ABSTRACT

Title of Document: PREVALENCE OF MRSA AND ANTIMICROBIAL RESISTANCE OF STAPHYLOCOCCUS AUREUS IN MARYLAND GROUND MEAT PRODUCTS

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The aim of this study was to evaluate the risk of exposure to antimicrobial-resistant *Staphylococcus aureus* from food-grade raw ground meat products in Maryland. Samples of ground beef (n = 198), pork (n = 300), and turkey (n = 196), were collected by random sampling from March-August, 2008. All isolates were tested for resistance to methicillin and confirmed *S. aureus* isolates (n = 200) were tested for susceptibility to 21 additional antimicrobials. Overall, turkey- and pork-derived isolates were more likely to be resistant to commonly used antimicrobials. One isolate from pork was confirmed to be the USA100 strain of MRSA and was resistant to 10 antibiotics. In addition, antibiotic-resistant non-*S. aureus* isolates were characterized and may represent a source for the transfer of resistance genes to *S. aureus*. Our findings suggest that meat production practices may impact the prevalence and antimicrobial resistance of *S. aureus* in ground meat.

PREVALENCE OF MRSA AND ANTIMICROBIAL RESISTANCE OF STAPHYLOCOCCUS AUREUS IN MARYLAND GROUND MEAT PRODUCTS

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1.1 Antibiotic Resistance: General Background

Although the development of antibiotics throughout the twentieth century has led to major advances in human and animal health, several factors have also led to antibiotic resistance. One part of the problem is that the discovery of new antibiotics has waned. Since 1970, there has only been one new class of antibiotics introduced, and on average, research and development of antibiotics takes 10 to 20 years (Lipsitch 2002). Therefore to maintain the efficacy of current antibiotics, humans have turned to combining them.

At the same time, with the introduction of antibiotics into healthcare, resistance has arisen. Up to 30% of *Streptococcus pneumoniae* and *Haemophilus influenzae*, bacteria that cause the major infectious disease pneumonia, are resistant to penicillin (Thornsberry 2002). Likewise, *Shigella dysenteriae*, a microbe which causes diarrheal disease, has become resistant to every available drug except ciprofloxacin within the past ten years (Green 2009). Ciprofloxacin belongs to a group of antibiotics called fluoroquinolones, which were banned for the treatment of poultry by FDA-CVM (the Food and Drug Administration Center for Veterinary Medicine) in 2005 because of quickly developing microbial resistance to the drug as a result of its use as a sub-therapeutic antibiotic in industry (FDA 2005). Similarly, after penicillin was discovered, levels of resistant *Staphylococcus aureus* in hospitals

were around 1%. This rose with increasing use of the antibiotic to 38% in 1947 and about 90% by 2000 (Greenwood 2000).

1.2 Use of Subtherapeutic Antibiotics in Livestock

Sources, or reservoirs, of antibiotic resistance have spread since antibiotics started being used. Originally, most cases were only found in hospital settings. However, reservoirs have expanded to many different environments. While resistant pathogens seem to pose a more imminent threat to humans, the fact that non-pathogenic organisms can also develop resistance adds to the problem. Through hospital waste, sewage, and other man-made materials, antibiotic resistance has spread through both pathogenic and non-pathogenic bacteria. Resistant non-pathogenic bacteria magnify the problem because they can pass resistance genes to pathogenic bacteria through processes such as conjugation.

One man-made source of resistance involves the use of antibiotics in agriculture. Subtherapeutic antibiotics are antibiotics administered at a low dose for an extended period of time in order to promote growth or prevent disease. In modern large-scale agriculture, poultry, swine, and other livestock are often given subtherapeutic doses of antibiotics as a prophylactic. When antibiotics are administered at subtherapeutic doses, the hardiest bacteria will survive treatment and reproduce to create an increasingly resistant population of bacteria. In surveys from both the 1980s and 1990s, about half of the antibiotics used in the United States were used in animal feed (Dupont 1987; Levy 2001).

Subtherapeutic doses of antibiotics given to livestock are used to increase growth rate and efficiency of feed utilization. Empirical data also showed that antibiotics reduce mortality and morbidity. Gary L. Cromwell's study suggested this shows that the use of subtherapeutic antibiotics is a necessary part of the diet of chickens and swine in order for farmers to continue producing enough meat to stay in business (2002). Giving livestock antibiotics helps ensure that they will not die prematurely and that they can eventually be sold as meat for human consumption. While there are numerous benefits for farmers who treat their livestock with antibiotics, this practice may cause antibiotic resistance in bacteria.

The subtherapeutic amounts of antibiotics given to livestock are not only capable of making bacteria on the farm resistant, but can also promote the spread of resistance to bacteria in the surrounding environment. As resistant bacteria from the livestock spread throughout the environment, the bacteria, through processes of gene transfer, can spread their resistance to other bacteria (Agersø 2002). This means that resistant bacteria from livestock may be easily spread throughout the environment and could potentially be spread to humans (DuPont 2007). It is unclear whether the use of subtherapeutic amounts of antibiotics is the sole factor in causing increased rates of MRSA in livestock, but it is most definitely a contributing factor.

Currently, a major concern associated with the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in farm animals is its prevalence among humans within and around heavily affected farming communities. A case study reports a mother with mastitis due to MRSA (Huijsdens 2006). Her baby daughter was found to have pneumococcal otitis media half a year later and subsequently

tested positive for MRSA. To investigate the source, family members and household animals, including the swine on the family farm, were tested for MRSA. Throat and nasal swabs were taken from family members and co-workers on the farm as well as from ten swine. Of those sampled, three family members, three coworkers, and eight swine were found to be MRSA positive. All of the pulsed-field gel electrophoresis (PFGE) non-typable MRSA isolates were found to be genetically identical, providing direct evidence of the clonal spread and transmission of MRSA between humans and swine. This is not only worrisome to farming communities, where direct swine-human contact can transmit these MRSA strains; this means that strains of MRSA that infect swine and other farm animals may be able to end up in the meat products that the general public consumes.

1.2.1 Antibiotic Use in Cattle

Antibiotics that have been commonly fed to cattle presently or in the past include virginiamycin, tetracycline, tylosin, and neomycin (McDonough 1999). *Enterococcus* from cows between 1998 and 1999 showed resistance to tetracycline (80%) and ampicillin (20%) (Butaye 2001). Similarly, *Salmonella dublin* from cows in Pennsylvania and New York were found resistant to ampicillin, chloramphenicol, tetracycline, and neomycin (McEwen 2002).

Resistant bacteria have not only been isolated from cattle directly, but also from the surroundings of cattle farms. For instance, cows that were fed resistant *E. coli* were found to excrete resistant bacteria for four months afterwards, while

bedding material and animals in close proximity to the cattle, including humans, harbored resistant bacteria for about four weeks (Marshall 1990).

1.2.2 Antibiotic Use in Poultry

In the 1980s, reports suggested that about 80% of poultry in the United States were given antibiotics subtherapeutically (Dupont 1987). Antibiotics used either in the past or currently include fluroquinolones, avoparcin, virginiamycin, and tetracycline. Resistance to the first three has been shown to be associated with resistance in the related drugs ciprofloxacin, vancomycin, and quinupristin/dalfopristin, which are used in humans. Even if usage of a drug has halted, resistance can remain in the farm environment for more than a year (Jacobs-Reitsma 1997; Kruse 1999).

Studies have been performed on poultry farms to test the resistance of different bacteria isolated from both poultry and the environment. Resistance was found in different species of *Staphylococci, Streptococci, Clostridium, Pseudomonas, Aeromonas, Campylobacter, Salmonella, Enterococci*, as well as *E. coli* (Jacobs-Reitsma 1997; Kelley 1998; Kruse 1999; Wiuff 2000; Levy 2001). In bacteria found in both the poultry and their waste, resistance to almost every class of antibiotics has been found, including resistance to multiple antibiotics, though resistance has been most commonly found to tetracycline and erythromycin (Yoshimura 2000). At the same time, resistance in the poultry-associated bacteria tends to decrease as expected after the use of a specific antibiotic is terminated. One study showed that the prevalence of vancomycin-resistant *Enterococcus* (VRE) decreased from 80% of

chicken carcasses to 5% following the ban on the agricultural use of a closely related antibiotic, avoparcin, in Denmark (Bager 1999).

1.2.3 Antibiotic Use in Swine

Common antibiotics used in pig farms include tylosin, sulfonamides, and tetracyclines (McEwen 2002). In different species of *Enterococcus*, high levels of resistance, including multiple antibiotic resistance, have been found for erythromycin, tetracycline, streptomycin, and ciprofloxacin (Mathew 1999; Werner 2000).

Although the United States has not put such a ban in place, several countries in Europe have stopped some or even almost all subtherapeutic use of antibiotics in swine farming (Aarestrup 2000; Aarestrup 2001). In Denmark, this occurred around 1999. Levels of resistance before this time and a few years after showed a decrease usually to about 50% of the original level or sometimes even less (Aarestrup 2001).

In 2007, a study conducted in the Netherlands took a representative sampling of Dutch pigs from nine different slaughter houses. MRSA was found to exist in 39%, with the problem existing in all nine slaughter houses. All of the test isolates were not only methicillin resistant, but resistant to many other antibiotics as well. The subtherapeutic treatment of the swine resulted in isolates which were universally resistant to tetracycline, with 23% also being resistant to both erythromycin and clindamycin and 36% to kanamycin, gentamicin, and tobramycin (De Neeling 2007).

1.3 Methicillin-Resistant *Staphylococcus aureus* (MRSA)

There is concern that subtherapeutic antibiotics fed to animals can promote the development and growth of methicillin-resistant *Staphylococcus aureus* (MRSA). *S. aureus* is an opportunistic bacterium that lives on the skin and in the nasal passages of people and animals. It can become resistant to methicillin by acquiring the *mecA* gene. Strains that are *mecA*-positive, indicated as MRSA, are the main cause of nosocomial infections worldwide. Person to person transmission in hospitals is the principal route of infection. However, according to recent publications, pets and farm animals and their caretakers can also act as reservoirs of MRSA (Corrente 2007).

An infection does not necessarily result in illness but can cause disease symptoms and occasionally even death if the bacteria infect an open wound or an immunodeficient individual such as a young child, a senior citizen, or hospital patient. It is important that, should symptoms arise, the person is immediately treated with safe and effective antibiotics; however, infections from methicillin-resistant strains of *S. aureus* do not readily respond to the first line of treatments. For this reason, doctors are forced to resort to stronger, more expensive, and harsher, intravenously administered drugs such as vancomycin to treat what was once an easily curable infection. In addition, patients suffer from prolonged sickness and hospital stays.

Staphylococci are a type of Gram-positive cocci that can be separated into coagulase-positive staphylococci and coagulase-negative staphylococci. The most common staphylococci which are found in humans are coagulase-negative *S. epidermidis* and coagulase-positive *S. aureus*. Both are opportunistic bacteria that

may cause skin infections such as pimples, although *S. epidermidis* infection is much less common. In addition, *S. aureus* often is found in hospital settings. Furthermore, *S. aureus* can produce toxins that cause food poisoning or septicemia (Le Loir 2003). It is due to these heat-stable toxins that even when cooked, food contaminated with *S. aureus* ingested by humans can result in illness.

Penicillin was discovered in 1928. Soon after its discovery, attempts were made to use it in medicine. By the 1940s, it was a commonly used antibiotic, with almost all bacteria including staphylococci susceptible to it. However, 59% of *S. aureus* from hospitals in 1948 were already resistant, and by 1950, almost all hospital strains of *S. aureus* were resistant to penicillin (Garrod 1971). Resistance has since spread past the hospital setting to the community. In several studies of *S. aureus* in the community, more than 90% of *S. aureus* exhibited resistance to penicillin or to penicillin and erythromycin (Greenwood 2000).

Some staphylococci were resistant to streptomycin, tetracycline, chloramphenicol, and novobiocin by 1953. With increasing levels of resistance to different types of antibiotics, new antibiotics had to be created. This led to the development of penicillin derivatives to fight *S. aureus* (Ruef 2004).

The record of methicillin, or rather penicillinase-resistant N-lactam drugs, in relation to *S. aureus* has been similar to other antibiotics. After its introduction in the 1950s, resistant strains appeared in hospitals by the 1960s (Garrod 1971). By 2000, studies performed in hospital settings in the United States showed that about half of all *S. aureus* from hospitals were resistant to methicillin. Resistant *S. aureus*, particularly MRSA, have thus emerged as a serious public health concern in the U.S.

Established risk factors for MRSA infection include recent hospitalization or surgery, residence in a long term care facility such as a nursing home, dialysis, and indwelling percutaneous medical devices and catheters.

Recently, MRSA has been of particular interest to the medical community. It is estimated that almost 100,000 serious MRSA infections arose in 2005, including nearly 19,000 deaths related to MRSA, compared to 17,000 deaths from HIV/AIDS (Klevens 2007). The first reported case of a MRSA infection in the United States occurred in 1968, seven years after its appearance in the United Kingdom (Barrett 1968). In 1972, 2% of hospitalized patients were infected with methicillin-resistant *S. aureus*. This proportion rose to 40% by 1997 (Lowy 1998). More recent reports have found further increases. Between 1999 and 2005, hospitalizations due to MRSA doubled (Klein 2007).

In general, recovery time with *S. aureus* complications is longer, placing burdens on both patients and hospitals. For patients with a general *S. aureus* infection compared to patients without *S. aureus* infections, in-hospital time was about three times as long; the total cost nearly three times as much; and in-hospital mortality nearly five times as high (Noskin 2005). Of these *S. aureus* infections, MRSA not only creates a still longer hospital stay, but also produces a higher mortality rate. Even in accounting for the general higher morbidity of patients with MRSA infections, the mortality rate for patients with MRSA infections is higher than that for patients with methicillin-susceptible *Staphylococcus aureus* (MSSA) infections (Blot 2002).

1.4 CA-MRSA and HA-MRSA: What is the difference?

So far, hospitals have been the main reservoir for MRSA. As of 2007, approximately 85% of United States MRSA infections were nosocomial, or hospital-associated infections (HA-MRSA), two thirds of which arose outside of the hospital and one third of which arose in the hospital. About 14% of infections are thought to be community-associated (CA-MRSA) (Klevens 2007). At the same time, CA-MRSA is becoming more common. HA-MRSA and CA-MRSA outbreaks involve different strains of MRSA with different microbiological and genetic properties (Naimi 2003).

CA-MRSA infection tends to occur in younger, healthy people without the risk factors associated with medical equipment and procedures. It more commonly involves serious skin and soft tissue infections and a severe form of pneumonia. CA-MRSA isolates are also more likely to be susceptible to ciprofloxacin, clindamycin, gentamicin, and trimethoprimsulfamethoxazole than HA-MRSA and possess different exotoxin gene profiles (Panton-Valentine Leukocidin genes) (Naimi 2003). However, the boundary between these two types of MRSA is becoming increasingly unclear as the incidence of CA-MRSA infections has dramatically increased in healthcare settings (Benoit 2008). Recent studies suggest that CA-MRSA is a growing epidemic. Cases of MRSA in pediatric head and neck *S. aureus* infections in the US increased from 11.8% to 28.1% from 2001 to 2006 (Naseri 2009).

Although prevalence of CA-MRSA is much lower than HA-MRSA, many strains of MRSA are resistant to almost all antibiotics except glycopeptides such as vancomycin (Greenwood 2000). This has led to outbreaks as well as some deaths

among groups of humans in regular close contact, such as those in day-care centers or sports teams (Kazakova 2001).

To treat MRSA infections, doctors have turned to stronger antibiotics such as vancomycin. Vancomycin is only used when all other drugs have failed because of the serious adverse effects associated with it (Edlund 1997; Von Drygalski 2007). However, vancomycin-resistant *S. aureus* (VRSA) was isolated in Japan in 1997 (McCormick 1998), and soon found elsewhere, including the United States (Srinivasan 2002). It has been suggested that VRSA has developed by acquiring the *vanA* operon (gene cluster) from vancomycin-resistant enterococci (VRE) (Noble 1992; Robinson 2005).

1.5 MRSA Contamination of Meat Products

Studies in Denmark, Canada, and the Netherlands have confirmed that the pig MRSA ST398 strain is transmissible from animals to humans (Lewis 2008). In 2008, infections with ST398 strains of MRSA, which are believed to be of animal origin, resulted in the treatment and even hospitalization of U.S. patients who had no recent contact with live animals suggesting that these strains may already be more common (Welinder-Olsson 2008). These patients may have been exposed to MRSA by handling raw meat products.

In the Netherlands, 2.5% of pork and beef products from 31 supermarkets and butcher shops were found to contain a non-typable MRSA (NT-MRSA) strain in 2008 associated with farming and most likely of animal origin (Van Loo 2007).

Meanwhile, the frequency of reported NT-MRSA in human cases in the Netherlands increased from 0% in 2002 to > 21% after intensified surveillance was implemented in 2006. NT-MRSA clustered geographically with pig farming, and carriers were most often pig or cattle farmers. The NT-MRSA strains belong to the clonal complex ST398 (Van Loo 2007).

Furthermore, the wide temperature, pH, and NaCl ranges in which the bacteria can survive and grow allows *S. aureus* to readily multiply in meat and dairy products (Le Loir 2003). Although contamination can be avoided through proper food preparation, *S. aureus* remains a leading cause of food-borne illness (Lund 2000). While enterotoxins cause illness for those consuming contaminated foods, the increasing resistance of *S. aureus* to antimicrobials also poses a risk to food handlers and the community as a whole (Pu 2009). Non-MRSA strains of *S. aureus* isolated from food products in Italy have been found to express resistance to some fluoroquinolones, tetracycline, erythromycin, gentamicin, bacitracin, oxacillin, penicillin, and/or ampicillin (Gundogan 2005; Pesavento 2007). The aim of the present study is to characterize antimicrobial resistance among *S. aureus* including MRSA recovered from raw retail ground meat products.

1.6 Study Objectives

We are interested in the role that contaminated meat products play in the transmission of MRSA. Until recently, meat products were not considered a significant source of MRSA; however, new studies conducted in Canada and Europe

have shown that MRSA is widespread in livestock, farmers, and meat (De Neeling 2007, Lewis 2008, Khanna 2008, Van Loo 2007). When farmers infected with either *S. aureus* or MRSA transfer the infection to their animals through routine handling, the bacteria may become even more resistant within the animals if livestock are fed a steady low dose of antibiotics. The animals then either pass the infection back to farmers or enter the food supply. Although ingested MRSA from meat would not lead to an infection (because the bacteria cannot survive in the intestinal tract), handling raw meat can lead to disease.

The United States meat supply should be monitored in order to determine the risk of MRSA infection from raw meat in addition to hospitals. Through resistance testing of *S. aureus* recovered from raw retail ground meat products, our research intends to contribute data and to encourage further studies concerning the link between the MRSA in livestock, the meat that consumers handle, prepare, and consume, and the recent surge in MRSA infections in humans.

Although this study has the obvious limitations that it will focus specifically on MRSA and *S. aureus* in three kinds of meat from retail stores in two Maryland counties, it will be one of the first few performed in the United States and should encourage more studies to be performed for a more integrative picture.

The idea that hospitals are reservoirs of MRSA has been around for years, but only more recently are researchers looking at other sources, such as animals. Examining the possibility of MRSA in consumer meat is important not only for direct consumer safety, but also for public health overall.

We thus aim to address the following question: How prevalent are antimicrobial resistant strains of *S. aureus* in retail raw ground turkey, beef, and pork products sold at three major grocery store chains in Montgomery and Prince George's Counties in Maryland? To answer this question, we screened 694 samples of meat (predominantly ground meat) from the grocery stores included in our study for antimicrobial resistant strains of *S. aureus*.

Chapter 2: Literature Review

2.1 Antibiotics Approved for Use in Livestock

Subtherapeutic antibiotic use is the administration of low levels of antibiotics to livestock and poultry through feed additives. Farmers have become increasingly dependent on using antibiotics in poultry and livestock feeds over the past half century. Antibiotics are incorporated into animal feed and drinking water for three main reasons: to prevent bacterial infection, to decrease the quantity of feed necessary to sustain the animals, and to increase the rate of growth (Dupont 1987). The antibiotics are administered in low doses in the feed which fosters the development of resistant strains of bacteria. With this low level of antibiotics present in the animal, resistant strains thrive because they lack competition from their less resistant counterparts.

Almost half the antibiotics sold in the United States every year, 2.1-2.5 million kilograms, are used subtherapeutically in animal feed. Nearly 80% of poultry, 75% of swine, 60% of feedlot cattle, and 75% of dairy calves have received antibiotics subtherapeutically at some point in their life. Antibiotics are mixed with feed at levels ranging from 2 grams/ton to 1000 grams/ton depending on the type, age, and activity of the animal. For instance, animals undergoing shipping, weaning, or severe environmental changes receive a greater dose of antibiotics (Dupont 1987).

As a result of the beneficial effects of antibiotics on livestock growth the United States Department of Agriculture has approved a myriad of antibiotics for subtherapeutic use in animals. Different antibiotics have been approved for use in cattle, swine, chickens and turkey. The majority of the antibiotics approved are used subtherapeutically in feed, while some drugs such as oxytetracycline can be administered by injection as well.

Antibiotics Approved by the USDA

Approved for Use in Dairy and Beef Cattle:

Amoxicillin	Lacalocid	Tylosin
Ampicillin	Monensin	Sulfabromomethazine
Bacitracin	Neomycin	Sulfachloropyridazine
Ceftiofur	Oxytetracycline (oral)	Sulfadimethoxine
Chlortetracycline	Oxytetracycline (injection)	Sulfaethoxypyridazine
Dihydrostreptomycin	Penicillin	Sulfamethazine
Erythromycin	Streptomycin	Sulfamethoxine
Furamazone	Tetracycline	
Gentamycin	Tilmicosin	

Approved for Use in Hogs:

Amoxicillin	Efrotomycin	Penicillin
Ampicillin	Erythromycin	Spectinomycin
Apramycin	Gentamycin	Streptomycin
Arsanilic acid	Lincomycin	Tetracycline
Bacitracin	Neomycin	Tiamulin
Bambermycins	Oleandomycin	Tylosin
Chlortetracycline	Oxytetracycline	Virginiamycin

Approved for Use in Chickens and Turkeys:

Bambermycin	Novobiocin	Streptomycin
Bacitracin	Oleandomycin	Tetracycline
Chlortetracycline	Oxytetracycline	Tylosin
Erythromycin	Penicillin	Virginiamycin
Gentamycin	Roxarsone	Fluoroquinolones
Neomycin	Spectinomycin	

Table 1: The USDA has approved the above antibiotics for use in cattle, hogs, chickens and turkeys.

Nearly every class of antibiotics used in humans is also used in animals (Schmidt 2002).

Healthy Animals, Unhealthy People? Popular Antibiotics on Farms and in Pharmacies

Antibiotic Class	Animal Species	Animal Use			Human Use	Bacterial Resistance
		Disease Treatment	Disease Prevention	Growth Promotion		
Aminoglycosides (gentamicin, neomycin, streptomycin)	cattle, poultry, sheep, swine	✓	✓		✓	✓
Beta-Lactams						
Penicillins (amoxicillin, ampicillin)	cattle, poultry, sheep, swine	✓	✓	✓	✓	✓
Cephalosporins (third generation)	cattle, poultry, sheep, swine	✓	✓		✓	✓
Ionophores	cattle, poultry, sheep		✓	✓		✓
Macrolides (erythromycin, tilmicosin, tylosin)	cattle, poultry, swine	✓	✓	✓	✓	✓
Polypeptides (bacitracin)	poultry, swine	✓	✓	✓	✓	✓
Fluoroquinolones (enrofloxacin)	cattle, poultry	✓	✓		✓	✓
Sulfonamides	cattle, poultry, swine	✓		✓	✓	✓
Tetracyclines	cattle, poultry, sheep, swine	✓	✓	✓	✓	✓

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Table 2: Antibiotic overlap amongst humans and livestock. Every family of antibiotics used in humans is used in animals as well (Schmidt 2002).

As evidenced by the preceding table, there is an overwhelming overlap between antibiotics used in humans and those used in animals. When animals receive these antibiotics at consistent low doses, the resistant strains of bacteria thrive in the absence of their non-resistant counterparts. Because humans then handle and ingest these animals and products produced by these animals, resistance to these drugs may be transferred to the human population.

2.1.1 Major Classes of Antibiotics Used in Animals

Aminoglycosides such as gentamicin and streptomycin are made up of a sugar and an amino group. Aminoglycosides disrupt protein synthesis in bacteria. They can interrupt the proofreading process and cause premature termination in protein synthesis. They may also inhibit ribosomal translocation such that the peptidyl-tRNA cannot move from the A-site to the P-site. Aminoglycosides also compromise the integrity of the bacterial cell membrane.

Beta-Lactams are antibiotic agents that contain a β-lactam nucleus and include penicillins and cephalosporins. Penicillins were the first class of antibiotics discovered. The β-lactam functional group interrupts the enzyme, DD-transpeptidase, which links peptidoglycan in bacteria. This compromises the bacterial cell wall. Peptidoglycan precursors accumulate which causes hydrolases and autolysins to activate. These digest the bacteria's peptidoglycan even further.

Macrolides such as erythromycin contain a macrolide ring which includes one or more deoxy sugars. Macrolides inhibit protein synthesis by inhibiting

peptidyltransferase and ribosomal translocation. Macrolides are often used to treat soft tissue and respiratory tract infections.

Polypeptide antibiotics such as bacitracin act by inhibiting proteins, but the mechanism of action is relatively unknown. In humans these are normally administered topically or inhaled into the lungs.

Flouroquinolones such as ciprofloxacin halt bacterial DNA replication by preventing bacterial DNA from unwinding. Because the DNA cannot relax from its condensed state it cannot be replicated.

Sulfonamides such as sulfamethoxazole contain a sulfonamide group. Sulfonamides competitively inhibit dihydropteroate synthetase (DHPS), which is necessary for the synthesis of the required nutrient folate in bacteria.

Tetracycline antibiotics have a four hydrocarbon ring structure. Tetracyclines inhibit translation by binding to the 30S ribosomal subunit and preventing amino-acyl tRNA from binding to the A site of the ribosome.

2.2 Economic and Animal Health Benefits of Antibiotic Use in Animals

To examine the effect of subtherapeutic antibiotic use on the efficiency of livestock growth, researchers examined two swine herds. One herd was administered antibiotics subtherapeutically while the other was used as a control and received no antibiotics in its feed. Conception rates between the experimental and control group dropped from 91% to 82% respectively and weaning weight decreased from 5.67 pounds to 5.37 pounds respectively. Additionally, the researchers note that these

results were obtained in a standardized environment. Because the barn used was likely much more sanitary than would be the case with non-experimental conditions, these growth discrepancies would likely be much more exaggerated in a real-life situation where disease and infection exposure would be greatly increased. It is evident from the results that antibiotics allow farmers to maintain larger, more efficiently grown herds with the subtherapeutic use. Without antibiotics, herds are more prone to infection, specifically when immature and undergoing the weaning process. Thus, it is clearly evident that there is a significant pressure on farmers to use antibiotics subtherapeutically when raising livestock (Cromwell 2002).

Furthermore, the two groups of swine initially harbored coliform bacteria which showed a very high resistance to tetracycline. The tetracycline resistance in the antibiotic treated herd began at nearly 100% and essentially maintained this level over the following years of study. The non-antibiotic herd which did not receive antibiotics over the duration of the experiments showed a decrease in resistance that continued over the years of the experiment (Cromwell 2002).

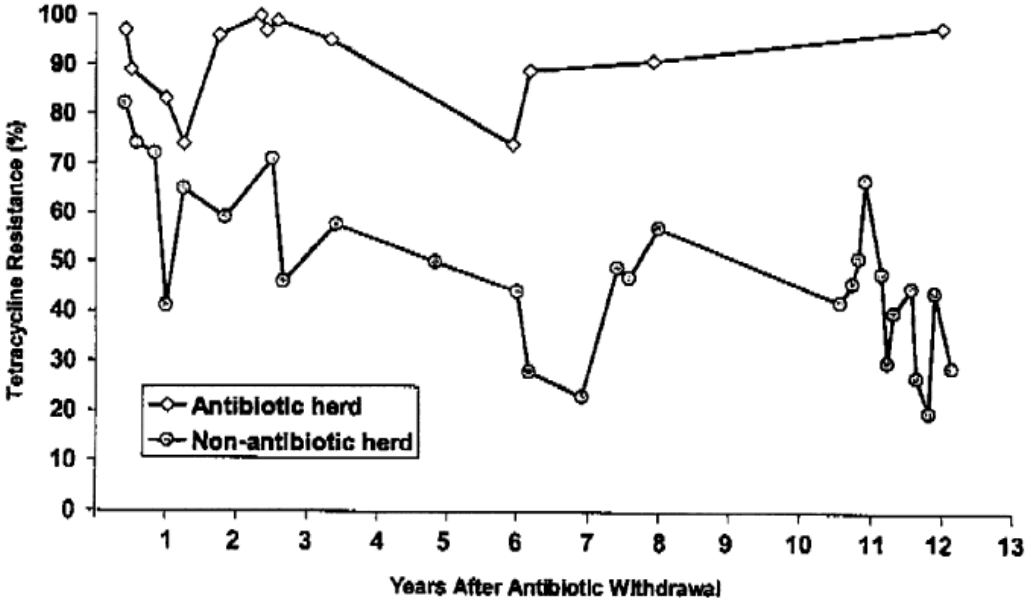

Figure 1: Level of tetracycline resistance in swine after antibiotic withdrawal. The level of resistance in the non-antibiotic herd drops after cessation of antibiotic usage (Close 2000).

Besides direct improvement in animal growth, administering antibiotics subtherapeutically can help prevent digestive disturbances, improve feed utilization within animals, and reduce nutrient wastes. One group of researchers found that there was an overall 3-5% improvement in nutrient utilization, a 2-8% improvement in growth rate, and a 2-5% improvement in feed conversion efficiency in pigs that had already finished growing, with more substantial improvements evidenced in piglets. The researchers concluded from this study that the monetary return from using antibiotics could result in a payout as high as 10 to 1 for farmers (Close 2000).

Unfortunately, this economic incentive encourages farms to continue to administer antibiotics subtherapeutically. Administering antibiotics to animals on an as-needed basis is less time consuming and costly but does not contribute the added

growth benefits that subtherapeutic administration of antibiotics provides. Alternatives do exist. Farmers can use natural acids and oils to help the digestive tract and digestive process of animals kill off formidable bacteria. This diet acidification helps to decrease intestinal bacterial growth within the animals. To destroy harmful bacteria within animal waste that may run off and contaminate the surrounding environment, farmers can soak animal waste in chlorinated water. However, farmers already employ this diet acidification technique in combination with subtherapeutic antibiotic use and thus gain increased benefits. Soaking waste in chlorinated water is often an additional step for farmers to take that many view as unnecessary.

2.3 Antibiotic Resistance Genes in the Farm Environment

Often, the subtherapeutic use of antibiotics not only contaminates meat and food products generated by animals, but also the environment surrounding farms that utilize this practice. Because bacteria are capable of picking up and expressing foreign genetic material, they can transfer antibiotic resistance amongst one another. Additionally, up to 95% of antibiotics given subtherapeutically to livestock are excreted into the surrounding environment unchanged. This creates a breeding ground for resistance to these antibiotics in lands and waters surrounding farms. In one study, researchers examined bacteria collected from river sediments and drinking water that had been processed at water treatment plants. They extracted DNA from bacteria and examined the levels of genes that showed resistance to tetracycline and sulfonamide, two antibiotics that are commonly administered subtherapeutically. They found that

the prevalence of resistance genes was hundreds or thousands of times higher in waters near urban and farm activity as opposed to 'pristine' bodies of water designated as controls (Choi 2007).

2.4 Antimicrobial Resistant Pathogens Associated With Farm Animals

In Australia, researchers sampled pig carcasses and pig meat to assess the level of resistance in bacteria that could be a threat to human health. A total of 231 swabs were taken of pig carcasses and 32 samples of pork meat were purchased, rinsed with sterile buffered peptone water, and isolations were taken from the initial bacterial suspensions. *Campylobacter* species, *Escherichia coli*, and *Enterococcus* species were tested for susceptibility to antibiotics. Resistance to tylosin, tetracycline, erythromycin, and lincomycin in *Campylobacter* species was common. In *E. coli* isolates, resistance varied greatly, but there was a prominent resistance to tetracycline in all the animal and meat isolates. In pig carcass swabs, resistance to ampicillin, spectinomycin, streptomycin, and sulphadiazine was found. There was widespread resistance to aminogylcosides, lincomycin and suphadiazine in *Enterococci* species (Hart 2004).

The *Campylobacter* species *C. jejuni* and *C. coli* are often dangerous pathogens for humans and can lead to a number of illnesses including enteritis, septicemia, and extraintestinal infections. Because erythromycin is often the first drug suggested for treatment of such infections, the high resistance *Campylobacter* species showed is troubling. The pronounced resistance *E. coli* showed to tetracycline was

expected and not worrisome given that tetracycline resistance is so common today that tetracycline is rarely used in clinical settings. However, this observation is evidence of the dangers of antibiotic resistance and its association with subtherapeutic use of antibiotics in livestock. The widespread resistance to antibiotics shown by *Enterococci* species could pose a serious threat to humans. *Enterococci* species are increasingly seen in hospital-acquired infections. This reservoir of antibiotic resistance in *Enterococci* species poses a threat to clinical treatment of such infections (Hart 2004).

2.4.1 Salmonella

In another study, researchers traced the death of a 62-year-old Danish woman and another individual to a resistant strain of *Salmonella* generated from a single Danish swine herd. Resistant strains of *Salmonella* have become increasingly prominent in industrialized nations. The definitive phage type 104 (DT104) strain of *Salmonella enterica* serotype *typhimurium* is often resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. However the DT104 strain is beginning to show resistance to fluoroquinolones, a family of antibiotics often used to treat *Salmonella* infections (Wegener 2000).

When the 62-year-old Danish woman arrived at the hospital, she complained of severe diarrhea that had persisted for nine days. Doctors quickly diagnosed her with food poisoning from *Salmonella* and prescribed ciprofloxacin, an antibiotic that usually cures even the most severe *Salmonella* infections. However, her infection did not resolve. The *Salmonella* created a perforation in her colon allowing the infection

to spread to the rest of her body along with other bacteria from her colon. Within four days the woman was dead. The *Salmonella* had gained resistance against ciprofloxacin. A microbiologist, Henrik Wegener, identified the *Salmonella* as the DT104 strain and traced it to a particular farm where swine herds were being administered enrofloxacin. Because ciprofloxacin and enrofloxacin belong to the same family of antibiotics, the subtherapeutic use of enrofloxacin in the swine generated a resistance to the entire family of compounds referred to as quinolones. This resistance prevented the quinolones from disrupting the mechanisms by which bacteria replicate DNA. A total of 25 individuals were infected with *Salmonella* as a result of the contaminated meat and two individuals died, including the 62-year-old woman (Ferber 2000).

This was one of the first cases where antibiotic resistance in a clinical setting was traced directly to consumption of antibiotic resistant bacteria in food. It is often difficult for researchers to directly prove that antibiotic resistance generated on a farm results in human infection. Researchers must first correlate a resistant strain with an antibiotic administered to the livestock. They then must prove that that strain survives the slaughter house and packaging process. The researcher must then solidify the fact that consumption of the meat actually leads to an infection that is not cured by the current antibiotic treatment. Wegener and his team had already identified a DT104 strain in five Danish patients that was resistant to seven drugs, rather than only five which was common to most other DT104 strains. They determined that all the infected individuals bought meat that was supplied by a particular slaughterhouse. They later found that one of the 37 herds supplied to the slaughterhouse was infected

with the resistant strain. The herd had not been directly treated with quinolones, but herds on nearby farms had been. Because *Salmonella* easily jumps from one herd to the next, Wegener hypothesized that this was the source of the resistant strain. DNA fingerprinting reinforced the idea that the drug-resistant genes in the pig herd were identical to those found in the patients (Ferber 2000).

In a similar study in Nebraska, Paul Fey and his team identified a 12 year-old boy infected with a *Salmonella* strain that showed resistance to ceftriaxone. Unfortunately, quinolones cannot be used in children infected with *Salmonella* because they impede bone growth in their developing bodies. So the spread of *Salmonella* strains resistant to ceftriaxone could have dire consequences for infected children because quinolones cannot be used for treatment (Ferber 2000).

2.4.2 Enterococci

Enterococci bacteria are part of the normal flora that inhabits the human gut. In immunocompromised individuals, these bacteria can often multiply unchecked and cause a chaotic response in the immune system. Vancomycin, a drug that is often used as a last defense against bacteria, has recently proved ineffective in hospital settings. This vancomycin resistance can be explained by its use in hospitals. However, vancomycin resistance is now being observed in the healthy, non-hospitalized population in Europe, and this could be a direct result of the subtherapuetic use of avoparcin in livestock. Avoparcin and vancomycin inhibit bacterial growth by deactivating an enzyme necessary for cell wall construction. *Enterococci* resistant to avoparcin also show resistance to vancomycin. If a person

consumes undercooked meat with bacteria resistant to avoparcin, this resistance can be transferred to strains of bacteria inhabiting the human gut. One group of researchers conducted a study to test for this transfer of resistance genes. They found that there were identical sequences of transposons with identical genes conferring resistance in people and in pigs. Because these transposons were different than those found in resistant *Enterococci* strains from cows, turkeys, and chickens, the resistance genes from bacteria in pigs were likely transferred to human gut bacteria through the consumption of undercooked meat (Ferber 2000).

Anthony van den Bogaard and colleagues in the Netherlands conducted a study in which they examined the prevalence of vancomycin-resistant strains of *Enterococci* in pigs, chicken and people in 1997 and 1999. In 1997, avoparcin was banned in Europe. In just 2 years, the prevalence of vancomycin resistant strains in all three species dropped by nearly 50% (Ferber 2000).

The meat processing step of meat preparation is often a source of bacterial contamination. To investigate cross-contamination of antibiotic resistance in the commercial cattle processing system, *Enterococcus* isolates were examined from 60 cattle that were commercially processed. Fecal and hide samples were collected immediately before the 60 cattle were shipped to the processing plant. Hide samples were taken at the plant before processing and after hide removal. Of the fecal samples, 53.9% were positive for *Enterococcus*, 77.8% of the hide samples tested positive for *Enterococcus* before shipment and 96.1% tested positive at the processing plant before hide removal. All 279 *Enterococcus* isolates were resistant to at least one antibiotic, while 179 (64.2%) of these isolates were resistant to at least six antibiotics

including chloramphenicol (100%), flavomycin (90.3%), lincomycin (87.8%), tylosin (78.5%), erythromycin (76.3%), tetracycline (58.9%), quinuprisin/dalfoprisin (47.7%), bacitracin (17.9%), streptomycin (9.0%), ciprofloxacin (1.4%), linezolid (0.7%), and salinomycin (0.4%). Pulsed-field gel electrophoresis was used to evaluate molecular similarities amongst the *Enterococcus* isolates. These results showed that the isolates recovered at the cattle farm were molecularly similar to those at the plant, suggesting that cross-contamination within the plant was responsible for the increased number of resistant *Enterococcus* isolates taken from the plant (Fluckey 2009).

2.4.3 E. coli

One group of researchers set out to observe increased antibiotic resistance in poultry workers. They compared the colonization rates of antibiotic resistant *E. coli* among poultry workers to those in the community. They collected health surveys and stool samples from 16 poultry workers and 33 non-farming community members in Marlyand and Virginia. *E. coli* isolates were taken from the samples and tested for susceptibility to ampicillin, ciprofloxacin, ceftriaxone, gentamicin, nitrofurantoin, and tetracycline. The researchers found that the poultry workers were 32 times more likely than members of the community to carry gentamicin-resistant *E. coli* and they showed an increased risk of carrying multi-drug resistant *E. coli* (Price 2007). Thus, these workers have an increased propensity to spread resistant bacteria to the rest of the community. It also exhibits the danger of this reservoir of antibiotic resistance within the chicken product itself. Although meat often undergoes treatments to eliminate bacteria, and although much of the remaining bacteria is killed in the

cooking process, these findings suggest that there is a potent reservoir of antibiotic resistance in poultry. If consumers improperly handle this meat, they, just as these poultry workers, are at risk for bacterial colonization by these resistant strains of *E. coli*.

2.5 *Staphylococcus aureus*: A Dangerous Pathogen

Staphylococcus aureus is an extremely common and potentially dangerous species of bacteria. It exhibits a spherical "cocci" shape when viewed under a microscope and it can be seen growing in "grape-like" clusters, which are typical of the *Staphylococcus* genus (Staphylococcus means "bunch of grapes" in Greek). It is a member of the phylum Firmicutes and the class Bacilli (Bauman 2007). *S. aureus* was first discovered by the surgeon Sir Clifton Smith in Aberdeen, Scotland in the year 1880. He isolated it from pus from surgical abscesses (Newsom 2008).

It is a member of the phylum Firmicutes, which means that less than 50% of its genome is made up of guanine and cytosine. Also characteristic of the Firmicutes phylum is the fact that *S. aureus* is Gram-positive, meaning that it has a thick cell wall made of peptidoglycan located outside of the cell membrane, it lacks a membrane outside of the cell wall, and it will stain purple, not pink, if one performs a Gram stain test. *S. aureus* is a facultative anaerobe. This means that it can survive and grow with or without the presence of oxygen, but it cannot utilize aerobic respiration, so it cannot use oxygen as a means of producing energy. When cultured, *S. aureus* normally produces large, round, golden-yellow colonies. This gold color is where *S.*

aureus gets its name as "aureus" means golden in Latin. *S. aureus* is known to produce the enzyme catalase, which converts highly reactive hydrogen peroxide molecules (a common byproduct of many metabolic processes) to water and oxygen, preventing damage to the cell. Testing for the presence of catalase is a useful way to distinguish *S. aureus* from other members of the Bacilli class such as *Streptococcus* and *Enterococcus*, which are catalase negative (Bauman 2007). Most *S. aureus* strains also produce the enzyme coagulase, which causes the coagulation of blood. This can be used to differentiate *S. aureus* from other members of the *Staphylococcus* genus such as *S. epidermidis*, which is coagulase negative. However, a small number of *S. aureus* strains have been shown to be coagulase negative, so the coagulase test is susceptible to false negatives (Matthews 1997). If *S. aureus* is grown on a blood agar plate, it is usually seen to be β-hemolytic, meaning that it can completely lyse red blood cells. This also serves to increase the virulence of the bacteria (Bauman 2007).

S. aureus often has a commensal relationship with humans and animals. It can grow on the skin and in the nasal passages and throats of humans and animals where it is part of the normal flora. According to one study, three general patterns are observed among healthy subjects: about 20% of people are persistent, long-term carriers; about 60% of people are intermittent carriers; and about 20% of people never carry *S. aureus*. In patients who repeatedly puncture the skin (such as hemodialysis patients or intravenous drug users) and patients with Human Immunodeficiency Virus (HIV) infection, higher rates of carriage are observed (Kluytmans J 1997).

Although *S. aureus* is part of the normal flora for humans and animals, it can cause infection if given the opportunity. *S. aureus* is a very versatile bacterium, and it

can cause many different kinds of medical problems depending on the site of its infection, the immune state of its host, and the toxins and enzymes that the infecting strain happens to produce. It is also one of the most common causes of nosocomial (hospital acquired) infections. These include many types of noninvasive, cutaneous, and systemic infections (Bauman 2007).

2.5.1 Food Poisoning

The only common non-invasive disease caused by *S. aureus* is food poisoning. The bacteria itself cannot survive thorough cooking or the harsh environment of the stomach, but many of the enterotoxins it produces can. Many types of food can be contaminated with *S. aureus*, including processed meats, custard pastries, potato salad, and ice cream. Unlike most forms of food poisoning, the food is often contaminated through contact with human skin rather than being native to the food itself. Food need only to be at room temperature for several hours for bacteria to grow and secrete toxins. If one ingests some of these toxins, the symptoms are the same as most forms of food poisoning. These include nausea, vomiting, diarrhea, headache, sweating, and abdominal pain. Symptoms usually appear within four hours of ingestion and disappear within twenty-four hours. Because the bacteria are killed during cooking or ingestion, no new toxin can be produced, and the disease will subside quickly (Bauman 2007).

2.5.2 Cutaneous Infections

S. aureus is capable of causing several different types of cutaneous (skin) infections. Some of these are isolated skin lesions. These include pimples (blockage in the skin's pores), abscesses (accumulations of pus in a tissue cavity in response to an infection), and folliculitis. Folliculitis is an infection of a hair follicle in which the base of the follicle becomes red, swollen, and filled with pus. A furuncle, or boil, is a raised, nodular extension of folliculitis into the surrounding tissue. If several furuncles coalesce, they form a carbuncle that extends deeper into the tissue, which triggers the body's inflammatory response (Bauman 2007).

2.5.3 Staphylococcal Scalded Skin Syndrome

S. aureus is also capable of causing infections that affect the entire skin. One such disease is called Staphylococcal Scalded Skin Syndrome (SSSS), which usually occurs in infants, but has been shown to occur in uncompromised adults as well (Opal 1988). This involves reddening of the skin, beginning near the mouth and spreading to the rest of the body, followed by large blisters filled with clear fluid that lacks bacteria or white blood cells. Due to the lack of bacteria in the blisters, the disease is not contagious. Within two days, the epidermis peels off in sheets as if it had been dipped into boiling water. SSSS can become much more serious if secondary bacterial infections occur in the denuded areas (Bauman 2007). The disease is not directly caused by bacteria but by the epidermolytic exotoxins A and B, which are both produced by *S. aureus*. A is produced by the bacterial chromosome, while B is produced by a plasmid (Opal 1988).

2.5.4 Impetigo

Another disease caused by *S. aureus* is impetigo. Impetigo usually affects children with underdeveloped immune systems, but it can affect adults as well. It is characterized by small, flattened, red patches on the face and limbs, which develop into pus-filled vesicles that eventually crust over. Unlike SSSS, the pus is filled with bacteria and white blood cells, a key means of distinguishing the two diseases. Also unlike SSSS, impetigo is highly contagious and can be spread through contact with the skin lesions of an infected person or through contact with a carrier of *S. aureus* (Stulberg 2002). *S. aureus* alone accounts for about eighty percent of all impetigo cases while about twenty percent also involve streptococci (Bauman 2007).

2.5.5 Cellulitis

S. aureus is also one of the most common causes of cellulitis. Cellulitis occurs when bacteria enter the skin by way of a cut, abrasion, or any other break in the skin. It is characterized by inflammation of the skin around the area of infection. Normally, this inflammation will go away on its own (although it may resurface if not properly treated), and, if not, it can be treated with antibiotics. However, more serious cases can result in debilitation and even death if left untreated. Also, cellulitis infection can allow the infecting bacteria the opportunity to invade lower layers of the skin where it can spread into the lymph nodes and the bloodstream (Bauman 2007).

2.5.6 Necrotizing Fasciitis

If *S. aureus* reaches the deep tissue layer known as the fascial lining and the subcutaneous tissue, it can cause more serious conditions such as necrotizing fasciitis (Vinh 2007). Since 2001, the occurrence of necrotizing fasciitis caused by MRSA has been increasing. One study concluded that between 2001 and 2006, of the 74 cases of necrotizing fasciitis observed at one hospital, MRSA was the causative organism in 39% of them (Lee 2007). Necrotizing fasciitis occurs when, after the bacteria have invaded the fascial lining, they release toxins that destroy skin and muscle tissue. The presence of warning signs such as pain and inflammation depends on how deep the bacteria are, but as the disease progresses, the skin will become swollen, and it may change color to violet and form blisters. These symptoms would be followed by the subsequent necrosis of the subcutaneous tissues. This disease often causes an enormous systemic inflammatory response that can endanger the patient's life. Necrotizing fasciitis is an extremely deadly disease with mortality rates noted as high as 73%. Treatment often requires both intravenous antibiotics and surgery to remove dead and infected tissue, and sometimes amputation and repeat explorations are necessary to make sure all infected tissue is removed (Trent 2002).

2.5.7 Toxic Shock Syndrome

Once *S. aureus* is introduced to deeper tissues of the body such as the blood, heart, lungs, or bones, it can cause a wide variety of potentially fatal infections. One such systemic infection is known as Toxic Shock Syndrome (TSS). This occurs when bacteria, growing in a wound, produce toxins that can be absorbed by the blood. TSS,

when caused by *S. aureus*, often presents in otherwise healthy individuals with high fever, low blood pressure, red rash, malaise, and confusion. If blood pressure falls too low, the patient can enter a state of shock, in which vital organs do not receive adequate blood supply. This can lead to stupor, coma, multi-organ failure, and ultimately death. TSS is usually treated with antibiotics and the removal of infected tissue, and patients usually recover in two to three weeks, but the TSS can be fatal in a matter of hours if the source of infection is not removed (McCormick 2001).

2.5.8 Bacteremia

S. aureus is also a common cause of bacteremia, which simply refers to the presence of bacteria in the blood. The blood is generally a sterile environment, so the presence of bacteria is always abnormal. Bacteria in the bloodstream is particularly dangerous because, through the blood, bacteria can travel anywhere in the body and cause infections. Bacteria can enter the blood through many pathways including, but not limited to, open wounds, contaminated medical devices such as intravascular catheters, furuncles, vaginal infections, intravenous drug use, and urinary tract infections. The presence of bacteria in the blood usually elicits a strong immune response, which can lead to sepsis. If the response is extreme, it can lead to septic shock, which can be fatal due to large disturbances in body temperature, respiration, heart rate, and white blood cell count. Bacteremia must be treated with antibiotics to avoid worsening of symptoms. Bacteremia is also the most common method for *S. aureus* to infect internal organs, such as the heart, lungs, or bones (Bauman 2007).

2.5.9 Pneumonia

One common internal infection caused by *S. aureus* is pneumonia. Pneumonia refers to an inflammation of the alveoli of the lungs as well as the alveoli abnormally filling with fluid. *S. aureus* can get into the lungs either through inhalation of airborne bacteria or through bacteremia. When the lungs' alveoli fill with fluid, it can severely impair the absorption of oxygen, and, in severe cases, it can impair breathing to the extent that patients are asphyxiated. Pneumonia can also cause respiratory failure by causing Acute Respiratory Distress Syndrome (ARDS), which is triggered by a combination of infection and immune response. It results in the lungs quickly filling with fluid and becoming stiff, which means that the patient will need mechanical respiration just to survive. Pneumonia can cause sepsis and septic shock. Another danger of pneumonia is that it can cause fluid to build up in the pleural cavity (the space surrounding the lungs), which will cause the infection to persist regardless of antibiotic therapy because antibiotics do not penetrate well into the pleural cavity. This fluid often has to be removed via thoracentesis, chest tubes, or, in severe cases, surgery. Bacteria in the lungs can also form abscesses, which can usually be treated by antibiotics but sometimes require surgery in severe cases (Hoare 2006). Pneumonia is a very deadly disease, especially when the cause is *S. aureus*. One study has shown that 50% of patients that contract pneumonia caused by *S. aureus* while on a ventilator will die (Combes 2004). Pneumonia is just one of many internal infections that can be caused by *S. aureus*.

2.5.10 Osteomyelitis

If *S. aureus* is able to invade bone tissue, either through bacteremia or a traumatic wound, it can cause swelling of the bone marrow and the surrounding bone. This is known as osteomyelitis. This is accompanied by extreme pain and high fever. In children, osteomyelitis usually occurs in the growing regions of long bones such as those found in the arms and legs, while in adults it is most often seen in the vertebrae. *S. aureus* is the most commonly isolated organism from all forms of osteomyelitis. Treatment usually requires prolonged antibiotic therapy and, sometimes, surgery and loss of limb (Bauman 2007).

2.5.11 Endocarditis

Perhaps the most serious infection that can be caused by *S. aureus* is endocarditis. This occurs when the bacteria infect the lining of the heart. This results in swelling, which causes a large drop in the rate of blood flow. To make matters worse, there are parts of the heart such as the valves that do not receive a dedicated blood supply, making it difficult for the body's immune defenses as well as antibiotics to reach the infected area. Treatment requires high doses of antibiotics for long periods of time and sometimes surgery to remove the infected material. This requires replacement of heart tissue with mechanical or bioprosthetic parts. Even with treatment, about fifty percent of endocarditis patients die (Bauman 2007).

2.5.12 Treatment

For many years, β-lactam antibiotics such as penicillin were used for the treatment of *S. aureus* infections. However, it is extremely rare to find *S. aureus* isolates that are still susceptible to those drugs. Now, the antibiotics that are resistant to the β-lactamase enzyme are required, such as methicillin (no longer used clinically), oxacillin, flucloxacillin, and gentamicin. These drugs, however, are suddenly showing weaknesses due to the rise of MRSA (Bauman 2007).

2.6 The Rise of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Antimicrobial-resistant, particularly methicillin-resistant, *S. aureus* (MRSA) has recently emerged as a serious public health concern in the U.S. *S. aureus* can easily become resistant to antibiotics because it is a very adaptive bacteria. Once *S. aureus* acquires the *mecA* gene, it becomes resistant to β-lactam antimicrobials such as methicillin, penicillin, oxacillin, and amoxicillin (Deurenberg 2006). Unfortunately, these are the antibiotics that are often used to treat *S. aureus* infections. The *mecA* gene creates a penicillin-binding protein which prevents the antimicrobials from attaching to the cell wall. As a result, the antibiotic cannot destroy the bacteria (Deurenberg 2006).

2.6.1 Increase in the Incidence of CA-MRSA

There are two main types of MRSA: Hospital Associated (HA) and Community Associated (CA). As the name implies, HA-MRSA is acquired through contact with the healthcare environment. Established risk factors for MRSA infection include recent hospitalization or surgery, residence in a long term care facility such as a nursing home, dialysis, and medical devices such as catheters (Naimi 2003). Individuals infected with CA-MRSA have not had contact with the healthcare environment prior to developing the infection (Chambers 2005). Additionally, CA-MRSA cases are commonly associated with groups of individuals who live in close quarters or frequently come in contact with other individuals' skin (Allen 2006). Another difference between the two infections is that CA-MRSA infects younger individuals. Twelve labs in Minnesota examined 1100 individuals that had become infected with MRSA. The average age for those individuals with CA-MRSA was 23, compared with an average age of 68 years old for individuals with HA-MRSA (Naimi 2003).

The majority of cases of CA-MRSA are skin infections. Panton-Valentine leukocidin genes are more often present in CA-MRSA than HA-MRSA. Panton-Valentine leukocidin genes produce cytotoxins that cause skin tissue to necrotize (Naimi 2003). Individuals infected with HA-MRSA experience skin infections as well as breathing and urine problems (Millar 2007). The range of antibiotics that are resistant to CA-MRSA and HA-MRSA is also different. HA-MRSA was more likely to be resistant to erythromycin, clindamycin, ciprofloxacin, and gentamicin. CA-MRSA, however, is likely to be susceptible to ciprofloxacin, clindamycin,

gentamicin, and trimethoprimsulfamethoxazole (Naimi 2003). The boundary between these two types of MRSA is becoming increasingly unclear as the incidence of CA-MRSA infections has dramatically increased in healthcare settings (Benoit 2008).

The steady increase in the number of individuals being infected with CA-MRSA has shifted the focus from HA-MRSA to CA-MRSA (Naimi 2003). Recent studies indicate that CA-MRSA is a growing epidemic. Cases of MRSA in pediatric head and neck *S. aureus* infections in the U.S. increased from 11.8% to 28.1% from 2001 to 2006 (Naseri I 2009). There are 8 types of S. *aureus*, which are labeled from USA100 to USA800 (Kurkowski 2007). Most HA-MRSA strains are typed as USA100, while CA-MRSA strains are typed as USA300 (Kurkowski 2007).

2.6.2 MRSA in Food

In 1995, a study conducted by Kluytmans et al. reported the first known case of humans becoming infected with MRSA after coming in contact with a contaminated banana at a hospital in the Netherlands. The banana became contaminated after being handled by a hospital employee. 27 patients and 14 employees became infected with MRSA, and 5 died (Kluytmans 1995). After this report, numerous studies emerged studying the link between MRSA and food contamination.

2.6.3 MRSA as a Zoonotic Pathogen

Over the past decade, numerous studies have emerged indicating a link between animals raised for food production and MRSA. Direct contact with animals

such as that involved in pig farming has been found to be a risk factor for increased nasal *S. aureus* colonization in France, as transmission of strains from pigs to farmers was frequent (Armand-Lefevre 2005). Similar studies in Denmark, Canada, and the Netherlands have confirmed that the pig ST398 MRSA strains are transmissible from animals to humans (Lewis 2008).

In another study conducted in Canada in 2007, it was shown that there is a link between farms that contain MRSA-contaminated pigs and the individuals that work on those farms. The pigs on these 20 farms were tested for *S. aureus* and then MRSA. The individuals were given a survey to assess their risk factors and were also swabbed for the MRSA bacteria. Of the 285 pigs that were tested, 71 tested positive for MRSA. Further, 5 of the 25 individuals that worked on the farm also tested positive for MRSA. More importantly, only the farms that had pigs that tested positive for MRSA had humans test positive for the MRSA as well; none of the individuals working on farms with non-contaminated pigs tested positive for MRSA. The surveys from the individuals that tested positive for MRSA did not reveal any risk factors that would indicate that the individual had contact with MRSA from human origins (Khanna 2008). However, there was no confirmation of the type of MRSA through lab procedures.

A logical link between pigs with MRSA on farms and pigs being slaughtered in slaughterhouses being contaminated with MRSA was studied. This is relevant because if the pigs are contaminated with MRSA at the slaughterhouse, they are likely to be contaminated when entering the human food supply. In a Netherlands study conducted in 2005, 540 pigs from 9 slaughterhouses were tested for S. *aureus*

and MRSA. 209 of those pigs tested positive for MRSA. All 9 slaughterhouses contained at least one pig with MRSA. The type of MRSA found on the pigs was determined to be non-typable (NT-MRSA). It is further explained that this form of MRSA is highly prevalent on farms throughout the Netherlands (De Neeling 2007).

The study explained that MRSA from the slaughterhouse, in addition to MRSA from the pig farms, contributed to the high number of positive samples. Because the number of pigs that were contaminated with MRSA in the slaughterhouses was so high, this study pointed out that individuals who are in physical contact with pigs, on farms or in slaughterhouses, for example, are more likely to have the MRSA strain than individuals who have never had contact with pigs (De Neeling 2007). This factor indirectly links the spread of MRSA bacteria with livestock that is raised for human consumption.

2.6.4 MRSA: A Foodborne Pathogen

Another study conducted in the Netherlands investigated the presence of MRSA in meats purchased at grocery stores. 2.5% of pork and beef products from 31 supermarkets and butcher shops were found to contain an NT-MRSA strain, which is probably of animal origin (Van Loo 2007). Through the two studies conducted in the Netherlands it can be observed that the same specific MRSA strain is present in the pigs on the farm, the slaughterhouse, and, finally, meat available for human consumption.

After a link between animals and MRSA became evident, researchers began to investigate the presence of MRSA in food products that came from animals. In 2005,

the first study to look at MRSA contamination in Italy's animal food products showed that animal food products could be a carrier for MRSA. A study conducted in Italy further tested samples that had tested positive for S. *aureus* from a previous study. The previous study had tested 1634 samples from animal food products like cheese and milk. Of those samples 160 had tested positive for S. *aureus.* These 160 samples were tested for MRSA, and 6 tested positive (Normanno 2007).

In 2004, Kitai et al. studied the presence of MRSA in ground chicken in Japan. 2 of the 714 samples tested positive for MRSA. Further tests were conducted to determine the origins of the 2 strains; they were determined to be of human origin. It was also determined that human contamination was probably responsible (Kitai 2004). While, this study did not conclude that there was a link between animals and the spread of MRSA to humans, it did show that meat found in grocery stores can be a carrier of MRSA and that this could be a public health concern (Kitai 2004).

A more recent study conducted in Baton Rouge, Louisiana in 2008 discovered MRSA in meat from different grocery store chains. In this study, 120 samples of meat were collected from three different grocery store chains. Of the 47 samples that were positive for *S. aureus*, 6 tested positive for MRSA. More specifically, the strains were determined to contain USA100 and USA300 genes (Pu 2009). USA100 is a strain of HA-MRSA, while USA300 is a strain of CA-MRSA (Kurkowski 2007).

While CA-MRSA can come from livestock, it was determined that the MRSA strains discovered in this study were USA300 and were therefore the result of human contamination. The study notes, however, that any type of MRSA contamination is a public health concern because it is in the food supply. It should be noted, however,

that the study was limited in the number of samples taken and by the geographic location of the samples (Pu 2009).

This was the first study to examine MRSA-contaminated meat from grocery stores in the United States. One of the limitations discussed with regards to the validity of the study was that the results were limited to the geographic area, and not representative of the entire United States. It is explained that more research across the United States needs to be conducted in order to better assess MRSA contamination in retail meat (Pu 2009). While all of the studies discussed above show a link between animal food products and MRSA, they are inconclusive about how the products became contaminated. It appears that most of the studies found MRSA contamination in the human food supply, but that the contamination did not come from animal origins.

In 2008, infections caused by ST398 strains of MRSA, which are believed to be of animal origin, resulted in the treatment and even hospitalization of U.S. patients who had no recent contact with live animals, suggesting that these strains may already be more common (Welinder-Olsson 2008). These patients may have been exposed to MRSA by handling raw meat products. Meanwhile, the number of human cases of NT-MRSA reported from 2002 to 2006 increased more than 21%. This occurred even though there was an increase in monitoring after 2006. There is a correlation between NT-MRSA and pig farming, where carriers are most likely pig and cattle farmers. The NT-MRSA strains belong to the clonal complex ST398 (Van Loo 2007).

Although contamination can be avoided through proper food preparation, *S. aureus* remains a leading cause of foodborne illness (Lund 2000). While enterotoxins

cause illness for those consuming contaminated foods, the increasing resistance of *S. aureus* to antimicrobials also poses a risk to food handlers and the community as a whole (Pu 2009). Non-MRSA strains of *S. aureus* isolated from food products in Italy have been found to express resistance to fluoroquinolones, tetracycline, erythromycin, gentamycin, bacitracin, oxacillin, penicillin, and/or ampicillin (Pesavento 2007).

2.6.5 Methods of Screening for MRSA Contamination in Food

When considering how to extract the bacteria from the meat samples, two different methods can be used. The first is to streak the meat sample, which has been stomached with buffered peptone water, directly onto Baird Parker plates. The second method is to, first, mix the sample with an enrichment broth for several hours and then streak the sample, which has also been liquefied with buffered peptone water using a stomacher, onto the Baird Parker plates. Using enrichment broth can help draw out more bacteria to be tested (Blanc 2003). Using the broth causes more bacteria to grow on the plates and leads to more samples testing positive for S. *aureus* (Davies 1997). Bocher et al., in a study that compared the direct plating method to the enrichment method, showed that two different types of semi-selective enrichment broth produced more positive samples of MRSA than the direct plating method (Bocher 2010).

However, there are two drawbacks to using the enrichment broth. The first is that it takes longer for the bacteria to grow (Davies 1997). The second and more important drawback is that not using enrichment allows the concentration of the bacteria cells in the meat to be determined. After growing the bacteria on the plates,

the species can be identified through a series of chemical, biological, and genetic tests.

Chapter 3: Methodology

3.1 Materials: Media Preparation

Buffered peptone water: Prepared by dissolving 20.0 g Buffered Peptone Powder (peptone 10 g, NaCl 5 g, Na_2HPO_4 3.5 g, KH_2PO_4 1.5 g; Difco, Becton and Dickinson, Cockeysville, MD) per liter of distilled water and then autoclaving (15 min. at 121ºC) the mixture to sterilize (final pH: 7.2 ± 0.2).

Baird-Parker Plates: Plates were made by dissolving 63.0 g Baird-Parker powdered base (tryptone 10 g, beef extract 5 g, yeast extract 1 g, sodium pyruvate 10 g, glycine 12 g, lithium chloride·$6H_2O$ 5 g, agar 20 g; Difco, Becton and Dickinson, Cockeysville, MD) per 950 ml distilled water. This solution was boiled, then autoclaved at 121ºC for 60 min (final pH: 7.0 ± 0.2). Once sterilized and cooled to 50ºC, the broth was enriched with 75 ml of egg yolk tellurite suspension (30% egg yolk suspension, 0.15% potassium tellurite; Difco, Becton and Dickinson, Cockeysville, MD) added per 1.5 L of base broth and the mixture was stirred until thoroughly mixed. The broth was then poured into plastic Petri dishes (100 x 15 mm; about 18 ml/plate) in a sterile environment, with an open flame, to form the BP plates. The plates were allowed to solidify at room temperature and were then stored, inverted, at 4ºC.

Tryptic Soy Agar (TSA) Slants: Prepared by adding 40.0 g Tryptic Soy Agar base (trypticase peptone 15 g, phytone peptone 5 g, NaCl 5 g, agar 15 g; Difco,

Becton and Dickinson, Cockeysville, MD) per liter of distilled water and boiling for 1 min. to dissolve. The solution was then autoclaved (121ºC, 15 min.), and 2 ml of solution was added to each of 500 5-ml glass test tubes. The test tubes were placed on their sides and allowed to cool, making a slanted agar formation within the tubes.

Brain-Heart Infusion Broth: Prepared by dissolving 469.5 g of Brain-Heart Infusion powder (calf brain infusion 200 g, beef heart infusion 250 g, proteose peptone 10 g, NaCl 5 g, Na_2HPO_4 2.5 g, dextrose 2.0 g; Bacto, Becton and Dickinson, Cockeysville, MD) in 1 L of distilled water, agitating over a gentle heat, and autoclaving for 15 min. at 121ºC (final pH: 7.4 ± 0.2).

Blood Agar Plates: Made by preparing tryptic soy agar plates infused with 5% sheep blood (infusion from beef heart 500 g, tryptose 10 g, NaCl 5 g, agar 15 g, distilled water 1.0 L, autoclaved at 121ºC for 15 min., then added 50 ml sheep blood (sterile, defibrinated) after cooling to 45-50ºC; final pH, 6.8 ± 0.2).

Mueller-Hinton (MH) Broth: Made of beef extract powder 2.0 g, Acid Digest of Casein 17.5 g, starch 1.5 g, distilled water 1.0 L; Difco, Becton and Dickinson, Cockeysville, MD.

3.2 Random Sampling of Ground Meat Products

A total of 700 meat samples (mostly ground) were collected and analyzed. Previous studies that were conducted (Pu 2009, Chao 2007) used anywhere from 30 to 220 samples of each type of meat. The team elected to select 700 samples for the study in order to stay within budget constraints as well as to ensure that the results were statistically significant and comparable to previous studies. These 700 samples were broken down as follows: 200 samples of ground turkey, 200 of ground beef, and 300 of ground pork. An additional 100 samples of pork were collected over the other two meat types because most previous studies (Pu 2009, Van Loo 2007) focused on pork. Since it had been previously established that MRSA is frequently transmitted between pigs and humans (Armand-Lefevre 2005, Lewis 2008, Welinder-Olsson 2008), we hypothesized that pork would be more likely to carry *S. aureus* and perhaps MRSA.

These 700 meat samples were collected through a random sample of grocery stores (n = 84) managed by three major chains in the Washington, D.C. area (Montgomery and Prince George's counties). Each week a total of seven fresh, refrigerated ground meat samples (two beef, two turkey, and three pork) were collected from five different stores. Thus, 35 samples were collected weekly from March 2008 to August 2008. To ensure random sampling and avoid regional, seasonal, or store chain bias, each of the potential stores was assigned a number and a computer program selected the five stores visited each week. During purchase of the meat, preference was given to packages with later expiration dates, ensuring that higher bacterial counts could not be attributed to expired meats. An effort was also

made to purchase different brands of meat (name brand, store brand, etc.), in order to obtain a broader view of all ground meats that the stores had to offer. Additionally, it was ensured that the time between purchase and laboratory processing of the meat was limited to a maximum of three days and prior to the expiration dates. During transport to the laboratory, all meat samples were kept in coolers on ice and immediately placed in a laboratory refrigerator (at 4ºC) so as to minimize any increase in bacterial counts from their "in-store" levels. Finally, all attempts were made to purchase only ground meats, as ground meats are more likely to have been contaminated during slaughter and processing, such as through the meat grinder. Also, a typical ground meat sample is made up of meat from several animals; therefore, only one contaminated animal, out of the dozens ground up in one package, is needed to potentially contaminate hundreds of packages of meat. However, the study was limited by what types of meat each store had in stock. When ground meat product was not available, a non-ground version, such as pork chop in lieu of ground pork, was substituted (102 non-ground pork and 8 non-ground beef samples were tested). In total, 694 meat samples were tested, after several samples were deemed unsuitable for testing (4 seasoned and 2 blended multi-animal meat samples were discarded).

3.3 Isolation and Identification of *S. aureus* from Ground Meat

The isolation and identification steps below were taken from general guidelines for identifying *S. aureus* provided by the U.S. FDA Bacteriological Analytical Manual (Bennett 2006).

3.3.1 Plating on Baird-Parker, a Selective Growth Medium

Buffered peptone water was used as a non-selective pre-enrichment treatment for the bacteria from the meat prior to plating. A 25.0 g (+/- 0.1 g) portion of each meat sample was added to 225 ml of buffered peptone water and the mixture was stomached for 5 minutes. All packages were opened with razor blades, which were sterilized between packages with 70% ethanol solution, and each meat sample was measured out using a sterile plastic spoon.

Baird-Parker (BP) plates were used to cultivate the stomached culture, selecting for members of the coagulase-positive *Staphylococcus* genus. A 1.0 ml aliquot of the stomached mixture was directly plated, using a bulb pipette and sterile glass bent streaking rod (sterilized with ethanol and flame between each use), onto three Baird-Parker agar plates (0.3 ml, 0.3 ml, and 0.4 ml, respectively), in a sterile environment. The liquid was allowed to absorb into the agar for 10 min., and then the plates were inverted and incubated at 35°C for 48 h. After incubation, the total number of colony forming units (CFU) on the plates was counted and recorded. For meat samples which produced 3 or more colonies, 3 colonies were chosen for purification. For those which produced less than 3 colonies, all colonies were purified in order to maximize the number of isolates retained. Since Baird-Parker agar with egg yolk tellurite enrichment selects for *S. aureus* (Becton 2009), colonies were not

chosen randomly. Larger (approximately 2-3 mm in diameter), dark (gray to jet-black) colonies were picked, particularly those with the characteristic egg-yolk-clearing halo. Presumptive colonies were picked using plastic inoculation loops and streaked onto a new set of Baird-Parker plates, in order to purify the single colony. These plates were then incubated at 35°C for 24 h.

Following incubation, tryptic soy agar (TSA) slants were prepared, and they were used for long-term storage and testing of the streaked *S. aureus* colonies. The TSA slants were inoculated with one bacterial colony each, using inoculation loops to streak the surface, and then penetrate the agar of each slant. The slants were then incubated for 24 h at 37ºC and subsequently stored at 4ºC.

3.3.2 Gram Staining

Gram stain tests were performed to determine whether the bacteria were Gram-positive or Gram–negative as well as the general shape of the bacteria. For the Gram-stain procedure, each of the presumptive *S. aureus* colonies was smeared onto a glass microscope slide (5 x 75 mm, with etched portion for labeling) with a drop of water on it to form a thin film, and then air dried and heat fixed using a Bunsen burner flame. The slide was then flooded with crystal violet staining solution, allowing contact with the stain for 30 s. The crystal violet was then decanted off, and the slide was rinsed gently with distilled water to remove excess dye. The slide was next flooded with fresh iodine solution, allowed to stain for 30 s, and then rinsed again with water. This was followed by a decoloring rinse and then another rinse of water. Finally, the slide was flooded with the counterstain safarin for 30 s, and once

more rinsed with distilled water. The slide was allowed to air dry, and then examined under a microscope with an oil immersion objective lens (100 x) and 10 x ocular lens. Samples that were not Gram-positive or did not form grape-like clusters or cocci when viewed under a microscope were ruled out as being part of the *Staphylococcus* genus to which *S. aureus* belongs.

3.3.3 Catalase and Coagulase Tests

Catalase testing was performed to differentiate catalase-positive staphylococci bacteria, such as *S. aureus*, from other cocci bacteria, such as *Enterococcus* or *Streptococcus*. During the test, 0.2 ml of a 3% hydrogen peroxide solution was pipetted into 5-ml test tubes. Presumptive colonies were picked, using a plastic inoculation loop, and smeared onto the wall of the tube just above the liquid meniscus. The tubes were capped and then tilted so that the solution covered the colony. A sample was deemed catalase-positive if any bubbling was observed in the liquid after 10 s.

Coagulase testing was performed to differentiate *S. aureus* from coagulase-negative staphylococci bacteria. For the procedure, 1.0 ml of brain-heart infusion (BHI) broth was pipetted into 5-ml test tubes. Colonies from each TSA slant were picked and used to inoculate each broth-filled test tube. The BHI culture suspensions were incubated on a shaker (225 rpm) at 35ºC for 24 h. Next, 0.5 ml of rabbit plasma serum with EDTA was placed in a 1.5 ml microcentrifuge tube, and then inoculated with 100 µl of the incubated broth. The microcentrifuge tubes were then incubated at 35ºC for 2 hours and checked periodically for growth. If the test was negative (no

clotting or coagulating of broth), then incubation was continued for up to 24 hr. Only firm and complete clots that stayed in place when tube was tilted or inverted were considered coagulase-positive results indicative of *S. aureus*.

3.3.4 Vitek Confirmation

For further confirmation of bacterial colonies suspected to be *S. aureus* from the previous isolation and identification procedures, a Vitek 2 Compact Immunodiagnostic Assay System (VIDAS; bioMerieux Vitek Inc., Hazelwood, MO) was used for staphylococcal speciation, following the manufacturers protocol (Pincus).

BioMerieux Gram-Positive cards (GP #21342) were used for testing. Each card has 64 microwells containing substrates used for 43 different biochemical tests (**Appendix E**) that measure various metabolic activities (growth, acidification, alkalinization, susceptibility). These tests help identify non-spore-forming, Gram-positive bacteria such as *S. aureus*. The cards test for 115 taxa from 22 different genera, specifically cocci bacteria.

Pure subcultures, 12-48 hours old, were obtained from the TSA slants of suspected *S. aureus* isolates. The pure subculture of each of these suspected isolates was made by transferring colonies from the TSA slants onto TSA plates made with 5% sheep blood (Blood Agar Plates, BAP). These subcultures were incubated at 35ºC for 18-24 h. A sterile pipette tip was then used to transfer several purified colonies from the slants to a clear polystyrene test tube (12 x 75 mm) containing 3.0 ml of sterile saline solution (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0). The turbidity of

these culture suspensions was adjusted to a standard of 0.50-0.63 (McFarland turbidity range) using a turbidity meter. The pure culture suspensions were then inoculated onto bioMerieux Gram-Positive (GP #21342) cards and sealed automatically by the Vitek System, using the pre-inserted transfer tube and culture cassette loading assembly. The cards were incubated online at a temperature of 35.5 ± 1.0ºC and read optically at 15 min. intervals for the entire incubation period. The optical readings measured either turbidity or colored products of substrate metabolism, depending on which microwell test was being observed. The Vitek System software automatically records and observes these optical readings and calculates the test results based on raw data collected and previously established thresholds. Test results are reported as either positive "+", negative "-", weakly positive "(+)", or weakly negative "(+)". Weakly negative and positive results indicate that these reactions were too close to the test threshold to be reported reliably. The software then analyzes the results of all 43 biochemical tests and compares them to biopattern profiles of each of the 115 Gram-positive bacteria tested for, reporting the confidence level of each positive identification.

3.4 Antimicrobial Susceptibility Testing

Pure cultures of each positive *S. aureus* isolate were streaked onto blood agar plates (BAP). These plates are a non-selective but differential growth and enrichment medium. Subcultures of the pure *S. aureus* isolates were streaked on the blood agar plates and incubated at 35ºC for 18-24 h.

Next, antibiotic susceptibility testing (AST) was done, and a 96-well plate pre-filled with varying levels of antibiotics was used for each sample. The panels used, TREK GPALL1F (SOP 530-018 R-3), contained antibiotics in the following ranges of concentrations: chloramphenicol (CHL) 2-16 µg/ml, erythromycin (ERY) 0.25-4 µg/ml, clindamycin (CLI) 0.5-2 µg/ml, daptomycin (DAP) 0.5-4 µg/ml, oxacillin + 2% NaCl (OXA+) 0.25-4 µg/ml, streptomycin (STR) 1000 µg/ml, ampicillin (AMP) 0.12-8 µg/ml, linezolid (LZD) 1 to 8 µg/ml, penicillin (PEN) 0.06-8 µg/ml, rifampin (RIF) 0.5-4 µg/ml, vancomycin (VAN) 0.25-32 µg/ml, trimethoprim/sulfamethoxazole (SXT) 0.5/9.5 – 4/76 µg/ml, levofloxacin (LEVO) 0.25-4 µg/ml, ciprofloxacin (CIP) 1-2 µg/ml, quinupristin / dalfopristin (SYN) 0.5-4 µg/ml, tigecycline (TGC) 0.03-0.5 µg/ml, nitrofurantoin (NIT) 32-64 µg/ml, tetracycline (TET) 2-16 µg/ml, moxifloxacin (MXF) 0.25-4 µg/ml, gentamicin (GEN) 500 µg/ml. The breakpoints, or concentrations at which growth indicates resistance to that particular antibiotic are as follows: CHL > 16 µg/ml, ERY > 4 µg/ml, CLI > 2 µg/ml, DAP > 4 µg/ml, OXA+ > 4 µg/ml, STR > 1000 µg/ml, AMP > 8 µg/ml, LZD > 8 µg/ml, PEN > 8 µg/ml, RIF > 4 µg/ml, VAN > 32 µg/ml, SXT > 4 µg/ml, LEVO > 4 µg/ml, CIP > 2 µg/ml, SYN > 4 µg/ml, TGC > 0.5 µg/ml, NIT > 64 µg/ml, TET > 16 µg/ml, MXF > 4 µg/ml, GEN > 16 µg/ml (**Appendix B)**. In addition, two wells were used for a D-test. A D-test can be used to detect CA-MRSA with inducible resistance. In these wells, erythromycin and clindamycin antibiotic discs were placed and growth around the discs observed. A flattened area of growth (D-shaped) near the clindamycin disc indicates inducible resistance to clindamycin. A cefoxitin (FOX) screen was also performed in one well, in which a cefoxitin disk was placed in the

well and the growth around it was observed, to confirm resistance to oxacillin resistance.

To perform the antibiotic resistance testing, several colonies of each sample from the blood agar plates were transferred to their own sterile 4 ml tube of Sensititre demineralized water, using a sterile 9" swab and vortexing well. The tube was then inserted into the nephelometer of a calibrated autoinoculator (Trek Diagnostic Systems, Cleveland, OH) to check if the inoculum was equivalent to the Sensititre 0.5 McFarland Latex Particle standard. Once the inoculum was standardized, enough of the suspension was added to a sterile 10 ml tube of Sensititre cation adjusted Mueller-Hinton broth to result in a final concentration of approximately 3×10^5 CFU/ml.

After vortexing the new solution, the autoinoculator was used to dispense 50 µl of cell suspension into each well of a Sensititre 96-well plate (TREK GPALL1F) which was then sealed. The plates were incubated at 35^{o}C for 16-20 h. After the incubation period, the growth on the plates was read manually using a microtiter plate holder fitted with a mirror that reflected the bottom of the plate. Wells with growth were recorded as positive while wells without were recorded as negative. Then the resistance of each sample to each antibiotic was evaluated based on the highest antibiotic concentrations that the samples could tolerate (see breakpoints above). The plates were read after the initial 16-20 h incubation for all of the antimicrobials. After another 4-6 h of incubation at 35^{o}C, a second reading for oxacillin and vancomycin only was performed.

3.4.1 Minimum Inhibitory Concentration Assay for Methicillin

In order to identify isolated bacteria as *S. aureus* or MRSA, isolates were also characterized using a methicillin agar dilution MIC (Minimum Inhibitory Concentration) assay. TSA plates were inoculated with pure isolates previously cultured on TSA slants and incubated at 35°C for 20 h. One colony was chosen from each TSA plate and diluted in 3 ml of distilled water using a turbidimeter in order to achieve a $10^6 – 10^7$ cells/ml concentration (0.05 – 0.09 range). The dilutions were then added onto a pin panel with the MRSA ATCC43300 strain as a positive control in each set of 35 isolates. MH agar plates were used for the MIC growth assay with 2% NaCl and the following concentrations of methicillin: 0.5 µg/ml, 1 µg/ml, 2 µg/ml, 4 µg/ml, 8 µg/ml, 16 µg/ml and 32 µg/ml. Two blank MH plates without methicillin were used as a negative control. Plates were incubated at 35°C for 20 h. The pin panel system allowed for testing in series of 35 strains simultaneously.

3.4.2 Polymerase Chain Reaction Screen for mecA Gene

PCR was used to screen isolates with methicillin MIC ≥ 8 µg/ml for the methicillin resistance gene (*mecA*) using the following primers: forward: 5'-GTAGAAATGACTGAACGTCCGATAA-3' and reverse: 5'-CCAATTCCACATTGTTTCGGTCTAA-3' (McClure 2006). These primers were designed to amplify a 301 bp *mecA* gene fragment.

25 µl PCR reactions were prepared. The forward and reverse primers were re-hydrated using nuclease-free distilled water (DI) to a concentration of 1 pmol/µl, vortexing to ensure through mixing and re-hydration. For each 25 µl reaction, 0.5 µl

forward primer and 0.5 µl reverse primer were added to 15.75 µl nuclease-free distilled water in 0.2 ml microcentrifuge PCR reaction tubes.

The DNA template was prepared using boiling methods. After purification of the *S. aureus* isolates, the bacterial strains were inoculated onto blood agar plates (BAP) and incubated 12-24 h overnight at 35°C. Two to three purified colonies were collected from each plate using a pipette tip and then swirled in a 1.5 ml microcentrifuge tube containing 0.5 ml DI water, vortexing thoroughly. The tubes were slightly vented and then boiled at 100°C for 15 min. The tubes were then centrifuged in a table-top microcentrifuge for 1-1.5 min. at 13,000 rpm (12000 x g). The upper level supernatant was collected and used as the DNA template for each sample. For each 25 µl reaction, 2 µl DNA template was used.

Once the DNA template was added to the primer-water solution in the PCR reaction tubes, the enzyme super-mix solution was prepared. The enzyme, Golden Taq (AmpliTaq Gold 360 DNA polymerase, Applied Biosystems Inc., Foster City, CA) was used for each reaction, and the enzyme and all corresponding components were kept on ice while the solution was prepared. A super-mix was prepared in a 1.5 µl microcentrifuge tube using 0.25 µl Golden Taq reaction enzyme (Applied Biosystems Inc., Foster City, CA), 2.5 µl 10X PCR reaction buffer, 2 µl dNTP mix and 1.5 µl $MgCl_2$ solution, for each reaction. The super-mix was stirred by pipette and mixed by inversion, and then 6.25 µl of the super-mix solution was added to each PCR reaction tube. The PCR tubes were tapped 2-3 times to mix and then spun down in a microcentrifuge to ensure proper mixing and to eliminate bubbles.

The tubes were then placed in a thermocycler. The PCR reaction conditions were as follows: denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, and elongation at 72°C for 1 minute for a total of 30 cycles.

Once the reaction was complete, agarose gel electrophoresis was performed on the PCR samples using a 1% agarose gel. The gel was prepared using 0.4 g electrophoresis-grade agarose powder and 40 ml TAE buffer (10 mM Tris-Cl, pH 8.5). The powder was added to the buffer in a 100 ml erylenmeyer flask and heated until dissolved and then cooled to about 60°C in a water bath. The gel was then poured into a standard size gel rack and a 12-well comb was inserted, and the gel was allowed to solidify at room temperature. Once cool, the gel was placed in a gel box containing enough 1X TAE buffer solution (0.04 M Tris-acetate, 0.001 M EDTA, pH 8.0) to cover the gel and fill the wells. Then approximately 10 µl of PCR reaction product was added to each well, after adding 1-2 µl DNA gel loading buffer. Approximately 1.5 µl of 100 bp DNA ladder was also run alongside the samples. The gel was run at 100 V for 30 min. Then the gel was removed and stained with an ethidium bromide solution in TAE for 20-30 min. This was followed by a destain in fresh distilled water for 45-60 min. The destained gel was then imaged using UV light photography (McClure 2006).

3.5 Strain Sub-Typing of MRSA by Pulsed-Field Gel Electrophoresis

A PFGE protocol developed by PulseNet for oxacillin-resistant *S. aureus* was used (CDC 2001). One colony from each of the test cultures was inoculated from the

TSA sheep blood plates into 5 ml of BHI broth in tubes (15 x 100 mm screwcap), and vortexed to mix. The tubes were then incubated at 37ºC for 24 h, with shaking (225 rpm). To prepare the plugs for the PFGE, 0.9% SeaKem Gold agarose in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8) was prepared in a 60ºC water bath. 200 µl of each cell culture was transferred to a 1.5 ml mirocentrifuge tube and vacucentrifuged at 13,000 rpm for 34 min to aspirate all the supernatant and form a pellet. The pellets were then resuspended in 300 µl of TE buffer and adjusted to a temperature of 37ºC in a water bath. 3 µl of lysostaphin (Sigma, St. Louis, MO) stock solution (1 mg/ml in 20 mM sodium acetate, pH 4.5) was then added to each tube, vortexing to mix. Then 300 µl of the agarose solution was added to each tube, mixing by pipette. The solution was then dispensed into the plug molds and cooled at 4ºC for 10 min. to solidify. Once solid, the plugs were removed from the mold and placed in a tube with 3 ml of EC lysis buffer (6 mM Tris HCl, 1 M NaCl, 100 mM EDTA, 0.5% Brij-58, 0.2% sodium deoxycholate, 0.5% sodium lauroylsarcosine) and incubated at 37ºC for at least 4 h. After 4 h, the EC buffer was removed and the plugs were washed 4 times with TE buffer and then stored at 4ºC.

The plugs were cut with a razor to fit the gel's well size (2 x 10 mm for 10-15 well gel), then placed in a tube with 200 µl water buffer mixture (10X Buffer stock diluted 1:10 with sterile, Type I water). After plug slices were equilibrated in the water buffer solution for 30 min, the buffer was pipetted off and into 150 µl of NEB Restriction Enzyme Buffer 4 (New England Biolabs, Beverly, MA.). Then 3 µl of restriction enzyme *Sma*I (New England Biolabs, Beverly, MA) was added to each

tube, to cleave the DNA. The reaction was incubated at room temperature for 15-30 min.

The samples were run on a 1% agarose gel in 0.5% TBE buffer on a CHEF DR-III PFGE system (Bio-Rad, Hercules, CA) using the following running parameters: volts 200 (6 v/cm), temp 14°C, initial switch 5 s, final switch 40 s, and 21 h. *Salmonella* H9812 was used to provide molecular size markers by using restriction enzyme *XbaI* (New England Biolabs, Beverly, MA). After the electrophoresis was completed, the gel was stained with ethidium bromide solution for 20-30 min. and followed by 3 times destain in fresh distilled water for 45-60 min. The destained gel was imaged using UV light photography (CDC 2001).

Chapter 4: Results

In order to evaluate the hypothesis that retail meat products can serve as a reservoir for MRSA, it was first necessary to isolate individual strains of *S. aureus* bacteria. Raw ground meat serves as an obvious reservoir for a variety of species of bacteria, but the focus of this study is the prevalence of *S. aureus* and any antibiotic resistance that may be found in the species. Therefore, first it was necessary to use selective media to grow only the relevant *S. aureus,* and second, to utilize a series of confirmation tests in order to remove any other species from the sample set. While Baird-Parker is selective media, it does not infallibly select only for *S. aureus,* but through the use of the Gram stain, catalase test, and the coagulase test, the unknown samples could be narrowed down.

4.1 Prevalence of *S. aureus* Contamination in Ground Meats

Of the 694 viable meat samples that were tested, 215 contained isolates that were suspected to be *S. aureus* using selective plating and positive results from the Gram stain, catalase, and coagulase tests. The 215 suspected *S. aureus* isolates were sent to the FDA for further confirmation using the Vitek Immunodiagnostic Assay System. The 215 isolates were narrowed down to the confirmed 200 positive *S. aureus* samples, an overall positive rate of 29%. The results of the Vitek

Immunodiagnostic Assay System as well as the Latex test for the 215 isolates are in **Appendix A,** with the eliminated 15 non-*S. aureus* isolates highlighted in yellow**.**

4.1.1 Prevalence of S. aureus Contamination by Meat Type

Table 1. *S. aureus* Positive Meat Samples by Meat Type

Meat Type	Total # of Samples	# of *S. aureus* Positive Samples	% *S. aureus* Positive
Beef	198	55	27.78
Turkey	196	110	56.12
Pork	300	35	11.67
Total	694	200	28.82

The general results for prevalence of *S. aureus* across all retail meat products can be broken down in various ways in order to provide a more complete picture of the state of contamination in the sampling area. The first is the evaluation of any differences or possible trends based on the type of meat from which the samples were isolated. The positive rates for each meat type are presented in the table above (**Table 1).**

A total of 694 beef, turkey, and pork samples were tested. Of the 694 samples, 198 were beef, 196 were turkey, and 300 were pork. Of the samples that were confirmed to be *S. aureus*, 55 came from ground beef, 110 from ground turkey, and 35 from pork. This is a positive rate of 27.78%, 56.12%, and 11.67%, respectively. More than half (56.1%) of the ground turkey samples were positive for *S. aureus*, and turkey is more than twice as likely to be contaminated by *S. aureus* than ground beef (27.8%) and nearly five times more likely than pork (11.7%) (**Table 1**). These

differences are clearly statistically significant (p-value < 0.0001) strictly in terms of the proportion of samples that are contaminated with *S. aureus* to some degree, but further analysis must be completed in order to help explain these findings. These results say nothing about the degree to which the samples are contaminated: the colony forming units per gram (CFU/g) of meat will address this specifically (section **4.2**). These results also say nothing about how resistant the *S. aureus* tends to be in each type of meat, which will be analyzed with minimum inhibitory concentration (MIC) tests (section **4.3**).

Due to the lack of availability of ground pork, pork loin was often bought as a substitute. This is one suggested explanation for the dramatically lower *S. aureus* positive rate in pork. The study was designed to focus on ground meat, as it was hypothesized that the meat has more opportunity for contamination in processing as well as through the inclusion of many different animals in each package, as opposed to coming from a single animal. The ground pork was distinguished from pork loin in order to evaluate this possible source of difference between the three meat types. The ground beef, ground turkey, ground pork, and pork loin are presented for comparison in the table below (**Table 2).**

Table 2. *S. aureus* Positive Meat Samples by Meat Type (Pork Loin)

Meat Type	Total # of Samples	# of *S. aureus* Positive Samples	% *S. aureus* Positive
Ground Beef	198	55	27.78
Ground Turkey	196	110	56.12
Ground Pork	194	26	13.40
Pork Loin	106	9	8.49
Total	694	200	28.82

This table shows that 106 of the 300 pork samples were not ground and the percentage of positive pork loin samples (8.49%) was in fact lower than the ground pork (13.40%). However, using a statistical z-test for two proportions with a 95% confidence interval, the null hypothesis that the positive rate of ground pork is less than or equal to that of pork loin cannot be rejected. This means that the two pork groups are too close in percentage to conclude statistically that ground pork has a higher contamination rate. In order to draw the conclusion that ground pork contains a higher *S. aureus* contamination rate, a significantly larger sample size is required that maintains this same trend. The 13.40% positive rate for ground pork remains significantly lower (p-value < 0.0001) than the positive rates for beef (27.78%) and for turkey (56.12%). Because there was no significant difference found between ground pork and pork loins, all further comparative analysis will refer to this meat group simply as "pork," with all of the pork products being compared directly with all of the turkey and beef products.

4.1.2 Prevalence of S. aureus Contamination by Grocery Store Chain

All of the stores for the three most popular grocery chains in Montgomery and Prince George's counties were placed into a random number generator in order to

select a wide area of coverage while eliminating any outside bias. If only the most convenient stores were selected, a complete profile of *S. aureus* prevalence and resistance in the local area could not be obtained. In order to provide more detail to this profile of the area, the *S. aureus* prevalence was also evaluated based on which of the three grocery chains the meat samples were bought from. If one chain has a significantly higher rate of contamination than the two others, this may suggest that the processing techniques used throughout the operation are introducing inordinate bacteria levels and need to be evaluated further. As the stores were selected using a random sample from all of the stores in Montgomery County and Prince George's County, the number of samples coming from each chain is somewhat different based on the total number of stores from that chain. The number of samples obtained from each chain and their corresponding positive rates are presented in the table below (**Table 3).**

Table 3. *S. aureus* Positive Samples by Grocery Chain

Store	Total # of Samples	# of *S. aureus* Positive Samples	% *S. aureus* Positive
A	357	109	30.53
B	127	30	23.26
C	210	61	29.05
Total	694	200	28.82

.

Of the 694 samples, 357 samples were taken from store A, 127 from store B, and 210 from store C. Of the 200 *S. aureus* positive samples, 109 came from store A, 30 came from store B, and 62 came from store C. This results in a positive rate of

30.53% for store A, 23.26% for store B, and 29.05% for store C. While these percentages seem very close to the overall positive rate of 28.88%, a two-sample z-test was performed to statistically determine whether the differences between the stores are significant. Using a 95% confidence interval, the null hypothesis that there is no difference between the positive rates at different stores could not be rejected. This means that the three stores are close enough in proportion of positive samples, that, statistically, they are not considered to be significantly different. Therefore, it cannot be concluded that contamination percentages are different at each grocery chain unless a larger sample size is obtained and the trend toward difference continues. Because no conclusion can be made that distinguishes one grocery chain's rate of contamination from the two others, further comparative analysis focuses on the type of meat as the distinguishing factor between sample groups.

4.2 Concentration of Bacteria in Meat: Colony Forming Units per Gram

As mentioned previously, *S. aureus* prevalence results do not address the degree to which the samples are contaminated, or exactly "how dirty" the *S. aureus* contaminated samples are. The colony forming units per gram (CFU/g) of meat shows more accurately how many bacterial colonies were present in the evaluated sample and subsequently grew into a distinct colony on the BP plate. The CFU/g data is presented below as a histogram (**Figure 1**) with all three meat types separated and shown on the same graph.

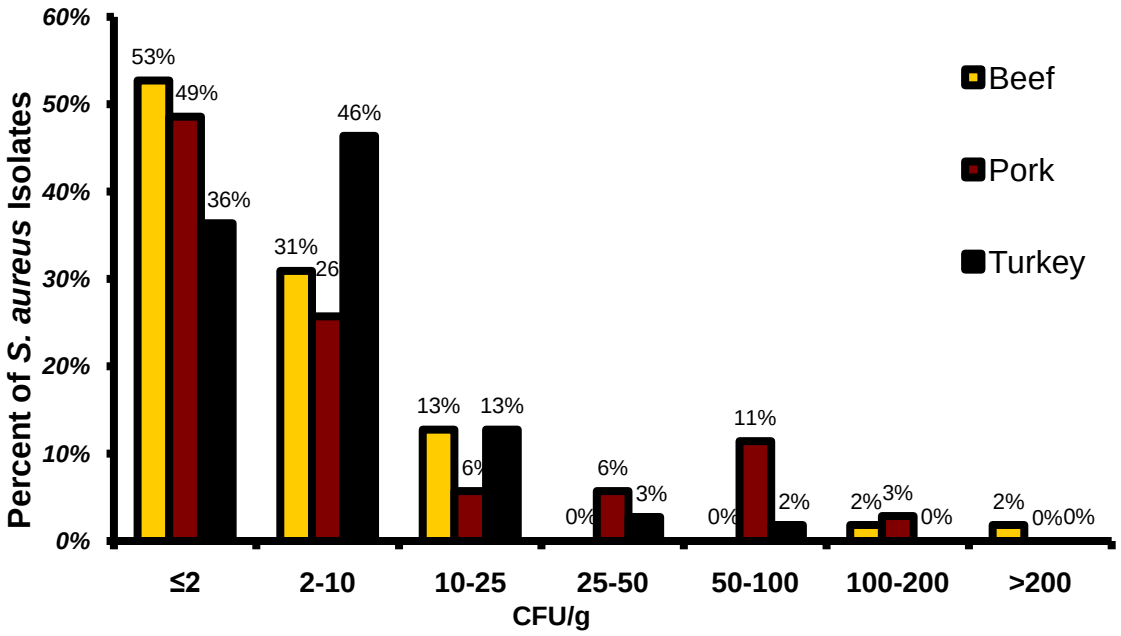

Figure 1. **CFU/g by Meat Type**. Histogram of CFU/g in *S. aureus* positive meat samples (n = 200) as determined by the total number of colonies on Baird-Parker plates inoculated with the meat sample buffer after incubation at 35°C for 48 hours. The CFU/g is shown in bins of increasing size on the horizontal axis. The percentage of turkey, pork, or beef samples that fall in each bin is shown on the vertical axis.

On the lower end (range of 0-10 CFU/g), the skew of *S. aureus* positive turkey samples toward having more CFU/g than pork or beef samples is observed (**Figure 1**). Most turkey samples (64%) had > 2 CFU/g, whereas only 47% of the beef and 51% of the pork samples contained > 2 CFU/g. However, on the high end, among *S. aureus* positive samples at concentrations of ≥ 25 CFU/g, the highest percentage of samples was pork. A full 20% of pork samples fall above the 25 CFU/g mark compared with 5% of turkey samples and only 4% of beef. The overall trend in CFU/g is that pork is more contaminated on average than turkey, which is overall more contaminated than the beef. However, it is important to note that only 11.6% of

pork samples had any *S. aureus* contamination at all, and only these contaminated samples are included in **Figure 1** above. Much higher percentages of the beef (28%) and turkey (56%) samples are included in the CFU/g figure, but on average they contain less bacterial colonies than the contaminated pork. This is an interesting trend that is repeatedly observed throughout the study; the trend being that while pork is not as often contaminated, in cases that it is the contamination tends to be more significant. One specific example of this is the only identified MRSA strain, which was isolated from a pork sample.

4.3 Antimicrobial Resistance in *S. aureus* Isolated from Ground Meat

Each *S. aureus* isolate was tested using antibiotic susceptibility testing (AST) with a 96-well plate. Each well is filled with varying levels of 20 different antibiotics in order to establish a resistance profile. The plate uses established minimum inhibitory concentration (MIC) values (**Appendix C**) in order to label an isolate as "resistant" or "susceptible." In other words, a series of concentrations of each antibiotic is used and growth is either observed or not observed at each concentration, the cutoff for MIC being the lowest concentration of antibiotic that prevents growth. The general results for the percentage of *S. aureus* isolates that were resistant to each of the 24 antibiotics are listed in the table below (**Table 4**).

Table 4. Prevalence of *S. aureus* Resistance to Profile of 20 Antibiotics

Antibiotic	Number of resistant *S. aureus*	Percentage Resistant	Antibiotic	Number of resistant *S. aureus*	Percentage Resistant
CHL	1	0.5%	**PEN**	**52**	26%
ERY	16	8%	**RIF**	0	0%
CLI	9	4.5%	**VAN**	0	0%
DAP	0	0%	**SXT**	0	0%
OXA+	1	0.5%	**LEVO**	0	0%
STR	0	0%	**CIP**	0	0%
DT1	12	6%	**SYN**	1	0.5%
DT2	12	6%	**TGC**	0	0%
GEN	3	1.5%	**NIT**	0	0%
AMP	**34**	17%	**TET**	**137**	68.5%
FOXS	1	0.5%	**MXF**	0	0%
LZD	0	0%	**GEN**	0	0%

Note: TREK GPALL1F (SOP 530-018 R-3) plate contained antibiotics chloramphenicol (CHL), erythromycin (ERY), clindamycin (CLI), daptomycin (DAP), oxacillin + 2% NaCl (OXA+), streptomycin (STR), ampicillin (AMP), linezolid (LZD), penicillin (PEN), rifampin (RIF), vancomycin (VAN), trimethoprim/sulfamethoxazole (SXT), levofloxacin (LEVO), ciprofloxacin (CIP), quinupristin / dalfopristin (SYN), tigecycline (TGC), nitrofurantoin (NIT), tetracycline (TET), moxifloxacin (MXF), gentamicin (GEN). Additionally, two wells were used for a D-test (DT1 and DT2). A D-test can be used to detect CA-MRSA with inducible resistance. These wells contain erythromycin and clindamycin antibiotic discs, with a flattened area of growth (D-shaped), near the clindamycin disc indicating inducible resistance to clindamycin. A cefoxitin (FOXS) screen was also performed in one well, in which a cefoxitin disk is placed in the well and the growth around it is observed, which acts as confirmation for resistance to oxacillin. Highlighted areas are the three most common types of resistance.

The most common drug resistance among *S. aureus* isolates (n = 200) was resistance to tetracycline, with 137 (68.5%) samples having an MIC above the established > 16 µg/ml break point. This was followed by 26.0% resistance to penicillin and ampicillin resistance in 17.0% of the isolates. All three of these are

very commonly used antibiotics in both humans and livestock. In the past, *S. aureus* infections were most commonly treated with penicillin, but the drug has become largely ineffective. Tetracycline is the most common antibiotic used subtherapeutically in livestock, therefore this result supports the assumption that subtherapeutic use encourages the development of resistant bacteria. This resistance has made its way into the animal meat that is handled prior to cooking and consumption. Ampicillin is in the penicillin family of drugs and has a very similar mechanism of action, making its similarly high resistance an expected result. Also as expected, none of the isolates were found to be resistant to vancomycin. Vancomycin often serves as a last resort in fighting an infection with MRSA, and therefore finding a vancomycin-resistant strain of *S. aureus* was highly unlikely.

4.3.1 Differences in Antimicrobial Susceptibility of Beef, Pork, and Turkey Isolates

In order to establish a better view of the resistance profile of *S. aureus*, once again the data was broken down by the type of meat each isolate came from. The differences in the rates of resistance across types of meat were found to be dramatic. The figure below, which displays the three most commonly found types of resistance (tetracycline, penicillin, and ampicillin), shows the differences that were found (**Figure 2).**

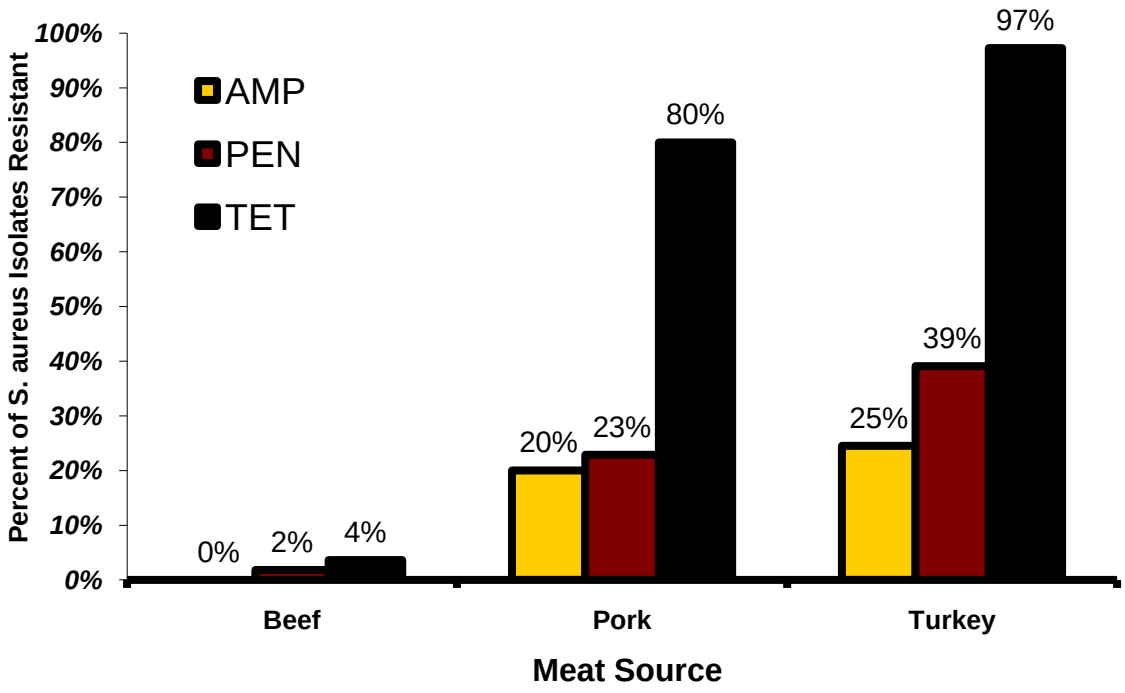

Figure 2. *S. aureus* Resistance to Antibiotics by Meat Source. Resistance of *S. aureus* isolated from ground beef (n = 55), pork (n = 35), and ground turkey (n = 110) to ampicillin (AMP), penicillin (PEN), and tetracycline (TET).

Colonies isolated from beef were found to have virtually no resistance to ampicillin, penicillin, or tetracycline at 0%, 2%, and 4%, respectively. Upon further analysis of the other 17 antibiotics, this was found to be no aberration: beef isolates hold virtually no resistance across the entire profile of antibiotics. Turkey and pork isolates were very different with 80% of pork and 97% of turkey samples being resistant to tetracycline. Both held similar resistance of 20% and 25% to ampicillin, and turkey isolates had nearly double the prevalence of resistance to penicillin (39% versus 23%). Differences in the prevalence of subtherapeutic use in turkeys and swine versus cattle are likely the main contributing factors in causing this difference, but there are likely others.

In order to expand the picture outside of tetracycline, penicillin, and ampicillin, similar analysis must be conducted on the 17 other antibiotics that were included in the profile. In order to compare across meat types, MIC50 and MIC90 values were found for all 9 antibiotics that had at least one resistant strain of *S. aureus.* The MIC is the lowest concentration of the antibiotic that results in inhibition of visible growth under standard conditions. MIC50 is the concentration required to inhibit the growth of 50% of the isolates in the study and similarly MIC90 is the concentration required to inhibit the growth of 90% of the isolates in the study. Once again, standard MIC cutoff values were employed for each antibiotic in order to determine the percent of resistant isolates. MIC50, MIC90, and percentage resistant are all displayed in the table below, with each shown for beef, turkey, and pork groups separately for comparison (**Table 5**).

Table 5: MIC50, MIC90, and Percentage Resistant for *S. aureus* Isolates

	MIC50 (µg/ml)	MIC90 (µg/ml)	Percent Resistant
AMP	**8**	**> 8**	**17**
Beef	0.12	4	0
Turkey	8	> 8	24.5
Pork	4	> 8	20
PEN	**8**	**> 8**	**26**
Beef	0.06	8	1.8
Turkey	8	> 8	39.1
Pork	4	> 8	22.9
TET	**> 16**	**> 16**	**68.5**
Beef	≤ 2	≤ 2	3.6
Turkey	> 16	> 16	97.3
Pork	> 16	> 16	80
CHL	**8**	**16**	**0.5**
Beef	8	16	0
Turkey	8	16	0.9
Pork	16	16	0

ERY	**0.5**	**1**	**8**
Beef	0.5	1	7.3
Turkey	0.5	0.5	1.8
Pork	0.5	> 4	28.6
CLI	**≤ 0.5**	**≤ 0.5**	**4.5**
Beef	≤ 0.5	≤ 0.5	0
Turkey	≤ 0.5	≤ 0.5	0
Pork	≤ 0.5	> 2	25.7
OXA+	**0.5**	**0.5**	**0.5**
Beef	≤ 0.25	0.5	0
Turkey	0.5	0.5	0
Pork	0.5	1	2.9
GEN	**≤ 2**	**≤ 2**	**1.5**
Beef	≤ 2	≤ 2	0
Turkey	≤ 2	≤ 2	0.9
Pork	≤ 2	≤ 2	5.7
SYN	**≤ 0.5**	**≤ 0.5**	**0.5**
Beef	≤ 0.5	≤ 0.5	0
Turkey	≤ 0.5	≤ 0.5	0
Pork	≤ 0.5	1	2.9

Note: DAP, STR, LZD, RIF, VAN, SXT, LEVO, CIP, TGC, NIT, and MXF all showed no resistance and are not listed in the table above.

The MIC50, MIC90, and resistance percentages for the other six antibiotics listed in the table above match the general trends observed in **Figure 2.** In nine out of nine cases above, isolates from pork require a greater than or equal concentration to prevent the growth of either 50% or 90% of samples. In other words, there is no antibiotic in the table above that has a higher MIC50 or MIC90 for beef than for pork. There is only one case, the MIC90 of erythromycin (ERY), for which beef is higher than turkey. Outside of the three previously mentioned antibiotics in **Figure 2,** pork has a greater prevalence for resistance than turkey in every case listed above. While the sample sizes are too small for each antibiotic to establish this as a conclusive trend, this data does prove that overall resistance in both turkey and pork isolates is

significantly higher than in beef isolates. Turkey isolates and pork isolates are both highly resistant to the common antibiotics penicillin (PEN), ampicillin (AMP), and tetracycline (TET), but at least one pork isolate was found to be resistant to a wider range of less common drugs including oxacillin (OXA+), gentamicin (GEN), quinupristin / dalfopristin (SYN), and erythromycin (ERY). Upon further analysis, this pork isolate was determined to be the only example of MRSA found in the study.

4.3.2 Multi-Drug Resistant S. aureus Isolates

A single outlier, such as a pork isolate that is resistant to 10 of the 20 antibiotics present on the plate, is best visualized using a multiple-antibiotic resistance profile. A multiple-antibiotic resistance profile is a histogram which plots the number of different antibiotics each isolate was resistant to. The data points on this histogram are once again separated by meat source and the result is shown in the figure below (**Figure 3**).

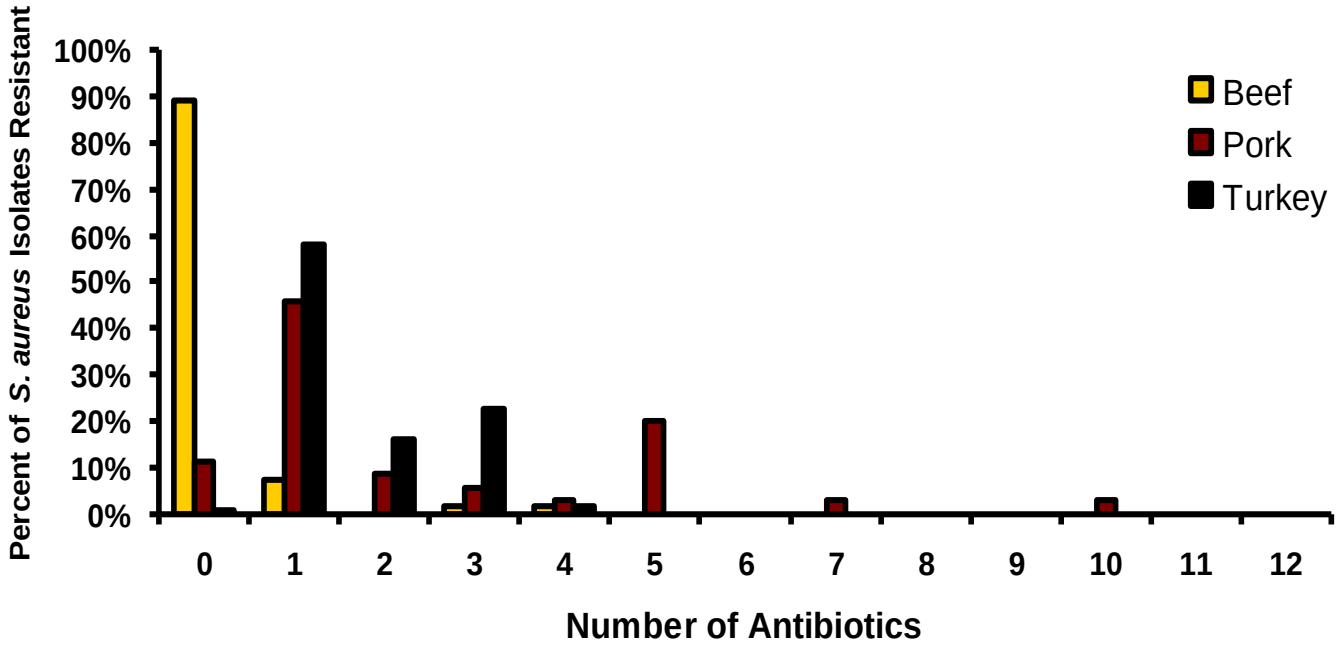

Figure 3. Multiple-antibiotic resistance profile of *S. aureus*. Multiple-antibiotic resistance profile of *S. aureus* isolates from beef (n = 55), pork (n = 35), and turkey (n = 110) presented as the percent of *S. aureus* isolates resistant to no more than the corresponding number of antibiotics.

The multiple-antibiotic resistance profile offers further confirmation of the observations made from both **Figure 2** and **Table 5.** Once again, for beef, 90% of the isolates were not resistant to a single antibiotic. The opposite is true of turkey, which had over 97% resistance to at least one antibiotic. Pork falls in the middle with 88% of isolates resistant to at least one antibiotic, but as it is easy to see on the histogram above, every outlying isolate came from pork. All isolates resistant to five or more antibiotics (n = 9) came from pork, and this is 25.7% of all pork isolates. This data offers further support for the trend that while fewer pork samples are contaminated with *S. aureus* than turkey or beef, those pork samples that are contaminated are more

likely to contain a greater number (CFU/g) of more highly resistant bacteria. This establishes pork as having the highest risk for being a reservoir of resistant *S. aureus* and the most likely of the three meat sources to serve as a possible avenue for MRSA infection. The pork isolate in **Figure 3** that falls in the category of being resistant to 10 different antibiotics was confirmed to be MRSA using several tests that will be detailed further in the following sections.

4.4 Methicillin-Resistant *S. aureus* Isolates from Ground Meat

Methicillin, the antibiotic of specific interest in examining MRSA (methicillin-resistant *Staphylococcus aureus),* was not one of the 20 antibiotics included in the resistance profile of the previous section. A separate set of dilutions was prepared in order to determine the MIC of methicillin for the 200 *S. aureus* isolates. The results of the methicillin MIC tests are displayed in the histogram below (**Figure 4**), with those samples falling in the category of ≥ 16 µg/ml and above being established as "resistant" by the standard MIC values (**Appendix B**).

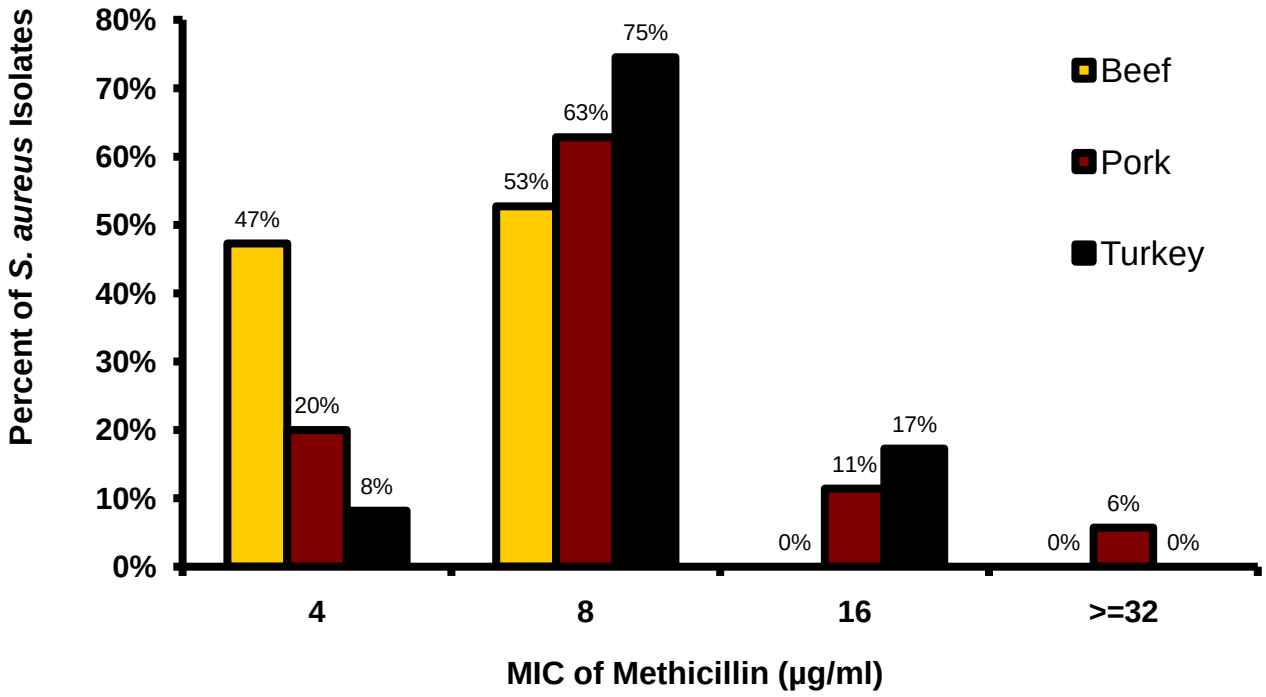

Figure 4. Methicillin MIC in *S. aureus*. Methicillin MIC of *S. aureus* isolates from ground beef (n = 55), pork (n = 35), and turkey (n = 110) samples.

Overall, there are 24 isolates that are resistant to methicillin, 19 coming from turkey and 5 from pork. This equates to 17% of turkey isolates and 17% of pork isolates, as can be seen above. Fitting the previous pattern, 100% of beef isolates were susceptible and 87.7% of the all *S. aureus* isolates were susceptible to methicillin (MIC ≤ 8 µg/ml). Two pork isolates once again served as outliers, having MIC ≥ 32 µg/ml. One of the two was the MRSA isolate and the other was a highly resistant *S. aureus* isolate that tested negative for the *mecA* resistance gene.

In order to determine if a sample is considered MRSA, genetic testing must be performed for the presence of a gene known as *mecA*. The *mecA* gene allows a bacterium to be resistant to antibiotics in the penicillin family. All antibiotics in this family have a β-lactam ring structure, which attacks and destroys enzymes in the bacterial cell well. The *mecA* destroys the β-lactam ring, thus preventing antibiotics such as methicillin from killing the bacterial cell. There is not only one strain of MRSA; in fact, many different strains are found in hospitals and even in the community. Hospital-acquired (HA-MRSA) strains are often resistant to a wider range of antibiotics and are more commonly found in older individuals. Community-acquired (CA-MRSA) strains spread from close contact and most often affect younger individuals. Previous studies have suggested that CA-MRSA can be spread through contaminated meat products, and thus it was suspected that CA-MRSA may be identified in this study. Through genetic analysis techniques and comparison to existing known MRSA isolates, it can be determined which strain of MRSA was isolated in this study.

4.4.1 PCR Confirmation of mecA-Positive Isolates Including MRSA Strain

Polymerase Chain Reaction (PCR) with primers specific to *mecA* was performed on all of the isolates that were found to be resistant to methicillin in the methicillin MIC tests. PCR amplified a specific DNA fragment, which in this case was *mecA*. It was determined that only one of the methicillin-resistant isolates actually contained the *mecA* gene. This isolate was one of the two pork isolates with

an MIC ≥ 32 µg/ml for methicillin, the same isolate that was established to be resistant to 10 different antibiotics. Since there was only one isolate that contained the *mecA* gene, but 24 that were resistant to methicillin, it seems obvious that there is more than one mode of resistance to methicillin-related antibiotics. There were 24 strains of *S. aureus* with methicillin resistance, but only one is technically considered to be methicillin-resistant *S. aureus*, or MRSA.

4.4.2 PFGE Typing of MRSA Isolate as USA100

Pulsed-field gel electrophoresis (PFGE) can be used to compare the DNA of the sample identified as MRSA to DNA from known MRSA isolates that have been isolated around the country. By cutting each strain's DNA with the same restriction enzyme (SmaI), the pattern of DNA fragments that appear on the gel can be compared to known patterns produced by previously isolated MRSA. Smaller DNA fragments move further down the gel than larger fragments, and thus by running all of the strain on a single gel, a simple comparison of the known to the unknown can be made. The PFGE analysis of the unknown MRSA isolate (409-1) is shown in the figure below (**Figure 5**).

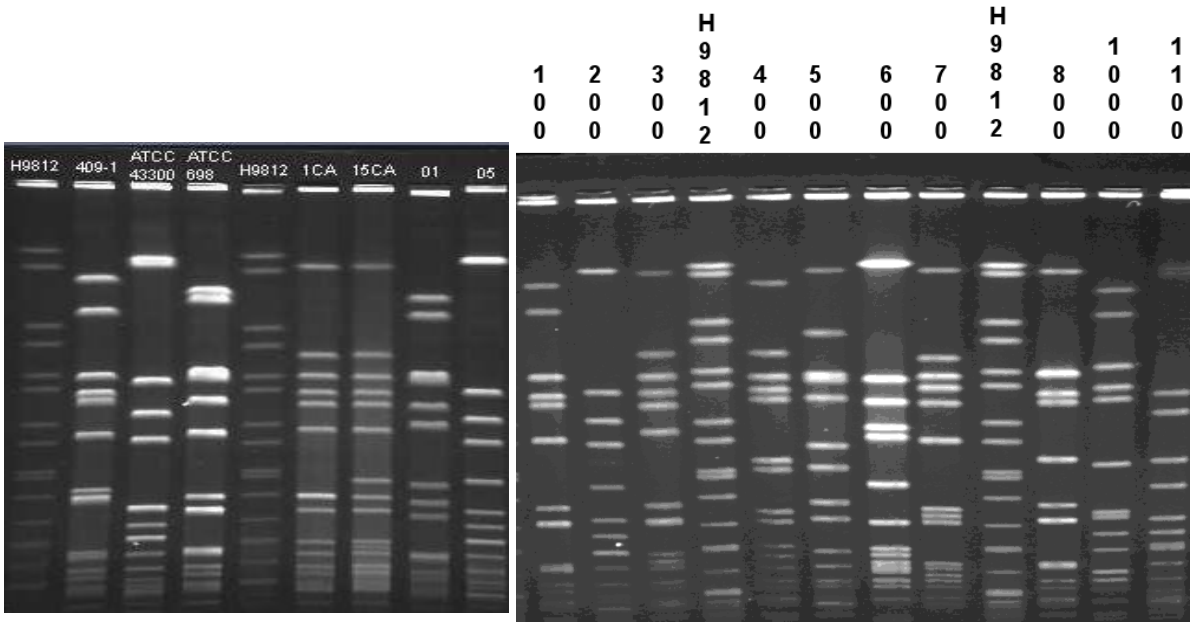

Figure 5. The unknown isolate, 409-1, matches 100. This is USA100, a known hospital-acquired (HA-MRSA) strain. H9812 is cut by XbaI, and the MRSA strains are cut by SmaI. 1CA and 15CA are HA-MRSA clinical strains from California, and 01 and 05 are HA-MRSA clinical strains from a Maryland hospital.

From the gel analysis above, it was determined that the pork isolate 409-1 matched the known USA100 isolate. USA100 is a well-established HA-MRSA strain, which contradicted the expectation that CA-MRSA would be found in the meat products. Because only one isolate was found, there is not enough data to make any conclusion regarding the presence of HA versus CA strains in the consumer meat supply.

4.4.3 Methicillin-Resistant Non-S. aureus Isolates

While the focus of this study was on *S. aureus*, some interesting non-*S. aureus* isolates were also identified. Only one *S. aureus* isolate contained the *mecA* gene and was therefore considered to be MRSA, but 17 other isolates, primarily

Staphylococcus epidermidis, were also found to be positive for *mecA* (**Appendix C**). Because these isolates are outside of the *S. aureus* species, they cannot be MRSA, but given the possibility of horizontal transfer of genetic material between bacteria, it is certainly possible that other species may serve as a source for this gene. Normally, *S. epidermidis* is not of much concern as it is typically a non-pathogenic bacterium, but horizontal transfer makes it a possible reservoir of dangerous genetic material. The complete antibiotic resistance profile for each of these 17 isolates is located in **Appendix C.** Additionally, there were 55 isolates of various species that were negative for *mecA* but nonetheless resistant to methicillin. Once again, this suggests alternate mechanisms for resistance to the antibiotic family. The antibiotic resistance data for each of these non-*S. aureus* isolates can be found in **Appendix D.**

Chapter 5: Discussion

5.1 Major Findings

Of the meat types collected, turkey yielded the greatest proportion of *S. aureus* positive samples: over half (56.6%) of the samples tested positive for *S. aureus*. Just over a quarter of the beef (28.5%) and 11.7% of the pork was *S. aureus* positive. This disproportionate *S. aureus* prevalence may be a result of animal-rearing conditions or meat-processing techniques which promote the growth of *S. aureus* or increase the likelihood of contamination.

The meat samples were collected from three prominent grocery store chains in Montgomery and Prince George's counties. Store A provided 31.4% of the *S. aureus* positive samples, Store B provided 22.6%, and Store C provided 29.5%. These minimal differences are likely due to the varying practices of the meat suppliers for each chain or, as demonstrated in the results, the differences may not be statistically significant.

5.1.1 Animal Husbandry Practices May Impact Antimicrobial Susceptibility

Overall, trends in antibiotic resistance of *S. aureus* isolates, CFU/g, and the prevalence of *S. aureus* colonization were observed across meat types but no such trends appear to exist across geographical locations or grocery store chains. Therefore, variation in these parameters is likely due to the animal husbandry and

meat-processing protocols that are characteristic to beef, pork, and turkey production rather than differences in meat handling further down the supply chain. For example, poultry and swine are more likely to be fed antibiotics at some point in their lifetime than beef cattle finished at a feed lot. Only 60% of feedlot cattle are fed subtherapeutic antibiotics, while nearly 80% of poultry and 75% of swine receive antibiotics as a feed additive (Dupont 1987).

In addition, poultry and swine are more likely to be fed antibiotics for a longer consecutive period of time than beef cattle. Beef cattle are usually raised on pasture or in pens at a cow-calf operation for either the first year of life or until they are weaned. They may then be transferred to a stocker operation where they are grazed for a few months on pasture and crop residue or may be shipped directly to a feedlot. Beef cattle normally spend only their last few months of life at a feedlot. At the feedlot, thin profit margins encourage antibiotic use as one of many strategies employed to maximize market weight. However, at the previous stages of life antibiotic use is often neither economical nor practical.

Forage is the main source of feed in cow-calf operations so adding antibiotics to the feed is not possible. Cows and calves are kept on pasture, rangeland, or crop residue when these are seasonally available and are usually fed harvested forage when supplementation is necessary due to snow or other unfavorable environmental conditions (Short 2001). These cattle often drink from streams, so adding the antibiotics to water is also not feasible. In addition, the calves lead relatively stress-free lives so antibiotics are not necessary to keep them healthy. When these animals

are shipped to feedlots, they are stressed by the transport and, because they are now maintained at a much greater density, diseases have the potential to spread rapidly.

Confined on the feedlot, antibiotics can be easily provided in water or processed feed. According to a 1995 USDA National Animal Health and Monitoring Service report, 58% of cattle on large feedlot operations housing 1000 or more animals receive antibiotics in their water at some point in their lives. However, only 28% of large feedlot operations used antibiotics in drinking water for 8 days or more in a cohort of animals and 25% of operations used antibiotics for less than 5 days. This indicates that antibiotics were used in order to control specific outbreaks on most feedlots and not as a routine water additive. Finally, of those feedlot operations that used antibiotics, only 7% used tetracycline, 30% used oxytetracycline, and 46% used chlortetracycline. No more than 1% of operations used penicillin, ampicillin, or similar antibiotics (NAHMS 1995).

By contrast, antibiotics are used throughout animals' lives at poultry and swine operations. As reported in USDA NAHMS Swine 2006, antibiotics in feed was the most common preventive practice used for grower / finisher pigs and second most common practice used for piglets after a routine iron injection. Almost 90% of nursery piglets received antibiotics in their feed and 78% continued to receive antibiotics at the grower / finisher phase (NAHMS 2007).

The differences in antibiotic susceptibility of *S. aureus* isolates from beef, pork, and turkey found in the present survey are consistent with differences in antibiotic use within these industries. Given the limited use of antibiotics in beef cattle, it is not surprising that nearly 90% of *S. aureus* isolates from ground beef were

susceptible to all the antibiotics tested. At the same time, it is important to note that most pork and turkey isolates were resistant to at least one antibiotic. Tetracycline is the commonly used antibiotic in livestock production. Accordingly, 97% of turkey *S. aureus* isolates and 80% of pork isolates were resistant to tetracycline. A significant portion of pork and turkey isolates were resistant to ampicillin, penicillin, and ampicillin as well. Although methicillin is not approved for use in livestock, it is an antibiotic within the same β-lactamase family to which penicillin and ampicillin also belong. Therefore, a gene that confers resistance to penicillin may also function to provide resistance to methicillin and/or other similar antibiotics.

5.1.2 Potential for Horizontal Transfer of Resistance Genes in S. aureus

Although only one *S. aureus* isolate carried the *mecA* gene, we found 17 non-*S. aureus* isolates which carried the *mecA* gene and were resistant to methicillin. These organisms are a possible source of horizontal transfer of the *mecA* gene to *S. aureus*.

As seen in previous studies of avoparcin and vancomycin resistance in *Enterococci* found in pigs and humans, when similar antibiotics are used in humans and animals with which we have close contact, resistance genes can be transferred between human and animal bacteria, speeding up the development of high levels of antibiotic resistance in the bacteria (Ferber 2000). The development of antibiotic resistance in bacteria normally takes a longer time if it is fostered exclusively by the selective pressure of antibiotic use in the host. However, horizontal transfer of genes is a shortcut that bacteria can take to develop resistance by sourcing resistance genes

from other strains that have already undergone the selective process over many generations.

Although horizontal transfer of resistance genes in bacteria most commonly occurs between strains of the same species, as was the case in *Enterococci*, it can also occur between strains of different species. As a strain moves between humans, animals, and the environment, it can pick up resistance genes from other bacteria and potentially develop multi-drug resistance. If different antibiotics are used in humans and livestock then resistance genes from animal bacteria would pose little risk to human health. However, when the same antibiotics are used, efforts at judicious use of an antibiotic in human medicine can be undermined by the spread of resistance genes from animal bacteria and vice versa.

In addition, because 95% of antibiotics given to livestock are excreted unchanged, bacteria living on people who have regular contact with animal waste or in the environment in close proximity to animal waste are constantly exposed to antibiotics and may develop resistance (Choi 2007). These people can then spread the resistant bacteria off the farm on their shoes or bodies.

In the present study, 15 out of the 17 non-*S. aureus* isolates harboring the *mecA* gene which confers methicillin resistance were identified to be strains of *Staphylococcus epidermidis*, a close relative of *S. aureus*. Because these bacteria species are closely related, horizontal transfer of resistance genes between them can be easily accomplished. Of the *mecA* positive *S. epidermidis* isolates, all but one were also resistant to erythromycin, 73% were resistant to oxacillin, 60% to penicllin, 73% to cefoxitin, and 53% were resistant to trimethoprim/sulfamethoxazole (Appendix C).

Therefore, although *S. epidermidis* rarely causes disease in healthy humans, antibiotic resistant strains of *S. epidermidis* in animals or meat products are an alarming potential source of antibiotic resistance genes, and *S. epidermidis* contamination of meat must be closely monitored. In addition, one *mecA* positive isolate which could not be identified by species was resistant to vancomycin, the last resort antibiotic currently available to treat MRSA. Although no vancomycin resistance was found in any *S. aureus* isolates, vancomycin resistance in this and other *mecA* negative non-*S. aureus* isolates may present a risk of *S. aureus* acquiring vancomycin resistance genes in the future.

5.1.3 Methicillin Resistance in Absence of mecA Gene

Methicillin resistance was identified by MIC testing. Though 29 of the 57 *S. aureus* positive beef isolates grew in a solution of 8 µg/ml, none grew in a solution of 16 µg/ml – the threshold for methicillin resistance. Twenty isolates of turkey and 4 isolates of pork showed methicillin resistance at a threshold of 16 µg/ml. The methicillin resistance observed in all of the turkey isolates and all but one of the pork isolates occurred in the absence of the *mecA* gene. This suggests methicillin resistance through a means other than the *mecA* gene.

It is interesting to note that 9 strains of *S. aureus* and 55 strains of non-*S. aureus* that lacked the *mecA* gene were resistant to methicillin. This indicates that there may be additional genes responsible for methicillin resistance in *S. aureus* and other organisms.

5.1.4 Antimicrobial Resistance in Non-S. aureus Meat Isolates

Of the 35 methicillin-resistant, *mecA*-negative, non-*S. aureus* isolates within this group that could be identified, almost half (n = 18) were *Enterococcus faecalis (*Appendix D*)*. This finding is consistent with previous studies in which *Enterococci* cultured from cattle feces and carcasses at the processing plant exhibited a high occurrence of antibiotic resistance with 64% of isolates being resistant to at least 6 antibiotics, usually including chloramphenicol, erythromycin, and tetracycline (Fluckey 2009). The methicillin-resistant *E. faecalis* isolated in the present study also had a high rate of multi-drug resistance with 100% resistant to oxacillin and cefoxitin, 94% of isolates resistant to clindamycin, 89% to quinupristin/dalfopristin, 56% to tetracycline, and 44% to erythromycin.

Interestingly, all but one of the 20 unidentified isolates resistant to methicillin were also resistant to vancomycin. The unidentified isolates also had an even higher rate of resistance to erythromycin (90%), clindamycin (100%), ampicillin (95%), linezolid (85%), penicillin (95%), and quinupristin/dalfopristin (95%) than the *E. faecalis* isolates but a lower prevalence of tetracycline and cefoxitin resistance. It will be important to conduct more experiments in order to identify these strains because of their exceptional multi-drug resistance and to determine whether these bacteria could transfer resistance genes to *S. aureus* or other species.

5.1.5 Prevalence of MRSA in Retail Ground Meats Lower Than Expected

Prior studies oriented towards MRSA have focused primarily on beef and pork products. In the Netherlands, for instance, pig and cattle farmers have been

reported to be more likely than the average person to be colonized by MRSA (NAHMS 1995). The significant methicillin resistance in *S. aureus* derived from turkey documented in this study was unexpected.

At a positive rate of only 0.14%, it appears that the prevalence of MRSA is lower in D.C. area meats than previously reported in European meats and Louisiana meats, although it must be noted that no enrichment was used in our cultures (Normanno 2007). We recovered MRSA from one meat sample out of the 698 collected. Although the detection of MRSA without enrichment of cultures in retail meat is significant, the prevalence of MRSA was lower than expected in light of the survey of retail meats in Louisiana in 2009 (Pu 2009).

Although the MRSA strain in this study was isolated from pork, ground turkey appears to pose the greatest risk of being colonized by antibiotic resistant *S. aureus*. However, pork isolates were overrepresented at the maximum values of CFU/g and multi-antibiotic resistance indicating that, although not as common, pork-derived *S. aureus* may be more dangerous. While most beef and turkey isolates were not resistant to erythromycin and clindamycin, over a quarter of pork isolates were resistant to at least one of these antibiotics.

In addition, fewer pork samples may have been found positive for *S. aureus* than turkey samples since some pork samples were not ground due to inconsistent availability of raw ground pork. Even so, the use of non-ground pork is not considered an extraneous variable in this study because no statistically significant difference was found in the *S. aureus* contamination rate of ground and non-ground pork.

5.1.6 Maryland Meat Consumers are Exposed to Multi-Drug-Resistant Bacteria

Consumer exposure to raw meat products could carry similar risks of infection with antibiotic resistant bacteria as are seen in farm and meat processing plant workers. Cross-contamination has been shown to allow bacteria from several animals to spread to processing plant surfaces (Fluckey 2009). People who handle raw meat may then be exposed to the same hazards as poultry workers who are more likely to carry multi-drug resistant bacteria such as *E. coli* (Price 2007).

The present study highlights the fact that consumers in Maryland handling raw ground pork and turkey are being exposed to multi-drug resistant *S. aureus*. Immunocompromised people or those with breaks in the skin on their hands who handle raw ground turkey and pork may be at risk for developing abscesses, folliculitis, cellulitis, impetigo, necrotizing fasciitis, or even bacteremia that could be difficult to treat with conventional antibiotic therapy because of antibiotic resistance within the causative agent.

5.2 Data Analysis in Light of Previous Research

While a ciprofloxacin resistant (MIC = 15 μg/ml) *S. aureus* strain has previously been isolated from a chicken in Canada with septicemia, none of the isolates in the present study expressed resistance to the second generation fluoroquinolone ciprofloxacin (MIC $\leq$ 1 μg/ml) or the third generation fluoroquinolone moxifloxacin (MIC $\leq$ 0.25 μg/ml) (18). This is not surprising since

neither drug is approved for veterinary use by the Food and Drug Administration, although ciprofloxacin is sometimes prescribed for dogs and cats.

In a study conducted in Ankara, Turkey, 80 *S. aureus* strains were isolated from 150 samples of chicken carcasses, lamb chunks, calf chunks, minced calf meat, and chicken giblets. Of these, 67.5% were resistant to methicillin and 53.8% to Penicillin G, both higher values than those encountered in the present study where we found 26% of all isolates to be resistant to penicillin. In parallel with our findings, Gundogan et al. also found a large proportion of poultry samples to be *S. aureus* positive, 43% of 60 chicken carcass samples. However, in contrast with our relatively low ground beef figures, Gundogan et al. reports 57% of 60 minced calf meat samples as *S. aureus* positive.

In addition, while no methicillin-resistant *S. aureus* beef isolates were found in the present study, 64.2% of Turkish minced calf meat isolates were methicillin-resistant according to a Kirby-Bauer diffusion test. Only 19% of our turkey isolates were methicillin-resistant while 76.4% of Turkish chicken carcass isolates were resistant. Overall, it is interesting to note that a similarly high frequency of methicillin and penicillin resistance was encountered by Gundogan et al. in *S. aureus* strains isolated from all of the different meat types sampled, indicating that these strains are likely of human origin and their antibiotic resistance profile is not related to the source animal. This result stands in stark contrast with our findings, where the resistance profile of *S. aureus* isolates was distinct for pork, turkey, and beef isolates. Furthermore, Gundogan et al. found a relatively low incidence of erythromycin resistance at 7.5%. At 8%, the overall rate of erythromycin resistance in *S. aureus*

was similarly low in our survey, but a disproportionately high number (29%) of pork isolates in the present study were resistant to erythromycin as opposed to 11% of beef isolates and 2% of turkey isolates (Gundogan 2005). None of the isolates in the aforementioned report were resistant to vancomycin and likewise we found no vancomycin resistance in our meat isolates.

Two investigations of *S. aureus* in Italian animal products produced findings distinctly different from ours. In 2005, Pesavento et al. isolated 42 *S. aureus* strains from 176 samples of beef, pork, and poultry over the course of one year and tested each for resistance to twelve antimicrobials (Chao 2007). Poultry and beef were equally likely to be *S. aureus* positive in this study while we saw a strong skew towards poultry. In addition, Pesavento et al. does not report any methicillin- or vancomycin-resistant strains. The meat isolates were most commonly resistant to ampicillin and oxacillin.

Pesavento et al. found 35.71% of *S. aureus* isolates to be resistant to oxacillin and 42.86% to ampicillin as opposed to 0.5% and 17% respectively in the present investigation. Beef isolates in the study had a high incidence of ampicillin resistance (58%) while we encountered no ampicillin-resistant isolates in beef. While almost all of our poultry isolates were resistant to tetracycline, Pesavento et al. reports poultry isolates to be rarely resistant to this antibiotic with an incidence of just 8%. Even in the small sample size collected by Pesavento et al., multiple ($\geq$ 3 antibiotics) resistances were reported in 13 strains (30.95%).

Another study in Italy, in which 1,634 samples of from milk, dairy products, meat, and meat products yielded 160 strains of *S. aureus*, also found 6 strains of

MRSA, all from dairy products such as milk and various cheeses. The lower overall *S. aureus* positive rate of the food products looked at in this study as opposed to the meat products we investigated may be due to geographical differences or, more likely, to differences in food types and food processing. The contamination rate of the milk and dairy products was also significantly higher than that of the meat products, but most biotypes were human (Bennett 2006). Interestingly, Normanno et al. reports that, although all MRSA isolates were resistant to at least two antibiotics including methicillin, none of the MRSA strains were resistant to tetracycline, erythromycin, or clindamycin, and only half were resistant to ampicillin, while we found resistance to all four of these antibiotics in our MRSA isolate. Some of our *S. aureus* isolates were also resistant to one or more of these antibiotics (Lin 2007). Furthermore, no vancomycin-resistant strains were reported.

A survey of 2,217 samples of beef, pork, veal, lamb, chicken, turkey, fowl and game products in the Netherlands found 264 MRSA strains. Turkey was by far the most likely to harbor MRSA (35% positive rate). Although the MRSA isolate in the present study came from pork, our findings are otherwise in agreement in that turkey was the most likely to be resistant to methicillin as well as ampicillin, penicillin, and tetracycline. In addition, we may not have detected the same incidence of MRSA because we did not use enrichment in our cultures as Boer et al. did (Boer 2005). Since no enrichment was performed in our protocol, comparison of MRSA quantity between our reports is not meaningful. Boer et al. isolated more MRSA from beef and turkey than from pork. In contrast, the single MRSA strain detected in our survey was

isolated from pork. In fact, the *S. aureus* positive rate reported by Boer at al. was higher for turkey and beef and actually lower for pork.

A study performed in Italian pigeon slaughterhouses tested the resistance of 59 *S. aureus* strains to 26 different antibiotics used for humans and for poultry (Normanno 2007). In agreement with their findings, most of our strains were susceptible to gentamicin, oxacillin, rifampin, and vancomycin. We found only 0.5% of isolates to be resistant to chloramphenicol compared to the 6.8% reported by Losito et al. Losito et al. likewise found a significant proportion of *S. aureus* isolates that were resistant to ampicillin and penicillin G at 15.2%. However, at 37.3%, the overall incidence of tetracycline resistance in *S. aureus* cultured from the pigeon slaughterhouses was significantly lower than our overall rate of 68.5% of resistance.

The only other study of *S. aureus* in U.S. retail meats thus far was conducted in Louisiana and involved the collection of 90 pork chop and 30 beef steak samples from 30 retail grocery stores of seven different chains (Pu 2009). The methodology used by Pu et al. is significantly different from the methods used in the present study. Pu et al. used whole cuts of meat rather than ground meat and wiped the outside of each meat package with alcohol in order to prevent contamination upon opening the package. Despite these measures, Pu et al. found a greater proportion of pork samples to be contaminated with *S. aureus* (45.6%) than the figure we present here (12%); however, this difference may be due to the fact that Pu et al. enriched their samples for 24 hours prior to spreading while we did not. The addition of enrichment may also be responsible for the greater overall incidence of MRSA that Pu et al. reports since enrichment allows the detection of very small initial quantities of bacteria (Pu 2009).

Pu et al. reports 22 isolates from 5 samples of pork and one sample of beef that were confirmed MRSA resulting in a 5% overall positive rate for MRSA with 5.6% of pork and 3.3% of beef samples testing positive. In order to confirm the identity of their MRSA isolates, Pu et al. used only the coagulase test, PCR for *S. aureus* genes, and PCR for the *mecA* gene characteristic of MRSA. Although through DNA testing Pu et al. were able to conclude that the found MRSA strains were likely of human origin just as the MRSA isolate found in the present study, the remarkable disparities in the prevalence and antibiotic resistance patterns of *S. aureus* isolates from different meat types in the present study indicate that these variations are a result of practices specific to the meat source such as animal husbandry and carcass processing standards.

In addition, it is important to note that Pu et al. found two isolates that had a methicillin-resistant phenotype but did not have the *mecA* gene just as we found 17 such isolates. This gives strength to our finding and provides evidence that it is not unique or related to flaws in our methodology.

5.3 Limitations

We now consider some of the limitations to our methodology. With regards to the project as a whole, there are limits to what we can infer considering the narrow focus that was necessary for a study of this length. For instance, only stores from a predetermined region in Central Maryland were sampled. Claims regarding the bacteria in retail meat from other regions cannot be made at this point. Also, the

length of the sampling period was not long enough to make reliable conclusions about bacteria characteristics changing over time, or from season to season. Additionally, no claims can be made for meats other than beef, pork, and turkey since these were the only types sampled. Ground meats were chosen since they contain a blend of meat, and therefore bacteria, from different parts of the animal and different animals. If there was significant contamination of consumer meat, it would likely be found in ground meat first. This focus means that claims about bacteria in whole cuts of meat cannot be made.

There were also certain procedural limitations of the study that may have affected our results. An important limitation was that we chose not to enrich our cultures. This choice saved time and allowed us to look more directly at the quantities of bacteria actually present in the meat samples; however, since enrichment allows for the detection of very small initial quantities of bacteria, we likely did not identify every sample that contained *S. aureus* or MRSA. Moreover, the results could not be compared explicitly to other studies that did use enrichment.

Another procedural limitation was the uneven period of time between buying the meat samples and processing them. The fact that this period of time existed at all means that at least some bacteria grew between the time the meat was bought and the time the meat was processed. A typical amount of time was a couple days, and this may have allowed significant bacterial growth. Also, since this time period varied from week to week, the results may have been skewed to some degree.

One of the main limitations of our survey design was that we could not culture meat immediately after purchase. Although expired meat was never used in this

study, meat was refrigerated for up to three days after purchase before being cultured. Because *S. aureus* does not grow appreciably at 4°C, refrigeration at this temperature should have prevented spoilage of the meat and proliferation of bacteria prior to sample processing in the laboratory. Another limitation was that, because no enrichment was used during the initial culture of meat samples, concentrations of bacteria less than 1 CFU/g but greater than 0 CFU/g could not be detected. However, the lack of enrichment allowed for the quantification of the concentration of presumed *S. aureus* in meat samples, lending an additional informative dimension to our data.

Other limitations came from the imperfect nature of the sampling process itself. Although the stores were chosen by random sampling, and the meat samples within each store were chosen as randomly as possible, this does not guarantee that the population of all stores in Central Maryland, or the population of all meat products within a given store, was adequately represented. To ensure adequate representation, all of the meat needed to be tested, but this of course was not possible, especially considering funds and time constraints. The random nature of the sampling was also limited by which stores were open and accessible that day, meaning that alternates stores sometimes had to be chosen. Thus store choice was not entirely random.

Moreover, since not all stores had the three meats (ground beef, ground turkey, and ground pork) available on the particular day of sampling, non-ground alternatives were often chosen. Since ground meats were assumed to have the highest bacteria counts, this complication likely reduced the overall total number of isolated

S. aureus. This complication was also unpredictable, perhaps making the study less representative. Although these sampling imperfections must have somewhat reduced the validity of the study, no random sample is perfect, and we estimate that these imperfections had little effect on our tentative conclusions.

Finally, this study was limited by our ability to process the samples accurately and handle problems and inconsistencies that occurred throughout the sampling period. For instance, some of the initial weeks of sampling differed from the others as we made mistakes and learned from them. For instance, we spread onto plates containing oxacillin in addition to the regular plates during the first few weeks. This was discontinued after it was realized that these plates did not help isolate more *S. aureus*. Since most of us had never performed the identification tests and some had never plated bacteria or streaked colonies, we had to learn the techniques as we sampled. Although tweaking the methods during the experiment is not scientifically valid, we believe these early variations did not affect the validity of the conclusions we have drawn. This is because our focus was on the overall meat and bacteria characteristics, not how the characteristics changed over time.

Throughout the sampling period, there were some growth plates that had far too many colonies to count. It is unknown whether this reflected the reality of the meat or if accidental contamination was introduced as we handled and processed the meat. This unknown was another limitation to our study, since it was hard to be consistent when counting and recording the number of colonies on such plates.

Because mostly ground meat was used, it is impossible to predict how many carcasses were initially contaminated or at which point in meat processing *S. aureus*

contamination occurred. Although some ground meat is ground from cuts in the grocery store, other packages are prepared at centralized packing plants prior to shipping as an already-ground portion.

5.4 Future Directions

Although PCR was performed in order to search for the gene which provides resistance against methicillin, the analysis of resistance to the other antibiotics studied was limited to phenotypic characterization. It would be interesting to conduct a genetic screen for genes responsible for the three other common types of resistance encountered in this survey – resistance to tetracycline, ampicillin, and penicillin. In addition, if some isolates are found to have a resistant phenotype but to be lacking the known resistance gene for a particular antibiotic, this finding would open the door for the discovery of novel resistance genes in *S. aureus*.

Because we still have all of our isolates in storage at -80°C, we would like to go back and classify the enterotoxins that the *S. aureus* isolates are capable of producing. This information would add the potential of food poisoning as a yet unexplored facet to our analysis of the risks involved in *S. aureus* contamination of ground meat. Unlike the risk of cutaneous or systemic infection which can be minimized through proper handling and cooking of raw meat, food poisoning is more difficult to prevent once contamination of the meat has occurred since the toxins which accumulate as the bacteria proliferate in the meat are heat stable and may not be broken down during the cooking process.

The central question which has been left unanswered due to the limited scope of the present study is exactly how and at what time the ground meat samples were contaminated with *S. aureus*. In order to answer this question, livestock raised for sale to suppliers of meat which service Washington D.C. area grocery stores must be nasally swabbed for the detection of any *S. aureus* that they may harbor during life. In addition, people working in direct contact with these animals must also be tested for *S. aureus* colonization since these bacteria are zoonotic and easily spread by contact between people and animals. Although the present study describes a theoretical risk for multi-drug resistant *S. aureus* infection associated with the handling of raw ground pork and turkey in Maryland, this risk needs to be directly assessed. Nasal swabs from consumers who are regularly exposed to raw ground meat while buying or preparing it should be compared to those consumers who do not regularly buy raw meat. In addition, raw ground meat kitchen preparation conditions can be simulated in a food science laboratory and swabs then taken of the kitchen environment to assess whether it can harbor antibiotic resistant *S. aureus* from meat products.

All other intermediate processing steps that the meat goes through prior to being packaged as ground meat must also be examined. *S. aureus* may be spread between animals during the stressful conditions of auction or transport. The slaughterhouse, meat processing plant, and shipping and packaging equipment are all additional points of potential contamination as well. Contamination may occur as a carcass-to-carcass or carcass-to-equipment transmission or when infected workers do not take precautions in washing their hands or covering their mouths when processing raw meat.

It would also be interesting to look at the difference between meat ground in the grocery store and pre-packaged ground meat in order to compare rates of *S. aureus* colonization and examine whether in-store equipment or personnel are a contributing factor. At every step of processing, it will also be important to investigate the prevalence of antibiotic resistance among isolates in order to elucidate the mode by which the *S. aureus* present in meat acquires resistance to specific antibiotics. As a corollary of this research, the effect of other types of processing on the amount and type of *S. aureus* colonization such as salting, slicing, and heating of meat can be explored.

Considering the narrow focus of this study, and taking into account some of the limitations discussed previously, we suggest future research that must be carried out in order to fully evaluate the presence of MRSA and the antimicrobial resistance of *S. aureus* in consumer meat products. Clearly, the next logical step would be to carry out a study similar to this one in a different geographic location. Then, the results of that study could be compared to the results of this one for a more general picture. Since this study only examined ground beef, ground turkey, and ground pork, other meats could be examined instead. It would be interesting to see the effects of organic vs. inorganic or even whole vs. ground as they pertain to *S. aureus* and MRSA presence in meat. This study was unable to suggest any seasonal or yearly variation in the bacteriological characteristics of grocery store meat, but a much longer longitudinal study might find some trends over time. Such data could potentially shed light on the paths that bacteria take to contaminate meat. If this were the case, many specific recommendations for consumer safety might be made.

There are also some specific questions that could be answered if the methodology from this study is altered somewhat. For instance, it would be useful to see if enriching the cultures significantly increases the number of isolated MRSA strains. A study that did use enrichment could be compared more easily to the existing research, most of which also used enrichment. Also, although the agar plates and other procedures used to select for *S. aureus* in this study were standard choices for this type of study, there are surely other choices that might yield very different results. It would be useful to see what different selection protocols might yield in terms of *S. aureus* counts and antimicrobial resistance.

In order to learn more about *S. aureus* and MRSA and how to protect consumers, completely different types of work must be done. For example, although this study found that only one *S. aureus* isolate carried the *mecA* gene, several non-*S. aureus* isolates carried the *mecA* gene and were resistant to methicillin. It is critical to know if these bacteria can pass their methicillin resistance to *S. aureus* through horizontal gene transfer of the *mecA* gene, thus creating MRSA. In addition, recall that this study found a few strains of *S. aureus* and many strains of non-*S. aureus* that lacked the *mecA* gene but were nonetheless resistant to methicillin. This suggests that there may be additional genes responsible for methicillin resistance in *S. aureus* and other organisms. Finding such genes should be the focus of future studies. The results of those studies would aid research such as this, since the very definition of MRSA relies on identifying its characteristic genes.

Finally, we would like to expand this study to investigate chicken meat and analyze the compiled data from completed and proposed experiments in order to

determine why the differences in contamination and antibiotic resistance between meat types which were discovered in the present study exist. This work will allow us to make further recommendations for reducing health risks associated with *S. aureus* infection and food poisoning.

5.5 Recommendations

Although the isolation of a single strain of MRSA does not indicate that the degree of MRSA contamination in retail meat poses a severe health risk, it does indicate that MRSA at least has a presence in U.S. consumer meat. Furthermore, this study has isolated a significant quantity of *S. aureus* from the tested samples and found significant resistance to certain other drugs. Based on this, we make the following recommendations.

No specific recommendations can be made at this time with regards to changes which can be made in food processing protocols. However, we have established a risk of infection with antibiotic resistant *S. aureus* and can make recommendations for measures which may mitigate this risk. Immunocompromised people should avoid handling raw ground turkey, pork, or beef, or wash their hands immediately afterwards and avoid touching their mouth, eyes, and nose. Any person with open sores or wounds on their hands should not handle raw meat without gloves. Personnel who provide food in hospitals, birthing centers, or homes for the elderly should be especially careful to prevent the contamination of surfaces with bacteria during food preparation. It may be advisable to mandate warnings on raw meat

packages which would inform consumers about the potential dangers of handling raw meat irresponsibly.

We provide some simple recommendations for consumer food safety. Although the presence of MRSA in consumer meat products is not cause for immediate alarm, there is significant risk if food is not cooked properly. This risk is greatest for ground meat, which was the focus of this study. We therefore recommend that ground meat be cooked throughout before consumption. Additionally, raw meat should be handled with care. We recommend that people with cuts or wounds on their hands avoid direct contact with raw meat, and that everyone who handles raw meat wash their hands afterwards and before eating. Finally, we recommend that surfaces which come in contact with raw meat be disinfected promptly after use.

Certain steps should be taken to minimize the overall bacteria count (CFU/g) in consumer meat. Since meat can be contaminated with bacteria at any step along the route from farm to consumer, it is important that safe and sterile practices be followed at each one. In particular, we recommend that meat be ground as late in the process as possible, ideally in the store where it is sold. Grinding meat effectively mixes the bacteria from different parts of the animal and even from different animals. If grinding is done early in the process, bacteria have more time to grow in more places. Next, any employee who handles meat should not just be trained in safe meat handling practices; they should also be educated about the health risks of meat with high levels of bacteria and the ways in which bacteria can contaminate meat.

In any slaughterhouse or processing facility, sanitation must be one of the highest priorities. We recommend the consideration of tighter regulations to enforce

this. These would include regulations on the sterility of equipment that comes in contact with meat, the meat handling practices themselves, and maintenance of safety during meat transportation. We recommend that tighter regulations also extend to farmers, who may have the greatest potential to introduce new types of bacteria to the meat supply through the routine handling of their animals, but who may otherwise have little motivation to ensure clean and sanitary practices.

All of the above recommendations, which are intended to reduce the overall amount of harmful bacteria in consumer meat, would serve to reduce also the presence of antibiotic-resistant bacteria. However, we add some additional recommendations that are meant to specifically reduce the spread of antibiotic resistance. Since the MRSA strain that was isolated in this study was identified as community-associated, it is likely that the initial source of contamination was a person (who did not necessarily become colonized or infected at a hospital). Given this, all of the recommendations described previously are further emphasized here.

In addition, we recommend that the use of subtherapeutic antibiotics in livestock be minimized. This means placing restrictions on what antibiotics or types of antibiotics farmers can administer to their animals. Since the antibiotics most frequently administered were the ones that had the highest levels of resistance among our isolates, we strongly recommend that the antibiotics most useful or necessary for treating human infections be banned for subtherapeutic use in farm animals.

Widespread tetracycline resistance in *S. aureus* as seen in turkey and pork derived isolates in this study is problematic not only because it eliminates tetracycline as a treatment option when an alternative to a β-lactamase is needed, but also because

it represents a high prevalence of tetracycline resistance genes in the farm environment and throughout the meat processing chain. These genes have the potential to be horizontally transferred to other important human and animal pathogens. Tetracycline is a preferred drug for treating chlamydia, Lyme disease, syphilis, and *Rickettsia* bacteria. It can also be used to treat tularemia, plague, anthrax, brucellosis, and Legionnaires' disease. Tetracycline is an important therapeutic agent against *E. coli* enteritis and pneumonia caused by *Pasteurella multocida* in cattle. In pigs it can also be used to control leptospirosis and in turkeys tetracycline is used to treat *Mycoplasma synoviae*, which is the causative agent for infectious synovitis in this species. Our ability to prevent and treat these infections in humans and animals in the future hinges on the continued efficacy of tetracycline, which is why it is so important to limit its routine use as a growth promoting feed additive in livestock.

Besides the importance of β-lactamase antibiotics in human medicine, they are also vital to veterinary food animal practice. Penicillin is used in cattle to fight bacterial pneumonia shipping fever complex caused by *Streptococcus* spp., *Corynebacterium pyogenes*, and *Staphylococcus aureus* and in swine erysipelas caused by *Erysipelothrix insidiosa*.

Farmers who raise turkey, beef, or pork must be educated about the prevention of *S. aureus* transmission between farm workers and animals and the process by which antibiotic feed additives could promote the development of antibiotic-resistant *S. aureus* in their animals. Practices such as disinfecting foot dips may limit the transfer of bacteria between farms, and rapid culling of sick animals in order to

minimize the exposure of farm workers to zoonotic bacteria may improve public health by keeping pathogens contained.

Appendices

Appendix A: Suspected *S. aureus* Isolates Tested at FDA for Confirmation (n = 215)

CVM #	SOURCE	STORE	Vitek Compact ID	GRAM STAIN	LATEX	CHL	ERY	CLI	OXA	GEN	AMP	VAN	TET
37760	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37761	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	1	<=2	>8	1	>16
37762	Beef	A	*S. aureus*	gram+ cocci	-	8	<=0.25	<=0.5	<=0.25	<=2	<=0.12	2	<=2
37763	Beef	A	*S. aureus*	gram+ cocci	-	8	<=0.25	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37764	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	4	1	>16
37765	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37766	Beef	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37767	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37768	Turkey	B	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	4	1	>16
37769	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	<=0.12	2	<=2
37770	Turkey	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	8	1	>16
37771	Pork	A	***S. warneri***	gram+ cocci	-	8	<=0.25	<=0.5	0.5	<=2	<=0.12	1	<=2
37772	Turkey	A	***Low discrimination***	gram+ rods	-	<=2	<=0.25	<=0.5	>4	<=2	1	1	<=2
37773	Beef	A	*S. aureus*	gram+ cocci	+	16	>4	<=0.5	0.5	<=2	1	1	<=2
37774	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37775	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37776	Turkey	C	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	8	1	>16
37777	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37778	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	8	1	>16
37779	Pork	B	*S. aureus*	gram+ cocci	+	16	0.5	2	1	<=2	>8	1	<=2

37780	Turkey	B	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	1	<=2	<=0.12	1	>16
37781	Beef	B	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	2	<=2
37782	Turkey	B	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	8	1	>16
37783	Turkey	B	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	8	1	>16
37784	Turkey	B	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	1	<=2	>8	1	>16
37785	Turkey	B	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37786	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	2	<=2
37787	Turkey	A	***S. hyicus***	gram+ cocci	-	16	>4	>2	0.5	<=2	>8	1	>16
37788	Pork	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	2	<=2	2	1	>16
37789	Beef	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37790	Beef	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37791	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37792	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37793	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	0.25	1	<=2
37794	Turkey	A	***Low discrimination***	gram+ cocci	-	16	>4	>2	0.5	<=2	>8	1	>16
37795	Turkey	A	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	<=0.25	<=2	8	1	>16
37796	Pork	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	2	1	>16
37797	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	2	<=2
37798	Beef	A	***S. saprophyticus***	gram+ cocci	-	8	<=0.25	<=0.5	1	<=2	0.25	1	<=2
37799	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37800	Pork	C	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	2	>16	>8	1	>16
37801	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37802	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37803	Pork	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37804	Turkey	A	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	>8	1	>16
37805	Pork	B	*S. aureus*	gram+ cocci	+	8	>4	<=0.5	0.5	<=2	8	1	<=2
37806	Beef	B	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	1	<=2	2	1	<=2
37807	Turkey	C	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	4	1	>16
37808	Turkey	C	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	<=0.25	<=2	>8	1	>16

37809	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37810	Beef	A	*S. aureus*	gram+ cocci	+	8	1	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37811	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37812	Turkey	A	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	>8	1	>16
37813	Beef	C	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37814	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37815	Turkey	C	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	8	1	>16
37816	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	4	1	>16
37817	Turkey	C	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	8	1	>16
37818	Pork	C	***S. caprae***	gram+ cocci	+	16	0.5	0.5	0.5	<=2	<=0.12	1	>16
37819	Beef	C	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	<=0.12	1	<=2
37820	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37821	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	2	<=2
37822	Beef	B	***S. saprophyticus***	gram + rods	+	4	<=0.25	<=0.5	<=0.25	<=2	<=0.12	1	16
37823	Pork	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	2	2	>16
37824	Turkey	A	*S. aureus*	gram+ cocci	+	8	>4	<=0.5	<=0.25	<=2	<=0.12	2	<=2
37825	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37826	Pork	A	***S. xylosus***	gram+ cocci	-	8	<=0.25	<=0.5	<=0.25	<=2	<=0.12	1	>16
37827	Beef	A	***S. warneri***	gram+ cocci	-	8	>4	<=0.5	0.5	<=2	<=0.12	1	>16
37828	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	4	1	>16
37829	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	4	1	>16
37830	Pork	B	***S. chromogenes***	gram+ cocci	+	4	<=0.25	<=0.5	0.5	<=2	<=0.12	0.5	<=2
37831	Pork	B	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	4	1	>16
37832	Beef	B	*S. aureus*	gram+ cocci	+	16	>4	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37833	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	<=0.12	1	<=2
37834	Turkey	A	***S. chromogenes***	gram+ cocci	-	16	>4	>2	<=0.25	<=2	2	2	<=2
37835	Pork	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	>16
37836	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	<=0.12	1	<=2
37837	Beef	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	<=0.12	1	<=2

37838	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	4	1	>16
37839	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	2	>16
37840	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37841	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	2	>16
37842	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	1	<=2	>8	1	<=2
37843	Pork	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	>8	1	>16
37844	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37845	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37846	Turkey	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	>8	1	>16
37847	Turkey	A	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	8	1	>16
37848	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37849	Beef	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	0.25	2	<=2
37850	Beef	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	0.25	2	<=2
37851	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37852	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37853	Turkey	B	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37854	Turkey	B	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	>8	1	>16
37855	Turkey	B	*S. aureus*	gram+ cocci	+	8	>4	<=0.5	<=0.25	<=2	0.25	1	>16
37856	Turkey	B	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	2	>16
37857	Turkey	B	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37858	Beef	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	2	<=2
37859	Turkey	C	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	8	1	>16
37860	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37861	Pork	B	*S. aureus*	gram+ cocci	+	16	>4	>2	0.5	<=2	2	0.5	>16
37862	Pork	B	*S. aureus*	gram+ cocci	+	16	>4	>2	0.5	<=2	2	0.5	>16
37863	Pork	B	*S. aureus*	gram+ cocci	+	16	>4	>2	0.5	<=2	2	0.5	>16
37864	Turkey	B	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37865	Turkey	B	*S. aureus*	gram+ cocci	+	>16	0.5	<=0.5	0.5	<=2	>8	1	>16
37866	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	<=0.12	1	<=2

37867	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	2	>16
37868	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37869	Pork	C	*S. aureus*	gram+ cocci	+	16	>4	>2	0.5	<=2	2	0.5	>16
37870	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37871	Turkey	C	*S. aureus*	gram+ cocci	-	16	1	<=0.5	1	<=2	0.25	2	<=2
37872	Pork	A	*S. aureus*	gram+ cocci	+	16	>4	>2	0.5	<=2	2	1	>16
37873	Pork	A	*S. aureus*	gram+ cocci	+	16	>4	>2	>4	>16	>8	1	>16
37874	Pork	A	*S. aureus*	gram+ cocci	+	16	>4	>2	0.5	<=2	>8	1	>16
37875	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37876	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	4	1	>16
37877	Pork	C	*S. aureus*	gram+ cocci	+	16	>4	>2	0.5	<=2	2	0.5	>16
37878	Pork	C	*S. aureus*	gram+ cocci	+	16	>4	>2	0.5	<=2	2	1	>16
37879	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	1	<=2	8	1	>16
37880	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	1	<=2	8	1	>16
37881	Beef	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37882	Turkey	C	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	2	1	>16
37883	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	1	>16	4	1	>16
37884	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	<=0.12	2	<=2
37885	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	4	1	>16
37886	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37887	Turkey	B	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37888	Beef	B	***S. capitis***	gram+ cocci	-	8	<=0.25	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37889	Turkey	B	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	8	1	>16
37890	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	<=0.12	1	<=2
37891	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	2	<=2
37892	Turkey	A	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	4	1	>16
37893	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37894	Beef	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	0.5	0.5	<=2
37895	Beef	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	4	1	<=2

37896	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37897	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37898	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37899	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	4	1	<=2
37900	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	2	<=2
37901	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37902	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37903	Beef	A	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	<=0.25	<=2	<=0.12	0.5	<=2
37904	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37905	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37906	Beef	A	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	<=0.25	<=2	<=0.12	0.5	<=2
37907	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37908	Turkey	C	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	4	1	>16
37909	Beef	C	*S. aureus*	gram+ cocci	+	16	1	<=0.5	0.5	<=2	8	1	>16
37910	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37911	Turkey	A	***S. fonticola***	gram – rods	-	4	>4	>2	>4	<=2	>8	>32	<=2
37912	Beef	C	*S. aureus*	gram+ cocci	+	16	>4	<=0.5	0.5	<=2	8	1	<=2
37913	Turkey	B	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37914	Turkey	B	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37915	Pork	A	*S. aureus*	gram+ cocci	+	16	1	<=0.5	<=0.25	<=2	<=0.12	2	>16
37916	Beef	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	<=0.12	1	<=2
37917	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	0.5	<=2
37918	Turkey	A	*S. aureus*	gram+ cocci	+	16	1	<=0.5	0.5	<=2	>8	1	>16
37919	Pork	A	*S. aureus*	gram+ cocci	+	8	1	<=0.5	0.5	<=2	2	1	>16
37920	Pork	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	>16
37921	Beef	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	8	1	<=2
37922	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37923	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37924	Pork	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	>16

37925	Turkey	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	8	1	>16
37926	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37927	Pork	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37928	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	0.5	>16
37929	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	1	<=2	>8	0.5	>16
37930	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	>8	1	>16
37931	Pork	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	>16
37932	Turkey	A	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	8	1	>16
37933	Turkey	A	***S. intermedius***	gram+ cocci	-	8	>4	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37934	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37935	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37936	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37937	Turkey	A	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	8	1	>16
37938	Pork	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	2	2	>16
37939	Pork	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	2	2	>16
37940	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37941	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37942	Turkey	C	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	8	1	>16
37943	Beef	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37944	Beef	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37945	Turkey	C	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	8	1	>16
37946	Pork	C	*S. aureus*	gram+ cocci	+	8	0.5	2	1	<=2	>8	1	<=2
37947	Beef	C	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	4	0.5	>16
37948	Beef	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	0.25	1	<=2
37949	Turkey	C	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	8	1	>16
37950	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37951	Turkey	C	***S. hyicus***	gram+ cocci	-	16	0.5	<=0.5	<=0.25	0.5	0.5	2	>16
37952	Pork	B	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	8	1	<=2
37953	Pork	B	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	<=2

37954	Pork	B	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	2	4
37955	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37956	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37957	Beef	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37958	Beef	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37959	Turkey	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	8	1	>16
37960	Turkey	A	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	<=0.25	<=2	<=0.12	1	>16
37961	Pork	C	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	4	4	>16
37962	Pork	C	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	<=0.25	<=2	4	2	>16
37963	Pork	C	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	0.5	<=2	4	2	>16
37964	Turkey	C	*S. aureus*	gram+ cocci	+	16	0.5	<=0.5	1	<=2	8	1	>16
37965	Turkey	C	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	8	1	>16
37966	Pork	B	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	0.25	2	>16
37967	Beef	C	*S. aureus*	gram+ cocci	+	8	>4	<=0.5	0.5	<=2	4	1	<=2
37968	Turkey	C	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	4	1	>16
37969	Turkey	C	*S. aureus*	gram+ cocci	+	8	<=0.25	<=0.5	0.5	<=2	8	1	>16
37970	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	0.25	1	<=2
37971	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	2	<=2
37972	Beef	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	<=0.25	<=2	<=0.12	1	<=2
37973	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
37974	Turkey	A	*S. aureus*	gram+ cocci	+	8	0.5	<=0.5	0.5	<=2	8	1	>16
		Key:	Not *S. aureus*										
			>Resistant										

Appendix B: Standard MIC Values for Antimicrobials (S = Susceptible, I = Intermediate, R = Resistant)

	MIC (µg/ml) Interpretative Standard		
Antibiotic name:	S	I	R
Ampicillin	<=0.25	-	>=0.5
Ampicillin/sulbactam	<=8/4	16/8	>=32/16
Cefamandole	<=8	16	>=32
Cefazolin	<=8	16	>=32
Cefdinir	<=1	2	>=4
Cefepime	<=8	16	>=32
Cefotaxime	<=8	16-32	>=64
Cefotetan	<=16	32	>=64
Cefpodoxime	<=2	4	>=8
Ceftriaxone	<=8	16-32	>=64
Chloramphenicol	<=8	16	>=32
Ciprofloxacin	<=1	2	>=4
Clindamycin	<=0.5	1 2	>=4
Daptomycin	<=1	-	-
Erythromycin	<=0.5	1 4	>=8
Gentamicin	<=4	8	>=16
Levofloxacin	<=2	4	>=8
Linezolid	<=4	-	-
Methicillin+2%NaCl	<=8	-	>=16
Moxifloxacin	<=2	4	>=8
Nitrofurantoin	<=32	64	>=128
Oxacillin+2%NaCl	<=2	-	>=4
Penicillin	<=0.12	-	>=0.25
Quinupristin/Dalfopristin	<=1	2	>=4
Rifampin	<=1	2	>=4
Tetracycline	<=4	8	>=16
Tigecycline	<=0.5	-	-
Trimethoprim/Sulfamethoxazole	<=2/38	-	>=4/76
Vancomycin	<=4	8 16	>=32

Appendix C: Non-*S. aureus mecA*+ Strains

17 strains

I.D. #	Identification	CHL	ERY	CLI	DAP	OXA	STR	DT1	DT2	GEN	AMP	FOXS	LZD	PEN	RIF	VAN	SXT	LEVO	CIP	SYN	TGC	NIT	TET	MXF	GEN
109-1	*S. epidermidis*	8	>4	<=0.5	<=0.5	4	<=1000	N-	N-	<=2	2	<=6	<=1	1	<=0.5	2	<=0.5	<=0.25	<=1	<=0.5	0.5	<=32	4	<=0.25	<=500
109-2	*S. epidermidis*	8	>4	<=0.5	<=0.5	2	<=1000	N-	N-	<=2	1	<=6	<=1	1	<=0.5	2	<=0.5	<=0.25	<=1	<=0.5	0.25	<=32	<=2	<=0.25	<=500
109-3	*S. epidermidis*	8	>4	<=0.5	<=0.5	>4	<=1000	N-	N-	<=2	2	>6	<=1	8	<=0.5	2	<=0.5	<=0.25	<=1	<=0.5	0.25	<=32	4	<=0.25	<=500
142-2	*S. sciuri*	16	>4	>2	2	>4	<=1000	P+	P+	<=2	8	>6	2	>8	<=0.5	2	<=0.5	0.5	<=1	4	0.12	<=32	>16	0.5	<=500
155-2-O	*Unidentified*	16	>4	>2	>4	>4	<=1000	P+	P+	<=2	0.5	<=6	>8	2	2	>32	<=0.5	<=0.25	<=1	>4	>0.5	>64	>16	<=0.25	<=500
209-2	*S. epidermidis*	8	>4	<=0.5	<=0.5	>4	<=1000	N-	N-	<=2	0.5	>6	2	0.25	<=0.5	2	<=0.5	<=0.25	<=1	<=0.5	0.25	<=32	4	<=0.25	<=500
369-2	*S. epidermidis*	8	>4	<=0.5	<=0.5	>4	<=1000	N-	N-	<=2	8	>6	2	8	<=0.5	2	4	<=0.25	<=1	<=0.5	0.12	<=32	<=2	<=0.25	<=500
369-3	*S. epidermidis*	8	>4	<=0.5	1	>4	<=1000	N-	N-	<=2	4	>6	2	8	<=0.5	2	>4	<=0.25	<=1	<=0.5	0.25	<=32	<=2	<=0.25	<=500
380-3	*S. epidermidis*	8	<=0.25	<=0.5	<=0.5	>4	<=1000	N-	N-	<=2	8	>6	2	4	<=0.5	2	4	<=0.25	<=1	<=0.5	0.5	<=32	4	<=0.25	<=500
397-1	*S. epidermidis*	8	>4	<=0.5	1	>4	<=1000	N-	N-	<=2	>8	>6	2	>8	<=0.5	2	>4	<=0.25	<=1	<=0.5	0.12	<=32	<=2	<=0.25	<=500
397-2	*S. epidermidis*	8	>4	<=0.5	1	>4	<=1000	N-	N-	<=2	>8	>6	<=1	>8	<=0.5	2	>4	<=0.25	<=1	<=0.5	0.12	<=32	<=2	<=0.25	<=500
397-3	*S. epidermidis*	8	>4	<=0.5	1	>4	<=1000	N-	N-	<=2	8	>6	2	>8	<=0.5	2	>4	<=0.25	<=1	<=0.5	0.12	<=32	<=2	<=0.25	<=500
398-2	*S. epidermidis*	8	>4	<=0.5	<=0.5	2	<=1000	N-	N-	<=2	1	<=6	2	1	<=0.5	2	>4	<=0.25	<=1	<=0.5	0.25	<=32	>16	<=0.25	<=500
556-1	*S. epidermidis*	8	>4	<=0.5	<=0.5	4	<=1000	N-	N-	<=2	4	>6	<=1	8	<=0.5	2	<=0.5	0.5	<=1	<=0.5	0.5	<=32	>16	<=0.25	<=500
555-1	*S. epidermidis*	0	>4	<=0.5	<=0.5	1	<=1000	N-	N-	<=2	2	<=6	<=1	2	<=0.5	2	<=0.5	<=0.25	<=1	<=0.5	0.25	<=32	<=2	<=0.25	<=500
562-1	*S. epidermidis*	8	>4	<=0.5	1	>4	<=1000	N-	N-	<=2	8	>6	2	>8	<=0.5	2	>4	<=0.25	<=1	<=0.5	0.12	<=32	<=2	<=0.25	<=500
606-2	*S. epidermidis*	16	>4	>2	<=0.5	>4	<=1000	P+	P+	<=2	2	>6	<=1	2	<=0.5	2	<=0.5	<=0.25	<=1	<=0.5	0.5	<=32	4	<=0.25	<=500

No.	CHL	ERY	CLI	DAP	OXA	STR	DT1	DT2	GEN	AMP	FOXS	LZD	PEN	RIF	VAN	SXT	LEVO	CIP	SYN	TGC	NIT	TET	MXF	GEN
No. (resistant)	0	16	3	1	13	0	3	3	0	2	12	1	8	0	1	8	0	0	1	5	1	4	0	0
% (resistant)	0	94.1	17.65	5.88	76.47	0	17.7	17.7	0	11.8	47.1	5.88	70.6	0	5.88	47.06	0	0	5.88	29.41	5.88	23.52	0	0

S. epidermidis	15
S. sciuri	1
Unidentified	1

Appendix D: Non-*S. aureus mecA*- Strains with Methicillin Resistance

55 strains

No.	Identification	CHL	ERY	CLI	DAP	OXA	STR	DT1	DT2	GEN	AMP	FOXS	LZD	PEN	RIF	VAN	SXT	LEVO	CIP	SYN	TGC	NIT	TET	MXF	GEN
126-2	*Unidentified*	4	>4	>2	>4	>4	<=1000	P+	P+	4	>8	>6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	0.25	<=32	8	<=0.25	<=500
13A-1M	*Unidentified*	8	>4	>2	<=0.5	>4	<=1000	N-	N-	<=2	2	>6	<=1	4	<=0.5	0.5	2	1	<=1	2	0.25	>64	<=2	<=0.25	<=500
526-1	*E. faecalis*	16	>4	>2	<=0.5	4	<=1000	P+	P+	<=2	>8	>6	<=1	>8	<=0.5	2	<=0.5	<=0.25	<=1	<=0.5	0.5	<=32	<=2	<=0.25	<=500
149-1-O	*S. agalactiae*	>16	>4	>2	>4	>4	<=1000	P+	P+	<=2	4	<=6	>8	>8	4	>32	<=0.5	<=0.25	<=1	>4	>0.5	>64	<=2	0.5	<=500
155-2	*Unidentified*	16	>4	>2	>4	>4	<=1000	P+	P+	>16	>8	<=6	>8	>8	4	>32	4	<=0.25	<=1	>4	>0.5	>64	>16	<=0.25	<=500
155-2#2	*S. intermedius*	8	>4	>2	>4	>4	<=1000	P+	P+	<=2	1	<=6	>8	4	4	>32	<=0.5	<=0.25	<=1	>4	>0.5	>64	>16	<=0.25	<=500
163-3	*E. faecalis*	8	<=0.25	>2	2	>4	<=1000	N-	N-	8	2	>6	2	4	2	2	<=0.5	2	<=1	>4	0.5	<=32	>16	0.5	<=500
166-1-O	*Unidentified*	8	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	<=6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	>0.5	>64	<=2	<=0.25	<=500
166-2-O	*S. intermedius*	8	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	<=6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	>0.5	>64	<=2	<=0.25	<=500
166-3	*E. faecalis*	8	0.5	>2	1	>4	<=1000	N-	N-	8	1	>6	2	4	2	2	<=0.5	2	<=1	>4	0.25	<=32	<=2	0.5	<=500
167-2-O	*Unidentified*	8	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	<=6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	0.5	64	>16	<=0.25	<=500
167-3-O	*Unidentified*	8	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	<=6	>8	>8	4	>32	<=0.5	<=0.25	<=1	>4	>0.5	64	>16	<=0.25	<=500
17-1	*E. faecalis*	8	1	<=0.5	1	>4	<=1000	N-	N-	8	1	>6	4	4	1	1	<=0.5	1	<=1	<=0.5	0.25	<=32	>16	<=0.25	<=500
174-1	*E. faecalis*	8	1	>2	2	>4	<=1000	N-	N-	16	2	>6	2	4	2	2	<=0.5	2	<=1	>4	0.25	<=32	<=2	0.5	<=500
175-2	*E. faecalis*	16	>4	>2	1	>4	>1000	P+	P+	>16	1	>6	2	4	2	2	<=0.5	1	<=1	>4	0.12	<=32	>16	<=0.25	>500
182-0	*K. kristinae*	<=2	>4	>2	<=0.5	>4	<=1000	P+	P+	16	8	>6	<=1	8	<=0.5	0.5	>4	<=0.25	<=1	<=0.5	0.25	>64	>16	<=0.25	<=500
197-6	*E. faecalis*	16	>4	>2	1	>4	>1000	P+	P+	>16	1	>6	2	2	2	2	<=0.5	2	<=1	>4	0.25	<=32	>16	0.5	>500
1A	*S. intermedius*	16	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	<=6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	>0.5	<=32	<=2	<=0.25	<=500
209-2	*S. epidermidis*	8	>4	<=0.5	<=0.5	>4	<=1000	N-	N-	<=2	0.5	>6	2	0.25	<=0.5	2	<=0.5	<=0.25	<=1	<=0.5	0.25	<=32	4	<=0.25	<=500
218-1-O	*E. faecalis*	8	>4	>2	2	>4	>1000	P+	P+	>16	2	>6	2	4	2	2	<=0.5	1	<=1	>4	0.5	<=32	>16	<=0.25	>500
236-1-O	*St. intermedius*	8	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	<=6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	>0.5	>64	<=2	<=0.25	<=500
269-3	*Unidentified*	4	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	>6	>8	>8	4	>32	<=0.5	<=0.25	<=1	>4	0.25	64	<=2	<=0.25	<=500
280-1-O	*E. faecalis*	8	1	>2	1	>4	<=1000	N-	N-	8	1	>6	2	4	1	1	<=0.5	2	<=1	>4	0.25	<=32	>16	0.5	<=500
292-1	*Unidentified*	16	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	<=6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	>0.5	>64	<=2	<=0.25	<=500
296-2	*K. kristinae*	8	<=0.25	<=0.5	<=0.5	>4	<=1000	P+	P+	<=2	4	>6	2	8	<=0.5	0.5	<=0.5	<=0.25	<=1	<=0.5	0.12	<=32	<=2	<=0.25	<=500
348-3	*S. haemolyticus*	4	<=0.25	<=0.5	<=0.5	<=0.25	<=1000	N-	N-	<=2	<=0.12	<=6	<=1	<=0.06	<=0.5	1	<=0.5	<=0.25	<=1	<=0.5	0.06	<=32	<=2	<=0.25	<=500
365-2	*Unidentified*	<=2	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	>6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	0.25	64	<=2	<=0.25	<=500
420-1	*E. faecalis*	8	>4	>2	1	>4	>1000	P+	P+	>16	1	>6	2	2	1	2	<=0.5	2	<=1	>4	0.5	<=32	>16	0.5	>500
438-3	*Unidentified*	<=2	4	>2	>4	>4	<=1000	N-	N-	16	>8	<=6	4	>8	<=0.5	>32	<=0.5	<=0.25	<=1	>4	0.25	<=32	<=2	<=0.25	<=500
439-2	*Unidentified*	4	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	>6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	0.5	<=32	<=2	<=0.25	<=500
476-1	*S. epidermidis*	8	>4	<=0.5	<=0.5	>4	<=1000	N-	N-	<=2	4	>6	2	2	<=0.5	2	>4	2	2	<=0.5	0.5	<=32	16	0.5	<=500
476-2	*S. epidermidis*	8	>4	<=0.5	<=0.5	>4	<=1000	N-	N-	<=2	0.25	<=6	2	0.25	<=0.5	2	>4	1	2	<=0.5	0.5	<=32	4	0.5	<=500
476-3	*S. epidermidis*	8	>4	<=0.5	<=0.5	>4	<=1000	N-	N-	<=2	4	>6	2	2	<=0.5	2	>4	2	2	<=0.5	0.5	<=32	>16	0.5	<=500
504-1	*S. epidermidis*	8	<=0.25	<=0.5	<=0.5	>4	<=1000	N-	N-	<=2	8	>6	<=1	>8	<=0.5	2	<=0.5	0.5	<=1	<=0.5	0.25	<=32	4	<=0.25	<=500

509-2	*Unidentified*	4	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	>6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	0.25	64	<=2	<=0.25	<=500
509-3	*Unidentified*	4	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	>6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	0.25	64	<=2	<=0.25	<=500
510-3	*Unidentified*	8	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	>6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	0.25	64	<=2	<=0.25	<=500
516-2	*E. faecalis*	8	1	>2	2	>4	>1000	N-	N-	16	0.5	>6	2	2	2	2	<=0.5	2	<=1	>4	0.25	<=32	>16	0.5	<=500
516-3	*E. faecalis*	16	>4	>2	1	>4	>1000	P+	P+	>16	1	>6	2	2	1	1	<=0.5	2	<=1	>4	0.12	<=32	>16	0.5	>500
526-1	*E. faecalis*	16	1	>2	1	>4	<=1000	N-	N-	8	1	>6	2	4	2	2	<=0.5	2	<=1	>4	0.25	<=32	<=2	0.5	<=500
532-1	*Unidentified*	4	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	>6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	0.25	64	<=2	<=0.25	<=500
534-3	*Staphylococcus cohni ssp urealyticus*	8	>4	<=0.5	<=0.5	>4	<=1000	N-	N-	<=2	>8	>6	2	>8	<=0.5	1	<=0.5	0.5	<=1	1	0.25	<=32	<=2	<=0.25	<=500
539-2	*E. faecalis*	16	>4	>2	1	>4	>1000	P+	P+	>16	1	>6	2	2	2	2	<=0.5	2	<=1	>4	0.25	<=32	>16	0.5	>500
539-3	*E. faecalis*	16	>4	>2	1	>4	>1000	P+	P+	>16	1	>6	2	2	2	2	<=0.5	2	<=1	>4	0.5	<=32	>16	0.5	>500
544-1	*S. epidermidis*	8	<=0.25	<=0.5	<=0.5	>4	<=1000	N-	N-	<=2	4	>6	2	4	<=0.5	2	4	<=0.25	<=1	<=0.5	0.5	<=32	8	<=0.25	<=500
553-1	*Unidentified*	4	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	>6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	0.25	64	<=2	<=0.25	<=500
553-2	*Unidentified*	4	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	>6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	0.5	64	<=2	<=0.25	<=500
553-3	*Unidentified*	4	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	>6	>8	>8	>4	>32	<=0.5	<=0.25	<=1	>4	0.25	64	<=2	<=0.25	<=500
571-1	*E. faecalis*	8	1	>2	2	>4	<=1000	N-	N-	16	1	>6	2	4	4	1	<=0.5	2	2	>4	0.25	<=32	<=2	0.5	<=500
571-2	*E. faecalis*	8	1	>2	2	>4	<=1000	N-	N-	16	1	>6	2	4	4	1	<=0.5	2	2	>4	0.25	<=32	<=2	0.5	<=500
584-2	*L. welshimeri*	16	<=0.25	>2	2	4	<=1000	N-	N-	<=2	0.5	>6	2	0.5	<=0.5	1	<=0.5	2	2	1	0.25	<=32	<=2	0.5	<=500
585-1	*L. welshimeri*	8	<=0.25	2	4	4	<=1000	N-	N-	<=2	1	>6	2	1	<=0.5	2	<=0.5	2	>2	2	0.5	<=32	<=2	0.5	<=500
598-1	*Unidentified*	<=2	2	>2	>4	>4	<=1000	N-	N-	8	>8	<=6	2	>8	<=0.5	>32	<=0.5	<=0.25	<=1	>4	0.25	<=32	<=2	<=0.25	<=500
61-1-M	*Unidentified*	<=2	>4	>2	>4	>4	<=1000	P+	P+	<=2	>8	<=6	>8	>8	4	>32	<=0.5	<=0.25	<=1	>4	0.12	<=32	<=2	<=0.25	<=500
650-3	*E. faecalis*	8	2	>2	1	>4	<=1000	N-	N-	8	2	>6	2	4	2	2	<=0.5	2	<=1	>4	0.25	<=32	<=2	0.5	<=500
No.		CHL	ERY	CLI	DAP	OXA	STR	DT1	DT2	GEN	AMP	FOXS	LZD	PEN	RIF	VAN	SXT	LEVO	CIP	SYN	TGC	NIT	TET	MXF	GEN
No. (resistant)		1	37	44	24	52	8	32	32	8	24	43	22	28	17	24	6	0	1	40	22	9	17	0	7
% (resistant)		18	67.3	80	43.66	94.55	14.55	58.2	58.2	14.55	43.6	78.2	40	50.9	30.91	43.6	10.91	0	1.82	72/73	40	16.36	30.91	0	12.73

E. faecalis	18
K. kristinae	2
L. welshimeri	2
Staphylococcus cohni ssp urealyticus	1
S. epidermidis	6
S. haemolyticus	1
S. intermedius	4
S. agalactiae	1
Unidentified	20

Appendix E: Vitek GP Card Substrates

Table 12. Test Substrates on GP Card.

Well	Test	Mnemonic	Amount/Well
2	D-AMYGDALIN	AMY	0.1875 mg
4	PHOSPHATIDYLINOSITOL PHOSPHOLIPASE C	PIPLC	0.015 mg
5	D-XYLOSE	dXYL	0.3 mg
8	ARGININE DIHYDROLASE 1	ADH1	0.111 mg
9	BETA-GALACTOSIDASE	BGAL	0.036 mg
11	ALPHA-GLUCOSIDASE	AGLU	0.036 mg
13	Ala-Phe-Pro ARYLAMIDASE	APPA	0.0384 mg
14	CYCLODEXTRIN	CDEX	0.3 mg
15	L-Aspartate ARYLAMIDASE	AspA	0.024 mg
16	BETA GALACTOPYRANOSIDASE	BGAR	0.00204 mg
17	ALPHA-MANNOSIDASE	AMAN	0.036 mg
19	PHOSPHATASE	PHOS	0.0504 mg
20	Leucine ARYLAMIDASE	LeuA	0.0234 mg
23	L-Proline ARYLAMIDASE	ProA	0.0234 mg
24	BETA GLUCURONIDASE	BGURr	0.0018 mg
25	ALPHA-GALACTOSIDASE	AGAL	0.036 mg
26	L-Pyrrolidonyl-ARYLAMIDASE	PyrA	0.018 mg
27	BETA-GLUCURONIDASE	BGUR	0.0378 mg
28	Alanine ARYLAMIDASE	AlaA	0.0216 mg
29	Tyrosine ARYLAMIDASE	TyrA	0.0276 mg
30	D-SORBITOL	dSOR	0.1875 mg
31	UREASE	URE	0.15 mg
32	POLYMIXIN B RESISTANCE	POLYB	0.00093 mg
37	D-GALACTOSE	dGAL	0.3 mg
38	D-RIBOSE	dRIB	0.3 mg
39	L-LACTATE alkalinization	ILATk	0.15 mg
42	LACTOSE	LAC	0.96 mg
44	N-ACETYL-D-GLUCOSAMINE	NAG	0.3 mg
45	D-MALTOSE	dMAL	0.3 mg
46	BACITRACIN RESISTANCE	BACI	0.0006 mg
47	NOVOBIOCIN RESISTANCE	NOVO	0.000075 mg
50	GROWTH IN 6.5% NaCl	NC6.5	1.68 mg
52	D-MANNITOL	dMAN	0.1875 mg
53	D-MANNOSE	dMNE	0.3 mg
54	METHYL-B-D-GLUCOPYRANOSIDE	MBdG	0.3 mg
56	PULLULAN	PUL	0.3 mg
57	D-RAFFINOSE	dRAF	0.3 mg
58	O/129 RESISTANCE (comp.vibrio.)	O129R	0.0084 mg
59	SALICIN	SAL	0.3 mg
60	SACCHAROSE/SUCROSE	SAC	0.3 mg
62	D-TREHALOSE	dTRE	0.3 mg
63	ARGININE DIHYDROLASE 2	ADH2s	0.27 mg
64	OPTOCHIN RESISTANCE	OPTO	0.000399 mg

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Glossary

Baird-Parker (BP) plates - Plates with media that is selective for members of the coagulase-positive *Staphylococci* genus.

Catalase - An enzyme that converts highly reactive hydrogen peroxide (a common byproduct of many metabolic processes) to water and oxygen, preventing damage to the cell. *Staphylococci* are catalase-positive.

Cellulitis - Occurs when bacteria can enter the skin by way of a cut, abrasion, or any other break in the skin. It is characterized by inflammation of the skin around the area of infection.

Coagulase - An enzyme that causes the coagulation of blood. *S. aureus* is coagulase-positive.

Colony forming units per gram (CFU/g) - An expression of the concentration of bacterial cells in a sample.

Community Associated MRSA (CA-MRSA) - Type of MRSA that is acquired without contact with the healthcare environment.

Folliculitis - An infection of a hair follicle in which the base of the follicle becomes red, swollen, and filled with pus.

Furuncle - A raised, nodular extension of folliculitis into the surrounding tissue. If several furuncles coalesce, they form a carbuncle that extends deeper into the tissue.

Hospital Associated MRSA (HA-MRSA) - Type of MRSA that is acquired through contact with the healthcare environment.

Methicillin-Resistant *Staphylococcus aureus* (MRSA) - *S. aureus* which has acquired the *mecA* gene, responsible for resistance to broad-spectrum antimicrobial β-lactams such as methicillin, penicillin, oxacillin, and amoxicillin.

Minimum inhibitory concentration (MIC) - The minimum concentration of an antimicrobial which inhibits the growth of a particular bacterium.

Nosocomial infection – Hospital-acquired infection.

Panton-Valentine leukocidin gene - A gene commonly associated with CA-MRSA that produces cytotoxins, which cause skin tissue to necrotize.

Polymerase Chain Reaction (PCR) – Test which can be used to screen bacteria for a specific gene.

Staphylococcal Scalded Skin Syndrome (SSSS) - A disease caused by *S. aureus* which involves reddening of the skin, beginning near the mouth and spreading to the

rest of the body, followed by large blisters filled with clear fluid that lacks bacteria or white blood cells.

Staphylococcus aureus - A potentially dangerous bacterium that lives on the skin and in the nasal passages of some people and animals.

Subtherapeutic antibiotics - Antibiotics administered at a low dosage to farm animals in order to prevent disease or promote growth.

Toxic Shock Syndrome (TSS) - A disease caused by *S. aureus* that occurs when bacteria, growing in a wound or mucus membrane, produce toxins that can be absorbed by the blood.

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