 2011). Also, rates of foodborne illness attributed to the pathogen *S. aureus* could be deceiving due to severe underreporting of illness by affected consumers due to the short onset (2 to 6h) and duration of symptoms (24-48h) (Mead *et al.* 1999, Bohach and Foster 2000). Since the main course of treatment for enteritis caused by SE is fluid therapy to prevent dehydration, patients may choose to suffer through milder symptoms while providing appropriate auto-replenishing of lost fluids. This makes accurate reporting of the intoxication difficult.

Foods contributing to *S. aureus* foodborne illness

American consumers have drastically changed their eating habits in the past twenty to thirty years. According to Doyle (2010), "About one-third of food dollars was spent eating away from home in 1970, whereas in 1996, 46% of food

dollars went to meals and snacks prepared outside the home." From this dramatic increase in food eaten outside of the home, it could be perceived that restaurants and food service establishments possess most of the burden of foodborne illness. Wieneke *et al.* (1993) however, indicated that most foodborne illness attributed to *S. aureus* or SE occurred in the home. It was concluded from this study that over 75% of food mediated intoxication was attributed to meat and poultry products (Wieneke *et al.* 1993). Ham, probably because of its high salt content and slow thermal processing times, appears to be a common meat source for *Staphylococcal* foodborne illness worldwide (Wieneke *et al.* 1993, Ward *et al.* 1997).

Fermented and dried sausages are also a potential ideal medium for *Staphylococcal* and enterotoxin contamination caused from improper fermentation rates (AMI 1997). Since *S. aureus* does not compete well with other microorganisms and is tolerant of a wide range of intrinsic factors (pH and a_w), processed meats serve as an ideal growth medium for *S. aureus* due to the lack of competing microflora (*Daly et al.* 1973, *Lowy* 2010).

Prevention of *S. aureus* foodborne illness

Since little medical treatment is available for patients experiencing symptoms of food intoxication, prevention of the affliction is a far better option. Prevention of *S. aureus* foodborne illness begins with sanitation. According to Bennett and Lancette (2001), *S. aureus* and SE in food is "generally an indication of poor sanitation." Many staphylococcal species have the ability to form biofilms

in a processing environment (Heilmann and Peters 2000). This makes sanitation more difficult.

Temperature control during storage and distribution of meat and meat products is critical to control the outgrowth of *S. aureus* and other pathogens (FDA 2001). Safe handling and prevention of temperature abuse during transport can prevent incidence of foodborne illness (Olsen *et al.* 2000, McCabe-Sellers 2004).

Sources of *S. aureus* contamination are numerous because of the ubiquity of the microorganism. Some common sources to contaminate meat and meat products include the following: livestock to be slaughtered, processing environments, food workers, home kitchens and consumers. With sources of the pathogen so numerous, personal hygiene is critical to prevent cross contamination of meat and meat products. Figure 2 graphically depicts the contributing factors attributing to foodborne illness in the United States from 1993-1997 (McCabe-Sellers 2004).

Antibiotic Resistant *S. aureus*

Methicillin-resistant *Staphylococcus aureus*, also known as MRSA, is a variant strain of *S. aureus* that is also commonly found in the nasal passage, mucous membranes and on the skin of humans (Wenzel and Perl 1995, Zetola *et al.* 2005). MRSA is resistant to antibiotic therapies normally administered to combat *S. aureus* infections. Some of the therapies used for *S. aureus* include methicillin (no longer used in human medicine), penicillin and other beta-lactam

Figure 2. Contributing factors to foodborne outbreaks from 1993-1997.
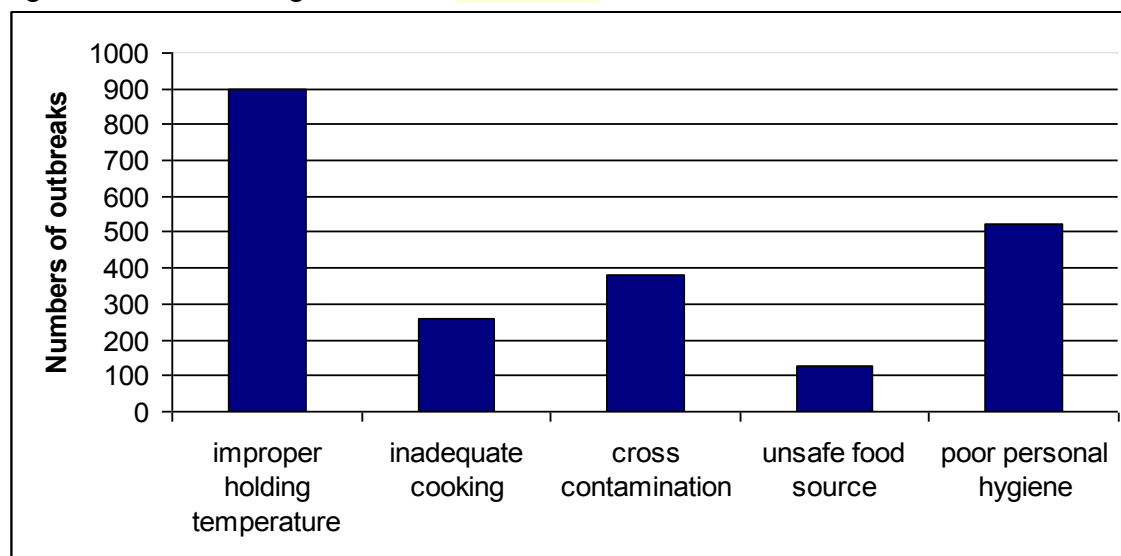
Source: McCabe-Sellers 2004, JADA 104(11):1708-1717.

antibiotics. 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.

Methicillin and other β-lactam antibiotics

Methicillin, like most β-lactam antibiotics, is in the penicillin class of drugs that act on pathogenic microorganisms by preventing cell wall synthesis and causing cell death. Methicillin, or meticillin, was developed by George Rolinson and Ralph Batchelor in 1959 by a UK company, Beecham Research Laboratories, LTD. (Rolinson *et al.* 1960, Dutfield 2009). The "new penicillin (BRL 1241)" was originally produced under the name "Celbenin" (methicillin) to treat penicillin resistant *S. aureus* infections (Knox 1960). Celbenin was soon replaced by oxacillin due to the severe nephritis and liver damage it caused in human

subjects. Although no longer utilized in human medicine, Celbenin (methicillin) is useful in determining drug sensitivities in laboratory studies.

β-lactam antibiotics work to inhibit bacterial cell wall synthesis by binding to transpeptidase enzymes located on the surface of most gram positive bacteria (Waxman and Strominger 1983). These transpeptidase enzymes, also known as penicillin binding proteins (PBPs), allow cross-linking of the D-alanyl-alanine peptide that forms the peptidoglycan layer of the cell wall. PBPs have a higher affinity for β-lactams and result in competition for binding sites on PBPs. Once the PBP binding site is blocked, inhibition of cross-linking of the peptidoglycan layer occurs resulting in bacterial cell death (Tomasz 1979). *S. aureus* strains resistant to this antibiotic action have genetically and conformationally different PBP sites that decreases the sensitivity to the antibiotic.

Mechanism for resistance

Resistance to an antibiotic by bacteria generally occurs through a change in the genetic make-up of the organism. Resistance genes can be translated vertically or horizontally. Vertical resistance results from a mutation to the genetic code responsible for, in the case of *S. aureus*, penicillin binding protein. This genetic mutation is in response to the antimicrobial agent being introduced into the organism's environment. Horizontal mutation occurs when genes carried by a plasmid are introduced from outside of the cell resulting in the bacteria incorporating the genetic code for resistance (Fuda *et al.* 2004, Mayer 2010).

Resistant strains of *S. aureus* possess the *Staphylococcal* chromosomal cassette (SCC) *mec* gene. The *mec*A gene encodes for a variant penicillin

binding protein (PBP-2α) which is conformationally different from PBP of *S. aureus* strains sensitive to β-lactam antibiotics (Enright *et al.* 2002). PBP-2α has a lower affinity for penicillin antibiotics than that of regular PBP and is therefore "refractory of the action all available β-lactam antibiotics" (Fuda *et al.* 2004).

History of MRSA

MRSA was first discovered in the UK in 1961, just two years after beginning clinical use of the drug methicillin (Enright *et al.* 2002). Discovery of MRSA in the United States did not occur until the late 1960's at Boston City Hospital (Barrett *et al.* 1968). Since its discovery more than fifty years ago, MRSA has emerged as one of the leading and most severe infectious microorganisms acquired from exposure in health care settings (CDC 2003). MRSA colonization and infections have recently evolved from being solely acquired in a health care setting to that of a community acquired (CA) 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 with MRSA (Wenzel and Perl, 1995).

CA-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 areas. Clinical signs include furunculosis, multiple infected boils, and necrosis of the skin (Zetola *et al.*, 2005). 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). Voss *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.

MRSA Confirmatory Testing and Identification

Multilocus sequence typing, or MLST, is commonly used to positively identify specific strains of unknown pathogens, including MRSA. This technology was proposed in 1988 by Maiden *et al.* (1998) as a "portable method" to confirm, identify and store sequence chromosomal DNA in a database for specific isolates (Maiden 2006). Polymerase chain reaction (PCR) is utilized to amplify a specific DNA section of internal genes (~ 450-500bp). For most bacteria, there are seven loci that are sequenced to generate a specific profile or sequence type for the unknown isolate. The allele sequence generated can then be compared to the database of known isolates to "unambiguously type" the pathogen (Urwin and Maiden 2003).

MRSA can also be positively identified by analyzing for presence of the *mec*A gene. The *mec*A gene, as previously discussed, is involved in the genetic encoding for a variant penicillin binding protein (2α) that inhibits the action of β-

lactam antibiotics. The problem with the *mec*A gene solely used for identification of MRSA is that some strains still show clinical signs for resistance to methicillin and other β-lactams in the absence of this gene and PBP2α (Bignardi *et al.* 1996). MLST results are more definitive, but are at a significant cost increase when compared to genetic testing for *mec*A.

A third confirmatory method for positively identifying MRSA is the *luk* gene. Panton-Valentine leukocidin, or PVL, is a virulence factor for some MRSA strains (CA-MRSAs). As previously discussed, leukocidin is a toxin secreted by the pathogen in two possible superantigen forms (lukS and lukF). These two exotoxins work synergistically to destroy white blood cells and by attacking soft tissues. According to Boubaker *et al.* (2004), PVL is carried by less than five percent of all known *S. aureus* isolates. This virulence factor has been linked to many antibiotic resistant strains of *S. aureus* causing CA-MRSA infections, necrotizing Pneumonia and other severe skin infections (Boubaker *et al.* 2004, Berglund *et al.* 2008).

MRSA in Food Producing animals

Although originally thought to be rare, MRSA has 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 *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, along 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.

In contrast to these results indicating a one-way zoonotic link between hog confinement operations and human disease, Price *et al.* (2012) describes an alternative theory to livestock and community associated MRSA in humans. This complicated genomic study clearly showed that a MRSA strain (CC 398) 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 (Price *et al.* 2012). This ground breaking genetic work on MRSA transmission is the first and only report of its kind to date.

Prevalence of MRSA in pork

More alarming than a bacterium becoming part of a pig's common nasal microflora is its potential effect on consumer perception and 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 foodborne pathogen, has been researched extensively in food for the intoxication it causes in humans. Very little, however, is known about its altered clone counterpart (MRSA) 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.* (2009) found MRSA in 5.6% out of 90 pork products sampled in Louisiana retail food stores. 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. This study concluded no significant difference between MRSA or MSSA prevalence in retail pork and no differences between conventional and alternatively raised (raised without antibiotics) production schemes with regard to MRSA prevalence. These results seem to coincide with the theory suggested by Price *et al.* (2012) of MRSA's bidirectional zoonotic pathway between humans and livestock.

One of the first experiments to test for more than just presence or absence and actually quantify the contamination level of MRSA on retail pork products was performed by Weese *et al.* (2010). This investigation of Canadian retail meat products reported a prevalence of 9.6% out of 230 pork samples examined. Of the 22 pork products analyzed, 32% were below the detection limit of the assay and therefore were not quantifiable. Sixty percent of the positive MRSA samples

were ≤ 20 cfu/g, while only 27% were ≥ 30 cells per gram of meat. It is interesting to note from this study that 91% of the MRSA positive samples were from two of the four Canadian provinces sampled, which further supports the idea that geographical location may impact the risk of MRSA exposure. Ironically, 67% of Canada's human population resides in these two provinces (Ontario and Quebec).

In a larger geographical study of five heavily populated US cities, MRSA was isolated in almost 27% of the retail pork samples examined (Waters *et al.* 2011). More concerning was the result that the MRSA-to-MSSA ratio was almost 2:1, which further conflicts MRSA prevalence results. The research from Waters *et al.* (2011) is the highest MRSA prevalence report of its kind around the world.

MRSA Causing Illness from Food

Although these studies in the North America 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 *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 shredded pork barbeque in Tennessee. The report from Tennessee is the first to note that MRSA is also

capable of causing intoxication from enterotoxin produced in food. SE production from MRSA in food could be a greater risk than typical MRSA infections from contact.

Disconnect Between Livestock & Human MRSA Strains

Since the discovery of MRSA ST398, the literature has focused in on this sequence type as being associated with livestock, especially pigs (Voss *et al.* 2005, Huijsdens *et al.* 2006, Khanna *et al.* 2007, Witte *et al.* 2007, Lewis *et al.* 2008, Smith *et al.* 2009 and Price *et al.* 2012). Although these findings of MRSA in pigs and pork indicate the presence and colonization of the pathogen, the results around the world do not necessarily parallel one another. For example, Weese *et al.* (2011) describes the "discordance between animal [strains of MRSA] and meat strains". This study revealed that all MRSA strains recovered from meat samples were USA 100 (also known as Canadian epidemic MRSA-2), a known human pathogen, not the livestock associated ST398 strain (Weese *et al.* 2011). In a more recent examination of MRSA sequence types recovered from pork in the United States, only 18% of strains recovered were ST398 (Price *et al.* 2012*)*.

The results from these investigations of MRSA in retail pork products in North America do not coincide with earlier work in Europe (VWA 2008, Boer *et al.* 2009). In a study from the Netherlands, de Boer *et al.* (2009) concluded that 97% of isolates recovered from pork were ST398, a strain known to be associated with swine and swine production in Europe and North America. This disconnect

is cause for further confusion of MRSA prevalence in humans, livestock and meat.

MRSA in the media

With all of the evidence of increased MRSA prevalence and new emerging strains of this "super bug", as described by New York Times columnist Nicholas Kristof (2009[a]), the question of how this is occurring is still of concern to both 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 2009[a]). Findings of MRSA in our food sources also make for media opinionated opportunities like a New York Times article entitled "Pathogens in Our Pork" (Kristof 2009[b]). Katie Couric (2010) soon followed the paper media driven frenzy to investigate answers from animal agriculture personnel in her CBS evening news report entitled "Animal Antibiotic Overuse Hurting Humans?" In this report, she interviewed employees at a large hog confinement operation, as well as members of the scientific community to give their opinion of "extra label" feeding of antibiotics to pigs to promote health and productivity (Couric 2010).

Prior to the Price *et al.* (2012) study, scientific literature and opinion filled media pieces alike seemed to indicate that modern food animal production practices were the only plausible explanation for the increased incidence of MRSA infections in our communities and now food. Regardless of the conflicting science, FDA still introduced a ban on the use of certain classes of human antibiotics (cephalosporin) in production animal agriculture (FDA 2012[b]). This

January 4, 2012 order went into effect April 5, 2012, and stops extra label uses of the drug in food producing animals for practices such as disease prevention. It is seemingly easy to overlook the fact that modern human medicine, not livestock production practices, created the original case for antibiotic resistance in *Staphylococcal* spp. more than forty years ago (Barrett *et al.* 1968).

Summary of Literature

Since its discovery in the early 1960's, MRSA has emerged as a main pathogen of interest to human health professionals. Infections from antibiotic resistant bacteria are increasing around the world and have also been reported in food producing livestock. MRSA prevalence has also been recently reported in retail meat products around the world at rates higher than originally thought; however, the source of this contamination is a topic of scrutiny. Although contamination levels reported in meat are not fully understood, the risk of contracting an infection from handling contaminated meat products is thought to be low. Foodborne illness from MRSA has been reported as well, which should not be surprising since most *S. aureus* strains produce toxins to some degree. The fact that resistant strains of bacteria prove to adapt to stressors in their environment and mutate genetically to allow survival of future generations is concerning to scientists and the general public. Although numerous studies have been performed on the presence of MRSA in livestock and meat, very little is known about this organism from a food safety perspective. One main question arises from all of these reports of MRSA in our food sources. Do normal cooking or heating methods "kill" the bacteria? Therefore 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)** to 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.

References

Altekruse, SF, Cohen, ML, Swerdlow, DL. 1997. Emerging
foodborne diseases. Emerg Infect Dis 3(3):285–293.

American Meat Institute. 1997. Good Manufacturing Practices for Fermented Dry
& Semi-Dry Sausage Products. http://www.meathaccp.wisc.edu/assets/
Heat_Treated _Shelf_Stable/AMIF_degreehours.pdf. [Accessed 30 November
2011].

APHIS Veterinary Services Centers for Epidemiology and Animal Health. 2007.
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. [Accessed 10 April 2009].

Arvidson, S. 2000. Extracellular Enzymes. In VA Fischetti, RP Novick, JJ Ferretti,
DA Portnoy and JI Rood (eds.). Gram-positive pathogens. Washington, DC. ASM
Press. pp 379-380.

Balaban, N and Rasooley, A. 2000. Review: *Staphylococcal* enterotoxins. Intl
Jour Food Micro 61(1):1-10.

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

Bean, NH, Griffin, PM, Goulding, JS and Ivey, CB. 1990. Foodborne disease
outbreaks, 5-year summary, 1983–1987. Morb Mortal Wkly Rep CDC Surveill.
Summ. 39(SS-1):15–57.

Bean, NH, Goulding, JS, Lao, C and Angulo, FJ. 1996. Surveillance for
Foodborne-Disease Outbreaks-United States, 1988–1992. Morb. Mortal Wkly.
Rep. CDC Surveill. Summ. 45(SS-5):1-66.

Bennett, RW and Hait, JM. 2001. BAM: *Staphylococcal* enterotoxins.
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAn
alyticalManualBAM/ucm073674.htm. [Accessed 8 July 2012].

Bennett, RW and Lancette, GA. 2001. BAM: *Staphylococcus aureus*.
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAn
alyticalManualBAM/ucm071429.htm. [Accessed 8 July 2012].

Bergey, DH. 1923. Bergey's manual of determinative bacteriology, 1st edition.
Baltimore. Williams and Wilkins Company.

Berglund, C, Prévost, G, Laventie, B, Keller, D and Söderquist, B. 2008. The genes for Panton Valentine leukocidin (PVL) are conserved in diverse lines of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*. Microbes Infect 10(8):878-884.

Beveridge, TJ. 2000. The gram-positive cell wall. In VA Fischetti, RP Novick, JJ Ferretti, DA Portnoy and JI Rood (eds.). Gram-positive pathogens. Washington, DC. ASM Press. pp 3-10.

Bhakdii, S and Tranum-Jensen, J. 1991. Alpha-toxin of *Staphylococcus aureus*. Micro Rev 55(4): 733-751.

Bignardi, GE, Woodford, N, Chapman, A, Johnson, AP and Speller, DCE. 1996. Detection of the *mec-A* gene and phenotypic detection of resistance in *Staphylococcus aureus* isolates with borderline or low-level methicillin resistance. Jour Antimicro Chemo 37(1):53-63.

Bohach, GA and Foster, TJ. 2000. *Staphylococcus aureus* Exotoxins. In VA Fischetti, RP Novick, JJ Ferretti, DA Portnoy and JI Rood (eds.). Gram-positive pathogens. Washington, DC. ASM Press. pp 367-378.

Boubaker, K, Diebold, P, Blanc, DS, Vandenesch, F, Praz, G, Dupuis, G and Teoillet, N. 2004. Panton-Valentine leukocidin and *Staphylococcal* skin infections in school children. Emerg Infect Dis 10(1):121-124.

Brookfield, DG. 1994. Bacterial Endocarditis. Chaser News. http://www.csun.edu/ ~ hcmth011/chaser/article2.html [Accessed 7 July 2012].

Buzby, JC and Roberts, T. 1997. Economic costs and trade impacts of microbial foodborne illness. World Health Stat Q. 50(1-2):57–66.

Byrne, J. 2009. 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. [Accessed 10 April 2009].

Cassman, EP. 1965. *Staphylococcal* enterotoxin. Ann. NY Acad. Sci. 128(1):124-131.

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

Centers for Disease Control. 2011. CDC estimates of foodborne illness in the United States. http://www.cdc.gov/foodborneburden/PDFs/FACTSHEET_A_FINDINGS_updated4-13.pdf. [Accessed 8 July 2012].

Centers for Disease Control. 2012[a]. FoodNet Surveillance – Burden of Illness. http://www.cdc.gov/foodnet/surveillance_pages/burden_pyramid.htm. [Accessed 8 July 2012].

Centers for Disease Control. 2012[b]. National Center for Health Statistics – Pneumonia. http://www.cdc.gov/nchs/fastats/pneumonia.htm. [Accessed 8 July 2012].

Chihara, S and Segreti, J. 2010. Osteomyelitis. Disease-a-Month 56(1):6-31.

Couric, K. 2010. Animal antibiotic overuse hurting humans? CBS Evening News – February 10, 2010. http://www.cbsnews.com/2100-18563_162-6191530.html. [Accessed 9 September 2010].

Daly C, La Chance, M, Sandine, WE, and Elliker, PR. 1973. Control of *Staphylococcus aureus* by starter cultures and chemical acidulation. JFS 38(3):426-430.

De Boer, E, Zwartkruis-Nahuis, JTM, Wit, B, Huijsdens, XW, de Neeling, AJ, Bosch, T, van Oosterom, RAA, Vila, A and Heuvelink, AE. Prevalence of methicillin-resistant Staphylococcus aureus in meat. Intl Jour Food Micro 134(1):52-56.

De Vos, P, Garrity, GM, Jones, D, Krieg, NR, Ludwig, W, Rainey, FA, Karl-Heinz Schleifer and Whitman, WB. 2009. Bergey's manual of systematic bacteriology. Volume three the Firmicutes. New York. Springer. p 392.

Doyle, MP. 2010. Food-borne disease. In AccessScience@McGraw-Hill. http://books.mcgraw-hill.com/EST10/site/spotlight/foodscience/articles/Food-borne_disease.pdf. [Accessed 9 July 2012].

Dugdale, DC, Vyas, JM and Zieve, D. Medline Plus. 2011. http://www.nlm.nih.gov/medlineplus/ency/article/000430.htm. [Accessed 7 July 2012].

Dutfield, G. 2009. Intellectual Property Rights and the Life Science Industries: Past, Present, and Future (2nd Edition). London. World Scientific Publishing Co. pp. 139-140.

Enright, MC, Robinson, DA, Randle, G, Feil, EJ, Grundmann, H, and Spratt, BG. 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc Natl Acad Sci 99(11):7687-7692.

Evenson, ML, Hinds, MW, Bernstein, RS and Bergdoll, MS. 1988. Estimation of human dose of staphylococcal enterotoxin A from a large outbreak of S*taphylococcal* food poisoning involving chocolate milk. Intl Jour Food Micro 7(4):311-316.

Food and Drug Administration. 2001. Chapter 4: Analysis of microbial hazards related to time/temperature controls of foods for safety. http://www.fda.gov/Food/ScienceResearch/ResearchAreas/SafePracticesforFood Processes/ucm094147.htm. [Accessed 8 July 2012].

Food and Drug Administration. 2012[a]. Bad Bug Book: Handbook of foodborne pathogenic microorganisms and natural toxins. http://www.fda.gov/downloads/Food/FoodSafety/FoodborneIllness/FoodborneIlln essFoodbornePathogensNaturalToxins/BadBugBook/UCM297627.pdf. [Accessed 3 July 2012].

Food and Drug Administration. 2012[b]. FDA to protect important class of antimicrobial drugs for treating human illness. FDA News release. http://www. fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm285704.htm. [Accessed 4 February 2012].

Food Safety and Inspection Service. 1998. Report to Congress. FoodNet: An active surveillance program for bacterial foodborne disease in the United States. http://www.fsis.usda.gov/ophs/rpcong98/rpcong98.htm. [Accessed 8 July 2012].

Fox, A. 2010. *Streptococcus pneumoniae* and *Staphylococci*. In Bacteriology – USC School of Medicine. http://pathmicro.med.sc.edu/fox/strep-staph.htm [Accessed 9 September 2010].

Fuda, C, Suvorov, M, Vakulenko, SB and Mobasherry, S. 2004. The basis for resistance to –lactam antibiotics by penicillin-binding protein 2 of methicillin-resistant *Staphylococcus aureus*. Jour Bio Chem 279(39):40802-40806.

Heilmann, C and Peters, G. 2000. Biology and pathogenecity of *Staphylococcus epidermis*. In VA Fischetti, RP Novick, JJ Ferretti, DA Portnoy and JI Rood (eds.). Gram-positive pathogens. Washington, DC. ASM Press. pp 442-449.

Holmberg, SD and Blake, PA. 1984. *Staphylococcal* food poisoning in the United States. New facts and old misconceptions. JAMA 251(4):487-489.

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

Huseby, M, Ke Shi, C, Brown, K, Digre, J, Mengistu, F, Seo, KS, Bohach, GA, Schlievert, PM, Ohlendorf, DH and Earhart, CA. 2007. Structure and biological activities of Beta-toxin from *Staphylococcus aureus*. Jour Bacteriol 189(23):8719-8726.

ICMSF. 1996. *Staphylococcus aureus*. In TA Roberts, AC Baird-Parker and RB Tompkin (eds.). Microorgansisms in Foods 5. Characteristics of Microbial Pathogens. London. Blackie Academic & Professional. pp 299-333.

Jones TF, Kellum ME, Porter SS, Bell M and Schaffner W. 2002. An Outbreak of Community-Acquired Foodborne Illness Caused by Methicillin-Resistant *Staphylococcus aureus*. *Emerg Infect Dis* 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.

Kim, JH. 2012. Food Safety: For safe and wholesome foods. http://crees.org/resources/files/forms/26.pdf. [Accessed 9 July 2012].

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.

Kloos, WE and Bannerman, TL. 1994. Update on clinical significance of coagulase negative *Staphylococci*. Clinical Microbiology Reviews 7(1): 117-140.

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

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

Knox, R. 1961. Correspondence. Brit Med Jour 1(5219):126.

Kollef, MH and Micek, ST. 2005. *Staphylococcus aureus* pneumonia: A "superbug" infection in community and hospital settings. CHEST 128(3):1093-1097.

Kristof ND. 2009[a]. Our Pigs, Our Food, Our Health. *New York Times* 11 March 2009. http://www.nytimes.com/2009/03/12/opinion/12kristof.html. [Accessed 20 April 2009].

Kristof ND. 2009[b]. Pathogens in Our Pork. *New York Times* 15 March 2009. http://www.nytimes.com/2009/03/15/opinion/15kristof.html. [Accessed 20 April 2009].

Lewis HC, Mølbak K, Reese C, Aarestrup FM, Selchau M, Sørum M and Skov RL. 2008. Pigs as Source of Methicillin-Resistant *Staphylococcus aureus* CC398 Infections in Humans, Denmark. Emerg Infect Dis 14(9):1383-1389.

Lowy, FD. 2010. *Staphylococcal* Infections. In DL Kasper and AS Fauci (eds.). Harrison's infectious diseases. New York. McGraw Hill Medical. pp 386-99.

Maiden, MCJ. 2006. Multilocus sequence typing of bacteria. Ann Rev Micro 60:561-588.

Maiden, MCJ, Bygraves, JA, Feil, E, Morelli, G, Russell, JE, Urwin, R, Zhang, Q, Zhou, J, Zurth, K, Caugant, DA, Feavers, IM, Achtman, M, Spratt, BG. 1998. Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci 95(6):3140-3145.

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

Mayer, G. 2010. Bacteriology-Chapter Six. Antibiotics-Protein synthesis, nucleic acid synthesis and metabolism. USC School of Medicine. http://pathmicro.med. sc.edu/book/immunol-sta/antibiot.htm. [Accessed 4 March 2011].

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illnesses and death in the United States. *Emerg. Infect. Dis.* 5:607-625.

McCabe-Sellers, BJ. 2004. Food safety: Emerging trends in foodborne illness surveillance and prevention. JADA 104(11):1708-1717.

Naber, CK. 2009. *Staphylococcus aureus bacteremia*: epidemiology, pathophysiology and management strategies. Clin Infect Dis 48(4):231-37.

Nilsson, IM, Hartford, O, Foster, T and Tarkowski, A. 1999. Alpha-Toxin and Gamma-Toxin Jointly Promote *Staphylococcus aureus* Virulence in Murine Septic Arthritis. Infect. Immun. 67(3):1045-1049.

Noble, WC, Valkenburg, HA and Wolters, CH. 1967. Carriage of *Staphylococcus aureus* in random samples of a normal population. Jour Hyg 65(4): 567-73.

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

Olsen, SJ, MacKinon, LC, Goulding, JS, Bean, NH and Slutsker, L. 2000. Surveillance for Foodborne Disease Outbreaks --United States, 1993-1997. MMWR 49(SS01)1-51.

Prescott, LM, Harley, JP and Klein, DA. 1996. The Bacteria: Gram-positive bacteria other than *Actinomycetes*. In EM Sievers, T Stanton, JL Wilde, LA Schrandt, JK Banowetz and L Hancock (eds.). Microbiology. Dubuque, IA. Wm. C. Brown Publishers. p 438-443.

Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, *et al. 2012. 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. 2009. Isolation and Characterization of Methicillin-Resistant *Staphylococcus aureus* Strains from Louisiana Retail Meats. *App Env Micro* 75(1):265-267.

Reaveley, DA and Burge. RE. 1972. Walls and membranes in bacteria. Advances in Microbial Physiology 7:1-81.

Rolinson, GN, Batchelor, FR, Stevens, S, Wood, JC and Chain, EB. 1960. Bacteriological studies on a new penicillin – BRL.1241. Lancet 276(7150):561-567.

Schmitz, FJ, Veldkamp, KE, Van Kessel, KPM, Verhoef, J and Strijp, JAG. 1997. Delta-Toxin from *Staphylococcus aureus* as a Costimulator of Human Neutrophil Oxidative Burst. Jour Infect Dis 176(6):1531-1537.

Sia, IG and Berbari, EF. 2006. Osteomyelitis. Best Practice & Res Clin Rheumatology 20(6):1065-1081.

Smith, JL and Marmer, BS. 1991. Death and injury in *Staphylococcus aureus* 196E: Effect of growth temperature. LWT Food Science and Technology 24(2): 169-*72.*

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

Stavropoulos, DN and Hornby, AS. 2008. Oxford English-Greek Learner's Dictionary. GN Stavropoulos (ed.). Oxford. Oxford University Press. p 207.

Tenover, FC and Gaynes, RP. 2000. Epidemiology of *Staphylococcus* infections. In VA Fischetti, RP Novick, JJ Ferretti, DA Portnoy and JI Rood (eds.). Gram-positive pathogens. Washington, DC. ASM Press. pp 414-421.

Takahashi, T, Satoh, I and Kikuchi, N. 1999. Phylogenetic relationships of 38 taxa of the genus *Staphylococcus* based on 16s rRNA gene sequence analysis. Intl Jour of Sys Bact 49(2):725-728.

Tomasz, A. 1979. The mechanism of the irreversible antimicrobial effects of penicillins: How the Beta-lactam antibiotics kill and lyse bacteria. Ann Rev Micro 33:113-137.

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/May 05/LDPM13001/. [Accessed 22 April 2009].

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. [Accessed 22 April 2009].

Urwin, R and Maiden, MCJ. 2003. Multi-locus sequence typing: A tool for global epidemiology. Trends Micro 11(10):479-487.

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

Voedsel en Waren Autoriteit (VWA). 2008. Prevalence of MRSA in meat, 2007 Factsheet. http://www2.vwa.nl/cdlpub/servlet/CDLServlet?p_file_id=25742. [Accessed 10 April 2009].

Vorvick, LJ. 2010. Medline Plus. http://www.nlm.nif.gov/medlineplus/ency/article/ 003239.htm. [Accessed 7 July 2012].

Vorvick, LJ and Zieve, D. 2010. Medline Plus. http://www.nlm.nih.gov/medline plus/ency/article/000823.htm. [Accessed 7 July 2012].

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

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

Ward, K, Hammond, R, Katz, D and Hallman, D. Outbreak of Staphylococcal Food Poisoning Associated with Precooked Ham-Florida, 1997. MMWR 46(50):1189-1191.

Waters, AE, Contente-Cuomo, T, Buchhagen, J, Liu, CM, Watson, L, Pearce, K, Foster, JT, Bowers, J, Dreibe, EM, Engelthaler, DM, Keim, PS and Price, LB. 2011. Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. Clin Infect Dis 52():1-4.

Waxman, DJ and Strominger, JL. 1983. Penicillin binding proteins and the mechanism of action of β-lactam antibiotics. Ann Rev Biochem 52:825-869.

WebMD. 2010. http://www.webmd.com/skin-problems-and-treatments/tc/impetigo-overview. [Accessed 7 July 2012].

Weese, JS, Avery, BP and Reid-Smith, RJ. 2010. Detection and quantification of methicillin-resistant *Staphylococcus* aureus (MRSA) clones in retail meat products. Letters in Appl Micro 51(3):338-342.

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

Wieneke, AA, Roberts, D and Gilbert, RJ. 1993. *Staphylococcal* food poisoning in the United Kingdom, 1969-1990. Epidemiol. Infect. 110(3):519-531.

Williams, REO. 1963. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. Bacteriol Rev. 27(1): 56-71

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

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

CHAPTER 3. SURVIVAL OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* DURING COMMERCIAL THERMAL PROCESSES FOR FRANKFURTERS, SUMMER SAUSAGE AND HAM

A paper to be submitted to Foodborne Pathogens and Disease

Jonathan A. Campbell[1], Joseph C. Cordray[1], James S. Dickson[1], Dennis Olson[1], Aubrey F. Mendonca[2] and Kenneth J. Prusa[2]

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 commercial thermal processing for ham, summer sausage and frankfurters is

sufficient to reduce the risk of MRSA as a potential food safety hazard.

[1]Department of Animal Science, Iowa State University, Ames, IA, USA.
[2]Department of Food Science & Human Nutrition, Iowa State University, Ames,
IA, USA.

Introduction

Methicillin-resistant *Staphylococcus aureus*, also known as MRSA, is a

variant strain of *S. aureus*, a known food pathogen and gram-positive cocci that

commonly inhabits the nasal passage, mucous membranes and skin of humans.

MRSA was first discovered in the United States in the 1960's at Boston City

Hospital (Barrett *et al.*, 1968), and since its discovery more than forty years ago,

MRSA has emerged as one of the leading and most severe infectious

microorganisms acquired from exposure in health care settings (CDC NNIS

system, 2003). According to a recent article in the Journal of the American

Medical Association, however, the incidence of invasive MRSA infections in the

United States is low (<1%), and prevalence varies by location (Klevens *et al.,*

2007). Incidence of MRSA colonization and 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 and 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 people and invasive infections 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) refutes popular views on antibiotic overuse in livestock production and explains 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). The recent research conducted evaluated various pork 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 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 majority of the literature suggests that MRSA contamination of meat products is very low (< 100 cfu/g detected).

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 *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 shredded pork barbeque in Tennessee. The report from Tennessee is the first to note that MRSA is also capable of causing classical *S. aureus* food poisoning from enterotoxin produced in food. The discovery that the strain causing the intoxication was MRSA was purely incidental.

These results bring into question the safety of meat products with the increased incidence of MRSA 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. 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. Cultures of each MRSA strain were
grown at 35°C in Trypticase Soy Broth (TSB; Difco™, Becton, Dickson & Co.,
Franklin Lakes, NJ, USA) for 24 hours. The four strains were then combined and
vortexed to create the mixed culture.

Frankfurter manufacture and inoculation. A blend of pork and beef
emulsified and 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 were ground through a 12.7mm plate using a Biro model 7552SS
(Biro MFG Co., Marblehead, OH, USA) grinder. The emulsion was produced
using a Krämer-Grebe model VSM65 (Krämer & Grebe GmbH & Co. KG.,

Biendenkopf-Wallau, Germany) vacuum bowl chopper using six knives at varying speeds. 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 as previously described, and 4 ml were mixed with 36g of the emulsion batter. The inoculated batter was aseptically stuffed into a plastic 50mL Corning® centrifuge tube (Corning Inc., Corning, NY, USA) using a sterile plastic syringe. The screw cap was secured onto the centrifuge tube and placed into a three-tube centrifuge cassette. The cassette was then placed into a Thermo / NESLAB™ model RTE-211 water bath / circulator (Thermo Scientific, Portsmouth, NH, USA) at 79.5°C with a 500g donut-style lead weight on top to keep the cassette submerged. 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 the internal temperatures of the sausage and to determine the presence of any naturally occurring mircoflora.

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 stomached with 90mL of 0.1% buffered peptone water (Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA) for 120 sec in a Seward model 400 lab blender stomacher (Seward Medical, London SE1 1PP, UK).The slurry was serially diluted as needed in buffered peptone water.

Appropriate dilutions (including the slurry contents) were surface 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. Colonies were enumerated at 24 and 48 hours and the results were recorded. Three independent replications of this experiment were performed.

Summer Sausage Manufacture and Inoculation. 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), Legg's cure (A.C. Legg, Calera, AL, USA) 6.25% nitrite curing salt (0.23% wt/wt), SAGA® 200 (*Pediococcus acidilactici)* (Kerry Seasoning, Beloit, WI, USA) 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 grinder (Biro MFG Co., Marblehead, OH, USA). The ground trim was then transferred to a Hollymatic® model 175 mixer grinder (Hollymatic Corporation, Countryside, IL, USA) 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 to the mixer grinder and mixed for an additional 2 minutes. The product was then reground 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 analysis.

A four strain mixed culture of MRSA was prepared as previously described, and 5mL of each individual strain was combined in a test tube (20 mL total volume). The mixed culture was vortexed and mixed with 180g of the sausage batter. The inoculated batter was aseptically stuffed into a 250mL glass 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 sausage was removed and the pH determined using a calibrated Accumet® model 15 pH meter (Thermo Fisher Scientific, Inc., Pittsburg, PA, USA). The fermented sausages were cooked in a Thermo / NESLAB™ model RTE-211 water bath / circulator (Thermo Scientific, Portsmouth, NH, USA) 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. An 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 sausages during cooking and chilling and to determine the presence of any naturally occurring mircoflora.

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 and stomached with 225mL of 0.1% buffered peptone water for 120 seconds using a Seward model 400 lab blender stomacher and the slurry was serially diluted as needed. Each dilution (including the slurry contents) was surface plated in duplicate on BPA-EY and incubated at

35°C for 48hrs. Colonies were enumerated at 24 and 48 hours and the results were recorded. Three independent replications of this experiment were performed.

Boneless Ham manufacture and inoculation. 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 Giulini Corp., Simi Valley, CA, USA) sodium 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 (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 four strain mixed culture was prepared as previously described and 3.5mL of each strain was combined in a test tube (14mL total volume). The mixed culture was vortexed and mixed with 1386g 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 outlined in Table 1.

The cooked hams were immediately 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), 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 mircoflora.

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 and homogenized with 450mL of 0.1% buffered peptone water for 120 seconds using a Seward model 400 lab blender stomacher and the slurry was serially diluted as needed. Each dilution (including the slurry contents) was surface plated in duplicate on BPA-EY and incubated at 35°C for 48hrs. Colonies were enumerated at 24 and 48 hours and the results were recorded. Three independent replications of this experiment were performed.

Data Analysis. Log_{10} 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 (LSD) 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, N.C., USA) at a significance level of $P < 0.05$.

Results

Frankfurters. Figure 1 graphically depicts the survival of MRSA in cooked, emulsified sausages. Table 2 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.

Fermented and cooked sausage (summer sausage). Figure 2 illustrates the survival of MRSA in fermented and cooked sausages. Table 3 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.

Boneless Ham. The results of the boneless ham experiment are shown in Figure 3. 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.

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 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). 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. 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.

Conclusions

The results of this study indicate that commercial procedures used for thermal processing and subsequent chilling of frankfurters, summer sausage and

boneless ham allowed for at least a 5.5 $\log_{10}$ reduction of MRSA at 70°C. This is important when considering the risk of foodborne illness from MRSA in processed meats, since the literature indicates that MRSA contamination at the retail counter is very low (< 100 cells per gram of meat). Reduced pH products and larger diameter processed meats showed an increased safety level with regard to survival of MRSA. It is important to consider good manufacturing practices for fermentation degree-hours in reduced 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 the main components for reduced risk of foodborne illness by a microorganism like MRSA.

Acknowledgements

Disclosure Statement

No competing financial interests exist.

References

American Meat Institute. Good Manufacturing Practices for Fermented Dry & Semi-Dry Sausage Products. October 1997. http://www.meathaccp.wisc.edu/assets/Heat_Treated _Shelf_Stable/AMIF_degreehours.pdf. [accessed 30 November 2011].

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. [accessed 10 April 2009]

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

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. [acessed 10 April 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.

Genigeorgis C, Riemann H, Sadler WW. Production of entertoxin-B in cured meats. Jour Food Sci 1969; 34:62-68.

Heiszler MG, Kraft AA, Rey, CR, Rust, RE. Effect of time and temperature of smoking on microorganisms on frankfurters. Jour Food Sci 1972; 37:845-849.

Ingham SC, Engel RA, Fanslau MA, Schoeller EL, Searls G, Buege DR, Zhu J. Fate of *Staphylococcus aureus* on vacuum-packaged ready-to-eat meat products stored at 21°C. Jour Food Protect 2005; 68:1911-1915.

Jones TF, Kellum ME, Porter SS, Bell M, Schaffner W. An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 2002; 8:82-84.

Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K *et al.* Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 2007; 298:1763-1771.

Kluytmans J, van Belkum A and Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Micro Rev 1997; 10:505-520.

Kristof ND. Our pigs, our food, our health. New York Times 11 March 2009. http://www.nytimes.com/2009/03/12/opinion/12kristof.html [accessed 20 April 2009].

Lewis HC, Mølbak K, Reese C, Aarestrup FM, Selchau M, Sørum, Skov RL. Pigs as Source of Methicillin-Resistant *Staphylococcus aureus* CC398 Infections in Humans, Denmark. Emerg Infect Dis 2008; 14:1383-1389.

Maree CL, Daum RS, Boyle-Vavra S, Matayoshi K, Miller LG. Community-associated methicillin-resistant *Staphylococcus aureus* isolates causing healthcare-associated infections. *Emerg Infect Dis* 2007; 13:236-242.

McCoy DW and Faber JE. Influence of food microorganisms on *Staphylococcal* growth and enterotoxin production in meat. Appl Microbiol 1966; 14:372-377.

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:e30092.

Palumbo SA, Smith JL, Kissinger, JC. Destruction of *Staphylococcus aureus* during frankfurter processing. Appl Env Micro 1977; 34:740-744.

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 3:1-6 e00305-11.

Pu S, Han F, Ge B. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* strains from Louisiana retail meats. *App Env Micro* 2009; 75:265-267.

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: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/May 05/LDPM13001/   [accessed 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. [accessed 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. [accessed 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; 13:1753-1755.

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

Witte W, Strommenger B, Stanek C, Cuny C. Methicillin-resistant *Staphylococcus aureus ST 398* in humans and animals, Central Europe. *Emerg Infect Dis* 2007; 13:255-258.

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.

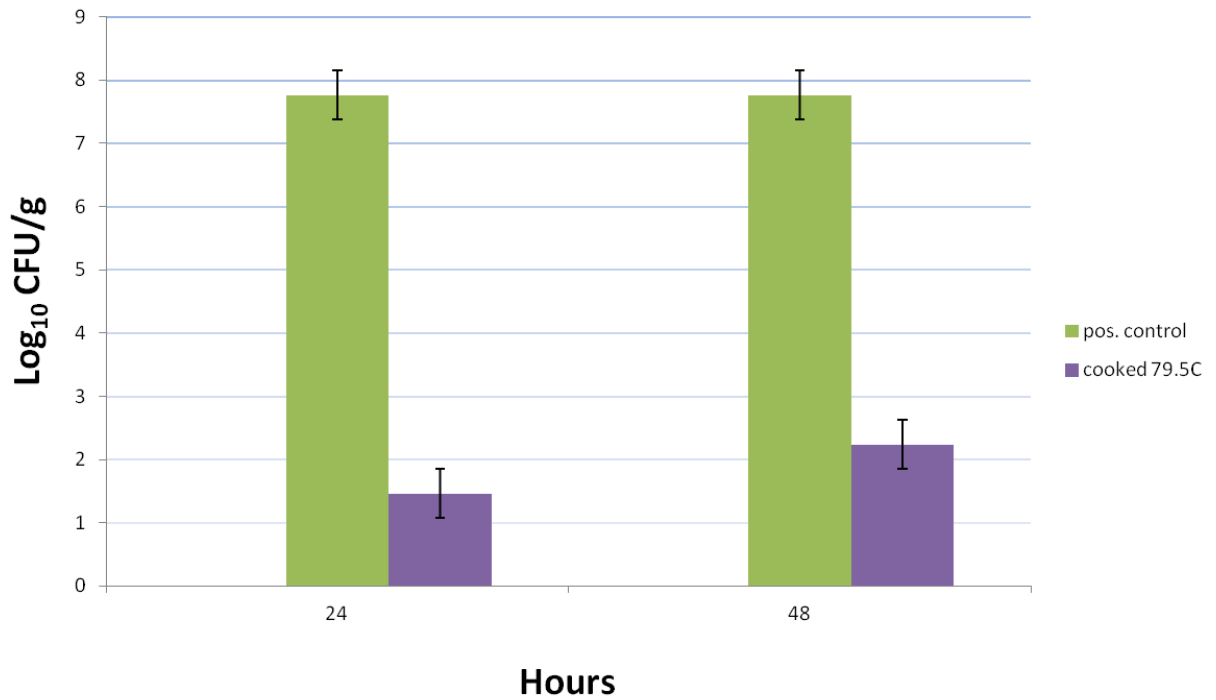**Figure 1. Survival of MRSA in Frankfurters Cooked to an Internal temperature of 70°C.**

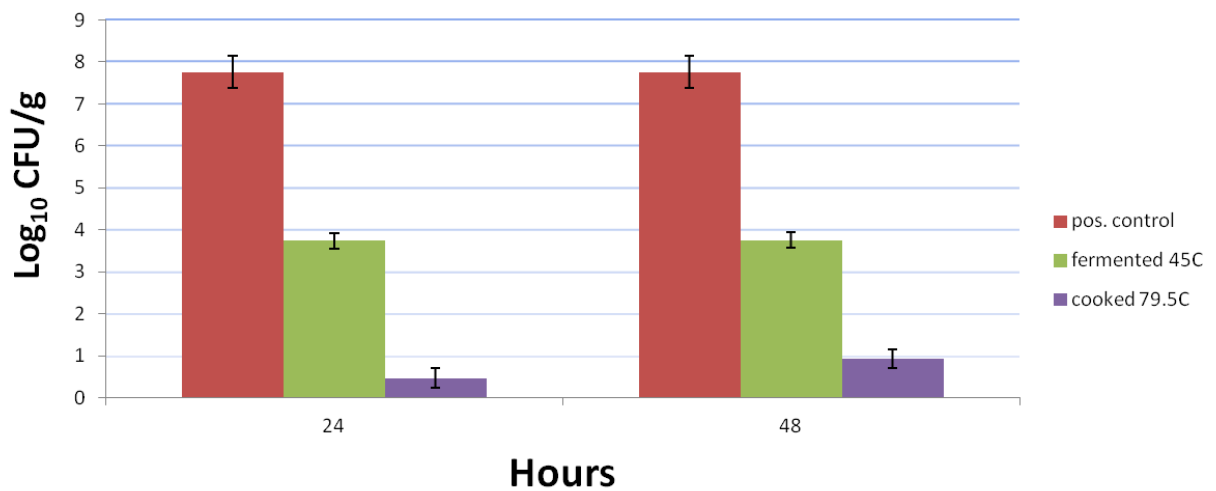**Figure 2. Survival of MRSA in Fermented and Cooked Summer Sausage.**

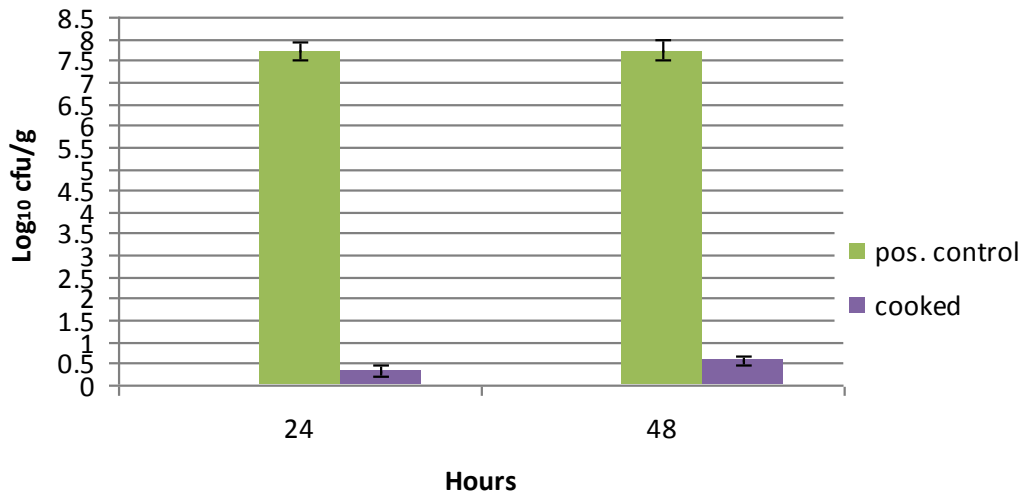Figure 3. Survival of MRSA in Boneless Ham Cooked to 70°C

Table 1. Boneless Ham Thermal Processing Schedule

Step	Time	D.B.	W.B.	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

Table 2. 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 3. 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 4. 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)

CHAPTER 4. SURVIVAL OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS DURING COMMERCIAL HEAT TREATMENT OF SLAB BACON AND CONSUMER PREPARATION OF SLICED BACON

A paper to be submitted to Journal of Food Protection

Jonathan A. Campbell[1], Joseph C. Cordray[1], James S. Dickson[1], Dennis Olson[1], Aubrey F. Mendonca[2] and Kenneth J. Prusa[2]

Abstract

With the knowledge that retail pork products may be contaminated with methicillin-resistant *Staphylococcus aureus* (MRSA), the risk of consumers contracting a MRSA infection or foodborne illness from processed meats, especially bacon, is uncertain. Therefore, a study was designed to investigate the survival of MRSA during heat treatment of slab bacon at a commercial process and during cooking of sliced bacon at the consumer level. Fresh pork bellies were injected with a curing solution, inoculated and heat treated to an internal temperature of 52°C. Three commercial brands of sliced bacon with similar "sell-by" dates and fat-to-lean ratios were also inoculated and cooked at a temperature of 177°C for 0, 2 and 5 minutes on each side. Heat treated slab bacon showed a log reduction of 1.89, which was significant ($P<0.05$) when compared to an uncooked, inoculated control. Cooked sliced bacon had a reduction of viable MRSA cells > 6.5 logs, and there was not a significant brand interaction ($P>0.05$).

_____

[1]Department of Animal Science, Iowa State University, Ames, IA, USA.
[2]Department of Food Science & Human Nutrition, Iowa State University, Ames, IA, USA.

Introduction

The popularity, sales and utilization of bacon made from pork bellies has increased dramatically in recent years in the United States. Sales of the top 20 retail bacon brands in the U.S. surged pass the $2 billion dollar mark in 2010 (1). The majority of retail bacon sold in the U.S. is uncooked, sliced and vacuum packaged for consumers to store and cook at home. According to the National Pork Board (8), bacon accounts for 19% of pork eaten in the home and is prepared in the home by pan frying on the stove top 45% of the time.

Unfortunately, another statistic of interest to pork producers and meat processors alike is the seemingly growing presence of antibiotic resistant pathogens detected throughout the pork production and distribution chain. MRSA is one of the main pathogens of interest linked to pork production, and a 2008 study outlined the risk for MRSA exposure and potential increased chance of infections in humans from swine production (7). Clinical signs of MRSA infections include furunculosis, multiple infected boils, and necrosis of the skin (17). A 2007 article in the Journal of the American Medical Association, however, reported that the extent of invasive MRSA infections in the U.S. is generally <1% (5). The connection between pig farming and MRSA exposure and carriage by humans is worldwide (15, 4, 11). Voss *et al.* (14) 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 in humans remain low, colonization and isolation of MRSA

strains linked to livestock production have increased dramatically since its discovery in the 1960's.

MRSA has also been detected in pork products in retail meat products around the world. An experiment in the Netherlands concluded that MRSA was prevalent in 33 out of 309 (10.7%) pork samples tested (2). Similar results were reported for retail pork products in Canada and the United States (10, 16, 9). Although these results from Europe and North America indicate the presence of MRSA in livestock and retail meats, the impacts these discoveries have on human health and food safety are not well understood.

Although the U.S. has seen an increase in both bacon sales and the prevalence of MRSA in retail pork products, very little is known about the risk of infection to consumers or foodborne illness caused by MRSA from consuming bacon. The objective of the following experiment was to investigate the survival of methicillin-resistant *Staphylococcus aureus* during commercial heat treatment of cured pork bellies and during cooking of sliced bacon at the consumer level.

Materials and Methods

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. Cultures of each MRSA strain were grown at 35°C in Trypticase Soy Broth (TSB; Difco™, Becton, Dickson & Co., Franklin Lakes, NJ, USA) for 24 hours. The four strains were then combined and vortexed to create the mixed culture.

Slab Bacon manufacture and inoculation. Fresh, skinless pork bellies ranging in thickness from 2.38 – 2.71 cm were obtained from swine harvested and fabricated at the Iowa State University meat laboratory. The bellies were injected to 12% over raw weight using the following brine ingredients: water (79.5%wt/wt), salt (12.75%wt/wt), sugar (4.25%wt/wt), Brifisol® 512 Super (BK Giulini Corp., Simi Valley, CA, USA) sodium 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 TSB. A four strain mixed culture was prepared as previously described 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 mixed culture 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.*(3)) prior to being heat treated 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 outlined in Table 1. The slab bacon was stabilized in a 4°C walk-in cooler to an internal temperature of 7.2°C in accordance with option 3 outlined in Appendix B, Compliance Guidelines for Cooling Heat-Treated Meat & Poultry Products (13).

Each of the six $25cm^2$ sections (approximately 50g) were excised using a sterile scalpel, aseptically transferred to a Whirl-Pak® filter stomacher bag (Nasco, Ft. Atkinson, WI, USA) and filled with 450mL of 0.1% buffered peptone water (BPW; Difco™,) (method modified from Burnham *et al.* (3)). 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 mircoflora. Figure 1 depicts the locations of the six $25cm^2$ sections on the belly surface.

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 as necessary in BPW. Appropriate dilutions (including the slurry contents) were surface 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. Colonies were enumerated at 24 and

48 hours and the results were recorded. Three independent replications of this experiment were performed.

Sliced Bacon inoculation and sampling. Three brands of commercially available sliced bacon, selected on the basis of similar "use by" dates and fat-to-lean ratios were purchased at local retail outlets in Ames, IA and brought back to the Iowa State University food safety research laboratory for testing and evaluation.

A four strain mixed culture was prepared as previously described, and 2.5mL of each strain was transferred into a test tube (10mL total volume) and vortexed. Two 25g slices of bacon from each brand were inoculated on both sides with 0.5mL of the mixed culture. Two degrees of doneness, "very crispy" and "less crispy" were used to evaluate the survival of MRSA during cooking of sliced bacon. "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 the same skillet for 2 minutes on each side. 10g of each brand of cooked bacon for the two doneness variables were aseptically transferred to a Whirl-Pak® filter stomacher bag and homogenized with 90mL of BPW for 120 seconds using a Seward model 400 lab blender stomacher. The slurry was serially diluted as necessary in BPW, surface plated in duplicate on BPA-EY and incubated at 35°C for 24 hours. Colonies were enumerated 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™) 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.

Data Analysis. Log_{10} values for surviving MRSA colonies were calculated in duplicate for each of three independent replications for sliced and slab bacon experiments. The detection limit for the assay was 50 cfu/g for both slab and sliced bacon experiments. The slab bacon and sliced bacon experiments utilized a randomized complete block design to interpret the data and block for location and side of the belly slab (lateral or medial) and brand of sliced bacon used in the study. A value of zero was entered when no growth was detected. Least significant differences (LSD) for the results were calculated using the general linear model procedure (PROC GLM) and the mixed effects procedure (PROC MIXED) of the Statistical Analysis Software program (SAS Institute Inc., Cary, N.C., USA) at a significance level of $P < 0.05$.

Results and Discussion

Slab Bacon. The results of the slab bacon study are illustrated in Figure 2, as well as the means summarized in Table 2. 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 3. The means of observed growth for the experiment are listed in Table 3. 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 4 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 4. 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.

Acknowledgements

References

1. American Meat Institute. 2010. Six Degrees of Bacon. http://www.meatami.com/ht/a/GetDocumentAction/i/59942 /. Accessed 29 June 2012.

2. de Boer, E., J. T. M. Zwartkruis-Nahuis, B. Wit, X. W. Huijsdens, A. J. de Neeling, T. Bosch, R. A. A. van Oosterom, A. Vila and A. E. Heuvelink. 2009. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *Intl. J. Food Micro.* 134:52–56.

3. Burnham, G.M., M.A. Fanslau, and S.C. Ingham. 2006. Evaluating microbial safety of slow partial-cooking processes for bacon: use of a predictive tool based on small-scale isothermal meat inoculation studies. *J. Food Prot.* 69:602–608.

4. Huijsdens, X. W., B. J. van Dijke, E. Spalburg, M.G. van Santen-Verheuvel *et al.* 2006. Community-acquired MRSA and pig-farming. *Annal. Clin. Micro. AntiMicro.* 5:26–28.

5. Klevens, R. M., M. A. Morrison, J. Nadle, S. Petit, K. Gershman *et al.* 2007. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA.* 298:1763–1771.

6. Lee, R. Y., G. J. Silverman and D. T. Munsey. 1981. Growth and enterotoxin A production by *Staphylococcus aureus* in the intermediate moisture range.

7. Lewis H. C., K. Mølbak, C. Reese, F. M. Aarestrup, M. Selchau, M. Sørum and R. L. Skov. 2008. Pigs as source of methicillin-resistant *Staphylococcus aureus* CC398 infections in humans, Denmark. *Emerg. Infect. Dis.* 14:1383–1389.

8. National Pork Board. 2007. Bacon Trends. http://www.porkretail.org/filelibrary/Retail/BaconTrends.pdf / Accessed 29 June 2012.

9. O'Brien, A. M., B. M. Hanson, S. A. Farina, J. Y. Wu, J. E. Simmering, S, E, Wardyn, B. M. Forshey, M. E. Kulick, D. B. Wallinga and T. C. Smith. 2012. MRSA in conventional and alternative retail pork products. *PLoS. ONE*. 7:e30092

10. Pu S., F. Han and B. Ge. 2009. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* strains from Louisiana retail meats. *App. Env. Micro.* 75:265–267.

11. Smith T. C., M. J. Male, A. L. Harper, J. S. Kroegar *et al*. 2009. Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in Midwestern US swine and swine workers. *PLoS. ONE.*4:e4248.

12. Taormina, P. J. and G. W. Bartholomew. 2005. Validation of bacon processing conditions to verify control of *Clostridium perfringens and Staphylococcus aureus*. *J. Food Prot.* 68:1831–1839.

13. 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_AppendixB.htm. Accessed 14 January 2010.

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

15. Voss, A., F. Loeffen, J. Bakker, C. Klaassen and M. Wulf. Methicillin-resistant *Staphylococcus aureus* in pig farming. 2005. *Emerg. Infect. Dis.* 11:1965–1966.

16. Weese, J. S., B. P. Avery, and R. J. Reid-Smith. 2010. Detection and quantification of methicillin-resistant *Staphylococcus* aureus clones in retail meat products. *Letters in Appl. Micro*. 51:338–342.

17. Zetola, N., J. S. Francis, E. L. Nuermberger and W. R. Bishai. 2005. Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging threat. *Lancet. Infect. Dis.* 5:275–286.
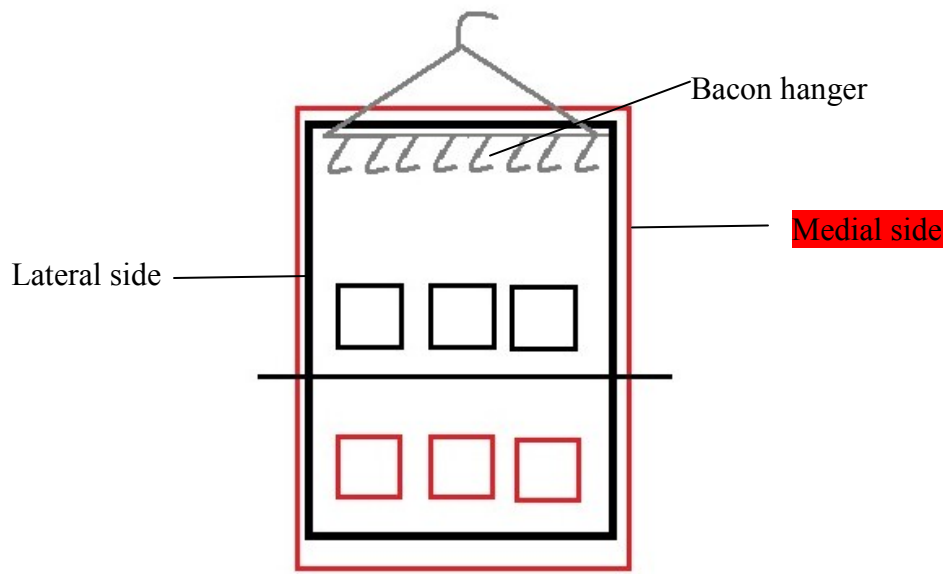
Figure 1. Schematic of bacon slab

Figure 1. Schematic of bacon slab

Figure 2. Survival of MRSA in slab bacon heat treated to 52°C

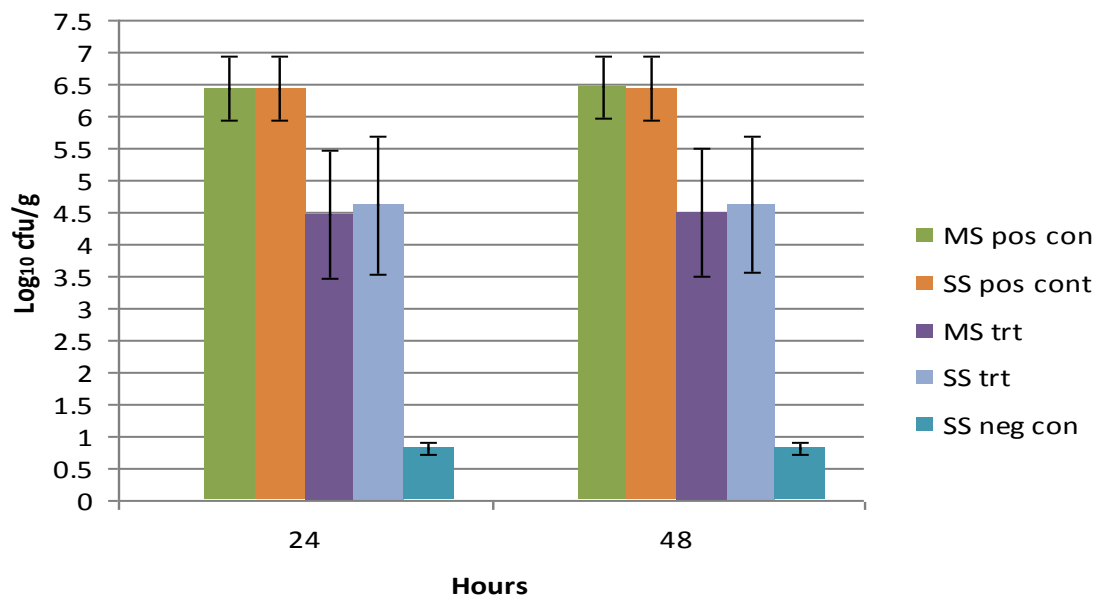Figure 2. Survival of MRSA in slab bacon heat treated to 52°C

Figure 3. Survival of MRSA in sliced bacon cooked at 177°C (350°F)
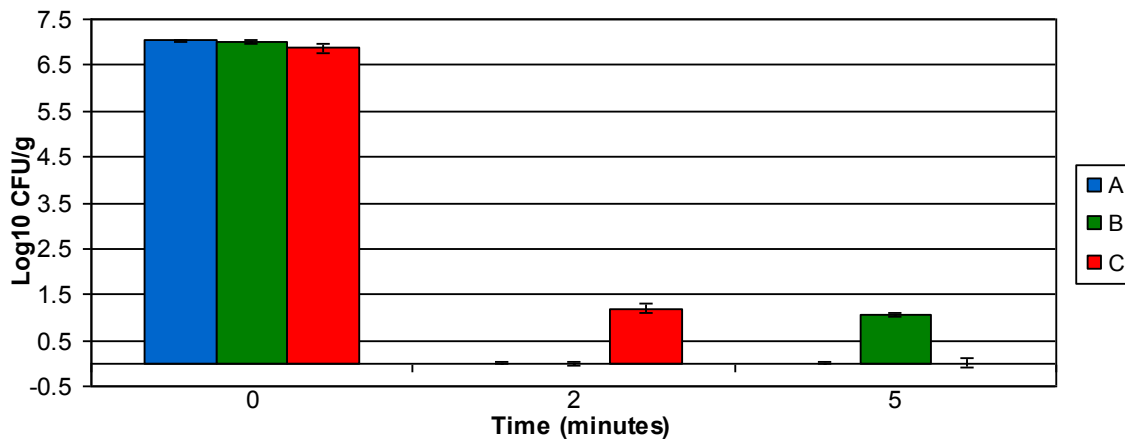

Figure 4. Percent positive MRSA in sliced bacon samples after cooking and BHI enrichment
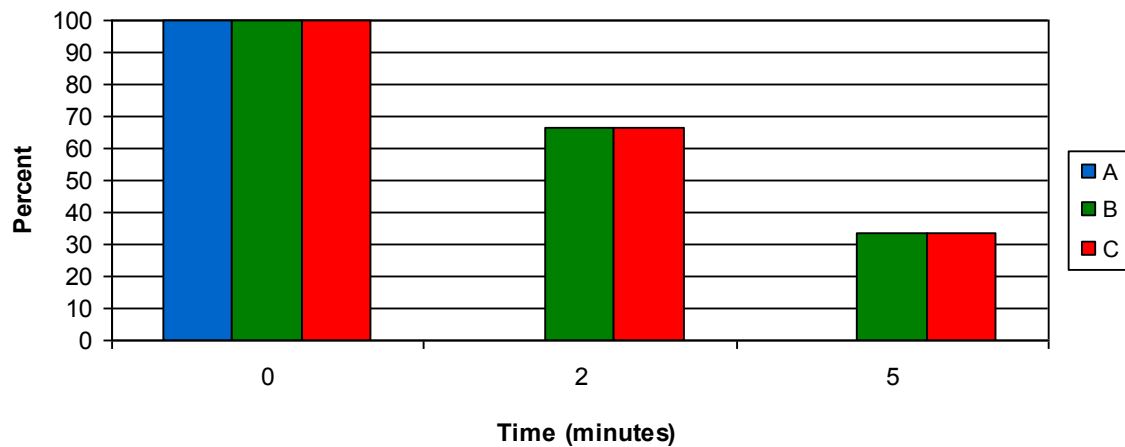
Table 1. Slab Bacon Thermal Processing Schedule

Step	Time	D.B.	W.B.	Humidity	IT (°F)	Dampers
Cook	2:00	104	86	48%		Auto
Cook	1:00	125	100	42%		Closed
Cook	1:30	125	0	0%		Auto
Cook	0:01	135	100	30%	126	Auto

Table 2. 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 3. 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 4. 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)

CHAPTER 5. GENERAL CONCLUSIONS

Consumers are constantly bombarded with the knowledge of potential pathogens in their environment through sometimes false and misleading modern media sources. As our world becomes more internet driven and scientists seek factual answers to consumer health concerns, caution must be taken in analyzing the true risk involved in a situation. For example, consumers should be well informed of the risk involved in eating undercooked meat and poultry products, yet this practice still occurs in modern societies today. Foodborne disease can devastate the credibility of an entire industry if appropriate controls for these risks are not in place. Primitive man understood that his food from a recent kill of a beast had a prolonged shelf life if he used fire to cook. Unfortunately, our world is much more complex than that of primitive human societies; however, we still face similar challenges with regard to the safety and security of our food supply.

Societies today are faced with the presence of genetically mutated microorganisms that threaten our health, food supply and modern way of life. Prior to this study, only the knowledge that antibiotic resistant *Staphylococcus aureus* was prevalent in meat and poultry products available to consumers at the retail counter. Furthermore, consumers were left to decide for themselves if this genetic mutation we call MRSA survived various cooking practices.

Results from this research indicate that methods used to commercially thermal process frankfurters, summer sausage and boneless ham to an internal temperature of 70°C dramatically reduce the presence of MRSA in these processed meat items. In addition to thermal processing, acid production during

fermentation and subsequent pH reduction to below 5.0 in summer sausage greatly reduced MRSA survival of viable cells. Although the results of the slab bacon heat treated to an internal temperature of 52°C indicate a higher rate of MRSA survival, the overall lethality of the heat treatment process could be sufficient to adequately reduce the risk of MRSA exposure to the consumer from MRSA populations reported in the literature. Regardless of the perceived risk to consumers, bacon sold at the retail level is handled as an uncooked item and labeled with safe handling instructions to consumers similar to that of raw meat items. As a result, studies were conducted to evaluate the effectiveness of consumers frying sliced bacon in the home. Results from the simulated consumer frying treatment indicate very low MRSA survival rates regardless of "doneness" level. Although MRSA proves to be a hardy organism with respect to antibiotic resistance, these studies indicate that MRSA is inhibited by various types and temperatures of thermal processing.

ACKNOWLEDGEMENTS

This dissertation is the result of many years of selfless assistance, guidance and personal sacrifice. I cannot begin to name all of the individuals who helped shaped my career path, and I apologize if I forget to name anyone specifically.

I would like to thank Dr. Joe Cordray, my major professor, for allowing me the opportunity to work fulltime as a meat science extension associate while I pursued my advanced degree in meat science at Iowa State University. Joe and I first worked together in 2006 at the American Cured Meat Championship in San Diego, CA. That opportunity was not by chance and would not have been possible without the help of Mr. Hugh Tyler, past AAMP president, and Dr. Jim Acton, professor emeritus, Clemson University.

Many thanks are due to my research mentor and office suitemate, Dr. Jim Dickson. Dr. Dickson definitely did not allow me to pursue this project because of my skills as a microbiologist. He instead saw a greater future vision for me as a meat scientist and avid researcher of food safety. His vast knowledge and personable disposition allowed me to seek answers to questions I had not previously pondered. I would also like to thank my outstanding committee members, Drs. Denny Olson, Aubrey Mendonca and Ken Prusa for their interest in my future as a PhD candidate and graduate of Iowa State University. I am also appreciative of the entire meat science faculty at Iowa State. You all played an important role in my development as your peer. I thank you all for your input and guidance during my doctoral studies here in Ames.

A special "thank you" is also due to Mr. Steve Niebuhr for being understanding of my work schedule and allowing me to complete this project in the food safety research lab. His guidance challenged me to learn and apply microbiology at a new level. I also value the experience of NOT contracting a MRSA infection from any mishaps that may or may not have occurred while working in the lab. At least it was an educational moment for us all, even the physician!

I could not begin to express my gratitude for the meat lab staff for your help in sourcing raw materials and assistance in organizing and prepping processing equipment. Thank you especially to Jeff Mitchell, Mike Holtzbauer, Steve Bryant and Matt Wenger. I am also appreciative of the numerous graduate and undergraduate assistants that help keep our meat lab running. I have been in your shoes. I know that most of you are just doing your jobs, but your hours of cleaning and sanitizing are greatly appreciated. We could not operate without you.

I would be remiss if I did not show appreciation to my fellow graduate students. Luckily for you, I have been in the PhD grad student role longer than any of you! I have far too many people to mention, but I am grateful for the interaction we have all had in one way or another.

Last, but in no way least, I leave you with this thought and guidance for my own life. I have listed many people who have physically and mentally assisted me in my studies. My life's spiritual verse however, is clearly outlined in Psalms 118:8. It simply states that "it is better to trust in the LORD than to put confidence

in man" (New King James Version). There are times in life when faith alone gets

us through our trials. I am most grateful that my faith is in something and

someone like Jesus. I choose this verse to guide me, because it is located in the

center of the Bible. We should all strive to put God in the center of our own lives.