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Determination of transfer of methicillin-resistant Staphylococcus aureus from retail pork products onto food contact surfaces and the potential for consumer exposure

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Determination of transfer of methicillin-resistant Staphylococcus aureus from retail pork products onto food contact surfaces and the potential for consumer exposure

by

Heather Snyder

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Microbiology

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Ames, Iowa

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Abstract

Methicillin-resistant *Staphylococcus aureus*, commonly referred to as MRSA, first emerged from *Staphylococcus aureus* in the 1960’s as an organism that was resistant to beta-lactam antibiotics such as penicillin. MRSA is able to colonize the skin, mouth and nares of both humans and animals without causing disease. However, if the bacteria gains entry via a cut or other skin abrasion, it can lead to more serious infections such as pneumonia, meningitis and septicemia. The three main types of MRSA infections currently recognized are hospital associated (HA), community associated (CA) and livestock associated (LA).

Recently, MRSA has also been discovered in retail meat products in the United States and other countries throughout the world. This discovery has raised concerns about the possibility of food being a vehicle for MRSA transmission and infection. It has been reported that MRSA can be found in meat processing facilities at any stage of production. Many studies have determined that pork and swine production are the major MRSA reservoirs. The current understanding of how MRSA may be transmitted in the food chain is limited thus preventing proper risk assessment to identify potential hazards to food handlers as well as to consumers.

The purpose of this research is to estimate the percent transfer of MRSA from retail pork products to food contact surfaces (cutting board or knife) and to estimate the risk of consumer exposure to MRSA via contact with contaminated products and contaminated food contact surfaces. In order to simulate how these events may occur; pork products were obtained from a local supplier and inoculated with a 4 strain MRSA mixed culture at levels of 10, 100, 1,000, 10,000 and 100,000 CFU/ml. Products were then analyzed to obtain initial bacterial populations. All products were vacuum-packaged and stored at 5°C for 2 weeks to simulate normal packaging and distribution. After 2 weeks, products were aseptically removed from the vacuum package and exposed to the transfer surfaces via 2 methods: light exposure to assess the percent transfer of MRSA from product to surface and heavy exposure to assess human exposure from contaminated products and surfaces. In light exposure, samples were laid on the transfer surfaces at room temperature for 5 minutes without any additional weight on the product and allowed to sit at room temperature before analysis. In heavy exposure, samples were laid on the transfer surface and a 500g lead donut was placed on top of the product as it was moved across a defined area of the transfer surface. The transfer surfaces then sat for 5 minutes at room temperature before being analyzed.
It was found that MRSA not only survived on the pork products during 2 weeks of cold storage but was able to transfer to food contact surfaces. This was true for all products tested across all 5 cell concentrations. These results suggest that even when cell concentration is low (≤ 10 CFU/cm²) human exposure is possible. Even though MRSA may only be present in very small quantities, more research is needed to determine the infectious dose of MRSA that may be in meat products in order to establish a risk assessment. Public education on safe raw meat handling and cooking practices should be continued as well as education of food handlers until there is a better understanding of the infectious dose of MRSA in raw meat products.
Chapter 1: Introduction

Thesis Organization
This thesis is divided into four chapters: the introduction, a literature review, the manuscript and closing summary. The literature review will first discuss methicillin-resistant *Staphylococcus aureus* in general and will look at how it became a health and food safety concern. The second section will cover MRSA in production facilities and how it can persist and end up on retail meat products. The third part will discuss the ability of MRSA to transfer to food contact surfaces and to skin. The literature review is designed to give background information of the characteristics of MRSA and to show why further research is needed in order to address the risk MRSA presents to community health. The manuscript covers research designed to address the need to discover the risk of the consumer becoming exposed to MRSA via contaminated retail meat products. The risk was estimated by first looking at the overall percentage of bacteria capable of transfer to food preparation surfaces followed by an assessment of transfer from a contaminated surface to skin, for these experiments the human skin model is used. The last chapter includes closing remarks and suggestions for further research.
Chapter 2: Literature review

Introduction. Methicillin-resistant *Staphylococcus aureus*, commonly referred to as MRSA, is a Gram positive cocci that is resistant to beta-lactam antibiotics and able to colonize the skin, mouth and nares of humans and animals without causing disease. However, more serious infections such as pneumonia, meningitis and septicemia can occur if the bacteria enter open wounds or abrasions in the skin. There are 3 main types of MRSA referred to in the literature: hospital acquired (HA), community acquired (CA) and livestock acquired (LA). MRSA first emerged from *Staphylococcus aureus* in the 1960's (28). Initially, MRSA was recognized as the bacteria associated with hospital acquired staphylococcal infections (28). Since 2000, MRSA infections acquired in the community, in those who are healthy and have not undergone hospitalization or long-term care, have become more common (44). To complete the trifecta is ST398, the primary strain of livestock acquired MRSA associated with pigs and other livestock which has been determined to be transmissible between swine and swine farmers (59). This review will first look at what defines MRSA and then discuss MRSA as it pertains to 3 main areas: carriage and infection in food production animals and humans, presence in production facilities and on retail pork products and transfer to surfaces such as cutting boards, knives and skin.

*Methicillin-resistant Staphylococcus aureus*. MRSA is a strain of *Staphylococcus aureus* that has developed resistance to methicillin and most other beta-lactam antibiotics. This class of antibiotics works to destroy bacteria by inhibiting penicillin binding proteins (PBP) which are found in the bacterial cell wall. When the cell wall is broken down, the bacterial cell cannot survive (28). Resistance is due to the acquisition of the mecA gene found on the staphylococcal chromosomal cassette. The mecA gene encodes the penicillin binding protein 2a (PBP2a) which, unlike PBP, is resistant to the action of beta-lactams and thus allows the bacteria to survive and persist in the host (41).

*MRSA in Humans*

As a testament to the adaptable nature of bacteria, MRSA emerged from *Staphylococcus aureus* in the early 1960’s not long after doctors began treating penicillin-resistant human staphylococcal infections with methicillin (57). This was the beginning of MRSA being almost synonymous with nosocomial infections. Today it is commonly referred to as HA-MRSA. Infections were not confined to hospitals, however. People in communities without a history of long term hospital care or surgical procedures were also presenting with MRSA infections which are now known as CA-MRSA (44). In the beginning many of these CA infections could be treated with various antibiotics but now are also
showing a surge in resistance to multiple antibiotics (50). In the United States, the two most common MRSA strains associated with human colonization and/or infection are USA100 or HA-MRSA (46) and USA300 or CA-MRSA (50).

Livestock-associated MSRA

The most recent MRSA strain to be identified is sequence type ST398 also called CC398 in some of the literature. This strain has been isolated in livestock in many countries throughout the world including the Netherlands, Denmark, Canada and the United States. (28) Most of the LA-MRSA isolated is ST398 which is also called non-typeable MRSA due to the inability of Smal to digest the cellular DNA (2). ST398 strain is primarily associated with pigs. Since pig farming is such a large and growing industry in many countries including the United States and swine are a reservoir for MRSA, carriage without infection should not be ignored.

While carriage in livestock is usually innocuous, it has been shown that MRSA can transfer to humans from swine and that among livestock animals, pigs are the primary reservoir (24). Further support to the evidence that swine are a reservoir was provided by Smith et al. 2009 (25) in a study showing a high colonization percentage of MRSA in Mid-western swine production facilities in the pigs and the workers. These findings seem to indicate that further research into the epidemiology of MRSA carriage, transmission and infection in both humans and food production animals is needed.

MRSA Carriage and Infection in Food Production Animals and Humans. Why should we be concerned about LA-MRSA? ST398 was not known to be present in humans before the early 2000’s and livestock was the putative source of those initial human isolates. This evidence was strengthened by molecular typing data (9). Nasal MRSA carriage was commonly found in pig farmers in the Netherlands (9). Colonization alone was not necessarily a health risk in and of itself but once colonized, the risk of developing a MRSA infection increased (58). Considering that it is not known how long colonization may persist or if it will persist as long as there is animal contact, the need for more epidemiological research is once again highlighted.

Transmission from Swine to Humans

After ST398 was isolated from several patients in the Netherlands in 2004, it became clear that close contact with swine was a major risk factor for ST398 colonization and possible infection in humans (61). Since then, other clinical cases of ST398 in humans have been reported (6, 9, 27, 45, 53). In one study, the isolates from the swine herd were found to be the same as the isolates from humans in contact with the pigs (1). In 2006, Huijsdens et al. (53) found that family members of pig farmers were
also colonized suggesting that ST398 could pass from human to human as well. It is important to note that there have also been cases in which patients from whom ST398 was isolated had no reported contact with animals (2, 14) indicating that animals may shed the organism in the environment and people with no known animal contact could become colonized or develop an infection through environmental contamination with ST398 (22). These findings infer that MRSA is a concern since it has demonstrated zoonotic capabilities, exhibits human to human transfer and environment to human transfer.

Implications of Antibiotic Use

One of the primary reasons the presence of MRSA continues to be of concern in animal and human health is the resistance to multiple antibiotics. It has been suggested that the extensive use of antibiotics in swine production has created a selection pressure on bacteria leading to multi-drug resistant strains (17). While the risk of human colonization with LA-MRSA has been defined through research, it is still unclear what risk factors may exist in pig populations. Even if infection rates among swine are low, the fact that such a large number of animals are colonized warrants careful monitoring. One study noted the isolation of methicillin-susceptible \textit{Staphylococcus aureus} ST398 in pigs and argues that this strain may have spread to other species through antibiotic use. Another study in pigs found that some treated with tetracycline had a higher prevalence of colonization with MRSA (37) but did not provide enough evidence to claim antibiotic use in animals as a risk factor for increased colonization with MSRA. Interestingly, a 2012 study has reported that there was no significant difference in amounts of MRSA found on pork raised conventionally versus pork raised without antibiotics. This finding is contrary to what has been found in the Netherlands (40). To date there is not enough substantial research to support or refute that hypothesis that antibiotic use in animals increases the likelihood of MRSA to transfer to and colonize humans. The matter is made more complex by the varying methods of raising swine that are currently in practice making it difficult to pinpoint the true origin of any existing risks. This, in turn, raises the question of whether colonized pigs are transferring MRSA to humans or if colonized humans are a source of MRSA transmission to the pigs.

Presence of MRSA in Production Facilities and on Retail Meat Products. Given that MRSA has been isolated from swine and humans (61) and that food production animals may be the main reservoir (33), it is not difficult to imagine that it may also be found in facilities that process swine and among employees who work in those facilities. Further, it is not unreasonable to assume that animals which are colonized at harvest may, in turn, be contaminated carcasses which could potentially become a contaminated retail pork product. On the other hand, one might also contend that
employees with previous MRSA nasal carriage could also be responsible for carcass and/or final product contamination. This portion of the literature review looks first at research that has investigated facilities but will focus chiefly on MRSA in retail meat products.

**Risks in Production Facilities**

Past studies have demonstrated that the amount of bacteria on carcasses may have a direct effect on humans becoming ill from the bacteria that is present therein. In 2007, Singer et al. (31) proposed a model which gave support to the idea that the amount of bacteria on a carcass could directly influence human health and that monitoring animal health would lessen the incidence of human illnesses related to carcass contamination (31). Similarly, another study demonstrated that a relationship exists between bacterial counts on carcasses, animal health and the human health risk (21). In this paper, it was determined that carcasses that required additional handling due to health problems increased the worker’s risk of contamination as well as the risk of cross-contamination in the environment. Clearly greater levels of bacteria on a carcass or carcasses of previously ill animals that have an increased bacterial load in lesions or other organs mean an increased likelihood of human illness and possibly environmental contamination due to the way in which these carcasses must be processed.

Bacteria on carcasses are not uncommon especially since many animals are colonized with their normal flora. MRSA ST398 found at facilities at the time of harvest may be due to colonized animals but MRSA may also come from human handlers and be a human strain. (24) Some early studies indicated that human flora could become established in the production environment and could, in turn, potentially contaminate carcasses and meat. The most common source of contamination was the hands of workers (15, 34). Thus contamination from personnel cannot be ruled out as another possible source of MRSA. However, concerning swine production, it is most often workers with daily contact to the animals who have been shown to be colonized by ST398 in the nasal cavity (19). This indicates that ST398, which is not very host specific, is more likely to be the culprit in production facilities. The caveat is that to date no evidence of how MRSA transmission occurs among animals and humans has been elucidated (19).

**MRSA in Retail Pork Products**

In addition to concerns about transmission of ST398 from animals to humans, current findings indicate that MRSA ST398 is present in retail meat products. There is little reported on carriage of MRSA from the live animal to the final retail pork product. This is sometimes complicated by the fact that the origin of contamination cannot always be determined. 26.9% of isolates found in a 2012
study were determined to be ST398 but their origin was not able to be identified (39). Still other studies from around the world have found MRSA at rates of 1.2% (35), 5% (52), 11.9% (16) and 2.5% (9). Although there have been no reported cases that implicated ST398 in human colonization or infection due to contact with a contaminated meat product to date, (35) it has been found that MRSA in food animals and in food was of the same clonal line, ST398, with increased prevalence in pork products (3). While this is not an automatic indicator of human infection potential, it does imply that producers and consumers of pork need to be aware that MRSA is present and given its tendency toward multiple-antibiotic resistance it deserves due consideration as a potential food-borne pathogen that poses a public health risk. A current German study reported that MRSA was isolated from all points in a fresh pork production facility and indicated that MRSA was isolated in 3% (2 out of 71 samples) of final products and all were related to ST398 (11). Though this seems a small amount, it demonstrates that MRSA on a live animal can persist to the final product to be shipped and sold. While the German study was instrumental in showing the potential for a contaminated product to reach the consumer, it is limited by the fact that this was only one facility with a small sample size.

It must be noted that when discussing the percent of MRSA found in retail meats, the number may indicate ST398 but it may also indicate common human strains such as USA 100 or USA 300 or a combination of human and animal isolates. This is evidenced by findings of USA 100 and USA 300 on meat products (52). Another paper stated that 32% of MRSA strains isolated were ST398 but that human isolates were also found (4). In a 2011 study of retail meat excluding pork, all MRSA isolates were USA 300 (13). However, as previously stated, there are no reported cases that implicated ST398 in human colonization or infection due to contact with a contaminated meat product. (35) Given this information, one may wonder why the presence of MRSA in food is being investigated at all.

While the current consensus among researchers is that MSRA is present in retail meat products in low quantities (5, 16, 24 38), there is also a general agreement that more research is needed to assess the true implications of MRSA in the food supply (5, 20, 24, 56). If MRSA is present in such small quantities on retail meats, why should there be a cause for concern? Especially since cooking to a proper temperature destroys the bacteria and food poisoning due to MRSA is very rare (24). So why is the presence of MRSA being investigated? ST398 can be transmitted from animals to humans (6) and it is resistant to many commonly used antimicrobial drugs (20). ST398 is known to cause human colonization and infection (9, 1). Additionally, ST398 seems to be transmitted more easily from animals to humans when compared to the transmission of USA 100 and USA 300 between humans (24). MRSA of any strain that is found in meat has the potential to contaminate a
food worker or the consumer via small cuts or wounds in the hands which may come from knives (4). This highlights the fact that consumption of contaminated meat is not as great a concern as is handling the contaminated product either in a food preparation setting or in the home.

Different methodologies of MRSA isolation

Though researchers agree that MRSA has been identified in retail meat products around the world, the problem that remains is the variance in percentage of MRSA found and the methods used to isolate MRSA. These factors hinder the discovery of the dose-response relationship and the true quantities of MRSA present (4). For example, Hanson et al. (35) found 3.6% MRSA in pork (2/55 samples) and overall 1.2% in retail meats in their study. They used surface swabs and one enrichment step. De Boer et al. (16) diluted and homogenized their samples and followed with two enrichments. They reported 10.7% (33/309) MRSA in pork and in their total of all meats sampled, 11.9% MRSA (264/2217 samples). Finally, Pu et al. (52) employed a rinse technique in which the product was left intact and two enrichments followed. Of the 120 meat samples collected in this study, the percent MRSA was determined to be 5.6%.

Due to these findings, it is evident that MRSA is present in retail meats yet no inferences can be made about actual levels of MRSA in meat across the board. It is difficult to make comparisons when methods are not standardized and when there are few if any reports of validation of medias that are effective at isolating MRSA from meats (4). Developing a dose-response model which would allow scientists to determine hazardous levels of MRSA in foods (3) is not as feasible without standardized culture methods.

Transfer of MRSA to Food Preparation Surfaces and Skin. So far this review has covered types of MRSA and the presence of these bacteria in animals and humans and the implications that colonized animals and humans can have on each other, the food processing environment and retail products in the community. This final portion of the review will touch on consumer awareness of proper food safety and how this awareness translates into behaviors during food preparation in the home followed by the ability of bacteria to survive, spread and attach to surfaces in the home kitchen. Much of the research done to date in this area has looked at other food borne pathogens such as Campylobacter jejuni, Salmonella spp. and Staphylococcus aureus but one might expect that MRSA, if present, will be capable of behaving in a similar fashion.
**Consumer Behaviors**

Through combined efforts of the CDC, USDA, FDA and health care providers around the world, one might think that the public is aware of the importance of thorough hand washing and proper food preparation. Yet studies have shown that just because the consumer knows the correct hygiene and food preparation measures does not mean that he/she will employ those methods in their own kitchen (42). Hands remain a large contributing factor in the spread of bacteria in the home and the kitchen may also be a source of food poisoning. Improperly washing hands after handling raw meat has been shown to be the cause of bacterial transfer to other surfaces around the kitchen (39). Interestingly, it seems that consumers worry more about aspects of their food that they cannot control such as how it is handled before it gets to the store as opposed to the aspects they can control such as the handling of raw meat at home and cooking to the recommended temperature. Consumers also tend to behave in a way that is indicative of how important or useful they feel certain protection measures are (39). The overall theme in the kitchen is a sort of cyclic transfer of bacteria that can occur if proper handling and hygiene is not observed.

**Pathogens in the kitchen**

*Staphylococcus aureus* and MRSA alike can survive over a wide range of pH, temperature and salt concentrations and show resistance to some disinfectants. These properties enhance the ability of the bacteria to survive on food and in the home. Research has shown that there are many ways in which bacteria can travel around in the kitchen. Early studies found a high percentage of cross-contamination due to improper hand washing as well as inadequate cleaning of cutting boards and knives (47) and pathogens are known to spread via those two items (12). These findings were confirmed by Kennedy et al. 2011 (39), who also identified knives and cutting boards as vehicles of cross-contamination. Kitchen sponges have also been determined to play a significant role in cross-contamination and *Staphylococcus aureus* harbored in a sponge can also transfer to hands and food contact surfaces (7).

**Potential transfer surfaces**

It has been suggested that surface type also plays a role in bacterial transfer though it is not completely agreed upon. Cross-contamination from a cutting board with cuts in it resulted in higher levels of contamination (48) while other studies found that surface texture did not impact transfer (18). In one experiment it was discovered that contaminated meat transferred substantial amounts of *Staphylococcus aureus* to stainless steel and polyethylene surfaces and that the total cells transferred on one surface when compared to the other was not significantly different (32). Still other experiments showed that not only a rough surface but pressure applied could aid in more transfer of
bacteria (23, 60). In spite of these findings, the fact remains that bacteria are able to transfer on surfaces around the kitchen and contaminate other objects, food and possibly the consumer.

Knowing that bacteria are capable of transfer from raw meat products to surfaces and from those surfaces to cooked products is part of the puzzle of how food borne illnesses can originate in the home. While the prevalence of bacteria in meat is crucial information, it is also important to know how much is initially present and how much is able to transfer to surfaces (54). In a study of the ability of raw chicken from the supermarket to transfer bacteria in a kitchen, the poultry was determined to have an initial mean of 2.6 Log10 MPN and after being placed on a cutting board the cells recovered from the board was 1.5 Log10 MPN giving 57.6% transfer (48). In another study poultry was inoculated with 7 Log CFU/g of *Staphylococcus aureus* and placed on stainless steel and polyethylene surfaces. The product was found to transfer 4 Log CFU/cm² *Staphylococcus aureus* to both surface types (32). Finally, Todd et al 2010 (7) also used *Staphylococcus aureus* to show bacterial cross contamination. Hands washed with contaminated water had initial counts of 2.2-4.3 Log CFU/cm² and were able to transfer 2.1-4.2 Log CFU/g. Ladies in this study were initially contaminated with 1.9-4.6 Log CFU/cm² *S. aureus* and transferred 1.2-4.3 Log CFU/g to food.

It has been reported that MRSA from food is rare (30, 49). However, it is not too much of a stretch to imagine that MRSA could spread or be transferred in a manner similar to other well-known food borne pathogens. While MRSA does not often produce the toxins which make *Staphylococcus aureus* a nasty agent of food borne illness, if it is present in sufficient quantity it still poses a health risk. Resistance can spread between bacteria and lead to the spread of pathogenic bacteria in the home (10). MRSA can contaminate surfaces as other pathogens do and can certainly colonize humans and cause infections in open sores or wounds on hands. Because *Staphylococcus aureus*, and to a lesser degree MRSA, are found on healthy people in the nose and areas often touched by the hands and can be found under fingernails, the presence of risk is nearly constant.

**Conclusions.** Though all of these studies were instrumental in elucidating the potential for pathogen transfer from animals and humans, through processing and to retail products and surfaces in the home, little is known about the true risk of MRSA in foods causing infection in the consumer. Due to methodological differences in sampling and testing it is difficult to quantify the amount of MRSA actually present and make accurate risk assessments. Overall, more research is needed to determine a dose-response relationship for MRSA from foods and food contact surfaces to the consumer and standardized methods would be a good place to begin.
While it may not currently pose a serious threat to human health in regards to being found in raw meats, its continued presence must not be ignored. Even though reports of infection due to MRSA from raw meats is very low, the bacteria possesses characteristics, most notably the ability to develop resistance to most antibiotics used to treat it, which make the need for continued research so compelling. In the mean time, educating the consumer on proper food handling and preparation in the home should be continued.
Chapter 3: Determination of transfer of methicillin-resistant Staphylococcus aureus from retail pork products onto food contact surfaces and the potential for consumer exposure

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Abstract

Methicillin-resistant Staphylococcus aureus (MRSA) is a pathogen that has developed resistance to beta-lactam antibiotics. MRSA was initially associated with hospital acquired infections but emerged in association with community and livestock acquired infections. Recently MRSA has been isolated at low levels in retail meat products in the United States and other countries. The objectives of this study were to estimate the percent transfer of MRSA from contaminated retail pork products to food contact surfaces over a range of initial contamination levels and to estimate the potential for human exposure to MRSA by coming in contact with those contaminated surfaces. Pork loins, bacon and pork sausage were inoculated with four strains of MRSA mixed culture, swabbed for initial bacterial populations, vacuum packaged and stored for two weeks at 5°C to simulate normal packaging and distribution. Polyethylene cutting boards, knives and pork skin as a model for human skin were contaminated with the inoculated product laying on the surface for 5 minutes. Polyethylene cutting boards and knives were also contaminated by placing a 500g lead donut on the product while it is dragged across the transfer surface. 5cmx5cm² areas were swabbed and bacterial populations of the inoculated pork products and contact surfaces were enumerated on Baird-Parker Agar and reported as Log10 CFU/cm². Percent transfer from the inoculated products to the cutting board ranged from 76% to 88% across all 5 cell concentrations. Percent transfer from inoculated products to the knife ranged from 53% to 87% across all 5 cell concentrations. Percent transfer from the inoculated products to the pork skin ranged from 71% to 91% across all 5 cell concentrations. Statistical analysis performed by SAS showed no significant differences in amounts of transfer between transfer surfaces and across cell concentrations. This research illustrates the potential for MRSA transfer to food contact surfaces and skin even at lower initial cell concentrations.
Introduction

Methicillin-resistant *Staphylococcus aureus* is a strain of *Staphylococcus aureus* that has acquired resistance to beta-lactam antibiotics. It first gained notice as the agent responsible for nosocomial infections referred to as hospital acquired (HA MRSA) and it has since been increasingly isolated from infections in the community (CA MRSA) among those with no history of long term hospitalization or surgical procedures (28). Within the last decade livestock acquired (LA MRSA) also known as ST398 has been isolated from healthy livestock, primarily swine. This strain has also been isolated from those who work in close contact with swine and some human cases have also resulted in infections (6,9). There have also been cases in those with no previous animal contact (2). Recently concerns have been raised from a food safety standpoint as ST398 and other MRSA strains have been isolated from raw retail meats (4, 16, 52). Though there have not been any documented cases of human infection with ST398 due to raw meats (40) the fact remains that MRSA strains including ST398 are present in retail raw meat. Therefore they serve as a possible source of bacterial infection that could enter open wounds on hands of food preparers in the food industry (5) and hands of the consumer in the home.

Contamination of retail meats with MRSA can occur in a number of ways. It may commonly be found on animals at harvest and the animals themselves may be the source. Research has shown MRSA to be present on carcasses and to carry through to the final products (11). In fact ST398 has been isolated in several studies as the source of contamination raw meat (16, 4, 35). Other findings point to humans as the source. On occasion, the MRSA contamination was found to be USA 100 or USA 300, common human strains (52). However, ST398 has demonstrated the ability to transfer between animals and humans more readily than USA 100 or USA 300 (24). Current research supports the possibility of more CA-MRSA infections due to contaminated retail products.

MRSA has demonstrated the ability to survive a range of pH, temperature, salt levels and to survive on raw meat that could reach the consumer. These attributes make MRSA a potential source of contamination and infection when found on meat prepared in the home. Just as MRSA is able to be carried through the stages of a production facility (11) it may also be carried around the kitchen. Several studies have reported that knives and cutting boards are major sources of cross contamination with bacteria during food preparation and that bacteria on these surfaces from raw meat may in turn contaminate hands (29, 39, 48). Research suggests that bacteria will transfer readily to food contact surfaces and to skin (32, 7). Despite these findings, in the case of MRSA, a
dose-response level is still not known so it is difficult to make a quantitative risk assessment regarding consumer (3).

The objectives of this study were to estimate the percent transfer of MRSA from pork to knives, cutting boards and skin over a range of initial contamination levels and to estimate the potential for human exposure from surfaces which have come become contaminated with MRSA.

**Materials and Methods**

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

**Bacterial Strains.** Four isolates of methicillin-resistant *Staphylococcus aureus* were used in this experiment. They are as follows: ST398 isolate from ground pork, ST398 isolate from pork chops (both provided by Dr. Catherine Logue, Iowa State University College of Veterinary Medicine; Ames, Iowa), an ST398 isolate from a 24 week old hog (Dr. Tara Smith, University of Iowa, College of Public Health Department of Epidemiology, Center for Emerging Infectious Diseases; Iowa City, Iowa), and ATCC strain BAA-44 (Dr. Brehm-Stecher, Iowa State University Department of Food Science and Human Nutrition; Ames, Iowa) was used as a reference organism. Dr. Logue’s laboratory performed the MLST (multi-locus sequence typing) on the ground pork and pork chop isolates to confirm they were MRSA ST398. Dr. Smith’s laboratory performed PFGE and MLST on the ST398 from the 24 week old hog.
Inoculum Preparation. Prior to inoculation, isolates were streaked onto Baird-Parker Agar (Difco, Becton-Dickinson, Sparks, MD) and Spectra MRSA Agar (Remel, Lenexa, KS) and incubated at 37°C for 24 hours. Black colonies on Baird-Parker were indicative of *Staphylococcus aureus* and denim colored colonies on Spectra MRSA was indicative of methicillin-resistant *Staphylococcus aureus*. All 4 isolates were black on Baird-Parker Agar and denim blue on Spectra MRSA Agar. Isolated colonies were also tested on the Staphylase Test (Oxoid Ltd., Basingstoke, Hants, UK) and the PBP2'Latex Agglutination Test (Oxoid Ltd., Basingstoke, Hants, UK) and were MRSA positive. All strains of methicillin-resistant *Staphylococcus aureus* were maintained in Tryptic Soy Broth (TSB; Difco, Becton-Dickinson, Sparks, MD). The day before inoculations, the cultures were transferred to TSB and grown aerobically at 37°C for 24 hours. On the day of inoculation, the four cultures were combined to form a mixed culture with a cell concentration of approximately $10^9$ CFU/ml.

Bacterial Culture Conditions. Baird-Parker agar with egg yolk tellurite supplement (BP-EYTA, Difco, Becton Dickinson, Sparks, MD) was prepared according to the manufacturer’s instructions. This media was chosen because it is commonly used for isolation and enumeration of *Staphylococcus aureus* (36). Since the identity of methicillin-resistant *Staphylococcus aureus* in this experiment had been determined with ancillary tests, BP-EYTA was used for these experiments.

Inoculation Procedure. Fresh boneless pork loins were purchased from a local supplier and held at 5°C until used for the experiments. Each of the 3 light and 3 heavy replications involved 6 pork loins, 5 loins were separately inoculated with 10ml of cell concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml diluted from the initial MRSA mixed culture. One loin was used as a negative control and swabbed with a speci-sponge (Nasco Whirl-Pak “Speci-Sponge” Bags, Fort Atkinson, WI) hydrated with 10ml Buffered Peptone Water, 0.01%(BPW, Difco, Becton-Dickinson) before and after the 2 week storage period to determine the presence of *Staphylococcus aureus* and/or MRSA, if any, prior to my inoculation of the products. One tenth of a ml was surface plated onto BP-EYTA in duplicate while another 0.1ml was surface plated onto Spectra MRSA in duplicate. No growth was observed after 24 hours incubation at 37°C. After inoculation, inoculated loins were held at 5°C for 30 minutes to allow attachment of the bacteria. A 5cm x 5cm area of each loin was swabbed with a speci-sponge hydrated with 10ml BPW. The loins were then each vacuum packaged and stored for 2 weeks at 5°C to simulate typical packaging and distribution. Swabs were taken to determine bacterial counts after inoculation but before storage. Swab bags were hand massaged for one minute, serially diluted in 9.0ml BPW tubes, surface plated on BP-EYTA in duplicate and incubated at 37°C for 24 hours.
Bacon was purchased from a local supplier and held at 5°C until used for the experiments. Each of the 3 light and 3 heavy replications involved 4 strips of bacon, 5 groups of 4 strips were separately inoculated with 10ml of cell concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml diluted from the initial MRSA mixed culture. One group of 4 strips was the negative control. All inoculated bacon was stored at 5°C for 30 minutes to allow attachment of the bacteria. The samples were swabbed and the bacterial populations enumerated as previously described.

Raw pork sausage was purchased from a local supplier and held at 5°C until used for the experiments. Each of the 3 light and 3 heavy replications involved 30g of sausage inoculated with 10ml of cell concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml diluted from the initial MRSA mixed culture. Each 30g sample was then divided up into 3 10g portions and formed into 2cm x 10cm x 5mm thick strips. One additional strip was formed to serve as the negative control. All inoculated sausage strips were held at 5°C for 30 minutes to allow attachment of the bacteria. Next, the 2cm x 10cm surface was swabbed with a speci-sponge. The sausage strips were then vacuum packaged and stored for 2 weeks at 5°C to simulate typical packaging and distribution. Swabs were taken to determine bacterial counts after inoculation but before storage. Bacterial populations enumerated as previously described.

**Sampling for Light Exposure.** The transfer surfaces consisted of: 5 cutting boards, 5 knives and 5 portions of pork skin set up to correspond to the 5 corresponding cell concentrations used in inoculation. The pork skin was sanitized underneath a UV light in a biosafety cabinet for 15 minutes and the cutting boards and knives were sterilized by autoclaving. All transfer surfaces were at ambient temperature (ca.22-24°C) the experiments. The loins were removed from the vacuum package with a sterile scalpel blade and divided into 3 sections, one section for each transfer surface. The individual sections were placed on the transfer surfaces for 5 minutes without any movement or additional pressure. After 5 minutes, the loins were moved and a 5cm x 5cm² area of each pork loin was swabbed with a speci-sponge rehydrated with 10ml BPW. A 5cm x 5cm² area of each cutting board and each pork skin section were also swabbed. The entire surface of each knife was swabbed, for knives the blade (14cm x 1.5cm) was swabbed. All swabs were hand massaged for one minute, serially diluted in BPW tubes to extinction, plated on BP-EYTA and incubated at 37°C for 24 hours. After transfer surfaces were swabbed, a 5cm x 5cm² x 5mm thick area of each loin was excised (samples weighed 15g +/- 0.5g) and placed in a filter stomacher bag (Nasco Whirl-Pak Bags, Fort Atkinson, WI) and diluted 1:10 (wt/vol) with BPW. The samples were homogenized for a minute in a stomacher (Seward Laboratory Blender 400, Tekmar Co., Cincinnati, Ohio) and serially diluted to extinction in BPW test tubes, plated on BP-EYTA and incubated at 37°C for 24 hours.
The previously described procedure was followed for the bacon and raw pork sausage with the following modifications. For the bacon, 4 slices of bacon were used to achieve a surface area of 5cm x 5cm² x 5mm thick which could be swabbed. Excised portions of bacon weighed 25g +/-0.5g depending on the thickness and fat content of the individual pieces of bacon. For the raw pork sausage a strip of sausage 2cm x 10cm x 5mm thick was formed by using a mold made by the Department of Engineering, Iowa State University, Ames, Iowa. The sausage was removed from the mold and the weight of each strip was determined to be 10g +/- 0.5g.

**Sampling for Heavy Exposure.** After the two week storage period, the vacuum packaged loins were set up as previously described for the light exposure. A 500g lead donut was then placed on the loin and the loin with weight was moved back and forth on a 10cm path on the transfer surface for 20 complete transits. One transit consisted of one back and forth movement along a 10cm path. After the surfaces were exposed to the inoculated loins they remained at ambient temperature for five minutes. The pork skin was applied to the surface with the 500g lead donut on top and the skin was passed along the same 10cm path for 20 back and forth transits. The surfaces and the pork skin were then swabbed with a speci-sponge rehydrated with 10ml BPW and after swab sampling the pork skin was placed in a Whirl-Pak bag and diluted to a 1:10 (wt/vol) with BPW. MRSA populations were enumerated as previously described.

**Quantitative Methods.** The numeric populations of MRSA were transformed to Log_{10} CFU using a commercial spreadsheet program (Microsoft Excel, Redmond, WA). For swabs the populations were reported as Log_{10} CFU/cm² of the surface area swabbed. For the meat samples and pork skin samples populations were reported as Log_{10} CFU/ml. Each combination of meat and cell concentration was independently replicated three times. The percent transfer of MRSA from the pork products to the surfaces was calculated by dividing the final Log_{10} CFU on the surface swab by the initial Log_{10} CFU population present on the product after inoculation (26). Average percent transfer was obtained by adding the percentages together and dividing by the total number of observations.

**Data Analysis.** Three independent replicate experiments were performed for each of the retail products: pork loins, bacon and raw pork sausage. The percentage transfer for each product and surface were analyzed by SAS (Statistical Analysis System version 9.2, SAS Institute, Inc., Cary, N.C.) with a general linear model using least square means and Q-Q plots (which indicated that the data were normally distributed.) A least square means test was performed to determine if there was
no significant difference in the ability of the bacteria to transfer among the surfaces: the polyethylene cutting board, the knife and the pork skin.

**Results.** The first part of this study, the light exposure, was designed to estimate the percentage of MRSA transfer from inoculated retail pork products to other surfaces across a broad range of initial concentrations. The heavy exposure was designed to demonstrate the ability of MRSA to transfer from a contaminated surface to skin using the pork skin model (55) across a broad range of initial concentrations. All samples were vacuum-packaged after inoculation and stored at 5°C to simulate normal packaging and distribution to suppliers. Before inoculation, all pork products used were analyzed and determined to have no detectable *Staphylococcus aureus*.

**Light Exposure**

The initial populations of MRSA across all replications on the day of inoculation was 4.8, 5.2, 6.5, 7.7 and 8.6 Log$_{10}$ CFU/cm$^2$ for intended inoculum concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml respectively. The area of loin sampled was 5cm x 5cm$^2$. For the designated 10 CFU/ml loin samples, transfer occurred on all 3 surfaces, the cutting board, knife and pork skin at 76%, 53%, and 80% respectively. However, for 2 of the 3 replications neither growth nor transfer was observed at 10 CFU/ml. This may have been due to the initial cell concentration being lower than expected; the cells in the inoculum may have been non-viable due to some stress not accounted for during incubation. For the remaining concentrations of 100, 1,000, 10,000 and 100,000 CFU/ml, average transfer percentages are displayed in Table 1.

The bacon light exposure initial populations of MRSA across all replications after inoculation averaged 5.3, 6.0, 7.3, 7.9 and 9.1 Log$_{10}$ CFU/cm$^2$ for designated inoculum concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml respectively. The area of 4 inoculated bacon slices sampled was 5cm x 5cm$^2$. The average percent transfer from the bacon to each of the 3 transfer surfaces is summarized in Table 2.

The raw pork sausage light exposure initial populations of MRSA across all replications averaged 5.6, 6.9, 8.0, 9.0 and 10.0 Log$_{10}$ CFU/cm$^2$ after inoculation for designated inoculum concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml respectively. For the sausage, molds (Figure 2) were made in order to form the sausage into standard portions that were 2cm wide, 5mm thick and 10cm in length and this is the area that was swabbed after inoculation. The average percent transfer from the sausage to each of the 3 transfer surfaces is summarized in Table 3.
The average percent recovery of MRSA from the products after two weeks of vacuum packaged storage at 4°C is summarized in Tables 4, 5 and 6 for pork loins, bacon and raw pork sausage respectively. These values are compared to the initial populations just after inoculation. The surfaces are listed to correlate the percent transfer from that product to the surface and the amount of bacteria remaining on the surface of the product at the time of sampling/transfer. Although there was no significant difference among transfer to the surfaces across the cell concentrations, the cutting board and the skin generally seemed to have more cells transferred but this may have been due to the total surface area differences in the knife swabs and the 5cm x 5cm² area of the cutting board and pork skin swabs.

Heavy Exposure
The initial populations of MRSA on the 5 pork loins averaged across all replications were 5.1, 5.7, 7.1, 7.9 and 9.0 Log₁₀ CFU/cm² for inoculum after inoculation for designated concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml respectively. The area of loin sampled was 5cm x 5cm². For the bacon, populations were as follows, 5.2, 5.7, 7.0, 7.8 and 8.9 Log₁₀ CFU/cm² for inoculum concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml respectively. The initial sausage populations were 5.4, 6.7, 8.3, 8.4 and 9.5 Log₁₀ CFU/cm² for designated inoculum concentrations of 10, 100, 1,000, 10,000 and 100,000 CFU/ml respectively. The results of the percent transfer to the pork skin from the cutting board and knife after those surfaces were exposed to the inoculated pork loin are summarized in Table 7. The results for the bacon and raw pork sausage are in Tables 8 and 9 respectively. The surface least square means test showed that among the 3 products, there was not a significant difference (P>0.10) in the ability of the inoculated product to transfer bacteria nor was there a significant difference (P>0.10) among the surfaces in the ability to transfer bacteria to the skin.

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

Findings in this study also show that the amount transferred from the product to the surface during light exposure increased as the initial inoculum amount on the products increased but the amount was not significantly different (P >0.10). While this was expected, other studies have described an inverse relationship in which a high initial bacterial load leads to a lower total amount transferred and lower initial bacterial counts will lead to a higher percent transfer due to bacterial interactions on the meat surface (26,29). However, one of these studies used raw poultry that was naturally contaminated (29) while this research used inoculated products. Also, pork product surfaces vary among type (loin, bacon, sausage) and may be quite different from the surfaces of raw products such as chicken and beef. Thus, bacterial interactions on inoculated pork and naturally contaminated poultry cannot be readily compared due to the differences in their surface type.

For the heavy exposure experiments, tables 7, 8 and 9 show that among the products and across the cell populations there was not a significant difference (P>0.10) in transfer to the pork skin from the contact surfaces. In the heavy exposure experiments, it was expected that the amounts of cell transfer would be higher if pressure, such as the 500g lead donut used here, was applied to the product while it was being moved across a surface. One study reported that applied pressure would facilitate bacterial removal from one surface to another resulting in higher transfer rates (26). It was also suspected that subsequent weighted skin contact on that surface would result in a high transfer. These percentages are lower than those from the light exposure when the contaminated product was simply placed on the pork skin for 5 minutes. However, a direct comparison cannot be made because heavy exposure was to determine if a contaminated surface could transfer bacteria to skin whereas the light exposure showed that direct skin contact on the contaminated product allowed bacterial transfer. Still, the results show that transfer occurs across all levels of initial contamination which indicates that risk of consumer contact and possible colonization or infection exists.

It is possible that some of the populations were lower than they actually may have been in the heavy exposure due to the movement of the product on the surface spreading out the cells away from the
area swabbed. Additionally, whole pork loins were inoculated with 10ml of inoculum and this may have added a dilution effect to the final cell counts and percentages as well.

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

Acknowledgements
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Figures

Figure 1a. Vacuum packaged retail pork products

Figure 1b. Vacuum packaged pork skin to serve as the human skin model
Figure 2. Sausage molds made to form sausage into strips.
### Table 1 Light Exposure: percent transfer to the 3 surfaces

<table>
<thead>
<tr>
<th>Pork Loin CFU/cm²</th>
<th>Cutting board swab (standard deviation)</th>
<th>Knife swab (standard deviation)</th>
<th>Pork skin swab (standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100,000</td>
<td>88 (6.08)</td>
<td>80 (6.93)</td>
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<td>76*</td>
<td>53*</td>
<td>80*</td>
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</table>

*indicates growth at this level was only detected at 1 of the 3 replications

### Table 2 Light Exposure: percent transfer to the 3 surfaces

<table>
<thead>
<tr>
<th>Bacon CFU/cm²</th>
<th>Cutting board swab (standard deviation)</th>
<th>Knife swab (standard deviation)</th>
<th>Pork skin swab (standard deviation)</th>
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<td>80 (7.37)</td>
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<td>10</td>
<td>72 (4.04)</td>
<td>69 (16.82)</td>
<td>71 (4.62)</td>
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### Table 3 Light Exposure: percent transfer to the 3 surfaces

<table>
<thead>
<tr>
<th>Sausage CFU/cm²</th>
<th>Cutting board swab (standard deviation)</th>
<th>Knife swab (standard deviation)</th>
<th>Pork skin swab (standard deviation)</th>
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<td>76 (16.07)</td>
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### Table 4 Average percent recovery of MRSA after 2 week storage at 4°C

<table>
<thead>
<tr>
<th>Pork Loin excised CFU/ml</th>
<th>Cutting board (standard deviation)</th>
<th>Knife (standard deviation)</th>
<th>Pork Skin (standard deviation)</th>
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</thead>
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<tr>
<td>10</td>
<td>86*</td>
<td>74*</td>
<td>84*</td>
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### Table 5 Average percent recovery of MRSA after 2 week storage at 4°C

<table>
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<tr>
<th>Bacon excised CFU/ml</th>
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<th>Knife (standard deviation)</th>
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### Table 6 Average percent recovery of MRSA after 2 week storage at 4°C

<table>
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<tr>
<th>Sausage excised CFU/ml</th>
<th>Cutting board (standard deviation)</th>
<th>Knife (standard deviation)</th>
<th>Pork Skin (standard deviation)</th>
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<td>90 (8.62)</td>
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### Table 7 Heavy Exposure: percent transfer from surface to human skin model

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<td>59 (8.96)</td>
<td>57 (12.02)</td>
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### Table 8 Heavy Exposure: percent transfer from surface to human skin model

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<th>Bacon Heavy CFU/cm²</th>
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</tbody>
</table>

### Table 9 Heavy Exposure: percent transfer from surface to human skin model

<table>
<thead>
<tr>
<th>Sausage Heavy CFU/cm²</th>
<th>knife to pork skin (standard deviation)</th>
<th>cutting board to pork skin (standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100,000</td>
<td>74 (5.03)</td>
<td>76 (3.61)</td>
</tr>
<tr>
<td>10,000</td>
<td>74 (5.20)</td>
<td>70 (8.74)</td>
</tr>
<tr>
<td>1,000</td>
<td>67 (5.30)</td>
<td>66 (10.07)</td>
</tr>
<tr>
<td>100</td>
<td>54 (6.51)</td>
<td>58 (2.08)</td>
</tr>
<tr>
<td>10</td>
<td>55 (3.78)</td>
<td>66 (9.02)</td>
</tr>
</tbody>
</table>
Chapter 4: Concluding remarks

The research presented in the literature review conveys the potential for food production animals and food to be potential vehicles for the transfer of MRSA not just to those in close contact with livestock but to the general public as well. The implication of this is the potential for both human to human transfer of MRSA and food borne outbreaks involving MRSA. The food outbreaks would differ from other outbreaks in that although the MRSA may come from the food, it may cause illness very different and potentially more severe than the illness due to food poisoning. The review also shows that lack of standardized methodologies for testing food products for MRSA has resulted in limited knowledge of the dose-response for these particular bacteria in foods.

The research manuscript has shown that MRSA is capable of transfer to surfaces commonly used in food preparation in the home and that it may also transfer from those surfaces to the skin thus presenting a risk to the consumer. The research shows that this transfer happens across a range of initial cell concentrations. This study could have looked at transfer with lower cell concentrations but the data provided herein could be used to develop a better model of the risk to the consumer if meat is contaminated with very low numbers, <10CFU/ml, of MRSA. Overall, a better understanding of the dose-response relationship is needed as well as more standard sampling and testing methods for MRSA in food, specifically foods of animal origin.