ABSTRACT

FERREIRA, JORGE PINTO. Methicillin-resistant *Staphylococcus aureus*: Epidemiology and Policy. (Under the direction of Dr. Maria Teresa Correa and Dr. Kevin L. Anderson.)

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged worldwide as a significant public health/antimicrobial resistance problem, both in human and veterinary medicine. Described for the first time in the 60’s, it was initially considered a nosocomial agent. Not much later, community-associated cases in people without health care risk factors were reported. In recent years, the so-called non-typable (NT)-MRSA, isolated normally from animals, has been challenging the epidemiological concepts that the scientific community had regarding this agent.

In this thesis we explored some of the connections between humans, animals (food and companion) and MRSA, from an epidemiological and policy point of view.

In chapter 1 our study about the presence of MRSA in North Carolina (NC) milk bulk tanks is presented. We analyzed a total of 125 Staphylococcus aureus isolates that had been collected from bulk tanks from 19 NC dairies, from 1997-2009. None of the *Staphylococcus aureus* tested were MRSA. Considering the small number of MRSA isolates found in the dairy arena and that milk is pasteurized, MRSA in dairy products seems to be currently a minor consumer or public health concern. Having in mind the importance of *S. aureus* as a human infectious disease agent, its highly contagious typical behavior among dairy cows, and the current gaps
in knowledge about the potential human:bovine connections, the epidemiology of MRSA (and other Staphylococcus spp.) in the dairy arena should represent a future area of attention.

The importance of companion animals as reservoirs of human MRSA infections is explored in chapter 2. We enrolled forty nine MRSA-positive human outpatients seen at Duke hospital [cases]. If they lived with companion animals and consented, a visit to their homes was scheduled and swabs were collected from their animal(s) to determine MRSA status. Dogs and cats owned by NCSU students and staff who were visiting a “wellness clinic” were used as controls. Occurrences of MRSA-positives were compared for human and animal case and control populations.

Four of 49 MRSA-infected patients (8.2%) had MRSA-positive companion animals. One MRSA-infected patient lived with a methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) animal. In contrast, no MRSA was found in the control population of 50 humans and 75 animals (45 dogs and 30 cats). Interestingly enough, one of the suggested trans-infection cases was between a hamster and a human, something that had never been described before (chapter 3).

These results suggest that companion animals of MRSA-infected patients can be culture-positive for MRSA, representing a potential source of infection or re-infection for humans. Further studies with a larger cohort should be performed.

Finally, chapter 4 addresses some of the MRSA policy related issues. Different policies have been tested or are currently being utilized to control MRSA. One of them involves the screening of patients in hospitals. In this report we present the results of the cost-benefit analysis of universal screening for MRSA at NCSU veterinary hospital. The savings
associated with this policy ($27,279,349) seem to overcome the costs (240,140/year) even after sensitivity analysis, suggesting that it should be implemented, from a societal point of view [net benefit of $27,039,209].

MRSA represents a complex challenge. There are still innumerous gaps in our knowledge about it. An holistic (“One Health”) perspective from a diverse range of professionals is needed to better understand its epidemiology and design the most adequate policies for its control.
Methicillin-resistant *Staphylococcus aureus*: Epidemiology and Policy

by

Jorge Manuel Pinto Ferreira

A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

Comparative Biomedical Sciences

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2011

APPROVED BY:

_______________________________  ______________________________
Maria Teresa Correa (chair)                                      Kevin Anderson (co-chair)

_______________________________              _______________________________
Thomas Birkland        Vance G. Fowler
DEDICATION

Para o meu Pai.

Um doutoramento é sem dúvida uma enorme lição. Um lição de ciência (neste caso) mas acima de tudo uma lição de Vida. Lembro-me de em miúdo ter uma espécie de um quadro no meu quarto em que se explicavam as etapas da nossa admiração pelos nossos Pais. Em resumo, dizia o referido quadro que começávamos por ver os nossos Pais como heróis, mais tarde começávamos a ter dúvidas quanto a este estatuto, por volta dos 30 anos começávamos a achar que afinal o Pai não sabia quase nada...mas mais tarde, por volta dos 50 ou 60 anos, olhávamos para trás e pensávamos: O meu pai foi sempre um génio. Eu é que não o percebi a tempo. Residir e estudar no estrangeiro, sendo eu próprio igualmente já Pai, aceleraou muitas destas etapas. Aos 30 e poucos anos aprendi a valorizar e respeitar tudo aquilo que extraordinariamente o meu Pai conseguiu ao longo da sua Vida.

Ser um Homem de sucesso como o meu Pai tem ainda muitíssimo mais valor porque o início do ser percurso não podia ter sido mais difícil. Acima de tudo não teve ele o seu...Pai ao seu lado. O doutoramento permitiu-me também perceber que este facto da vida do meu Pai terá tido consequências psicológicas. Em si e em mim. Felizmente (haverá mesmo coincidências, ou algo muito mais poderoso que isso que todos desconhecemos e a quem vamos dando diferentes nomes?) os que me rodearam durante estes 4 anos deram-me o apoio de que necessitei. Bem hajam.

“O que eu gostava mesmo era de te ver um dia como um Sr. Doutor, Joca”, pois bem querido Pai, agora já o sou. Este grau académico é dedicado a ti.
DEDICATION

To my Father.

A doctoral program is, without any doubts, an enormous lesson. A lesson of Science (in this case) but above all a Life lesson. I remember having, as a child, a painting in my room that represented the different stages that we go through in our lives regarding our admiration of our fathers. In summary, it was said that we start by looking at our fathers as the biggest of the heroes. Later, we start having doubts about this status/position, and around 30 many think that their father actually is not that smart. However, when we reach 50 or 60, we look back and think: My father was always a genius. I just was not smart enough to realize it on time. Living and studying abroad, being myself also already a Father, perhaps accelerated many of these stages. At 34 I have learned to respect everything that my Father extraordinarily was able to achieve during his life.

Being a successful man has even more value because…he never had his own father to support and guide him. During my doctoral studies, I realized that this fact probably had some psychological consequences. In my Father and in myself. Fortunately (are there really coincidences, or something much more powerful that none of us knows what it is and keep giving it different names?) those that surrounded me during these 4 years gave me all the support that I required.

“What I really would like to see would be you as a Doctor, Joca”. Well dear Father, now I am a Doctor. This academic degree is dedicated to you.
BIOGRAPHY

Jorge Pinto Ferreira: In May 2011, as a Fulbright scholar, completed his Comparative Biomedical Sciences Ph.D. program (concentration areas of Population Medicine and Veterinary Public Health) at North Carolina State University, College of Veterinary Medicine (Raleigh, NC, USA). Dissertation topics: MRSA – Epidemiology and Policy. Did an internship at the World Health Organization in Geneva (Summer 2009) and developed a public health project in Uganda (Summer 2010). He received a Master of Science Degree in Food Science and Technology from the Escola Superior de Biotecnologia, Universidade Católica Portuguesa (2007, Porto, Portugal) and a Doctor of Veterinary Medicine Degree from Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto (2001, Porto, Portugal). He has worked as a dairy cattle veterinarian in Portugal (where he is originally from) and later developed and managed a milk quality laboratory. Research interests: global health policy, MRSA, human-animal trans-infection of antimicrobial resistant organisms. Career goal: International Organizations.
ACKNOWLEDGEMENTS

My parents, Daniel Aires Ferreira and Maria Auzenda Pinto Ferreira. For their continuous support (financial and moral) without which I would have not been able to pursue this career goal. And mom – thank you for all you lovely letters!

My daughter, Carolina: for her smile. For her phone calls telling me about all her new boyfriends. For her handmade gifts. For her love.

My girlfriend, Holly Durham. For the wonderful moments that we spent together, either travelling, organizing parties or simply at home. For sharing with me her friends and family. For everything that I have learned from her. For all her support and love.

My sister, Daniela. For being on the other side when I most needed her. For always being on the other side when I most needed her. And I did need her.

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Dr. Maria Correa, the chair of my committee and supervisor at NCSU. For teaching me not only Epidemiology or Public Health, but much more important than that a life attitude that smiles when problems show up.

Dr. Kevin Anderson, co-chair of my committee and supervisor at NCSU. For emphasizing the importance of the literature review for a good research project and for being a very good (and fast!) editor of my papers and thesis.

Roberta Lyman, research specialist in the milk and mastitis laboratory at NCSU. For all her help and assistance with the laboratorial work involving the animal samples, and for reviewing and editing a variety of the texts that I had to write during my doctoral studies.

Dr. Birkland, William T. Kretzer Professor and the head of the NCSU Public Policy School of Public and International Affairs, and member of my committee. For introducing me to the world of public policy, that I ended up finding out to be the “best kept secret inside of me”. It was a pleasure to take all the policy classes and I hope to pursue this area in my career.

Thank you for you program Dr. Birkland.

Ellen Condelli. She knows why. And I will always remember why. I promise.

My former team handball coach, Serafim Borges. For our e-mail correspondence where we shared so many thoughts and feelings about areas so different as national and international politics, education, history, economy and above all, our common passion – Sporting Clube de Portugal!

My Friends, for their support and friendship that allowed me to keep balanced.

NCSU librarians and Tammy Ball, for being some of the most competent and responsible professionals that I had the chance to work with during my doctoral studies.
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Fulbright Program, without which none of this would have happened in the first place. Thank you for providing me this education and life opportunity.

NCSU CVM, the institution that awarded me my doctoral degree.

Duke University, Duke Sanford Policy School and Duke Global Health Institute. For having received me as a visiting researcher and have provided me the chance to do an internship at the World Health Organization in Geneva and to develop a field public health project in Uganda.
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CHAPTER 1: METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)
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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged globally as a significant public health/antimicrobial resistance problem both in human and veterinary medicine. It has been well known as a nosocomial agent, later also as a community-associated pathogen. In recent years, the so-called non-typable (NT)-MRSA (also called Livestock-associated MRSA, LA-MRSA) has become an additional focus of concern.

Literature review of MRSA and dairy cattle indicates few reports of MRSA in dairy cattle. Work from our laboratory supports previous studies indicating that MRSA is rare in milk of dairy cattle in the US. Dairy cattle-human trans-infection has been reported in a limited number of instances. Further investigation is needed to determine the direction of infection and several other critical epidemiological issues.
Introduction

The domestication of food animals and the development of antimicrobials can be considered among some of the most significant achievements of humanity’s agriculture history. However, the development of antimicrobial resistance, both in humans and animals, might lead to a new era in terms of animal agriculture. Driven largely by human population growth, income growth and urbanization (1), there are current concerns about the expected significant increased needs at the global level for animal protein. At this point it is unclear what should be the most correct strategies to achieve this level of production. Superbugs, as described in lay terms and mass media, represent an important example of consumers’ potential concerns. Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is an example. It has emerged worldwide as a significant public health/antimicrobial resistance problem, both in human and veterinary medicine. It has been well known as a nosocomial agent, later also as a community-associated pathogen, an in recent years, the so-called non-typable (NT)-MRSA (other designations: Livestock-associated MRSA, LA-MRSA or MRSA Sequence Type (ST) or Clonal Complex (CC) 398), has become another focus of concern.

The overuse of antimicrobials has been repeatedly emphasized as the main selection pressure for bacterial resistant strains (2). According to the Food and Drug Administration, 41,328 Kg (of active ingredient) of cephalosporins and 610,514 of penicillins were sold for the food animal industry in the US in 2009 (3). The use of antimicrobials as growth promoters has been a matter of intense debate (4), but it is not a common practice in the dairy industry. Antibiotic treatments are a common practice on dairy farms around the world.
Worldwide, the dairy industry uses a significant volume of antimicrobials (5). But the situation in dairy cattle challenges the straightforward/simplistic idea that the overuse of antimicrobials is the sole cause for the development of multi-resistant strains. Despite use of antimicrobials, most reports on MRSA prevalence on dairy farms found low values (Table 1). At the same time, studies have found no major significant difference in the prevalence of resistant strains between traditional and organic dairy farms (6).

Mastitis is one of the main reasons for the use of antibiotics in the dairy industry. It still represents the most important cause for economic loss for the dairy industry (7). Of the wide variety of pathogens that have been isolated as causative agents of mastitis, *Staphylococcus aureus* remains a common and economically significant cause of mastitis in dairy cattle and has known zoonotic and public health significance. *S. aureus* is recognized as a major contagious mastitis agent worldwide, but this does not seem to be true of MRSA (Table 1). Some strains of *S. aureus* produce enterotoxins that are associated with food poisoning (8), and there is increasing concern about the antibiotic resistance of *S. aureus*, specifically to methicillin or β-lactam antibiotics (i.e. MRSA).

Although the hyper-production of β-lactamase has been suggested as the resistance mechanism (7), methicillin resistance in *S. aureus* is most commonly the result of the production of the novel penicillin-binding protein (PBP)-2a, which has a decreased binding affinity for β-lactam antibiotics. PBP-2a requires 2-10 times higher penicillin concentrations for inactivation than PBP-2 and 20 times higher than PBP-1 (9). PBP-2a production is encoded by the chromosomal gene mecA found on a large mobile genetic element called the
staphylococcal chromosomal cassette mec (SCCmec). There are currently six major SCCmec types (I, II, III, IV, V and VI) and several subtypes, based on the combination of the cassette chromosome recombinase (ccr) gene complexes and mecA regulatory genes, mecI and mecRI (10). The presence of the mecA gene in MRSA is the specific molecular characteristic that differentiates MRSA from Methicillin Susceptible Staphylococcus aureus, also known as MSSA. Staphylococcus fleuretti, a commensal Staphylococcus species of animals, has been recently reported as the highly probable origin of the mecA gene (11).

The first MRSA occurrence was reported by Jevons in England in 1961 (12) soon after the introduction of beta-lactamase resistant penicillins in human medicine (1959). A cow with mastitis, in Belgium, was the first reported MRSA infection in an animal in 1972 (13). Despite this historical background, there are many unknowns concerning “MRSA dairy epidemiology” and the transmission of MRSA between animals and humans. Moreover, the risk factors involved in trans-infection and the direction of transmission between cattle and humans are not clearly understood. In this paper, we present an overview of the literature that has been published about MRSA and dairy cattle. The potential connections between the bovine and human isolates (Figure1) are also explored in this paper. Suggestions for future research in this area are included.
MRSA prevalence in milk and other dairy products

Literature about MRSA and the dairy industry is scarce. In table 1, we summarized the literature concerning the isolation of MRSA in milk and other dairy food samples. For this review we focused on papers published in the last three decades independently of the study design. We found that most of the studies reviewed were prevalence reports or case-series studies. For the most part, the sampling scheme was convenient.

Table 1: Reports of MRSA in bovine milk, mastitis samples and other dairy products.

<table>
<thead>
<tr>
<th>Publication Year</th>
<th>Authors</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>2000</td>
<td>(7) De Oliveira et al. (2000)</td>
<td>determined the minimum inhibitory concentrations for 811 strains of S. aureus isolated from cases of bovine mastitis in 11 countries (Denmark, England, Finland, Germany, Iceland, Ireland, Norway, Sweden, Switzerland, United States and Zimbabwe). Only 12 strains could be phenotypically classified as MRSA, but they were all mecA negative.</td>
</tr>
<tr>
<td>2003</td>
<td>(14) Lee et al. (2003)</td>
<td>found an isolation percentage of 1.34% when analyzing 894 milk samples collected from 2001 to 2003 in the Republic of Korea.</td>
</tr>
<tr>
<td>2003</td>
<td>(15) Guerin-Faublee et al. (2003)</td>
<td>analyzed 119 isolates of S. aureus collected between 1998 and 2000 in France from cows with clinical mastitis. No MRSA was found.</td>
</tr>
<tr>
<td>2004</td>
<td>(16) Farzana et al.</td>
<td>analyzed 50 raw milk samples collected in 1992 in Pakistan. S. aureus was present in all the samples. 10% of the isolates (8 of 77) were methicillin-resistant.</td>
</tr>
<tr>
<td>2005</td>
<td>(17) Kwon et al. (2005)</td>
<td>found an isolation rate of 0.18% of MRSA in 9,055 milk samples with more than 500,000 somatic cells/mL collected in 1999, 2000 and 2003 in the Republic of Korea.</td>
</tr>
<tr>
<td>2006</td>
<td>(18) Turutoglu et al. (2006)</td>
<td>analyzed 103 S. aureus isolates from milk samples collected from cases of mastitis in herds in Turkey during the years of 2002-2004. 18 of the isolates were resistant to methicillin (17.5%).</td>
</tr>
<tr>
<td>2007</td>
<td>(19) Nunes et al. (2007)</td>
<td>determined the antibiotic susceptibility of 30 isolates of S. aureus responsible for subclinical bovine mastitis in Portugal. No MRSA was found.</td>
</tr>
<tr>
<td>2007</td>
<td>(20) Monecke et al. (2007)</td>
<td>identified two MRSA from 128 S. aureus isolates from cows in Germany and Switzerland.</td>
</tr>
<tr>
<td>2007</td>
<td>(21,22) Normano et al. (2007)</td>
<td>analyzed 437 raw milk, 702 heat-treated milk, 1578 cheese, 87 curd, 194 ricotta cheese, 350 ice cream and 349 other dairy products collected between 2003 and 2005 in Italy. The isolation rate of MRSA was of 0.16%.</td>
</tr>
<tr>
<td>2007</td>
<td>(23) Moon et al. (2007)</td>
<td>analyzed 3,047 bovine mastitic milk samples from 153 dairy farms in the Republic of Korea, collected from 1997-2004. 21 (2.5% of 840) S. aureus and 19 (2.4%) coagulase negative staphylococci were resistant to methicillin.</td>
</tr>
<tr>
<td>2008</td>
<td>(24) Wang et al. (2008)</td>
<td>analyzed 72 bovine S. aureus isolates obtained from 12 dairy farms of Inner Mongolia of China and found no MRSA.</td>
</tr>
<tr>
<td>2008</td>
<td>(25) Graveland et al. (2008)</td>
<td>explored the spread of MRSA in veal calf production in the Netherlands, having found surprisingly high prevalences: 32% of the farmers, 8% of the family members and 28% of the calves were MRSA positive.</td>
</tr>
<tr>
<td>2009</td>
<td>(26) Cui et al.</td>
<td>could not find MRSA from 276 cattle nasal swabs and 47 cattle workers collected on four Chinese provinces.</td>
</tr>
<tr>
<td>2009</td>
<td>(27) Studies have also been done using bulk tank milk samples such as the study by Virgin et al. (2009) where the herd MRSA prevalence in US dairy herds was estimated. In this study, bulk tank milks (n=542) were tested and no positive MRSA was found (27).</td>
<td></td>
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Table 1
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<tr>
<th>Year</th>
<th>Reference</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>2010</td>
<td>(28) Vanderghaeghen et al.</td>
<td>found that nearly 10% of the <em>S. aureus</em> isolated from bovine subclinical and clinical mastitis (118 isolates from 118 different farms in Belgium, collected from 2006 to 2007) in their study were MRSA, a much higher prevalence than previously published. This suggests that about 10% of the Belgian farms with <em>S. aureus</em> mastitis have a MRSA problem.</td>
</tr>
<tr>
<td>2010</td>
<td>(29) Spohr et al. (2010)</td>
<td>analyzed the occurrence of MRSA in 3 dairy farms in Germany with a history of clinical and subclinical MRSA mastitis. Herds were tested twice. In the first investigation, the range the proportion of positive MRSA cows was 5.1-16.7%, and in the second one 1.4-10.0%. MRSA was isolated from the noses of 4 out of 7 calves in this study.</td>
</tr>
<tr>
<td>2010</td>
<td>(30)</td>
<td>100 samples of bulk tank milk and 200 samples of raw-milk cheese were tested in Switzerland. No MRSA found.</td>
</tr>
<tr>
<td>2010</td>
<td>(31)</td>
<td>139 non-duplicate <em>S. aureus</em> isolates collected in France between 2007 and 2008 were analyzed by Haenni et al. (2010). One isolate was classified as MRSA.</td>
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Related work done in our laboratory

In 2006 we analyzed 357 *S. aureus* isolates recovered from milk samples that had been submitted to our laboratory for diagnostic bacteriologic testing from 24 dairy herds in North Carolina (NC) and Virginia, USA. We found very few resistant isolates: 86% (308 of 357) were susceptible to all antimicrobial tested and no MRSA was found (32). In order to screen for the presence of MRSA in NC dairies over time, we analyzed *S. aureus* isolates from our collection of about 3,800 bovine *S. aureus* isolates. Of these, a total of 125 *S. aureus* isolates had been collected from bulk tanks from 19 NC dairies and were further tested. These represented samples collected over a 13-year period from 1997-2009 and the isolates had been kept frozen for variable period of time at -75°C in 15% glycerol (0.225 mL of glycerol and 1.275 mL of culture grown overnight in brain heart infusion). This convenient sample included bulk tanks representing a cross-section of dairy herds in NC. None of the *S. aureus* tested were MRSA. All the isolates were phenotypically susceptible to
cefoxitin, showing inhibition zones greater than 22 mm. The mecA gene could not be detected and amplified in any of the PCR reactions.

**Analysis and discussion of studies results**

Until recently, most reports indicated that MRSA was an agent of minor importance and low prevalence in dairy samples (Table 1). However, comparing isolation rates of different studies is difficult since there is no standardization of sampling and diagnostic methods. In fact, there is not even an agreement upon the definitions of CA and LA-MRSA (33). Our bulk tank *S. aureus* results agree with those of Virgin et al. (2009). Both suggest that the prevalence of MRSA on dairies in the US is very low or undetectable. We used isolates that had been collected during a 13 year time period, which suggests prevalence has not changed over time. One of the main limitations of our study was that the samples in our database were not randomly collected and potentially do not represent true prevalence. We can only speculate that negative results throughout so many years could mean that MRSA was not there. Also, bulk tank milk samples were used, which might have resulted in a significant number of false negatives, as single bulk tank culture sensitivity has been reported as not being higher than 60% (34).

The study by van Griethuysen et al. (2005) raises the concern of the potential loss of the mecA gene during storage of MRSA isolates (35). In this study, the mecA gene was lost in 36 (14.4%) of 250 MRSA isolates after two years of storage at -80°C with the Microbank system (Pro-lab Diagnostics, Austin, Tex.). These authors hypothesize that MRSA isolates consist of heterogeneous populations (with mecA positive and mecA negative cells), with the mecA negative cells resisting storage conditions better. Our *S. aureus* isolates had not been
tested for the presence of the *mecA* gene before storage so we cannot predict the influence that storage had on them.

Recent reports of increased MRSA prevalence (25,28,29) are intriguing. One potential explanation might be the increased interest in this topic by the scientific community. On the other hand, the differences in prevalence between the European and American studies might be explained by the different agricultural systems: more traditional, family based, with multiple animal species in Europe (facilitating the transfer of genetic material between species (29)), as opposed to the situation in the US, with larger and more industrial herds. Cattle turnover is higher in the US than in Europe, where cows are traditionally kept longer. Also, in Europe, national surveys are being conducted (particularly in the Netherlands, in the swine industry), that might lead to the finding of an increased prevalence.

Different antimicrobial national policies and regulations could also explain the different prevalence rates. To make possible an accurate comparison of antimicrobial policies, national public health institutions (human and veterinarian) should annually collect data on kilograms of active antimicrobial agents used by species, route of administration and purpose of use (therapeutic, prophylaxis or growth promotion). Grave et al. recently compared the sales of veterinary antibacterial agents between 10 European countries (5). The authors found a wide variation (18-188 mg/kg) in the usage between countries and concluded that the difference could not be explained only by differences in the animal species demographics. Speculative explanations include, for example, different animal husbandry
practices, pharmaceutical drugs availability in the market or veterinary prescription habits (5).

**Human MRSA and cattle MRSA**

The connection between MRSA, dairy cattle and people (Figure 1) has recently been brought to the attention of the scientific community. For example, Juhasz-Kasznayitzky et al. (2007) reported that MRSA (ST1) were isolated from cows with subclinical mastitis and from a person who worked with these animals. The bovine and human isolates were undistinguishable by phenotyping and genotyping methods, possibly representing the first documented case of direct transmission of MRSA between cows and humans. The direction of transmission could not be determined (36).

![Diagram illustrating the potential ways that can explain the transfer of MRSA from cattle to human and vice-versa. (Coagulase-negative Staphylococci, CNS).](image_url)

**Figure 1:** Diagram illustrating the potential ways that can explain the transfer of MRSA from cattle to human and vice-versa. (Coagulase-negative Staphylococci, CNS).
Although MRSA ST398 has been considered as a lineage with limited virulence and ability to spread among humans, some recent human MRSA ST398 reports have been published (37,38). It is important to emphasize that, since less than 5% of the population in industrialized countries is involved in farming, a significant spread to the general population of CC398 from this reservoir should not be expected (39).

In Italy, a recent case of MRSA ST398 necrotizing fasciitis in a dairy farmer might represent another case of MRSA cattle-to-human transmission (40). This study lacks information about MRSA colonization of animals on the farm, and this assumption is only based on the absence of other risk factors (40). Similarly, also in Italy, MRSA ST 398 was recovered from a patient with a chronic leg ulcer (41). This patient was living in a semi-rural area but did not report direct contact with livestock or pet animals (41). Similar findings in Finland (isolation of human Clonal Complex (CC) 398 MRSA from individuals without connection with animals) seems to indicate that there are unrecognized sources of this CC in the community (42). Nevertheless, ST 398 seems to be more adapted to animals than to humans, and in comparable conditions is less transmissible than non-ST398 in (Dutch) hospitals (43).

**Trans-infection risk factors**

From a prevention standpoint, it is important to know which factors increase the risk of MRSA carriage in people and other animals. This knowledge could be essential for the development of appropriate biosecurity measures and public or agricultural policies applied to farm or hospital environment. Hanselman *et al.* (2006) isolated MRSA from 27 out of 417 (6.5%) attendees at the Annual American College of Veterinary Internal Medicine
Conference held in Baltimore, Maryland in 2005 (44). In this study, colonization was more common for large-animal (15/96 or 15.6%) personnel than for small-animal personnel (12/271 or 4.4%). Large animal veterinary practice was, in fact, the only variable significantly associated with colonization, with an odds ratio (OR) close to 3. In a study by Van Loo et al. (2007), done in the Netherlands, contact with cattle had an OR of 20 (45), indicating that those who have contact with cattle are 20 times more likely to be infected with MRSA. These studies suggest that colonization/infection with MRSA might be an occupational hazard for dairy farmers and veterinarians (44).

**Direction of transmission**

“Who infects whom?” This is a question that still represents a challenge for the scientific community. In 1975, when current molecular epidemiological tools were not available, Devriese suggested a human origin for the 68 MRSA isolates from Belgian dairy herds (46). Lee (2003), considering the rate of methicillin resistance among human *S. aureus* isolates in Korea (over 50%) and the low incidence of MRSA in animals (including cattle) suggested that the animal isolates may have originally come from humans (14). Turkyilmaz et al. analyzed 16 isolates of *S. aureus* recovered from mastitic bovine milk in Turkey. Fourteen of the 16 isolates were classified as ST239- SCCmec type III, a lineage that is associated with hospital-associated clones (47). This seems to suggest a human origin of the bovine isolates, transferred initially from the hospital (47). A human origin was also suggested by the results of the study by Haenni et al. (31).
Nearly 35 years have passed between the Devriese et al. (1975) and Haenni et al. (2010) studies, and they both suggest the same conclusion: the origin of bovine MRSA isolates is human. However, direction of infection is still unknown.

**MSSA, CNS, MRSP and MRSA: is there a link?**

Considering that the horizontal transfer of genetic material (48) between bacteria has been claimed to be the most significant way for the spread of antibiotic resistance (49), it is important to analyze the published literature about the potential connections between MRSA, Methicillin-Susceptible *Staphylococcus aureus* (MSSA) and other staphylococci. The relative importance of MRSA as a pathological agent differs between human and veterinary medicine. While MRSA has been recognized as responsible for more human deaths in the US than other infectious diseases, including HIV/AIDS (50), methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), also a coagulase positive Staphylococcus, is of major concern to veterinarians. There is the possibility of misdiagnosis of this agent in clinical microbiology laboratories.

In dairy cattle, *S. aureus* is a major contagious mastitis agent. Despite the fact that dairy cows have been treated with penicillin for decades (51) the relatively low resistance rates observed among *S. aureus* isolates from bovine mastitis (32) may be explained by the limited possibility of acquiring resistance genes in a virtually sterile compartment, such as the udder, where no indigenous bacterial flora is present (52). On the other hand, a variety of coagulase-negative staphylococci (CNS) are considered important minor mastitis agents. Importantly, CNS have been suggested as a source of SCCmec in the farming environment
(10) and the transfer of SCCmec from CNS to *S. aureus* can turn sensitive strains into resistant ones (10,53), i.e., MRSA.

Two recent human reports suggest the highly probable transfer of methicillin resistance between Staph. spp. *Staphylococcus haemolyticus* (a coagulase negative staphylococcus) was the probable donor of the SCCmec element to *S. aureus*, that led to a MRSA outbreak in a neonatal intensive-care unit in Sweden (54). Chlebowicz *et al.* reported the *in vivo* conversion of MRSA ST-398 to MSSA during a “community-acquired” human infection in which a mother and her daughter suffered from pneumonia and umbilicus phlegmone respectively (55).

At this point, the potential exchange of genetic material between CNS and *S. aureus* can speculatively be considered as one of the reasons for the increased prevalence reported in some recent publications. Considering the usual highly contagious spread of *S. aureus* in dairy farms, this fact can be of particular importance.

**Comparison of human and bovine isolates**

Using various molecular tools, other authors have studied the similarity between human and bovine isolates. Feßler *et al.* investigated the genetic relatedness of twenty-five MRSA ST 398 isolates from cases of bovine clinical mastitis (collected from 17 dairy farms in Germany) and two isolates from farm personnel (56). The two human isolates were indistinguishable geno-typically (Apal PFGE, *spa* typing, SCCmec typing and direct repeat unit (*dru*) typing) and phenotypically (broth microdilution antimicrobial resistance pattern) from the mastitis isolates of the same farm (56). Hata *et al.* first reported MRSA isolation from bovine milk in Japan. These authors analyzed 4 bovine MRSA isolates obtained
between May 1998 and May 2005 in Japan and evaluated their relationship with 9 human MRSA isolates: 3 of the bovine isolates showed identical genotypes to the human isolates (57).

Brodly et al. (2008) revealed that the human MRSA252 strain uniquely shares multiple DNA sequence blocks with three different etiological agents of contagious bovine mastitis (including *S. aureus*) but not with other human isolates (58). Turutoglu *et al.* (2009) sequenced the mecA genes of 3 MRSA isolated from bovine mastitis cases and found a very high homology with human MRSA isolates; all three bovine mecA genes were identical to those found in human MRSA isolates, except for a one-base substitution at nucleotide position 757 (59). Besides isolating MRSA from individual cows, calves and bulk tank samples (table 1), Spohr *et al.*, were able to find MRSA in 7 of the 9 tested herds personnel (nasal and oropharyngeal swabs). All isolates were the same spa type, spa-type t011. In the study by Haenni *et al.* (2010) (table 1), the MRSA isolate identified had identical characteristics to the human Geraldine clone (ST5, spa-type t002, and the same virulence genes, resistance pattern and SCCmec cassette type I).

The similarity between human and bovine isolates is surprising. Moreover when one considers that dairy cows are food producing animals and as such they have limited contact with humans compared to companion animals. The published literature seems to suggest that food (milk and other dairy products) may not be the most bridge between dairy cattle and humans (Figure 1). The role of wildlife (2) and the importance of environmental contamination and airborne transmission of MRSA between farms and neighboring residential buildings is currently unknown (60). Alvarado *et al.* (2009) evaluated the
concentration and seasonal variation of airborne fungi and (antibiotic resistant) bacteria in a dairy farm in southwest US (61). *S. aureus* was the predominant bacteria present, and more than half of the *S. aureus* found were resistant to one or more antibiotics (61). This is where we are today, without an explanation for the similarity of bovine and human MRSA isolates.

**Conclusions**

Despite an interesting historical background, MRSA references associated with dairy literature are scarce. Considering the small number of MRSA isolates found in the dairy arena, MRSA in dairy products seems to be a minor consumer or public health concern. The common use of pasteurization and the low levels of MRSA found in raw milk should also be seen as a reason for little concern (22,29). Recent and sporadic isolation of MRSA and related staphylococci from cattle in countries other than the US, and the similarity between some of the human and animal isolates found provide rationale for monitoring MRSA occurrence in cattle.

Considering the importance of *S. aureus* as a human infectious disease agent, its highly contagious typical behavior among dairy cows, and the current gaps in knowledge about the potential human:bovine connections (Figure 1), the epidemiology of MRSA (and other Staph. spp) in the dairy arena should represent a future area of attention by the scientific community.

Future research should include longitudinal studies, addressing the persistence of MRSA colonization in humans and cattle and exploring all the potential sources and reservoirs. It is critical that these studies are done as collaborations between human and veterinary medicine institutions. The legal framework that regulates these types of
collaborations must be made more reasonable and encouraging for researchers. International organizations should provide guidelines for the ideal uniformization of methods and protocols. Molecular characterization of MRSA isolates (SCC mec typing, spa typing, mecA PCR, MLST, PFGE) must be done to make it possible to track the path of dissemination of MRSA. Cost benefit analysis addressing surveillance and antimicrobial policy analysis should complement the epidemiological studies.

**Material and Methods**

*S. aureus* identification was performed in accordance with routine laboratory techniques, including typical colony morphology, gram stain, catalase and coagulase tests. DNA extraction was performed according to the UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010) instruction manual, with the exception of the initial step. *S. aureus* were plated on Trypticase Soy Agar (TSA) and incubated for 18 hours at 36 - 37 °C. A loop-full of this culture was scraped into 2 mL collection tube, to which 400 μL of MicroBead solution (Mo Bio Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010) and 20 μL of lysostaphin solution (1 mg/mL) were added. This mixture was incubated at 37 °C for 2 hours. Polymerase Chain Reaction (PCR) amplification of the mecA gene was performed as described by Lee (62). Antibiotic resistance pattern (Ampicillin, Cephalothin, Erythromycin, Cefoxitin, Novobiocin, Penicillin G, Ceftiofur, Sulfamethoxazole trimethoprim, Streptomycin, Tetracycline, Sulfisoxazole and Pirlimycin) was determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines. According to these guidelines, an isolate is classified as susceptible to cefoxitin (the antibiotic used to test “methicillin-resistance”), if the inhibition zone is \( \geq 22 \) mm.
References


(3) Food and Drug Administration, Department of Health and Human Services. Summary Report on Antimicrobials Sold or Distributed for Use in Food-Producing Animals. 2009.


(48) de Vries LE, Christensen H, Skov RL, Aarestrup FM, Agerso Y. Diversity of the tetracycline resistance gene tet(M) and identification of Tn916- and Tn5801-like (Tn6014) transposons in *Staphylococcus aureus* from humans and animals. J Antimicrob Chemother 2009 Sep;64(3):490-500.


CHAPTER 2: TRANSMISSION OF METHICILLIN-RESISTANT
S. AUREUS BETWEEN COMPANION ANIMALS AND INFECTED HUMAN PATIENTS PRESENTING TO OUTPATIENT MEDICAL CARE FACILITIES

Abstract

Methicillin-resistant S. aureus (MRSA) is a significant pathogen in both human and veterinary medicine. The importance of companion animals as reservoirs of human infections is currently unknown. The companion animals of 49 MRSA-infected outpatients (cases) were screened for MRSA carriage, and their bacterial isolates were compared with those of the infected patients using Pulsed-Field Gel Electrophoresis (PFGE). Rates of MRSA among the companion animals of MRSA-infected patients were compared to rates of MRSA among companion animals of pet guardians attending a “veterinary wellness clinic” (controls). MRSA was isolated from at least one companion animal in 4/49 (8.2%) households of MRSA-infected outpatients vs. none of the pets of the 50 uninfected human controls. Using PFGE, patient-pets MRSA isolates were identical for three pairs and discordant for one pair (suggested MRSA trans-infection p-value= 0.1175). These results suggest that companion animals of MRSA-infected patients can be culture-positive for MRSA, representing a potential source of infection or re-infection for humans. Further studies are required to better understand the epidemiology of MRSA human animal trans-infection.
Introduction

The epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) is dynamic \(^1,^2\). First identified in the 1960s, MRSA was initially considered a nosocomial pathogen. Beginning in the late 20\(^{th}\) century, a specific clone of MRSA, known as USA300, emerged as a leading cause of community-acquired infection \(^3^–^5\). Recently, another strain of MRSA, Sequence Type 398 (ST-398), has been shown to be strongly associated with livestock \(^6\), accounting for up to 20\% of all human cases of MRSA infection in the Netherlands \(^7\).

During this time, a growing number of reports have described probable transmission of *S. aureus* and MRSA, in particular, between humans and companion animals (table 4). Little is known, however, about the potential role of companion animals in the transmission of MRSA to humans. For example our understanding regarding direction of transmission, persistence of colonization, rate of animal-human transmission, trans-infection risk factors, animal population or breeds with increased risk to be carriers of MRSA and the significance of companion animals as reservoirs for human MRSA infections are all incomplete.

In the current study, we sought to investigate the significance of pets/companion animals as sources of MRSA infection or re infection for human outpatients by evaluating the frequency of MRSA transmission between MRSA-infected outpatients and their companion animals. Our results suggest that this reservoir might be more significant than currently considered.
Materials and methods

This cross-sectional study was a collaboration between a medical school and a veterinary college and was approved by Institutional Review Boards and Animal Care and Use Committees at both participating institutions.

Ascertainment of Cases and Control Groups

Between January and May 2010, MRSA-positive patients seen as outpatients at a large southeastern United States hospital were identified. Other inclusion criteria were an age of 18 years or older, ability to speak in English and residence within a 50 miles radius from the hospital. The health care providers of the patients meeting these criteria were contacted by study personnel to obtain permission to contact the individuals. If the health care provider consented, patients were contacted by phone to determine if they had companion animals. If patients lived with companion animals and consented to participate in the study, a household visit was scheduled to obtain nasal and rectal swabs from the animals to determine their MRSA status. A short questionnaire was given to the animal guardians on the day of the visit. The goal of this questionnaire was to identify trans-infection risk factors. Forty nine patients, 76 dogs, 25 cats, 3 hamsters and 3 horses were included in the study population (table 1). Thirteen adult family members voluntarily participated in this study, answering the questionnaire and self-collecting nasal swabs to determine their MRSA status.

Companion animals presenting to a veterinary institution wellness clinic and their guardians served as a control population (table 1). Animals were voluntarily taken to this
clinic mainly for prophylactic vaccinations, being otherwise generally healthy. The control population included 50 people and 45 dogs and 30 cats (table 1).

**Table 1**: Descriptive statistics of the cases and controls of this study.

| STUDY POPULATIONS | HUMAN | | |
|--------------------|-------|-------|
|                     | CASES | CONTROLS |
| Female              | 23    | 41     |
| Male                | 26    | 9      |
| Median age          | 49    | 28.5   |
| Mean age            | 47.86 | 32.2   |
| Asian               | 0     | 1      |
| Black               | 6     | 0      |
| Hispanic            | 1     | 1      |
| White               | 42    | 48     |
| Total:              | 49    | 50     |

<table>
<thead>
<tr>
<th>ANIMAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
</tr>
<tr>
<td>Cats</td>
</tr>
<tr>
<td>Hamsters</td>
</tr>
<tr>
<td>Horses</td>
</tr>
<tr>
<td>Total:</td>
</tr>
</tbody>
</table>

**Microbiological identification of MRSA isolates**

The clinical human MRSA isolates from the patients were collected from the Clinical Microbiology Laboratory of the medical school integrated in this project and stored (-80 °C) until required for additional use.

*Staphylococcus* spp. identification was performed in accordance with routine laboratory techniques, including typical colony morphology, gram stain, catalase and coagulase tests. *S. aureus* and *S. pseudintermedius* diagnosis was confirmed by multiplex
PCR. Resistance to oxacillin and cefoxitin was determined using standard disk diffusion. S. aureus isolates were classified as MRSA if the inhibition zone was less than or equal to 21 mm for cefoxitin or less than or equal to 10 mm for oxacillin. Oxacillin was used to determine susceptibility of the S. pseudintermedius isolates. When the inhibition zone was less than or equal to 17 mm, they were considered resistant.

meca PCR was performed on the human and animal MRSA isolates. Genetic relatedness was evaluated by use of pulsed field gel electrophoresis (PFGE) and spa typing, as previously described. Statistical analysis was performed with SAS 9.2 (SAS Institute, Cary, NC, USA).

Results

A total of 49 MRSA-infected outpatients (cases) and 50 uninfected (human) controls participated in the study (table 1). Overall, the human control population was younger and more frequently female. The animal case population was larger than the control population (total of 107 vs 75 animals) and included more dogs than the animal control population (76 vs. 45) (table 1).

Four out of the 49 (8.2%) human cases with culture-confirmed MRSA infections lived with a companion animal (2 dogs, 1 cat, 1 hamster) from which MRSA was isolated. One of the patients diagnosed with MRSA lived with a methicillin-resistant Staphylococcus pseudintermedius (MRSP) positive dog.

No MRSA or MRSP was found in the 13 family members of the MRSA-infected patients that voluntarily participated in this study, or in the 50 humans or 75 animals of the control population.
Using PFGE, three of the human:animal MRSA pairs were identical and one was discordant (Figure 1). Three of the four human:animal MRSA isolates pairs were classified as spa type 2 and clonal complex 5 (table 2).

**Figure 1:** PFGE comparison of the MRSA human and animal pairs. Three were identical and one discordant.

**Table 2:** Summary of the classification of the MRSA isolates, using spa typing.

<table>
<thead>
<tr>
<th>patient : animal pair</th>
<th>CDC classification</th>
<th>spa typing</th>
<th>clonal complex</th>
<th>Pair similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>patient 533 cat 533</td>
<td>USA 100 USA 100</td>
<td>type 2 type 2</td>
<td>cc 5 cc 5</td>
<td>identical</td>
</tr>
<tr>
<td>patient 547 dog 547</td>
<td>USA 300 not a common CDC-designated isolate</td>
<td>type 1 type 176</td>
<td>cc 8 cc 5</td>
<td>Non identical</td>
</tr>
<tr>
<td>patient 598 hamster 598</td>
<td>not a common CDC-designated isolate</td>
<td>type 2 type 2</td>
<td>cc 5 cc 5</td>
<td>identical</td>
</tr>
<tr>
<td>patient 609 dog 609</td>
<td>not a common CDC-designated isolate</td>
<td>type 2 type 2</td>
<td>cc 5 cc 5</td>
<td>identical</td>
</tr>
</tbody>
</table>
MRSA-infected case subjects were significantly more likely than controls to report (table 3):

a) Having children in the household
   (44.9% vs 8%; OR 4.28; 95% CI [1.67; 10.98])

b) Having a family member diagnosed with MRSA in the previous year
   (16.33% vs 2.04%; OR 9.37; 95% CI [1.12; 78.05])

c) Having been hospitalized in the previous year
   (31.25% vs 8%; OR 5.23; 95% CI [1.59; 17.18])

d) The presence of an immunocompromising condition
   (57.1% vs 6%; OR 20.89; 95% CI [5.71; 76.42])

e) Treatment with an antibiotic in the previous year
   (77.55% vs 36%; OR 6.14; 95% CI [2.53; 14.89])

Table 3: Univariable analysis of the variables potentially associated with MRSA carriage and human:animal transmission. “Don’t know” or “missing“ answers were excluded from the analysis. Legend: FM= family member; HCW=health care worker; AB=antibiotic.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n ; %)</th>
<th>Controls (n ; %)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you have a FM who is HCW?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7 (14.28%)</td>
<td>17 (34%)</td>
<td>0.32</td>
<td>[0.12, 0.87]</td>
</tr>
<tr>
<td>No</td>
<td>42 (85.71%)</td>
<td>33 (66%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you have a FM who is a veterinarian?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1 (2.27%)</td>
<td>9 (18%)</td>
<td>0.11</td>
<td>[0.01, 0.87]</td>
</tr>
<tr>
<td>No</td>
<td>43 (97.72%)</td>
<td>41 (82%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are there children in the household?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22 (44.9%)</td>
<td>8 (16%)</td>
<td>4.28</td>
<td>[1.67, 10.98]</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has a FM been treated with AB in the past year?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22 (44.9%)</td>
<td>14 (29.79%)</td>
<td>1.92</td>
<td>[0.83, 4.45]</td>
</tr>
<tr>
<td>No</td>
<td>27 (55.1%)</td>
<td>33 (70.21%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has a FM been diagnosed with MRSA in the past year?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8 (16.33%)</td>
<td>1 (2.04%)</td>
<td>9.37</td>
<td>[1.12, 78.05]</td>
</tr>
<tr>
<td>No</td>
<td>41 (83.67%)</td>
<td>48 (97.96%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Table 3 Continued</td>
<td>Were you hospitalized in the past year?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Yes</td>
<td>15 (31.25%)</td>
<td>4 (8%)</td>
<td>5.23</td>
<td>[1.59, 17.18]</td>
</tr>
<tr>
<td>No</td>
<td>33 (68.75%)</td>
<td>46 (92%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you been diagnosed with a disease or take medication that affects your immune condition?</td>
<td>Yes</td>
<td>28 (57.14%)</td>
<td>3 (6%)</td>
<td>20.89</td>
</tr>
<tr>
<td>No</td>
<td>21 (42.86%)</td>
<td>47 (94%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are you a HCW?</td>
<td>Yes</td>
<td>8 (16.33%)</td>
<td>3 (6%)</td>
<td>3.06</td>
</tr>
<tr>
<td>No</td>
<td>41 (83.67%)</td>
<td>47 (94%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aware of recent (past month) contact with person or animals MRSA positive?</td>
<td>Yes</td>
<td>7 (14.29%)</td>
<td>5 (10%)</td>
<td>1.5</td>
</tr>
<tr>
<td>No</td>
<td>42 (85.71%)</td>
<td>45 (90%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Were you treated with any AB in the past year?</td>
<td>Yes</td>
<td>38 (77.55%)</td>
<td>18 (36%)</td>
<td>6.14</td>
</tr>
<tr>
<td>No</td>
<td>11 (22.45%)</td>
<td>32 (64%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do any of your animals have currently sores?</td>
<td>Yes</td>
<td>7 (14.28%)</td>
<td>6 (12%)</td>
<td>1.22</td>
</tr>
<tr>
<td>No</td>
<td>42 (85.71%)</td>
<td>44 (88%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Were any of your animals hospitalized in the past year?</td>
<td>Yes</td>
<td>5 (10.20%)</td>
<td>6 (12%)</td>
<td>0.83</td>
</tr>
<tr>
<td>No</td>
<td>44 (89.80%)</td>
<td>44 (88%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are any of your animals allowed to go outdoors?</td>
<td>Yes</td>
<td>24 (48.98%)</td>
<td>11 (22%)</td>
<td>3.4</td>
</tr>
<tr>
<td>No</td>
<td>25 (51.02%)</td>
<td>39 (78%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are any of your animals allowed to move freely in the house?</td>
<td>Yes</td>
<td>36 (74%)</td>
<td>46 (92%)</td>
<td>0.24</td>
</tr>
<tr>
<td>No</td>
<td>13 (26%)</td>
<td>4 (8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are any of the animals allowed to lick human faces?</td>
<td>Yes</td>
<td>21 (42.86%)</td>
<td>37 (74%)</td>
<td>0.26</td>
</tr>
<tr>
<td>No</td>
<td>28 (57.14%)</td>
<td>13 (26%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are any of the animals allowed to sleep where humans sleep?</td>
<td>Yes</td>
<td>31 (63.27%)</td>
<td>37 (74%)</td>
<td>0.61</td>
</tr>
<tr>
<td>No</td>
<td>18 (36.73%)</td>
<td>13 (26%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you have contact with your animals everyday?</td>
<td>Yes</td>
<td>42 (85.71%)</td>
<td>45 (88.89%)</td>
<td>1.5</td>
</tr>
<tr>
<td>No</td>
<td>7 (14.29%)</td>
<td>5 (11.11%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Our results provide further evidence into the potential significance of companion animals as a source of infection and/or re-infection of humans/outpatients. These findings are particularly important, as MRSA is the most common identifiable cause of soft tissue infection in the US and it is estimated that about 75 million dogs and 88 million cats are owned in the US. Because companion animals are increasingly seen and treated as family members by their guardians, the opportunity for transmission between humans and pets is only likely to increase.

Our results are consistent with previous reports. Weese et al. (2006) studied the transmission of MRSA in veterinary clinics and in the households, after the identification of an MRSA positive animal. These authors described 6 cases. MRSA was isolated from 16% (14/88) of household contacts or veterinary personnel and in all of the 6 cases it was possible to find at least one human isolate identical to the animal (initial) one. More recently, Faires et al. evaluated both the rate of MRSA transmission from infected animals to humans and vice-versa. When the MRSA-infected animal was initially identified, at least one MRSA-colonized person was identified in over one-quarter (6/22; 27.3%) of the study households. By contrast, only one of the 8 (12.5%) study households of MRSA-infected humans contained a MRSA-colonized pet. By evaluating about 5 times the number of MRSA-infected humans as Faires et al. and finding a similar companion animal MRSA colonization rate (~8%), the current study externally validates the findings of the previous study. Our results clearly demonstrate that MRSA transmission between infected patients and
companion animals occurs. As summarized in Table 4, such transmission between humans and animals has been previously implicated as potential cause of recurrent MRSA infections.

**Table 4:** Previous reports of suggested human-pet (dogs and cats) MRSA trans-infection.

<table>
<thead>
<tr>
<th>Authors and year of publication</th>
<th>Ref.</th>
<th>Study design</th>
<th>Description and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scott et al., 1988</td>
<td>(8)</td>
<td>case report</td>
<td>The source of a MRSA outbreak on a rehabilitation geriatric ward was believed to be a patient shedding MRSA-contaminated skin scales. A cat that had been resident on the ward for 5 months was the most probable vehicle of the MRSA spread.</td>
</tr>
<tr>
<td>Cefai et al., 1994</td>
<td>(9)</td>
<td>case report</td>
<td>A staff nurse, his wife and their dog were all colonized with the same MRSA strain (EMRSA 1) that was found in two different patients that staff nurse worked with.</td>
</tr>
<tr>
<td>Manian, 2003</td>
<td>(10)</td>
<td>case report</td>
<td>Recurrent MRSA infections in a patient with diabetes and in his wife were only prevented after decolonization of the family’s apparently healthy dog. All MRSA isolates revealed similar PFGE patterns.</td>
</tr>
<tr>
<td>van Duijkeren et al., 2004</td>
<td>(11)</td>
<td>case report</td>
<td>First case reported of human-to-animal transmission of MRSA in the Netherlands. A nurse (that was screened due to an outbreak in a 190-bed nursing home), her daughter and their healthy pet dog were all colonized with the same (indistinguishable PFGE pattern) strain of MRSA.</td>
</tr>
<tr>
<td>van Duijkeren et al., 2005</td>
<td>(3)</td>
<td>case report</td>
<td>First transmission reported of a Panton-Valentine leucocidin (PVL) – MRSA strain between humans and an animal: a 51-year-old female patient remained a MRSA carrier even after several treatments and further sampling revealed that her husband, her son and their dog were all colonized with indistinguishable MRSA isolates.</td>
</tr>
<tr>
<td>Baptiste et al., 2005</td>
<td>(12)</td>
<td>case series</td>
<td>In Liverpool, the same MRSA strain (EMRSA-15) was found in dogs and staff members of a veterinary hospital.</td>
</tr>
<tr>
<td>Leonard et al., 2006</td>
<td>(13)</td>
<td>case report</td>
<td>In Ireland, indistinguishable MRSA were isolated from five dogs with wound infections after surgical procedures and from one veterinary surgeon.</td>
</tr>
<tr>
<td>Vitale et al., 2006</td>
<td>(14)</td>
<td>case report</td>
<td>First reported case of isolation of USA 300 MRSA in a household pet (a cat with multiple recurrent dermatologic problems) and its owner.</td>
</tr>
<tr>
<td>Sing et al., 2008</td>
<td>(15)</td>
<td>case report</td>
<td>MRSA spa-type t131 and ST80 (extremely rare in humans) were suspected to be transmitted from an apparently healthy cat to a woman with recurrent multiple deep abscesses and her family members.</td>
</tr>
<tr>
<td>Nienhoff et al., 2009</td>
<td>(6)</td>
<td>case series</td>
<td>Three MRSA positive dogs were identified in a prevalence study in Germany. 2 of them were further investigated. In one case, the guardian was a veterinarian specializing in swine diseases who was also MRSA positive (ST398). In the other, the dog owner’s elderly mother-in-law that lived in the same household and received nursing care at home was also MRSA positive, with the same spa type t014 and MLST type ST225.</td>
</tr>
</tbody>
</table>
Previous publications have described cases where human MRSA could not be linked with traditional MRSA sources in the community or health care facilities. This challenges the accepted epidemiology of MRSA and suggests that there are currently unrecognized/unknown sources of MRSA. Finding 5 out of 8 (62.5%) MRSA isolates that were not identical to any of the most common (and previously described by the Centers for Disease Control (CDC)) HA or CA MRSA clones seems to reinforce this idea.

Not finding MRSA in any of the humans or animals of the control population was surprising. Veterinarians have been described as a professional group with increased risk of carrying MRSA. Different prevalence studies have found very diverse prevalence values in small/companion animals. To our knowledge, prevalence in companion animals has never been determined in North Carolina, which makes it hard to evaluate the absence of MRSA in the animal control population.

Our study has limitations. Finding MRSA in both out patients and their companion animals is suggestive of inter species transmission of this agent. However, we can only speculate about transmission and there is the possibility that both parts became infected from different sources. Direction of transmission also cannot be determined. Finding 3 identical human:animal MRSA pairs is not statistically significant (p=0.1175) considering a reasonable significance level and therefore a larger sample size should be considered in future studies. The most ideal control population would have been the one formed by outpatients diagnosed with methicillin sensitive Staphylococcus aureus (MSSA) living with companion animals, with the same number of both humans and animals in the study and
control populations (a 1:1 ratio). Using the population of animals and their guardians that attended a wellness clinic was, therefore, a convenient and more readily available choice. We still believe, however, that this gave us an estimate of the prevalence of MRSA co-existence at the household level in healthy humans and animals in the general population.

**Other Staphylococcus spp. trans-infection**

The primary goal of this project was to study human-animal MRSA transmission. Increased attention has, at the same time, been given by the scientific community to other staphylococcus species (spp.) trans-infection 23-26. More recently, a novel staphylococcus has been identified: *Staphylococcus pseudintermedius* 27. Since *S. pseudintermedius* is coagulase positive, the possibility of misdiagnosis in clinical microbiology laboratories is possible and has to be taken into consideration 25, 28. Our finding of a human infected with MRSA living with an MRSP animal should be investigated in future projects. The exchange of genetic material between different species of staphylococcus has been repeatedly reported and emphasized 26, 29, 30, and its significance for human infections is currently unknown.

**Challenges and future research**

One of the most challenging aspects of this project was the enrollment of patients. Reasons for this included: difficulty in reaching the health care providers and patients, the non-existence of companion animals in the household, residences being outside the 50 mile radius, the inexistence of financial compensation to the participants, and patient or medical team declining participation.

Future research should focus on the dynamics of transmission. Longitudinal studies with multiple samplings of animals and humans will be critical in addressing questions
regarding direction of transmission and duration of colonization. Environmental samples should also be taken at the household level to identify other potential sources of reinfection. Staphylococcus diagnostic protocols should be carefully reviewed to make sure that the recently discovered coagulase positive staphylococci are included in the differential diagnosis list. Staphylococci should be characterized at the molecular level with different techniques (PFGE, multiplex PCR, multi locus sequence typing, spa typing) to allow a better comparison with different studies and traceability of the isolates origin.

Conclusions

Nearly 10% of MRSA outpatients lived with a MRSA pet. When faced with chronic and or recurrent MRSA cases, physicians should consider the possibility of household pets as MRSA source. Patients should be informed of this possibility. Unnecessary close contact should be avoided and heightened hygiene practices should be instituted. Sampling/swabbing of all the human and animals in a household seems appropriate to identify unrecognized sources and break potential cycles of re infection especially in cases involving immunocompromised patients. It is critical that medical and veterinary institutions partner and collaborate in researching this topic. The legal/institutional approval that regulates this type of partnerships should be expedited to encourage them. MRSA epidemiology is a perfect example of an infectious disease agent whose control requires a “One Health” approach.
Acknowledgements

The Clinical Microbiology Laboratory staff of Duke medical school that partnered in this project for their help with the recovery of the human MRSA isolates and continuous support, availability and collaboration in this project; Lawrence Park, for his assistance with statistical analysis and Thomas Rude for his assistance with laboratory analysis involving the human samples.

Funding sources

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North Carolina State University, College of Veterinary Medicine

Dr. Ferreira stipend was partially supported by Fulbright program.
References


CHAPTER 3: TRANSMISSION OF METHICILLIN-RESISTANT

*STAPHYLOCOCCUS AUREUS* (MRSA) BETWEEN HUMAN AND HAMSTER

Abstract

Transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) between humans and animals is increasingly recognized. We newly document that the transmission of MRSA between human and hamster is also possible.
Case report

We describe a case of suggested transmission of MRSA between a human and a pet hamster. This finding was one of the results of a project where MRSA-positive patients seen as outpatients at a large southeastern United States hospital were identified and contacted to determine if they had pets. If they had pets and consented to participate in the study, a visit was scheduled to obtain samples from pets to determine their MRSA status. The study developed as collaboration between a medical school and a veterinary college and was approved by Institutional Review Boards and Animal Care and Use Committees at both participating institutions.

The index patient was a 28 year old Caucasian male with advanced cystic fibrosis who had undergone initial bilateral lung transplant and repeat left lung transplantation. He also had chronic sinusitis that required three previous surgical procedures, diabetes mellitus, renal insufficiency, and presented with postnasal drip, cough, clear rhinorrhea, and headaches. He was diagnosed with chronic rhinosinusitis and underwent endoscopic ethmoidectomy, sphenoidotomy, and partial resection of bilateral nasal turbinates. Pre surgical culture of the patient’s sinus contents yielded MRSA and he was therefore contacted.

The clinical MRSA isolate from the patient was collected from the Duke Clinical Microbiology Laboratory and stored (-80 °C) until required for additional use. After written informed consent was provided by the patient, nasal and rectal swabs were collected from three hamsters at the patient’s residence. Nasal swabs were also collected from the patient’s housemate. Swabs from the animals were processed within a 24-hour time period at a
microbiology laboratory in the NCSU College of Veterinary Medicine Population Health and Pathobiology Department.

*S. aureus* identification was performed in accordance with routine laboratory techniques. Swabs were rolled on Trypticase Soy Agar plates (containing 5% sheep blood) and Mannitol Salt agar (BD, NJ, USA) and incubated at 35C-37C for 24 and 48 hours. Colonies with typical *S. aureus* colony morphology were further analyzed using gram stain, catalase and tube coagulase tests. *S. aureus* diagnosis was confirmed by multiplex PCR, targeting thermonuclease (*nuc*) gene locus (11). Resistance to oxacillin and cefoxitin was determined in the *S. aureus* isolates by disk diffusion. *S. aureus* isolates were classified as MRSA if the inhibition zone was less than or equal to 21 mm for cefoxitin or less than or equal to 10 mm for oxacillin (3).

Nasal and rectal swabs from one hamster (female, 1.5 years) yielded MRSA. The other two hamsters and the housemate were *S. aureus* culture-negative. *mecA* PCR was performed on the human and hamster MRSA isolates, and we evaluated their genetic relatedness using pulsed field gel electrophoresis (PFGE) and spa-typing as previously described (2, 8). The *mecA* gene was detected in both the hamster and patient MRSA isolates. PFGE banding patterns of the human and hamster MRSA were identical to each other (Figure 1) but not equivalent to the most common hospital-acquired or community-associated MRSA types previously described by CDC. All the isolates were spa type 2, clonal complex 5.
Figure 1: PFGE gel image comparing human and hamster Smal DNA digestion patterns.

MRSA is a significant problem for both human and veterinary medicine. MRSA infection has been described in several different animal species, and MRSA transmission between humans and different species has also been suggested (1, 4-7, 10, 12-14). Most of our current knowledge on this topic is based on anecdotal reports and several of the details of this interspecies exchange of MRSA are still unknown.

*S. aureus* has been previously isolated from hamsters (9). However, to the best of our knowledge there is no previous report of isolation of MRSA in a hamster. At the same time, this manuscript documents the first reported case of suggested MRSA transmission between a human being and a hamster.

The genotype of the hamster and human MRSA isolates were identical by PFGE banding patterns. The presence of MRSA with identical PFGE genotype on both the patient and his hamster strongly implies that hamsters are capable of carrying MRSA, and thus can potentially transmit it to pet owners. Conversely, patients who are colonized with MRSA may be also capable of transferring MRSA to hamsters.

The MRSA positive hamster was acquired from the same source (a pet store) as the other two hamsters. In the household, the MRSA-positive hamster was housed in the same cage as her sister but separately from the other hamster. The three hamsters had daily contact.
with each other. Patient would daily feed and hold/play with the hamsters, but was not responsible for cleaning their cages. He reported that he would always disinfect his hands with alcohol-based hand sanitizer after touching the hamster(s).

In the current case, we believe that the hamster most likely became a carrier following the patient, who was at high risk for long-term MRSA carriage given his immunocompromised state and co-morbidities. However, the hamster was not MRSA screened at the time of acquisition and had been living with the patient for about one year and four months before the patient had his first (blood) MRSA positive culture. Our assumption on the direction of transmission is therefore speculative. The possibility that both the hamster and the patient obtained their infection from a third party or perhaps fomite cannot be excluded.

We recognize that our study has other limitations. The hamster died while we were developing the study, which prevented us from collecting additional nasal swab samples so we were unable to estimate the duration of colonization. On the other hand, the patient had multiple MRSA positive samples (blood, sinus contents, nasal swabs, bronchoalveolar lavage) for a total period of approximately one year and four months, which included some months after the hamster’s death.

Despite these limitations, this report makes an important observation: MRSA exchange between humans and hamsters is possible. Should testing of MRSA positive patients’ pets be recommended? At this point we recommend MRSA positive patients to be informed that their companion animals can be a potential source of infection or re infection.
In the presence of a MRSA positive human or animal, heightened hygiene practices should be instituted and unnecessary close contact should be avoided. Screening of household pets might be indicated in situations of recurrent MRSA infections despite adequate treatment or when in the presence of immunocompromised patients. We speculate that the clinical significance of the findings are important for immune-compromised patients who keep pets in close proximity but at this point we cannot determine the prevalence or clinical significance of this phenomenon.

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References


CHAPTER 4: METHICILLIN-RESISTANT *STAPHYLOCCUS AUREUS* (MRSA) SCREENING AT A SMALL ANIMAL UNIVERSITY VETERINARY HOSPITAL: DO THE BENEFITS EXCEED THE COSTS?

Abstract

Antimicrobial resistance (AMR) has been recognized as one of the top three threats to human health. Methicillin-resistant *Staphylococcus aureus* (MRSA) is an example of a resistant organism. It has been reported globally and is a significant pathogen both for human and veterinary medicine. Different policies have been tested or are currently being utilized to control AMR/MRSA. One of them involves the screening of patients in hospitals. In human hospitals, the results of screening strategies are not uniform. Very little research has been done in this area of veterinary medicine. In this report we present the results of the cost-benefit analysis of universal screening for MRSA at NCSU veterinary hospital. The savings associated with this policy ($27,279,349) seem to overcome the costs ($240,140/year) even after sensitivity analysis, suggesting that it should be implemented, from a societal point of view [net benefit of $27,039,209].
**Introduction**

In times where there is intense debate in the American society about the entire health care system structure and the budget deficit with it associated, it seems critical to bring up in the public agenda cost-benefit studies, looking for the best use of public resources. Antimicrobial resistance (AMR) and methicillin-resistant *Staphylococcus aureus* (MRSA) are areas to which particular attention should be given. Here is why.

AMR has been recognized by the World Health Organization (WHO) as one of the top three threats to human health. In particular, Methicillin-resistant *Staphylococcus aureus* (MRSA) has been increasingly reported as an emerging and significant public health/antimicrobial resistance problem. It represents a disease burden 100-fold higher than that of tuberculosis (TB) (1) and has an annual mortality rate that exceeds that of HIV-AIDS (2).

Economically, AMR represents a negative externality (3). Although there is no consensus and accurate data regarding the economic costs of AMR, it is estimated that the range of annual costs are between $100 million USD and $30 billion USD, as a result of poor response to treatment, longer hospitalization and the use of more expensive treatments (4,5). Annual US health care costs associated with MRSA infections are estimated to be approximately $6 billion (6).

MRSA is still seen mainly as a significant human pathogen (2). However, multiple reports have described it in different animal species (7). More recently, much particular attention has been given to the suggested trans-infection between humans and animals with this agent (8-18).
The relationship between humans and small animals is of particular interest and concern. The use of antibiotics in food animals has been restricted for a long time, to prevent transfer of cross resistance. However, there has been no restriction on antibiotic use in small animals, which are still treated with the same classes of drugs as humans. North Carolina has actually been a leading region in the World recognizing the importance of the “One Health” concept, according to which human and animal health cannot be seen separately.

It is estimated that nearly 75 million dogs and 88 million cats are owned in the US, with pets being present in around 63% of American households. Animals are increasingly seen as “family members”, which translates into closer contact and sharing of the same environment and spaces (19). The increased proximity between humans and animals can also represent increased risk of trans-infection with resistant organisms (15), or the horizontal gene transfer between species (20).

Policies with the goal of controlling MRSA spread between humans and animals (pets) are necessary. MRSA screening of small animals and staff at veterinary hospitals entrance can be an alternative, as MRSA colonization is associated with an increased risk of MRSA infection (21) and can constitute up to 70% of the patient reservoir for cross-transmission (22). Active surveillance specimens can identify the reservoir of asymptomatic colonized patients who would otherwise go unrecognized and unisolated, and who would, therefore, be able to spread MRSA to other hospitalized animals, to their own owners and to the hospital staff. Stopping this cycle of transmission at animal hospitals has the potential to stop the spread of this bacteria to the communities and later to human hospitals. Even more than that, it might actually be a way to control the spread of other resistant organisms, particularly methicillin-
resistant *Staphylococcus pseudintermedius* (MRSP). The potential transfer of the *mecA* gene between MRSA and *S. pseudintermidius* (a recently described Staph spp.(23)) isolates explains this.

The resistance in MRSA strains is most commonly mediated through the expression of the *mecA* gene as penicillin protein binding 2a (PBP-2a). PBP-2a has a decreased affinity for β-lactam antibiotics, making them ineffective. This gene is found on a large *mobile* genetic element called the staphylococcal chromosomal cassette mec (SCCmec) and the possibility of horizontal *mecA* gene transfer between different Staphyloccci spp. has been reported (25-27). The significance of this phenomenon is currently unknown. *Staphylococcus pseudintermedius* has currently mainly significance for veterinary medicine. However, the first human cases of MRSP have already been reported (28,29) as well as cases of suggested trans-infection (30).

**MRSA policies**

Previous studies have achieved diverse conclusions regarding the cost-benefit (CB) of MRSA surveillance methodologies (1,31-33). Very little research as been done on this topic on veterinary medicine.

In the Netherlands, a national MRSA “search and destroy” policy has been implemented. This policy demands active screening, strict isolation, and decolonization of carriers on admission to health care facilities. This policy may explain the fact that the Netherlands has a MRSA prevalence of 1.0%, one of the lowest in Europe (34).
In the UK, it is mandatory to report MRSA infections in hospitals (35). In fact, the European Consensus Conference on the role of screening and decolonization in the control of MRSA infection recommended that in an environment where MRSA is endemic, universal or targeted screening of patients to detect colonization was considered to be an essential pillar of any MRSA control program (36).

In the US, Illinois, Pennsylvania, New Jersey, Minnesota, California, Washington and Maine have legislation requiring MRSA active surveillance (37). Other states have proposed MRSA active surveillance legislation in 2010 (Hawaii, Massachusetts, New York, South Carolina and Tennessee) (37). However, the Association of Professionals in Infection Control and Epidemiology (APIC) and Healthcare Epidemiology of America (SHEA) do not support legislation to mandate use of active surveillance cultures to screen for MRSA (38).

In North Carolina, human hospitals have different policies in place (e.g. Duke University screens high risk populations, Rex implements a screening procedure in the case of outbreaks and Pitt County Memorial Hospital does universal screening). University of North Carolina hospitals have performed universal screening of all the persons admitted to intensive care unit (ICU) for a year, and could not find a benefit in this procedure, and therefore discontinued it. Ohio State University Vet school is one of the few that has a MRSA policy in place. Currently NCSU vet hospital does not have a MRSA policy in place.

The objective of this paper is to provide a cost-benefit (CB) analysis of MRSA universal (all animals admitted and staff in the vet hospital) surveillance at the NCSU small animal hospital. This topic is of particular current relevance as this school is about to open a new small animals hospital.
Hospital characterization

NCSU small animal hospital provides 12 different specialty services\(^1\) and is divided in 4 parts:

1. General Hospital (up to 70 Patients)
2. Intermediate care (up to 32 Patients)
3. Intensive Care Unit (up to 18 Patients)
4. Triage/Emergency (up to 9 Patients)

**TOTAL:** up to 129 patients

The following table represents the number of people that work or visit the hospital.

**Table 1:** NCSU small animal hospital “staff”. Total “staff” = 632 (65+60+144+28+320+15)

<p>| | |</p>
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NCSU small animal hospital</td>
<td></td>
</tr>
<tr>
<td># veterinarians</td>
<td>65</td>
</tr>
<tr>
<td># residents/interns</td>
<td>60</td>
</tr>
<tr>
<td># technicians</td>
<td>144</td>
</tr>
<tr>
<td># administrative staff</td>
<td>28</td>
</tr>
<tr>
<td># students</td>
<td>320</td>
</tr>
<tr>
<td># clients</td>
<td>23,256/year</td>
</tr>
<tr>
<td># visitors</td>
<td>480/year</td>
</tr>
<tr>
<td># housekeeping</td>
<td>15</td>
</tr>
<tr>
<td><strong>TOTAL:</strong></td>
<td><strong>24,368</strong></td>
</tr>
</tbody>
</table>

The following sections will provide the necessary background/information for some of the numbers that will be used in the CB analysis.

How many animals and humans will be MRSA positive?

Comparing MRSA prevalences (either human or animal) is controversial. Geographic realities are very different, but the US and Japan seem to be among the countries with the highest prevalences. However, there are several regions in the world where data are scarce.

\(^1\) Cardiology, Dermatology, Oncology, Dentistry, Ophthalmology, Soft tissue, Surgery, Orthopedics, Internal Medicine, Nutrition, Behavior, Emergency/Critical Care.
In addition, there is usually no use of control groups or randomized trials, and the sampling and laboratory techniques and the populations studied are not uniform. Methodology standardization is clearly needed.

- **Human prevalences**

  Gorwitz et al. (2008) studied the changes in the prevalence of nasal colonization with *S. aureus* in the general population in the US in 2001-2004 and concluded that the prevalence of colonization with *S. aureus* decreased from 32.4% in 2001-2002 to 28.6% in 2003-2004 (p < 0.01), whereas the prevalence of colonization with MRSA increased from 0.8% to 1.5% (p < 0.005)(39).

  Veterinarians and those with close animal contact have been described as a population group with increased risk of carrying MRSA. Hanselman et al. (2006) isolated MRSA from nares of 6.5% (27/417) attendees at an international veterinary conference (40). Loeffler et al. (2005) isolated MRSA from 17.9% (14/78) of a small animal hospital staff in the UK (41,42).

  For this CB analysis, we will assume a prevalence of 10% in the veterinary population (nurses, vets and students).

- **Animal prevalences**

  MRSA was isolated from 0.5% (1/193) of dogs admitted to the Ontario Veterinary College Veterinary Teaching Hospital (42), which seems to be in accordance with the low prevalence found in other studies. Loeffler et al. (2005) isolated MRSA from 4/45 (8.9%) hospitalized dogs in a small referral animal hospital in the UK (41). The prevalence in cats
seems to be lower, perhaps reflecting the less intense contact with humans and other animals, making transfer of genetic material less common (41).

To the best of our knowledge the MRSA prevalence in companion animals in NC has never been estimated. Considering that NCSU small animal hospital is a referral hospital, the prevalence of MRSA in the admitted animals might be higher than in other studies. For this CB analysis, a 4% prevalence in admitted and hospitalized small animals will initially be assumed. Different values are tested in the sensitivity analysis section.

**MRSA diagnosis**

Timely and correct diagnosis of MRSA carriers is essential to an effective isolation policy, as it seems reasonable to expect that those humans that have been diagnosed with MRSA will have increased awareness and conscious behavior about infection control.

At NCSU clinical microbiology laboratory, in 2010, the following resistant Staphylococci spp. were diagnosed: 7 MRSA; 66 MRSP; 20 Coagulase negative Staphylococci; 7 *S. epidermidis* and 5 isolates of other Staph. spp., in a total of 105 resistant isolates in one year. About 80% of these isolates came from the dermatology service.

Nasal swabs are the most common MRSA sampling method, as the anterior nares are the most frequent site of MRSA colonization (43)(44). The sensitivity of single nasal swabs for detection of MRSA colonization is, however, unclear (45), but previous work suggests that a single culture from this site may have a sensitivity of approximately 85% (43)(44). For this CB analysis, only nasal swabs will be considered, although in an ideal scenario, at least nasal and rectal swabs should be collected (46).
In terms of laboratory methods, standard culture methods for the identification of MRSA are labor-intensive, require at least 48 hours to complete (6), and previous studies suggest that these cultures have a sensitivity of only about 18% (47). Polymerase chain reactions (PCR) tests for *S. aureus* colonization have better sensitivity than culture-based assays, but they may yield more false-positive results (48).

The ideal diagnostic test would be an easy-to-use real-time PCR assay suitable for specific detection of MRSA in nasal specimens in less than 1h (e.g. the IDI-MRSA system (GeneOhm Sciences, San Diego, CA, USA). This would save isolation time and the need for isolation infrastructures. However, to the best of our knowledge, there is not a test with such characteristic that would at the same time diagnose or differentiate between MRSA and MRSP. Therefore, despite the recognized disadvantages, for this cost-benefit analysis we will consider a diagnosis being performed with traditional culture techniques and disk diffusion tests, followed by multiplex PCR to accurately differentiate the different Staph. spp. (49). Considering manufacturers prices, a cost of $5.00/sample submitted for diagnose will be considered.

**Decolonization procedure**

Studies by Weese & Rousseau (2005) and Hanselman *et al.* (2008) suggest that transient contamination of the nares, instead of true contamination, cannot be dismissed and that otherwise healthy animals (dogs and horses) can eliminate MRSA colonization within a few weeks (42,45). This has particular importance when considering the implementation of a MRSA policy, because it suggests that a policy involving only surveillance, segregation and enhanced infection control precautions, without unnecessary use and exposure to antibiotics,
can potentially be effective, at least in animals. The efficacy of isolation in humans has been previously suggested (50).

Colonization with S. aureus in humans can occur soon after birth and at any given time (51), but the duration of MRSA colonization (or decolonization) is unclear (52). When identified, the most commonly used protocol to decolonize a human MRSA carrier involves topical (nasal) treatment with mupirocin (twice a day during five days). In addition, because multi-site carriage is known to be a risk factor for decolonization failure, whole body decolonization with baths using chlorhexidine is usually also recommended. However, the role of decolonization in the control of MRSA spread is also not clear.

The animal isolation and segregation approach will be the approach taken in the CB analysis considered in this paper, with only the hospital staff being considered eligible for decolonization treatment. Owners would receive educational materials on how to prevent trans-infection between them and the animals at home.
Cost-benefit analysis

This analysis can be done from several different perspectives: the hospital administration, the hospital staff, the animal/veterinary one, the animal guardian/hospital client one or from the viewpoint of society as a whole. This last option will be taken.

Similar outcomes are expected in the human and animal sides.

Expected outcomes:

✓ Decreased number of animal MRSA cases, specifically surgical wound infections, at NCSU vet hospital

✓ Decreased number of human NCSU staff MRSA cases

✓ Decreased number of MRSA human (pet owners) dermatology cases at hospitals

✓ Decreased use of second and third antibiotic therapeutic choices (human and vet hospitals)

✓ Decreased use of antibiotics in NCSU small animal hospital

✓ Increased savings in antimicrobial drugs

✓ Decreased hospitalization time

✓ Increased savings in caregiver time (veterinarians, technicians)

✓ Increased client satisfaction
Because MRSA is a significant skin pathogen (it causes 60% of skin infections seen in emergency rooms in the US (53)), the decreased number of human MRSA dermatology cases will be the focus of this analysis and the monetized outcome.

**Costs**

This surveillance program has the following costs associated:

- **Nasal swabs** (and disposable gloves) = $2.00/each

  Staff (632 people) will be swabbed once a year; Assuming that each client brings a single animal to the hospital (=23,256 animals/year)

  TOTAL “swabbed population” = 23,256 + 632 = 23,888 x $2.00 = $47,776 (swabs cost)

- **MRSA/MRSP diagnosis**: $5.00/each

  TOTAL “diagnosed population” = 23,888 x $5.00 = $119,440 (diagnosis cost)

- **Technicians’ time** = A technician salary is about $30,000/year + 25% benefits ($7,500) = $37,500; A year of work has 2080 hours x 60 min. = 124,800 min./year.

  37,500/124,800 = $0.30/minute x 10 min (estimated time to perform the surveillance policy) = $3.00/patient

  Technicians salary/time cost = 23,888 x $3.00 = $71,664 (salary cost)

- **MRSA decolonization treatment for staff** = $20.00/each

  Assuming 10% of the staff will be MRSA positive = 10% x 632 = 63.2

2 Nasal swabs, PCR tests and decolonization treatment costs have been estimated according with the purchase prices from the manufacturers.
63 x $20.00 = $1260 (decolonization cost)

Total cost of the MRSA surveillance policy to NCSU hospital = $240,140.00 / year

Savings

NCSU small animal hospital itself would not directly save any money. Reduction in costs associated with infections are opportunity costs, as fixed healthcare costs do not change if (MRSA) cases are lowered as it results in spare capacity in the hospital which can be used for other patients. The actual costs may in fact increase overall (35). The main financial advantage of such a policy is for the animals’ owners and society overall.

Engemann et al. (2003) estimated that the mean cost per MRSA case of skin and soft tissue infections (SSI) in patients undergoing surgery at Duke University Medical center and Duke Regional Hospital was $92,363. After adjusting for duration of surgery, hospital, length of hospitalization before infection, length of ICU stay before infection, renal disease and diabetes mellitus, the mean cost of these SSI cases attributable to methicillin-resistance was US$ 13,901(4). The 10-year average Consumer Price Index (CPI) for medical costs is around 4% per year, so inflating this value to 2011 dollars:

To calculate the associated savings, the following formula adapted from Lucet et al. (2003) can be used:

\[
\text{Savings} = \text{cost} \times \text{prevalence of MRSA carriage} \times \text{# patients who may acquire MRSA} \times \text{Probability (infection)}
\]

\[
\text{Savings} = $18,292.77^a \times 0.04^b \times 93,204^c \times 0.4^d = $27,279,349
\]

- **a)** estimated cost of treating a MRSA skin problem
- **b)** estimated prevalence of MRSA in admitted animals
- **c)** # clients (23,256) x 4 (family members) = 93,024
- **d)** 0.4 (according with Lucet et al., 2003)

**Sensitivity analyzing**

The following table (Table 2) shows the different values of savings that would be achieved considering different values of MRSA prevalences in animals and different probabilities of infection development.

**Table 2: Sensitivity analyzing**

<table>
<thead>
<tr>
<th>Prevalences</th>
<th>Savings</th>
<th>Probability (develop. inf.)</th>
<th>Savings</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>$6,819,837</td>
<td>0.10</td>
<td>$6,819,837</td>
</tr>
<tr>
<td>0.05</td>
<td>$34,099,186</td>
<td>0.25</td>
<td>$17,049,593</td>
</tr>
<tr>
<td>0.10</td>
<td>$68,198,373</td>
<td>0.5</td>
<td>$34,099,186</td>
</tr>
</tbody>
</table>
Discussion

The savings associated with this policy ($27,279,349) seem to overcome the costs (240,140/year), even after sensitivity analysis, suggesting that it should be implemented [net benefit of $27,039,209].

The potential disadvantages of a screening policy should however be remembered. These include: isolation (with unknown psychological and physical harms), delays in treatment, inappropriate allocation of resources or diminished health care quality (31,32). On the other hand, an (animal) false negative result might lead an owner to have diminished precautions with hygiene or infection control.

This analysis has however several limitations. The significance of the pet reservoir for MRSA human infection is not clear. Previous research by our group found that about 10% of MRSA outpatients lived with a companion animal from which it was possible to isolate at a colony of a methicillin-resistant Staphylococcus. Multiple assumptions have to be done has epidemiology answers are missing to questions like: What is the transmission risk between animals and humans when an animal is infected? In other words, it is not known how many humans an animal index MRSA carrier case infects. What is the prevalence of MRSA in companion animals in NC? Are there high risk populations/breeds of animals?

In a future review, the equine part of the NCSU vet hospital should also be included, although the most common MRSA equine types in North America are different from the human ones, and therefore equine MRSA does not seem to be a major public health problem (40). The cost of having the animals isolated during the hospitalization time has not been considered. Mortality benefits from avoiding infections have not been included as well as the
quality of life and survival effects (QALYs) of this MRSA intervention. Several expected outcomes were not monetized. We assumed a 100% efficacy on decolonization procedure, which is not realistic.

Ideally, a pilot longitudinal prospective study should be conducted in the future. Hospital environmental sampling should also be performed to evaluate the significance of this reservoir (54). Considering that 80% of the methicillin resistant Staph. spp. have been isolated from dermatology, this service could be the focus of a screening policy.

Conclusions

Antimicrobial resistance and MRSA control represent a challenge to the scientific community. Its complexity suggests that will never be one ideal policy to will be always effective, everywhere. A combination of multiple smaller policies seems to be the most probable combination to achieve success. This study results suggests that screening of animals in veterinary hospitals might be one of those effective policies. From the veterinary hospital economical perspective, this policy should not be implemented as in fact it would mean an increase in costs. However, there is a critical point that should be kept in mind: NCSU is a public institution, a public education institution, a public public health education institution. It has therefore ethical and societal responsibilities that should not be ignored.
References


Study title: Methicillin-resistant Staphylococcus aureus (MRSA) Trans-infection Between Humans and Animals: Pilot Study.

Purpose of the study

1) Determine the clinical significance of animals as reservoirs in human-animal MRSA trans-infection.

2) Compare human and animal MRSA isolates to determine percent homology. (We hypothesize a high degree of concordance between the isolates from the infected humans and the carrier animals)

3) Determine MRSA prevalence in apparently healthy animals. (We speculate this to be less than 5%)

4) Identify potential trans-infection risk factors.

Background & significance

*Staphylococcus aureus* (*S. aureus*) is a human and animal pathogen. It is a gram-positive bacterium that can cause diverse and severe clinical conditions in both human beings and animals. *S. aureus* that are resistant to the antibiotic methicillin (or similar ones), are known as methicillin-resistant *Staphylococcus aureus* (MRSA).

MRSA has been increasingly reported as an emerging and significant public health problem worldwide (1), both in human and veterinary fields.

It is one of the most prevalent pathogens that cause nosocomial infections (2), and infection with MRSA is associated with increased mortality, morbidity and hospitalization time (3-5). It has been estimated that about 5,500 people died per year (1999-2005) in the US due to MRSA infections (6). Antimicrobial resistance results in an annual cost of $100 million to $30 billion, as a consequence of poorer response to treatment, longer hospitalization, and the use of more expensive treatments (4, 5).

MRSA strains are resistant to β-lactam antibiotics, including all penicillinase-stable β-lactams, with resistance most commonly mediated by the *mecA* gene. This gene resides on a large mobile genetic element called Staphylococcal Chromosomal Cassette mec (SCC mec). It encodes for a penicillin-binding protein (PBP 2a) which is expressed in the bacterial
wall and has a low affinity for β-lactam antibiotics, making these antibiotics ineffective against these bacteria.

Jevons (1961) reported methicillin-resistance as early as 1961, but the possibility that dogs and cats could act as a source for zoonotic staphylococcal infections in humans was suggested even before that, in 1959 (7).

Several recent reports found similar or indistinguishable MRSA isolates in humans and animals suggesting trans-infection between species (8-21). However, finding similar isolates does not establish causality, and the direction of infection is unknown. It seems that animals are most often infected by humans, as opposed to pets serving as a reservoir for MRSA (22). However, the study by Manian (2003) showed that pets can act as a source of re-infection for humans.

This topic is of particular and timely interest, as the relationship between pets and their guardians has changed. Pets are increasingly seen and treated as family members and allowed a high degree and frequency of contact with humans (23). The significance of this routine contact with household pets on the global epidemiology of MRSA is still unknown (24), and there are limited data concerning the risk factors (Hanselman et al., 2009, article in press).

Published literature suggests that the prevalence of MRSA colonization in the general small animal population is low (22). A UK study (16) failed to isolate MRSA from 55 dogs sampled in the community over a two month period and only 1/255 dogs was positive in another study (25). However, US studies have identified MRSA at much higher frequency than in Europe (Nuttall et al., 2008): 15% of healthy cats (26) and 5% of healthy dogs (27). In the US and Canada the predominant human strain, Canadian epidemic MRSA-2 (USA 100), is most common in animals (8, 22).

Data on the prevalence of MRSA in apparently healthy pets and about the ease of transmission between animals and humans are needed to establish preventive measures against infection/re-infection. In this project we will be working mainly with dogs and cats, but, if outpatients have other animals, we will also sample them.

**Design & procedures**

The initial focus of this project will be outpatients attended at Duke Medical Center or one of the Duke clinics who have a positive culture for *S. aureus* where an antibiotic susceptibility test was performed (indicating that the isolate is clinically significant).

Our intent is to initially enroll 50 Duke patients with MRSA and their family members and their animals. Procedures for sampling involve the collection of nasal swabs.
from patient family members (or farm workers) and nasal, rectal and skin lesions (if present) swabs from animals as follows:

1. For 50 Duke patients: Each patient will be asked to provide information about the study to adult family members with whom they live. If the family members are willing to volunteer for the study, they will be asked to provide consent. Consenting family members (or farm workers) will be asked to have one nasal swab collected and to complete a questionnaire. We anticipate a total of 50 patients and a total of approximately 150 family members. They will also be asked to consent to allowing nasal, rectal or skin lesion (if present) samples to be taken from their pets or other animals.

2. When necessary, animals will be appropriately restrained (see IACUC for further description) by Patrick Brinson (NCSU animal sciences senior student and all animals in the household will be sampled by Dr. Ferreira (veterinarian and NCSU+Duke graduate student). A nasal and a rectal swab will be collected from each animal, and from potential skin lesions. We estimate that the 50 household will have no more than 100 animals total.

Consent process. Dr. Ferreira will get daily access to the Staphylococci isolates analyzed the day (days) before at Duke medical center clinical microbiology laboratory. Together with these plates, he will also get a printed list of the patients that were diagnosed with MRSA, in the previous day (days). Dr. Ferreira will screen these plates and records (using the Duke e-browser system) to verify if patients meet the initial inclusion criteria (briefly: outpatients, MRSA diagnosis, leaving in a 50 miles radius from Duke, 18 or older; see following section for further details)

Dr. Ferreira will then contact the attending physician (by e-mail or phone) to ask for permission to approach the patient. If permission is received, Dr. Ferreira will contact the patient, briefly explain the project, and ask for oral consent to participate in the study. If the outpatient gives oral consent to participate in the study and meets the inclusion criteria, a sampling date will be coordinated.

After a written consent form is signed (patients will keep a non-signed copy), nasal swab samples will be collected from the subject at the place of residence. Samples will also be collected from the pets or other animals by Dr. Ferreira.

The nasal swab procedure will be performed by removing the swab from the transport tube and inserting the swab 2 cm (about \(\frac{3}{4}\) inch) into one naris. The swab will be rotated against the anterior nasal mucosa for three seconds. This procedure will be repeated using the same swab in the second naris. The swabbed specimen will be returned to the transport tube, labeled with a preprinted label, and transported to the laboratory.

For the animal population only, the rectal sampling procedure is performed by inserting a Culturette swab into the rectum, then rotating the swab to collect fecal material,
and carefully removing the swab from the rectum. The swabbed specimen will be inserted in the transport medium, labeled with a preprinted label, and transported to the laboratory.

If present, skin lesions (in patient family members and animals) will also be swabbed. These are minimally invasive sampling techniques and they are not considered painful procedures. Therefore we expect to need only minimum physical and short-time restraint of the animals. When required, Patrick Brinson, NCSU animal sciences senior student, will be asked to restrain the animals appropriately (please see IACUC submitted documents for further details).

A short questionnaire will be given to the pet/animal owner the day of the visit. The goal of this questionnaire is to identify trans-infection risk factors. Following are some examples of questions we will ask MRSA cases (non-exhaustive list): human case-pet contact (e.g. dog allowed to lick the face of the patient), animal(s) recent health history (e.g. skin sores not responding to treatment in small animals), frequency of hand washing following contact with pets and information on whether other family members have been tested for MRSA.

Animal samples will be analyzed at the Milk and Mastitis laboratory of North Carolina State University (NCSU), managed by Dr. Kevin Anderson. Human samples will be analyzed at the Duke Infectious Disease laboratory, managed by Dr. Vance Fowler.

*S. aureus* isolates from both humans and animals will be further characterized and compared using molecular techniques described elsewhere such as Polymerase Chain Reaction (PCR) of the *mecA* gene and Pulsed Field Gel Electrophoresis (PFGE). All the PFGE analysis will be performed at Dr. Fowler’s lab.

**Subject recruitment and selection**

**Identification of patients with infections due to Staphylococcus aureus.** Health Insurance Portability and Accountability Act (HIPAA) compliant procedures will be used to identify subjects with *S. aureus* infections for the proposed study. Each workday, the research staff will receive notification from staff working on the culture bench of the Duke Clinical Microbiology Laboratory (DCML) of all patients at DUMC with at least one culture positive for *S. aureus*. This system allows immediate notification of potential subjects with *S. aureus* infections. A Waiver of Consent and HIPAA Authorization will be obtained from the Duke Institutional Review Board (IRB). The research personnel will determine the patients’ eligibility using criteria (below), then contacts the patients’ physician (preferably the attending) for permission to approach the patient about study participation.
Inclusion criteria for cases are as follows:

- Adult (equal to or greater than 18 years of age) with culture-confirmed Staphylococcus aureus infection;
- Attended as outpatient at Duke or is his/her family member/farm worker;
- Live within a radius of 50 miles from Duke Medical Center;
- Have animals at home;
- English speaker;
- Allow sampling of animals using the previously described sample collection procedures.

Ascertainment of subjects with Staphylococcus aureus infections. These cultures are obtained as a matter of routine clinical care from patients at Duke with suspected bacterial infection by their healthcare-practitioners, in order to confirm a diagnosis (e.g., MRSA soft tissue abscess), and guide treatment decisions (e.g., based on antimicrobial susceptibility of the infecting pathogen). Decisions regarding treatment are always made based on the features of the individual patient for whom these decisions are being made; however, the majority of the patients from whom cultures were obtained are expected to receive treatment.

All demographic groups of patients will be equally considered for inclusion in this study as long as they meet the previously defined criteria.

Control Population

A control population for this study is being derived from an IRB and IACUC approved protocol at NC State University. We will use the dogs and cats attended at NCSU small animals wellness clinic as a control population for the project. Our goal is to have a 1:1 match (for each subject dog/cat sampled: a control dog/cat will be sampled). We have been working with dogs and cats that are owned by MRSA patients at Duke, and are interested in having at least an estimate of, for example, the MRSA prevalence in dogs owned by apparently healthy people. All the procedures (including consent forms and questionnaires) used will be similar to the cases enrolled at Duke with minor modifications to the forms as for the original "study population". NCSU and Duke will both store a copy of all the human and animal MRSA bacterial isolates. At Duke the data and specimens will be stored for possible future research under e-IRB # Pro00008031, Bloodstream Infections Database/Repository.

Risk/benefit assessment

Nasal, rectal and skin lesions swabs used to diagnose MRSA are considered minimally invasive and painless techniques. Minimum physical restraint may be required in order to complete sample collection procedures for animals.
There are no direct benefits to study participants. However, if interested, they will get
the results of their animals. One of the main long range benefits to participants is the
prevention of re-infection. Considering this, patients will get an official results report form
(regarding both animal and human results). To this results report (see attachment), a brief
explanation about “What to do if your animal is positive/negative” will be given. Study
participants will be able to find out if their animals can be the source of their (re)-infection
and which of their behaviors increase the risk of getting infected. This information will allow
them to take measures to prevent re-infection.

The loss of confidentiality is a risk that subjects face from the data collection part of
this project. This risk will be minimized by storing patient identifiers in an encrypted
(coded) format such that all patients will remain anonymous if any publication originate from
this data. Confidentiality is maintained by the use of password access to the data with the
password known only to the project staff and the principle investigator, Dr. Fowler.

Data storage & confidentiality

Data will be stored at Duke with one faculty assigned to keep patient information
confidential. Patient identifiers will be removed in all files that are shared with other faculty.
The project results will be retained in the research record for at least six years, or until after
the project is completed, whichever is longer. At that time, either the research information
will be destroyed or information identifying the subjects will be removed from such project
results at DUHS.

Data & Safety monitoring

Data quality assurance is incorporated in the study at all levels by having a small
number of individuals involved and trained in the collection and interpretation of data. One
person is doing all sample collection. All faculty involved have worked together in the
development of the spreadsheets, questions, coding and editing.

Data Analysis & Statistical Considerations

Statistical analysis: Data will be classified into nosocomial or community-acquired
MRSA infections and associated with the results from the sampled animals. Frequency
distributions for different known MRSA risk factors paired with strain isolation and
identification will be obtained. Contingency table and non-parametrical statistical tests (e.g.
Chi-square or Fisher’s exact test using a liberal p-value ≤ 0.10) will be used to determine, for
example, the proportion of MRSA human cases that have the same MRSA strain as their
pets, or if the same MRSA strain is isolated from community-acquired human cases and
dogs.
This is a pilot study, which will provide initial data for a study with higher statistical power.

The data collection part of this study will last 4 to 6 months: January-June 2010.

**Costs to the subjects, and compensation**

The subjects will have no costs or compensation as a result of their participation.
November 19, 2009  
Dr. Vance Fowler  
Box 102359, DUMC  

Dear Dr. Fowler,  

Re: MRSA TRANS-INFECTION BETWEEN HUMANS AND ANIMALS (PILOT STUDY)  
Protocol Registry Number A329-09-11  

On November 19, 2009, the Duke University & Duke University Medical Center Institutional Animal Care and Use Committee (IACUC) reviewed and approved the above referenced protocol. The terms of this approval are as follows:  

Approval Period: The date of approval for this protocol is November 19, 2009. Approval is for three years from this date, contingent upon annual reporting of protocol activity.  

Approved Number of Animals: The species and numbers of animals approved for the full three-year period of this study are:  
100 DOGS; 100 CATS; 5 HORSES; 5 CATTLE; 5 SWINE; 5 SHEEP/GOATS  

The Committee agreed to process amendments for new species as minor with veterinary review.  

Annual Reporting: Continued approval during this three year period is contingent upon the timely submission of Annual Review reports. The report forms are available / downloadable from the animal program website. The web address is: http://vetmed.duke.edu/index_of_forms.html. Annual Review reports must be received and approved by the anniversary date of the original approval for continued approval of your protocol. All personnel listed on the approved protocol must be current on employee health surveillance and training requirements; supervisors can assess the status of employees health and safety status on the CESO web site (see Management Reports). The Office of Animal Welfare Assurance will alert you to the approaching annual report date so that you may respond promptly with your report.  

Protocol Access: Information contained in your animal use protocol is considered privileged. The policy that governs access to the file can be viewed here: http://vetmed.duke.edu/documents/iacuc/pdf/policy_on_IACUC_review_and_approval_practices- 
protection_of_protocol_information.pdf  

Renewal of the Protocol: Federal requirements dictate a complete new review of continuing studies at the end of the three-year approval period. If you desire continuation of the protocol beyond the current (3 year) approval, you will need to submit a renewal application for review and approval by the IACUC. This renewal application must be a de novo submission; the IACUC cannot consider the current document in its present form. The Office of Animal Welfare Assurance will alert you at approximately the 32nd month of your current approval period. At that time, please prepare a new application for animal use and submit it before the applicable deadline. Please use the most current protocol template, also available on the animal program web site. Without renewed IACUC approval, ongoing research under the retiring protocol must be halted. Any remaining animals must be: 1) turned over to DLAR for disposal; 2) transferred to another approved protocol; or 3) transferred to the DLAR holding protocol (you cannot use any animals in active research being held on the DLAR protocol). With IACUC approval of a new protocol submission, a new protocol registry number will be assigned. Any cage cards for existing cages of animals should be transferred to the new protocol number. You will need to contact DLAR so that they can prepare cage cards for you, and your staff should replace the retiring cage cards with the cards that reflect the new registry information. Failure to promptly transfer the animals to the new protocol registry number may result in the placement of new cage cards by DLAR staff with assessment of a service charge.  

THE DUKE UNIVERSITY ANIMAL CARE & USE PROGRAM IS COMMITTED TO ADVANCING HEALTHCARE FOR HUMANS AND ANIMALS THROUGH COMPASSIONATE CARE AND PROGRESSIVE ANIMAL USE.
Principal Investigator Responsibilities: Use of animals for research, testing, teaching, production, or exhibition must be in accordance with the USDA Animal Welfare regulations, PHS Policy on Humane Care and Use of Laboratory Animals, the NIH/NRC Guide for the Care and Use of Laboratory Animals, AAALAC accreditation guidelines, and Duke University Institutional Animal Care and Use Committee (DIUACUC) care and use policies. These references materials are available for review on the animal program web site at http://vetmed.duke.edu.

Personnel Performing Work Under This Approval: All personnel working with animals must be enrolled in an appropriate occupational health and safety program. The Duke University Occupational Health Program is available to all Duke students, employees and staff. Enrollment forms are available from the animal program web site. If you determine that additional personnel should be associated with this approved activity, then you must notify the IACUC of these personnel changes to the protocol, including changes in roles for existing personnel, and the addition or deletion of animal care and use personnel. These changes must be approved by the IACUC before personnel can begin work with animals. Except for a change in the Principal Investigator, a Minor Amendment form should be used for this purpose, also available on the animal program web site at http://vetmed.duke.edu/index_of_forms.htm.

Amendment of the protocol: Approval for any change to the protocol (whether Significant or Minor) must be obtained from the IACUC prior to implementation of the change. Forms for requesting either a Minor or Significant Changes are available / downloadable from the animal program web site at http://vetmed.duke.edu/index_of_forms.htm.

Post-Approval Monitoring of Protocols: Duke University and Duke University Medical Center is fully committed to quality animal care and compassionate animal use in an atmosphere of progressive animal based research. To fulfill our legal, ethical, and moral obligations under federal regulations, funding commitment, and accreditation principles, the institution will perform post-approval monitoring of approved activities:

A. All animal use areas are inspected every 6 months by a subcommittee of the IACUC. When this activity is required, the Office of Animal Welfare Assurance may contact your staff and determine a convenient schedule to visit your laboratory. While these visits are usually announced, the IACUC has the obligation to perform unannounced visits on occasion.

B. A second method of meeting public expectation of animal research management is through the Office of Animal Welfare Assurance’s Compliance Liaison Program (CLP). These individuals assure research integrity for the institution while facilitating your research needs and goals. The institution’s Liaisons may perform either scheduled or un-announced visits to the animal research environment. While the goal is a fully compliant audit, any correction of issues discovered during a compliance visit will be facilitated by the CLP. The Liaisons will partner with your laboratory to keep your research fully productive and your adherence to the plethora of rules and regulations fully engaged.

At any time, please visit the animal program web site for the latest in program information. You are also encouraged to use the IACUC’s Email address IACUC@Duke.edu for all of your correspondence and communication needs.

Please do not hesitate to contact me if there is anything that we can do to facilitate your research.

Sincerely,

James D. Reynolds, Ph.D.
Chairman, IACUC

The Duke University Animal Care & Use Program is committed to advancing healthcare for humans and animals through compassionate care and progressive animal use.
NORTH CAROLINA STATE UNIVERSITY
APPLICATION FOR VERTEBRATE ANIMAL USE
(Revised by IACUC office 06-30-2009)

NOTE: BEFORE COMPLETING THIS FORM, READ INSTRUCTIONS AT http://www.ncsu.edu/iacuc/forms.html

If this application covers a clinical study involving privately owned animals, please attach a copy of the client consent form. If this application covers animal use for teaching, please attach a copy of the course syllabus. Handwritten forms will not be accepted.

Title of project: MRSA human animal trans-infection pilot study

The IACUC Administrator will review a copy of your funding application. Please provide the following information:

Submitted to (Name of Funding Agency, if applicable):

Agency Deadline:

If the Committee has approved this use previously, please indicate:

The IACUC ID# of the previous protocol

And expiration date:

A COPY OF THIS APPROVED PROTOCOL WILL AUTOMATICALLY BE SENT TO THE ANIMAL HOUSING FACILITY NAMED IN THE CONFIDENTIAL SECTION OF THIS DOCUMENT.

APPROVAL IS RENEWABLE ANNUALLY FOR UP TO AN ADDITIONAL TWO YEARS. RENEWAL IS ACCOMPLISHED THROUGH COMPLETION OF THE 2-PAGE ANNUAL RENEWAL FORM (http://www.ncsu.edu/iacuc/forms.html). CONTINUATION OF THE APPROVED ANIMAL USAGE BEYOND THREE YEARS REQUIRES COMPLETION OF A NEW APPLICATION/PROTOCOL FORM AND COMPLETE IACUC REVIEW.

ANY CHANGES TO THIS APPROVED PROTOCOL REQUIRE THE SUBMISSION OF A PROTOCOL AMENDMENT FORM (http://www.ncsu.edu/iacuc/forms.html). CHANGES SHOULD NOT BE IMPLEMENTED PRIOR TO IACUC REVIEW AND APPROVAL.

Please retain a copy and, as appropriate, submit a copy with your application to various University offices through which applications must be routed, or send a copy directly to the review group or project officer in the Funding Agency for your project.

Date of Final Review and Approval: APR 1 4 2010 Expiration Date: APR 1 3 2011

Chairman, Institutional Animal Care and Use Committee

This institution has an Animal Welfare Assurance on file with OLAW (#A3331-01).
From: Carol Mickelson, IRB Coordinator
North Carolina State University
Institutional Review Board

Date: May 19, 2010

Project Title: MRSA Human Animal Trans Infection Pilot Study
(‘Control’ at NCSU)

IRB#: 1417-10

Dear Dr. Ferreira;

The project listed above has been reviewed by the NC State Institutional Review Board for the Use of Human Subjects in Research, and is approved for one year. **This protocol will expire on May 18, 2011 and will need continuing review before that date.**

NOTE:

1. You must use the attached consent forms which have the approval and expiration dates of your study.

2. This board complies with requirements found in Title 45 part 46 of The Code of Federal Regulations. For NCSU the Assurance Number is: FWA00003429.

3. Any changes to the protocol and supporting documents must be submitted and approved by the IRB prior to implementation.

4. If any unanticipated problems occur, they must be reported to the IRB office within 5 business days by completing and submitting the unanticipated problem form on the IRB website.

5. Your approval for this study lasts for one year from the review date. If your study extends beyond that time, including data analysis, you must obtain continuing review from the IRB.

Sincerely,
Carol Mickelson
NC State IRB
Consent to Participate in a Research Study  
*Title: MRSA Trans-infection Between Humans and Animals: A Pilot Study*

You are being asked to take part in this research study because you and/or your family members have a methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Research studies include only people who choose to take part. Please read this consent form carefully and take your time making your decision. As your study doctor or study staff discusses this consent form with you, please ask him/her to explain any words or information that you do not clearly understand. We encourage you to talk with your family and friends before you decide to take part in this research study. The nature of the study, risks, inconveniences, discomforts, and other important information about the study are listed below.

**WHO WILL CONDUCT THE STUDY?**
Dr. Jorge Ferreira will conduct the study and will be in touch with your regular physician as needed.

**WHY IS THIS STUDY BEING DONE?**
The purpose of this study is to better understand how humans and their animals infect each other with bacteria called methicillin-resistant *Staphylococcus aureus* (MRSA). This is a microbe (bacteria) that can infect both humans and animals and potentially cause serious infections in both. Infections passed between animals and people have been reported—however, very uncommonly.

**WHAT IS INVOLVED IN THE STUDY?**
If you agree to be in this study, you will be asked to sign and date this consent form. You will be given a copy of the consent form to keep.

Nasal swab samples will be collected from you. If you are a family member of a Duke patient, the samples will be collected from you at your home. Nasal, rectal and skin lesions (if present) swabs will also be collected from your animals by Dr. Ferreira. Collecting the swabs is simple, quick, and not painful. It involves putting a soft cotton tipped swab just inside the cavity and lightly rolling the swab around. Therefore, we expect to need only minimum physical and short-time restraint of the animals. Only animals that have been vaccinated are eligible for this study. We may ask for your help to restrain your animals but another research staff will be available to assist with restraining animals.

A short questionnaire may be given to you on the day of the visit. You will complete the un-shaded areas of the questionnaire. A medical record review will be performed to obtain the information in the shaded areas of the questionnaire. The goal of this questionnaire is to better understand factors that may be involved in the potential passing of MRSA infections between animals and people, and vice versa. Some examples of the questions we will ask relate to human case-pet contact, e.g., dog allowed to lick the face of the patient, animal(s) recent health history, e.g., skin sores not responding to treatment in small animals, frequency
of hand washing following contact with pets, and information on whether other family members have been tested for MRSA.

Duke University will maintain these samples indefinitely or until the samples are exhausted. These samples are unavailable for clinical (diagnostic) purposes. Duke University will assert all rights of ownership in the samples. Research done with your sample may help to develop new products in the future. Duke University and/or the developers will assert all rights arising from use of the sample. In this event, there is no provision to provide financial compensation to you.

**RIGHT NOT TO PARTICIPATE OR WITHDRAW**

You may choose not to participate in this study. If you agree to participate, you may withdraw from the study at any time. If you withdraw from the study, no new data about you will be collected other than the data needed to keep track of your withdrawal.

Your decision not to participate or to withdraw from the study will not involve any penalty or loss of benefits to which you are entitled. Nonparticipation or withdrawal from this study will not affect your job status if you are a Duke employee and will not affect your grades of you are a Duke student. If you do decide to withdraw, we ask that you contact Dr. Vance Fowler or Dr. Jorge Ferreira in writing. The mailing address is: DUMC, Department of Medicine, P O Box 102359, Durham, North Carolina 27710.

If you decide to withdraw your permission to use your samples in this research, please contact Dr. Fowler in writing and let him know that your are withdrawing your permission for your samples to be stored and used for research. His mailing address is above. At that time, we will ask you to indicate in writing if you want your unused samples destroyed or if your samples (with all identifying information removed that would link the sample to you) could be used in research.

**HOW LONG WILL I BE IN THIS STUDY?**

Data and sample collection for this study will be completed over a period of four (4) to six (6) months. The length of your involvement will depend on how long it takes to set up a date to collect the required specimens and complete the questionnaire. Collecting the swabs and completing the questionnaire should take no longer than 45 minutes.

**WHAT ARE THE RISKS OF THE STUDY?**

Although the physical risks in this study from collecting samples are low, there is rare (1 in 1000) risk of transmission of infections. Precautions will be used by study staff to prevent the transmission of infections from one household member to the other (and animals).

Sampling techniques used for the animals are minimally invasive and are not considered painful procedures. Therefore only minimum physical and short-time restraint of the animals is expected. There may be minimal risk associated with this procedure. For example, the
animals may become agitated, injured; or the animal may attempt to escape. However, no major health complications are expected, with the exception of the small risk of bleeding. From the questionnaire and collection of information from your medical records, there is the potential risk of loss of confidentiality. Every effort will be made to keep your information confidential; however, this can not be guaranteed. Some of the questions we will ask you as part of this study may make you feel uncomfortable. You may refuse to answer any of the questions and you may take a break at any time during the study. You may stop your participation in this study at any time.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?
If you agree to take part in this study, there is no direct medical benefit to you.

We hope that in the future the information learned from this study will benefit other people with your condition; specifically to prevent passing infection between people and animals. If you are a family member of a Duke patient, the laboratory results of the swab samples will be mailed to the address provided on the questionnaire. Laboratory results for your animals will also be mailed to this address.

WILL MY INFORMATION BE KEPT CONFIDENTIAL?
Study records that identify you will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of Duke University Health System (DUHS). For records disclosed outside of DUHS, you will be assigned a unique code number. The key to the code will be kept in a locked file and a password-protected computer.

Your records may be reviewed in order to meet federal or state regulations. Reviewers may include representatives from the Food and Drug Administration and the Duke University Health System Institutional Review Board. If any of these groups review your research record, they may also need to review your entire medical record. If this information is disclosed to outside reviewers for audit purposes, it may be further disclosed by them and may not be covered by the federal privacy regulations.

The study results will be retained in your research record for at least six years, or until after the study is completed, whichever is longer. At that time either the research information not already in your medical record will be destroyed or information identifying you will be removed from such study results at DUHS. Any research information in your medical record will be kept indefinitely.

While the information and data resulting from this study may be presented at scientific meetings or published in a scientific journal, your identity will not be revealed.
WHAT ABOUT SECONDARY USE OF SPECIMENS?
Your (and your animal’s) specimens may be kept and shared with other investigators for future research purposes. This is optional and whether or not you choose to permit this, it will not affect your participation in the rest of this study. The samples may be stored for study of disorders unrelated to the ones in you. Such usage will be strictly confidential in that no identifying information about you is provided to the researcher.

If you are willing to permit your samples to be stored for future research, you will be asked to sign a separate consent form.

WHAT ABOUT RESEARCH RELATED QUESTIONS, PROBLEMS, OR INJURIES?
Immediate necessary medical care is available at Duke Medical Center in the event that you are injured as a result of participation in this research study. However, there is no commitment by Duke University, Duke Health System, Inc., or your Duke Physicians to provide monetary compensation or free medical care to you in the event of study–related injuries.

If necessary, attempts will be made to aid you in finding a veterinarian. In doing so, we cannot guarantee the quality of care of your animal/pet. Further, there is no commitment by Duke University Health System, Inc., or your Duke physicians to provide monetary compensation or free veterinary care for your animals in the event of study-related injuries or illness in your animals/pets.

For questions about the study or a research-related injury, or if you have complaints, concerns or suggestions about the research, contact Dr. Vance Fowler at 919-684-4487 during regular business hours and at 919-970-5471 after hours, on weekends, and holidays.

If your animals are confirmed to have an MRSA infection, we advise you to speak with your veterinarian. Those animals might infect other family members.

If you have questions concerning your rights as a research subject, or to discuss problems, concerns or suggestions related to the research, or to obtain information or offer input about the research, contact the Duke University Health System Institutional Review Board (IRB) Office at (919) 668-5111.

WHAT ARE THE COSTS AND COMPENSATION?
There will be no additional costs to you as a result of being in this study. However, routine medical care for your condition (care you would have received whether or not you were in this study) will be charged to you or your insurance company.

There will be no compensation for your participation in this study.
STATEMENT OF CONSENT
"The purpose of this study, procedures to be followed, risks and benefits have been explained to me. I have been allowed to ask questions, and my questions have been answered to my satisfaction. I have been told whom to contact if I have questions, to discuss problems, concerns, or suggestions related to the research, or to obtain information or offer input about the research. I have read this consent form and agree to be in this study, with the understanding that I may withdraw at any time. I have been told that I will be given a signed and dated copy of this consent form.

________________________________________________________________________  ___________
Signature of Subject       Date

________________________________________________________________________  ___________
Signature of Person Obtaining Consent       Date
This form was approved for use by North Carolina State University and is valid for use from May 18, 2010 to May 18, 2011

This project is a collaboration between NCSU veterinary teaching hospital and Duke University hospital.

You are being asked to be part of this research project, as a member of a control (normal) population. To our knowledge, neither you nor your animal has been diagnosed with MRSA.

Please read this consent form carefully and take your time making your decision. Please ask the project staff to explain any words or information that you do not clearly understand. We encourage you to talk with your family and friends before you decide to take part in this research study. The nature of the study, risks, inconveniences, discomforts, and other important information about the study are listed below.

WHO WILL CONDUCT THE STUDY?

Dr. Jorge Ferreira will conduct the study, under supervision of Dr. Kevin Anderson, Dr. Maria Correa and Dr. Vance Fowler.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to better understand how humans and their animals potentially can infect each other with bacteria called methicillin-resistant Staphylococcus aureus (MRSA). This is a microbe (bacteria) that can infect both humans and animals and potentially cause serious infections in both. Infections passed between animals and people have been reported—however, very uncommonly. We are studying the potential trans-infection between sick people (Duke patients) and their pets and now would like to compare our findings to healthy people and pets (control population).

WHAT IS INVOLVED IN THE STUDY?

If you agree to be in this study, you will be asked to sign this consent form and another one (as an animal owner).

After all written consent forms are signed (you will keep a copy), nasal swab samples will be collected from you, by yourself. Collecting the swabs is simple, quick, and not painful. It involves putting a soft cotton tipped swab just inside each nostril and lightly rolling the swab around.

A short questionnaire will be given to you on the day of the visit. You will complete the un-shaded areas of the questionnaire. The goal of this questionnaire is to better understand factors that may be involved in the potential passing of MRSA infections between animals.
and people, and vice versa. Some examples of the questions we will ask relate to human-pet contact, e.g., dog allowed to lick the face of the person, animal(s) recent health history, e.g., skin sores not responding to treatment in small animals, frequency of hand washing following contact with pets, and information on whether other family members have been tested for MRSA.

Duke and NCSU University will maintain these samples indefinitely or until the samples are exhausted. Duke and NCSU University will assert all rights of ownership of the samples. These samples will not be used for clinical (diagnostic) purposes but research done with your sample may help to develop new products in the future. Duke and NCSU University and/or the developers will assert all rights arising from use of the sample. In this event, there is no provision to provide financial compensation to you.

If you withdraw from the study, your samples will be destroyed, and no further research will be done with them.

**RIGHT NOT TO PARTICIPATE OR WITHDRAW**

You may choose not to participate in this study. If you agree to participate, you may withdraw from the study at any time. If you withdraw from the study, no new data about you will be collected other than the data needed to keep track of your withdrawal.

Your decision not to participate or to withdraw from the study will not involve any penalty or loss of benefits to which you are entitled. If you do decide to withdraw, we ask that you contact Dr. Kevin Anderson or Dr. Jorge Ferreira in writing. The mailing address is: NCSU, Population Health and Pathobiology, 4700 Hillsborough St. Raleigh, NC 27605.

**HOW LONG WILL I BE IN THIS STUDY?**

The length of your involvement should take no longer than 25 minutes.

**WHAT ARE THE RISKS OF THE STUDY?**

Sampling techniques used are minimally invasive and are not considered painful procedures. No major health complications are expected. Bleeding is an unexpected potential complication that could occur in a very small percent of cases.

From the questionnaire and collection of information from your medical records, there is the potential risk of loss of confidentiality. Every effort will be made to keep your information confidential; however, this can not be guaranteed. Some of the questions we will ask you as part of this study may make you feel uncomfortable. You may refuse to answer any of the
questions and you may take a break at any time during the study. You may stop your participation in this study at any time.

You might find out that you or your animal(s) are “MRSA positive”. In this case, you will first be contacted by phone by Dr. Ferreira. You will also get the results letter like any other study participant.
Please be aware that a “MRSA positive” result might only mean that you and/or your animal(s) are carriers. This is not the same as having an active infection by MRSA. A small percentage (less than 5%) of the normal human and animal populations are MRSA carriers. Carriers are people (or animals) that have a specific microbe without showing any signs of disease caused by it.
We strongly advise you to discuss the results with “your veterinarian” and “your physician”. This is a research project and the project staff will not be available to provide you with any further medical assistance.

**ARE THERE BENEFITS TO TAKING PART IN THE STUDY?**

If you agree to take part in this study, there is no direct benefit to you. We hope that the information that we will get with this study will help prevent future MRSA human–animal trans-infection cases.

**WILL MY INFORMATION BE KEPT CONFIDENTIAL?**

Study records that identify you will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records. You will be assigned a unique code number. The key to the code will be kept in a locked file and a password-protected computer.

Your records may be reviewed in order to meet federal or state regulations. Reviewers may include representatives from the Food and Drug Administration and the Duke University Health System or North Carolina State University Institutional Review Board. If any of these groups review your research record, they may also need to review your entire medical record. If this information is disclosed to outside reviewers for audit purposes, it may be further disclosed by them and may not be covered by the federal privacy regulations.

The study results will be retained in your research record for at least six years, or until after the study is completed, whichever is longer.

While the information and data resulting from this study may be presented at scientific meetings or published in a scientific journal, your identity will not be revealed.
WHAT ABOUT SECONDARY USE OF SPECIMENS?

Your (and your animal’s) specimens may be kept and shared with other investigators for future research purposes. This is optional and whether or not you choose to permit this, it will not affect your participation in the rest of this study. The samples may be stored for study of disorders unrelated to the ones in you. Specimens that are authorized for future use will be stored at NCSU and Duke. Such usage will be strictly confidential in that no identifying information about you is provided to the researcher.

Please check one of the options, and initial on the side:

__ YES, I allow my samples and my animal’s samples to be used for secondary purposes.

__ NO, I do not allow my samples and my animal’s samples to be used for secondary purposes.

WHAT ABOUT RESEARCH RELATED QUESTIONS, PROBLEMS, OR INJURIES?

Immediate necessary medical care is available at Duke Medical Center in the event that you are injured as a result of participation in this research study. However, there is no commitment by Duke University, North Carolina State University, Duke Health System, Inc., or your Duke Physicians to provide monetary compensation or free medical care to you in the event of study–related injuries.

For questions about the study or a research-related injury, or if you have complaints, concerns or suggestions about the research, contact Dr. Jorge Ferreira at 919-757-3448.

If your animals are confirmed to have a MRSA infection, we advise you to speak with your veterinarian. Those animals might infect human beings. If your own swabs are MRSA positive we advise you to speak with your physician.

If you have questions concerning your rights as a research subject, or to discuss problems, concerns or suggestions related to the research, or to obtain information or offer input about the research, contact the North Carolina State University Institutional Review Board (IRB) Office at (919) 515-4514.

WHAT ARE THE COSTS AND COMPENSATION?

There will be no additional costs to you as a result of being in this study.
There will be no compensation for your participation in this study.

STATEMENT OF CONSENT
"The purpose of this study, procedures to be followed, risks and benefits have been explained to me. I have been allowed to ask questions, and my questions have been answered to my satisfaction. I have been told whom to contact if I have questions, to discuss problems, concerns, or suggestions related to the research, or to obtain information or offer input about the research. I have read this consent form and agree to be in this study, with the understanding that I may withdraw at any time. I have been told that I will be given a signed and dated copy of this consent form.

__________________________________________  ___________
Signature of Subject       Date

__________________________________________  ___________
Signature of Person Obtaining Consent    Date
North Carolina State University/Duke University
MRSA human-animal trans-infection pilot study
Animal Owner Consent Agreement

I agree to enter my animal(s) into the “MRSA human-animal trans-infection pilot study”. I recognize that nasal and rectal swab samples will be collected from my animals at my place of residence/farm. Swabs will be collected by Dr. Ferreira (veterinarian) and Patrick Brinson (NCSU animal sciences senior student) will help him restraining the animals. The nasal swab procedure will be performed by removing the swab from the transport tube and inserting the swab 2 cm (about ¾ inch) into one naris. The swab will be rotated against the anterior nasal mucosa for three seconds. This procedure will be repeated using the same swab in the second naris. If the animal is too small for this procedure, a perinasal swab will be collected. The swabbed specimen will be returned to the transport tube, labeled with a preprinted label, and transported to the laboratory. The rectal sampling procedure will be performed by inserting a Culturette swab into the rectum, then rotating the swab to collect fecal material, and carefully removing the swab from the rectum. The swabbed specimen will be inserted in the transport medium, labeled with a preprinted label, and transported to Dr. Fowlers’ (Duke University) laboratory where they will be further analyzed. All the materials that will be used are disposable to prevent transmission of MRSA between animals and households. These are minimally invasive sampling techniques and they are not considered painful procedures. Therefore only minimum physical and short-time restraint of the animals is expected. There may be minimal risk associated with this procedure. For example, the animals may become agitated, injured; or the animal may attempt to escape. However, no major health complications are expected, with the exception of the small risk of bleeding. In that case, I will contact my veterinarian_____________________. As an alternative choice NCSU veterinary hospitals are available to give my animal(s) medical assistance, at my own expenses.

The funding for this study is provided by Dr. Fowler discretionary account. I recognize that there will be no financial compensation for myself for being part of this study. The activities above have been approved by the Institutional Animal Care and Use Committee of Duke University and NCSU.

I understand and consent to the aforementioned.

Signed/Date _________________________
Printed Name _________________________
Witnessed/Date_______________________

Patient Name: _____________________    Case Number: __________________
OWNER CONSENT AGREEMENT

MRSA human animal trans infection – pilot study

As the owner or authorized agent for the owner of ________________________________ insert name of animal/pet you are being asked to have your animal/pet participate in a clinical study being performed at North Carolina State University Veterinary Teaching Hospital. You and your animal will be considered the control population of this study. In other words, neither you or your animals have been diagnosed with MRSA. The data collected from your pet(s) will be compared with that of data collected from pets owned by MRSA patients. Please read the following and ask as many questions as needed to understand what your participation involves, before giving your consent to your animal’s/pet’s participation by signing and dating the statement at the end of this document.

PURPOSE OF STUDY

The purpose of this study is to better understand how humans and their animals potentially might infect each other with bacteria called methicillin-resistant Staphylococcus aureus (MRSA). This is a microbe (bacteria) that can infect both humans and animals and potentially cause serious infections in both. Infections passed between animals and people have been reported—however, very uncommonly. Data from control populations are essential to compare sick and healthy human and animal populations.

EXPECTED DURATION OF PARTICIPATION

The collection of samples and data for purposes of this study will occur during a single visit to the CVM VTH. However, you may be contacted after this visit to obtain additional follow-up information pertaining to the study.

PROCEDURES

If you choose to enroll your animal/pet in the study, the following procedures will be performed:

After all written consent forms are signed (you will keep a copy), nasal, rectal and skin lesions (if present) swabs will also be collected from your animals by Dr. Ferreira. Collecting the swabs is simple, quick, and not painful. The nasal swab procedure will be performed by removing the swab from the transport tube and inserting the swab 2 cm (about ¾ inch) into one nostril. The swab will be rotated against the interior of the nostril for three seconds. This procedure will be repeated using the same swab in the second nostril. If the animal is too small for this procedure, a perinasal swab will
be collected. The swabbed specimen will be returned to the transport tube, labeled with a preprinted label, and transported to the laboratory. The rectal sampling procedure will be performed by inserting a Culturette swab into the rectum, then rotating the swab to collect fecal material, and carefully removing the swab from the rectum. Skin lesions will be swabbed by rotating the swab for three seconds in the lesion area. The swabbed specimen will be inserted in the transport medium, labeled with a preprinted label, and transported to the laboratory where they will be further analyzed.

We expect to need only minimum physical and short-time restraint of the animals. Only animals that have been vaccinated against rabies are eligible for this study. Research staff will be available to assist with restraining animals, if necessary.

Your animal/pet will be humanely treated at all times and all investigative procedures will be performed using the customary methods applied to all other client-owned patients at North Carolina State University Veterinary Teaching Hospital.

POSSIBLE DISCOMFORTS AND RISKS

Sampling techniques used for the animals are minimally invasive and are not considered painful procedures. Therefore, only minimum physical and short-time restraint of the animals is expected. There may be minimal risk associated with this procedure. For example, the animals may become agitated, injured; or the animal may attempt to escape. However, no major health complications are expected. Bleeding is an unexpected potential complication that could occur in a very small percent of cases.

TREATMENT AND POTENTIAL BENEFITS

There may be no direct benefit to your animal/pet from its participation in this study.

DISCLOSURE OF RESULTS

The results of the study tests will be reported to you.

The identity of any individual animal in the study will not be included in the presentation of the results of the study, unless you are contacted and provide written authorization to do otherwise.

VOLUNTARY PARTICIPATION AND RIGHT TO WITHDRAW

Participation in this study is entirely voluntary. Electing to not participate will not affect the quality of care provided to your animal/pet, and you may withdraw your animal/pet at any time without prejudicing its present or future care. Your animal/pet may be withdrawn from the study if your veterinarian finds it necessary and/or in your animal’s/pet’s best interest. If your animal/pet is withdrawn from the study for any reason, its progress may continue to be
followed and clinical data may continue to be collected from your animal’s/pet’s medical records without additional authorization

UNFORSEEN RISKS

In any clinical trial, some currently unknown risks may be identified. The investigator(s) will inform you of any new risks or changes in the way the study will be conducted as soon as any such information becomes available.

UNIVERSITY COMMITTEE ON ANIMAL RESEARCH

This study has been approved by the Institutional Animal Care and Use Committee (IACUC) at North Carolina State University (Protocol # insert protocol number). Questions regarding this review can be directed to the IACUC office at (919) 515-7507.

CONTACT PERSON(S) FOR THE STUDY

The principal investigator of this study is Kevin L. Anderson. If any questions should arise, he can be contacted at 919 513 6245 and Kevin_anderson@ncsu.edu. Alternatively you may contact Jorge Pinto Ferreira, graduate student at 919 757 3448 and jmferrei@ncsu.edu with any study related questions.

CONSENT

By signing below, I authorize my animal/pet to participate in the proposed study described above. I have been fully informed about the proposed study. I understand that any procedure, even routine blood sampling, has the potential for complications. I understand that only properly trained personnel will be allowed to participate in the proposed study. I will not hold the College of Veterinary Medicine or North Carolina State University responsible for complications resulting from participation in the study. I will receive a copy of this signed consent form.

Owner/Agent’s Name (please print): __________________________
Owner/Agent’s Signature: ________________________________
Date ____________________
Witnessed: ________________________________
Date ____________________

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Methicillin-resistant Staphylococcus aureus (MRSA) Trans-Infection Between Human and Animals: Pilot Study

Sponsoring Investigator: Vance Fowler, MD

A Duke University and NC State University Collaborative Project

Case Report Form (Patient)
MRSA Trans-Infection Between Human and Animals Study
Case Report Form (Patient)

Instructions: Complete the un-shaded sections at the time of the interviews with the participants and family. Shaded sections are to be completed by performing a review of the Duke medical record.

Date of Assessment: _____/_____/_____ (month/day/yyyy)

Unique Study Number: PT __ __ __ __ Medical Record Number: __________

PARTICIPANT’S DEMOGRAPHICS

Last Name: __________________________________________

First Name: __________________________________________

Address: ____________________________

Phone number: ____________________________

City ____________ State_____Zip______

(______) _______ ______- ________

Date of Birth: ___/___/____

(month/day/yyyy)

Age: __________

Gender (Circle only one) 1. Male 2. Female 3. Unknown

Race (Circle only one) 1. Unknown/Not reported

2. White

3. Black or African-American

4. Hispanic

5. Native American Indian or Alaskan native

6. Asian

7. Native Hawaiian or other Pacific Islander

8. More than one race
List family members who have consented to participate in the research:

<table>
<thead>
<tr>
<th>Name</th>
<th>Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PARTICIPANT’S MEDICAL INFORMATION

Were you recently (past 12 months) hospitalized at Duke?  Yes  No

Admission Date: ___/___/____ (month/day/yyyy)

Reason for admission: __________________________________________________________

Do you have any of the following conditions or disease that might weaken or compromise your immune system?  Yes  No

- Diabetes
- Rheumatoid Arthritis
- Lupus
- Cancer
- Organ transplant
- HIV/AIDS
- Specify other: ________________________________________________

Are you a healthcare worker?  Yes  No

Are you a veterinarian?  Yes  No

What is your occupation?

Are you aware of any recent contact you have had with any person or animal with MRSA infection?  Yes  No
**PARTICIPANT’S MEDICAL INFORMATION (cont.)**

Did you recently (≤ 1 year) take any antibiotic treatment?  
Yes  No  

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Dose</th>
<th>*Frequency (Check one)</th>
<th>Route (Circle one)</th>
<th>Start date (dd/month/year)</th>
<th>End date (dd/month/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>QD</td>
<td>1. By Mouth</td>
<td><strong>/</strong>/____ (dd/month/yyyy)</td>
<td><strong>/</strong>/____ (dd/month/yyyy)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BID</td>
<td>2. IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TID</td>
<td>3. Other (specify)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Q4H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*QD=Once a day, BID=Twice a day, TID=Three times a day, Q4H = Every 4 hours

**FAMILY MEMBERS’ INFORMATION**

Are any of your family members healthcare workers?  
Yes  No  

Are any of your family members veterinarians?  
Yes  No  

Are there children in the household?  
Yes  No  

How many children are there in the household?  
________
Indicate the age categories of the children in the house?
(Check all that apply)
   ____ 1. Neonatal (Birth – 1 month)
   ____ 2. Infants (1 month – 1 year)
   ____ 3. Toddlers (1 – 3 years)
   ____ 4. Preschool (3- 6 years)
   ____ 5. School age (6- 11 years)
   ____ 6. Adolescent (11-21 years)

Were other members of your family recently (\(\leq\)1 year) tested for MRSA?       Yes       No

Has any family member received antibiotic treatment in the past 12 months? Yes       No

Have any member of your household been diagnosed with MRSA in the past twelve months? Yes       No

How many members have had suspected or confirmed MRSA infections?
HUMAN-ANIMAL CONTACT

On the next three pages you are being asked to list any animals that you have at home, take care of, or have considerable contact with. List only animals that are available for sampling.

We would like to know about your animals’ health and the closeness of your contact with your animals. Having this in mind, please answer the following questions for each animal listed on the next three pages.
**HUMAN-ANIMAL CONTACT – Animal #1**

*We would like to know about your animals’ health and the closeness of your contact with your animals. Having this in mind, please answer the following questions for each animal.*

<table>
<thead>
<tr>
<th>Species (Check one for each)</th>
<th>Pet’s Name/ID</th>
<th>Pet’s Age in Years</th>
<th>Pet’s Gender (Check one)</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>__1. Dog</td>
<td></td>
<td></td>
<td>__1. Male</td>
<td></td>
</tr>
<tr>
<td>__2. Cat</td>
<td></td>
<td></td>
<td>__2. Female</td>
<td></td>
</tr>
<tr>
<td>__3. Horse</td>
<td></td>
<td></td>
<td>__3. Unknown</td>
<td></td>
</tr>
<tr>
<td>__4. Other (specify)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Animal Medical Information**

Does animal #1 have wounds or open sores?  
Yes  No

Was animal #1 recently (≤1 year) hospitalized?  
Yes  No

**List animal #1’s hospitalizations below:**

<table>
<thead>
<tr>
<th>Pet’s Name/ID</th>
<th>Date of hospitalization</th>
<th>Reason for admission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em><strong>/</strong></em>/____</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(dd/mon/yyyy)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em><strong>/</strong></em>/____</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(dd/mon/yyyy)</td>
<td></td>
</tr>
</tbody>
</table>

**Human-Animal Contact**

Where is this animals kept?  
1. Indoors  2. Outdoors  3. Indoors and outdoors  
(Circle one)

Is the animals allowed to move freely in the house, i.e., animals are allowed to move freely on beds, couches, or other furniture?  
Yes  No

Is the animals allowed to lick human faces?  
Yes  No

Is the animals allowed to sleep where humans sleep?  
Yes  No

How often do you have contact with this animal?  
How often do you wash your hands following contact with this animal? 1. Always 2. Sometimes 3. Never (Circle one)

### HUMAN-ANIMAL CONTACT – Animal # 2

*We would like to know about your animals’ health and the closeness of your contact with your animals. Having this in mind, please answer the following questions for each animal.*

#### Animal Demographics

<table>
<thead>
<tr>
<th>Species (Check one for each)</th>
<th>Pet’s Name/ID</th>
<th>Pet’s Age in Years</th>
<th>Pet’s Gender (Check one)</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>__1. Dog</td>
<td></td>
<td></td>
<td>__1. Male</td>
<td></td>
</tr>
<tr>
<td>__2. Cat</td>
<td></td>
<td></td>
<td>__2. Female</td>
<td></td>
</tr>
<tr>
<td>__3. Horse</td>
<td></td>
<td></td>
<td>__3. Unknown</td>
<td></td>
</tr>
<tr>
<td>__4. Other (specify)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Animal Medical Information

<table>
<thead>
<tr>
<th>Does animal #2 have wounds or open sores?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Was animal #2 recently (≤1 year) hospitalized?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

*List animal #2’s hospitalizations below:

<table>
<thead>
<tr>
<th>Pet’s Name/ID</th>
<th>Date of hospitalization (dd/month/yyyy)</th>
<th>Reason for admission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>/</strong>/____</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(dd/month/yyyy)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>/</strong>/____</td>
<td></td>
</tr>
<tr>
<td>(dd/month/yyyy)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Human-Animal Contact

Where is this animal kept? 1. Indoors 2. Outdoors 3. Indoors and outdoors (Circle one)

Is the animals allowed to move freely in the house, i.e., animals are allowed to move freely on beds, couches, or other furniture? Yes No

Is the animals allowed to lick human faces? Yes No

Is the animals allowed to sleep where humans sleep? Yes No
How often do you have contact with this animal? (Circle one)  
1. Always  
2. Sometimes  
3. Never

How often do you wash your hands following contact with this animal? (Circle one)  
1. Always  
2. Sometimes  
3. Never

---

**HUMAN-ANIMAL CONTACT – Animal # 3**

*We would like to know about your animals’ health and the closeness of your contact with your animals. Having this in mind, please answer the following questions for each animal.*

### Animal Demographics

<table>
<thead>
<tr>
<th>Species (Check one for each)</th>
<th>Pet’s Name/ID</th>
<th>Pet’s Age in Years</th>
<th>Pet’s Gender (Check one)</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>_1. Dog _2. Cat _3. Horse _4. Other (specify)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Animal Medical Information

Does animal #3 have wounds or open sores?  
Yes  No

Was animal #3 recently (≤1 year) hospitalized?  
Yes  No

*List animal #3’s hospitalizations below:*

<table>
<thead>
<tr>
<th>Pet’s Name/ID</th>
<th>Date of hospitalization</th>
<th>Reason for admission</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>/<strong>/</strong></em></td>
<td>dd/mon/yyyy</td>
<td></td>
</tr>
<tr>
<td><em>/<strong>/</strong></em></td>
<td>dd/mon/yyyy</td>
<td></td>
</tr>
</tbody>
</table>

### Human-Animal Contact

Where is this animal kept?  
1. Indoors  
2. Outdoors  
3. Indoors and outdoors (Circle one)

Is the animals allowed to move freely in the house, i.e., animals are allowed to move freely on beds, couches, or other furniture?  
Yes  No

Is the animals allowed to lick human faces?  
Yes  No
Is the animals allowed to sleep where humans sleep?  

Yes  No

How often do you have contact with this animal?  (Circle one)  


How often do you wash your hands following contact with this animal?  (Circle one)  


**EARLY WITHDRAWAL DATA**

*Date of Assessment:__ __/__/__ __/__/__ __ (dd/mon/yyy)*

Unique Study Number: ______________  Medical Record Number: ______________

Participant’s Initials ___ ___ ___

Patients’ participation may be terminated prior to completion of the study for the following reasons. **If subject is terminated early, check all that apply:**

( ) 1. Subject choice
( ) 2. Adverse Event(s)  
( ) 3. Clinical Failure
( ) 4. Protocol Violation
( ) 5. Termination of the study by the investigator
( ) 6. Other: Specify_______________________
Methicillin-resistant Staphylococcus aureus (MRSA) Trans-Infection Between Human and Animals: Pilot Study

**Sponsoring Investigator:** Vance Fowler, MD

*A Duke University and NC State University Collaborative Project*

*Case Report Form (Family Member)*
MRSA Trans-Infection Between Human and Animals Study
Case Report Form (Family member)

**Instructions:** Complete the un-shaded sections at the time of the interviews with the participants and family. Shaded sections are to be completed by performing a review of the Duke medical record.

Date of Assessment: _____/_____/______ (month/day/yyyy)

Unique Study Number: FM______ Medical Record Number: __________

**FAMILY MEMBER’S DEMOGRAPHICS**

Family Members’s Relationship to Patient (Check only one):

- __1. Spouse
- __2. Parent
- __3. Sibling
- __4. Child
- __5. Other. Please specify:____________________

<table>
<thead>
<tr>
<th>Last Name:</th>
<th>First Name:</th>
</tr>
</thead>
<tbody>
<tr>
<td>__________________________</td>
<td>__________________________</td>
</tr>
</tbody>
</table>

Address: __________________________

Phone number: __________________________

City ______ State____ Zip_____ (___ ___) ___ ___- ___ ___ ___

Date of Birth: _____/____/____

(month/day/yyyy)

Age: __________

**Gender (Circle only one)**

- 1. Male
- 2. Female
- 3. Unknown

<table>
<thead>
<tr>
<th>Race (Circle only one)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Unknown/Not reported</td>
</tr>
<tr>
<td>2. White</td>
</tr>
<tr>
<td>3. Black or African-American</td>
</tr>
<tr>
<td>4. Hispanic</td>
</tr>
<tr>
<td>5. Native American Indian or Alaskan native</td>
</tr>
<tr>
<td>6. Asian</td>
</tr>
<tr>
<td>7. Native Hawaiian/Pacific Islander</td>
</tr>
<tr>
<td><strong>FAMILY MEMBER’S MEDICAL INFORMATION</strong></td>
</tr>
<tr>
<td>----------------------------------------</td>
</tr>
<tr>
<td><strong>Were you recently (past 12 months) hospitalized at Duke?</strong></td>
</tr>
<tr>
<td><strong>Reason for admission:</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Do you have any of the following conditions or diseases that might weaken or compromise your immune system?</strong></td>
</tr>
<tr>
<td>Diabetes</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
</tr>
<tr>
<td>Lupus</td>
</tr>
<tr>
<td>Cancer</td>
</tr>
<tr>
<td>Organ transplant</td>
</tr>
<tr>
<td>HIV/AIDS</td>
</tr>
<tr>
<td>Specify other:</td>
</tr>
<tr>
<td><strong>Are you a healthcare worker?</strong></td>
</tr>
<tr>
<td><strong>Are you a veterinarian?</strong></td>
</tr>
<tr>
<td><strong>What is your occupation?</strong></td>
</tr>
<tr>
<td><strong>Are you aware of any recent contact you have had with any person or animal with MRSA infection?</strong></td>
</tr>
</tbody>
</table>
FAMILY MEMBER’S MEDICAL INFORMATION (cont.)

Did you recently (≤ 1 year) take any antibiotic treatment?  Yes  No

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Dose</th>
<th>*Frequency (Check one)</th>
<th>Route (Circle one)</th>
<th>Start date</th>
<th>End date</th>
</tr>
</thead>
<tbody>
<tr>
<td>_QD</td>
<td></td>
<td>_QD</td>
<td>1. By Mouth</td>
<td><strong>/</strong>/____</td>
<td><strong>/</strong>/____</td>
</tr>
<tr>
<td></td>
<td></td>
<td>_BID</td>
<td>2. IV</td>
<td><strong>/</strong>/____</td>
<td><strong>/</strong>/____</td>
</tr>
<tr>
<td></td>
<td></td>
<td>_TID</td>
<td>3. Other (specify)</td>
<td><strong>/</strong>/____</td>
<td><strong>/</strong>/____</td>
</tr>
<tr>
<td></td>
<td></td>
<td>_Q4H</td>
<td>(dd/mon/yyyy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>_Other</td>
<td>(dd/mon/yyyy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>_Other</td>
<td>(specify)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*QD=Once a day, BID=Twice a day, TID=Three times a day, Q4H = Every 4 hours
HUMAN-ANIMAL CONTACT

On the next three pages you are being asked to list any animals that you have at home, take care of, or have considerable contact with. List only animals that are available for sampling.

We would like to know about your animals’ health and the closeness of your contact with your animals. Having this in mind, please answer the following questions for each animal listed on the next three pages.
**HUMAN-ANIMAL CONTACT – Animal # 1**

*We would like to know about your animals’ health and the closeness of your contact with your animals. Having this in mind, please answer the following questions for each animal.*

<table>
<thead>
<tr>
<th>Animal Demographics</th>
<th>Pet’s Name/ID</th>
<th>Pet’s Age in Years</th>
<th>Pet’s Gender (Check one)</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species (Check one for each)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>__1. Dog</td>
<td></td>
<td></td>
<td>__1. Male</td>
<td></td>
</tr>
<tr>
<td>__2. Cat</td>
<td></td>
<td></td>
<td>__2. Female</td>
<td></td>
</tr>
<tr>
<td>__3. Horse</td>
<td></td>
<td></td>
<td>__3. Unknown</td>
<td></td>
</tr>
<tr>
<td>__4. Other (specify)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Human-Animal Contact**

How often do you have contact with this animal? (Circle one)


How often do you wash your hands following contact with this animal? (Circle one)

**HUMAN-ANIMAL CONTACT – Animal # 2**

*We would like to know about your animals’ health and the closeness of your contact with your animals. Having this in mind, please answer the following questions for each animal.*

<table>
<thead>
<tr>
<th>Animal Demographics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong> (Check one for each)</td>
<td><strong>Pet’s Name/ID</strong></td>
</tr>
<tr>
<td>__1. Dog</td>
<td></td>
</tr>
<tr>
<td>__2. Cat</td>
<td></td>
</tr>
<tr>
<td>__3. Horse</td>
<td></td>
</tr>
<tr>
<td>__4. Other (specify)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Human-Animal Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>How often do you have contact with this animal? (Circle one)</td>
</tr>
<tr>
<td>How often do you wash your hands following contact with this animals? (Circle one)</td>
</tr>
</tbody>
</table>
We would like to know about your animals’ health and the closeness of your contact with your animals. Having this in mind, please answer the following questions for each animal.

<table>
<thead>
<tr>
<th>Animal Demographics</th>
<th>Pet’s Name/ID</th>
<th>Pet’s Age in Years</th>
<th>Pet’s Gender (Check one)</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species (Check one for each)</td>
<td>_1. Dog</td>
<td>_2. Cat</td>
<td>_3. Horse</td>
<td>_4. Other (specify)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>__1. Male</td>
<td>__2. Female</td>
</tr>
</tbody>
</table>

**Human-Animal Contact**

How often do you have contact with this animal? (Circle one)

1. Always  
2. Sometimes  
3. Never

How often do you wash your hands following contact with this animals? (Circle one)

1. Always  
2. Sometimes  
3. Never
EARLY WITHDRAWAL DATA

Date of Assessment: ____/____/____ (dd/mon/yyyy)

Unique Study Number: _______________ Medical Record Number: _______________

Participant’s Initials ___ ___ ___

Patients’ participation may be terminated prior to completion of the study for the following reasons. **If subject is terminated early, check all that apply:**

( ) 1. Subject choice
( ) 2. Adverse Event(s)
( ) 3. Clinical Failure
( ) 4. Protocol Violation
( ) 5. Termination of the study by the investigator
( ) 6. Other: Specify___________________
Methicillin-resistant Staphylococcus aureus (MRSA) Transmission Between Human and Animals: Pilot Study

Sponsoring Investigator: Vance Fowler, MD

A Duke University and NC State University Collaborative Project

Case Report Form (control population)
MRSA Trans-Infection Between Human and Animals Study
Case Report Form (Wellness clinic animal owner)

Instructions: Complete the un-shaded sections at the time of the interviews with the participants and family. Shaded sections are to be completed by performing a review of the Duke medical record.

Date of Assessment: _____/_____/_____ (month/day/yyyy)

Unique Study Number: CP __ __ __ __

PARTICIPANT’S DEMOGRAPHICS

Last Name: ____________________________________________

First Name: ___________________________________________

Address: _____________________________________________

Phone number: _______________________________________

City __________ State____ Zip____ (______) ____-______

Date of Birth: ____/____/____ (month/day/yyyy)

Age: __________

Gender (Circle only one) 1. Male 2. Female

Race (Circle only one) 1. Unknown/Not reported
2. White
3. Black or African-American
4. Hispanic
5. Native American Indian or Alaskan native
6. Asian
7. Native Hawaiian or other Pacific Islander
8. More than one race
PARTICIPANT’S MEDICAL INFORMATION

Were you recently (past 12 months) hospitalized?  

Yes  No

Admission Date: ___/___/____ (month/day/yyyy)

Reason for admission:

_________________________________________________________

Do you have any of the following conditions or disease that might weaken or compromise your immune system?  

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lupus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organ transplant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specify other:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Are you a healthcare worker?  

Yes  No

Are you a veterinarian?  

Yes  No

What is your occupation? ________________________________

Are you aware of any recent contact you have had with any person or animal with MRSA infection?  

Yes  No

Were you recently (≤1 year) tested for MRSA?  

Yes  No
PARTICIPANT’S MEDICAL INFORMATION (cont.)

Did you recently (≤ 1 year) take any antibiotic treatment?  
Yes  No

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Dose</th>
<th>*Frequency (Check one)</th>
<th>Route (Circle one)</th>
<th>Start date</th>
<th>End date</th>
</tr>
</thead>
<tbody>
<tr>
<td>QD</td>
<td></td>
<td><strong>QD</strong></td>
<td>1. By Mouth</td>
<td>/ / / (dd/mm/yyyy)</td>
<td>/ / / (dd/mm/yyyy)</td>
</tr>
<tr>
<td>BID</td>
<td></td>
<td><strong>BID</strong></td>
<td>2. IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TID</td>
<td></td>
<td><strong>TID</strong></td>
<td>3. Other (specify)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q4H</td>
<td></td>
<td><strong>Q4H</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*QD=Once a day, BID=Twice a day, TID=Three times a day, Q4H = Every 4 hours
Are any of your family members healthcare workers?    Yes  No
Are any of your family members veterinarians?      Yes  No
Are there children in the household?      Yes  No
How many children are there in the household?     _________

Indicate the age categories of the children in the house? (Check all that apply)
  _____ 1. Neonatal (Birth – 1 month)
  _____ 2. Infants (1 month – 1 year)
  _____ 3. Toddlers (1 – 3 years)
  _____ 4. Preschool (3- 6 years)
  _____ 5. School age (6- 11 years)
  _____ 6. Adolescent (11-21 years)

Were other members of your family recently (≤1 year) tested for MRSA?        Yes  No
Has any family member received antibiotic treatment in the past 12 months?    Yes  No

Have any member of your household been diagnosed with MRSA in the past twelve months? Yes  No
How many members have had suspected or confirmed MRSA infections?  _________
HUMAN-ANIMAL CONTACT

*We would like to know about your animals’ health and the closeness of your contact with your animal(s). Having this in mind, please answer the following questions for each animal listed on the next three pages.*
HUMAN-ANIMAL CONTACT – Animal # 1

We would like to know about your animals’ health and the closeness of your contact with your animals. Having this in mind, please answer the following questions for each animal.

<table>
<thead>
<tr>
<th>Animal Demographics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species (Check one for each)</td>
</tr>
<tr>
<td>__1. Dog</td>
</tr>
<tr>
<td>__2. Cat</td>
</tr>
<tr>
<td>__3. Horse</td>
</tr>
<tr>
<td>__4. Other (specify)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Medical Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does animal #1 have wounds or open sores?</td>
</tr>
<tr>
<td>Was animal #1 recently (≤1 year) hospitalized?</td>
</tr>
</tbody>
</table>

List animal #1’s hospitalizations below:

<table>
<thead>
<tr>
<th>Pet’s Name/ID</th>
<th>Date of hospitalization</th>
<th>Reason for admission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong><strong>/</strong></strong>/____</td>
<td></td>
</tr>
<tr>
<td>(mon/dd/yyyy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong><strong>/</strong></strong>/____</td>
<td></td>
</tr>
<tr>
<td>(mon/dd/yyyy)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Human-Animal Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Where is this animals kept?</td>
</tr>
<tr>
<td>Is the animals allowed to move freely in the house, i.e., animals are allowed to move freely on beds, couches, or other furniture?</td>
</tr>
<tr>
<td>Is the animals allowed to lick human faces?</td>
</tr>
<tr>
<td>Is the animals allowed to sleep where humans sleep?</td>
</tr>
<tr>
<td>How often do you have contact with this animal? (Circle one)</td>
</tr>
</tbody>
</table>
How often do you wash your hands following contact with this animal?
(Circle one)

1. Always
2. Sometimes
3. Never

HUMAN-ANIMAL CONTACT – Animal #2

We would like to know about your animals’ health and the closeness of your contact with your animals. Having this in mind, please answer the following questions for each animal.

Animal Demographics

<table>
<thead>
<tr>
<th>Species (Check one for each)</th>
<th>Pet’s Name/ID</th>
<th>Pet’s Age in Years</th>
<th>Pet’s Gender (Check one)</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>__1. Dog</td>
<td></td>
<td></td>
<td>__1. Male</td>
<td></td>
</tr>
<tr>
<td>__2. Cat</td>
<td></td>
<td></td>
<td>__2. Female</td>
<td></td>
</tr>
<tr>
<td>__3. Horse</td>
<td></td>
<td></td>
<td>__3. Unknown</td>
<td></td>
</tr>
<tr>
<td>__4. Other (specify)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Animal Medical Information

Does animal #2 have wounds or open sores?
Yes  No

Was animal #2 recently (≤1 year) hospitalized?
Yes  No

List animal #2’s hospitalizations below:

<table>
<thead>
<tr>
<th>Pet’s Name/ID</th>
<th>Date of hospitalization</th>
<th>Reason for admission</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>/</strong>/______</td>
<td><strong>/</strong>/______</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mon/dd/yyyy</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>/</strong>/______</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(mon/dd/yyyy)</td>
<td></td>
</tr>
</tbody>
</table>

Human-Animal Contact

Where is this animal kept?
1. Indoors
2. Outdoors
3. Indoors and outdoors (Circle one)

Is the animals allowed to move freely in the house, i.e., animals are allowed to move freely on beds, couches, or other furniture?  
Yes  No

Is the animals allowed to lick human faces?  
Yes  No

Is the animals allowed to sleep where humans sleep?  
Yes  No
How often do you have contact with this animal? (Circle one) 1. Always 2. Sometimes 3. Never

How often do you wash your hands following contact with this animal? (Circle one) 1. Always 2. Sometimes 3. Never

**HUMAN-ANIMAL CONTACT – Animal # 3**

*We would like to know about your animals’ health and the closeness of your contact with your animals. Having this in mind, please answer the following questions for each animal.*

<table>
<thead>
<tr>
<th>Animal Demographics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species (Check one for each)</td>
</tr>
<tr>
<td>__1. Dog</td>
</tr>
<tr>
<td>__2. Cat</td>
</tr>
<tr>
<td>__3. Horse</td>
</tr>
<tr>
<td>__4. Other (specify)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Medical Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does animal #3 have wounds or open sores?</td>
</tr>
<tr>
<td>Was animal #3 recently (≤1 year) hospitalized?</td>
</tr>
</tbody>
</table>

*List animal #3’s hospitalizations below:*

<table>
<thead>
<tr>
<th>Pet’s Name/ID</th>
<th>Date of hospitalization</th>
<th>Reason for admission</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>/</strong>/____</td>
<td>(mon/dd/yyyy)</td>
<td></td>
</tr>
<tr>
<td><strong>/</strong>/____</td>
<td>(mon/dd/yyyy)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Human-Animal Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Where is this animals kept? (Circle one)</td>
</tr>
<tr>
<td>Is the animals allowed to move freely in the house, i.e., animals are allowed to move freely on beds, couches, or other furniture?</td>
</tr>
<tr>
<td>Is the animals allowed to lick human faces?</td>
</tr>
</tbody>
</table>
Is the animals allowed to sleep where humans sleep? Yes No

How often do you have contact with this animal? (Circle one) 1. Always 2. Sometimes 3. Never

How often do you wash your hands following contact with this animal? (Circle one) 1. Always 2. Sometimes 3. Never

**EARLY WITHDRAWAL DATA**

*Date of Assessment:* ___/___/____/____ (month/day/yyyy)

Unique Study Number: ______________ Medical Record Number: ______________

Participant’s Initials ___ ___ ___

Patients’ participation may be terminated prior to completion of the study for the following reasons. **If subject is terminated early, check all that apply:**

( ) 1. Subject choice
( ) 2. Adverse Event(s)
( ) 3. Clinical Failure
( ) 4. Protocol Violation
( ) 5. Termination of the study by the investigator
( ) 6. Other: Specify_______________________
Dear (patient’s name),

Thank you for participating in the research study entitled MRSA Trans-infection Between Humans and Animals: A Pilot Study (e-IRB # Pro00018484). The laboratory results of the swab sampling that was done on your animal(s) as part of this project demonstrated evidence of MRSA.

The investigators in this study provide the informational results as described above for you and/or your animals. Our intent is to use currently accepted techniques to identify and classify the bacteria involved. In the case where a MRSA is identified in either an animal or person, we cannot be your physician and/or your veterinarian. Our knowledge and awareness of MRSA in people and animals is constantly changing, so we are not in a position to give you advice on treatment, prevention, and other relevant areas. You will need to work directly with your physician and/or your veterinarian for any questions/concerns dealing with persons and/or animals, respectively. Your physician and/or veterinarian will be in the best position to deal with these issues.

Please see the enclosed handouts for information about MRSA. Please contact your animal doctor for further diagnosis and treatment.

Again, thank you for participating in this research project.

Sincerely,

Kevin Anderson, DVM, PhD.
Professor College of Veterinary Medicine, North Carolina State University

Vance Fowler, MD, MHS
Associate Professor
Department of Medicine, Duke University Medical Center
March XX, 2010

Animal owner’s name
Street address
City, state, and zip

Dear (Animal owner’s name),

Thank you for participating in the research study entitled *MRSA Trans-infection Between Humans and Animals: A Pilot Study*. The laboratory results of the swab sampling that was done as part of this project demonstrated **evidence of MRSA**.

Please be aware that a “MRSA positive” result might only mean that you and/or your animal(s) are carriers. This is not the same as having an active infection of MRSA. A small percentage (less than 5%) of the normal human and animal populations are MRSA carriers. Carriers are people (or animals) that have a specific microbe without showing any signs of disease caused by it.

The investigators in this study provide the informational results as described above for you and/or your animals. Our intent is to use currently accepted techniques to identify and classify the bacteria involved. In the case where a MRSA is identified in either an animal or person, we cannot be “your physician” and/or “your veterinarian.” Our knowledge and awareness of MRSA in people and animals is constantly changing, so we are not in a position to give you advice on treatment, prevention, and other relevant areas. You need to work directly with your physician and/or your veterinarian for any questions concerns/questions dealing with persons and/or animals, respectively. Your physician and/or veterinarian will be in the best position to deal with these issues.

Please see the enclosed handouts for information about MRSA. Please contact your animal doctor for further diagnosis and treatment.

Thank you again for your participating in this research project.

Sincerely,

__________________________

(Kevin Anderson, DVM, PHD, Professor)  (Vance Fowler, MD, MHS, Associate Professor)

College of Veterinary Medicine, North Carolina State University  Department of Medicine, Duke University
MRSA and Animals Q & A

What should I do if my animals/pets become infected with MRSA?

• Contact your animal doctor if your animals/pets are infected with MRSA.
• Follow your animal doctor’s instructions. Always complete the full course of treatment, even if your animal/pet looks better.

How should I care for my animals/pets to prevent the spread of MRSA?

• Wash your hands with soap and warm water after handling animals or pets.
• If you have to touch infected sites such as a sore on your animal, use gloves and wash your hands immediately.
• Avoid contact with your pet's face. MRSA often lives in the nose and the site of infection.
• Avoid contact with nasal discharge, saliva, and wounds.
• If your pet has a MRSA infection, limit overall contact with your pet until the infection has resolved.

What should I do if I'm worried about MRSA and dog bites?

• Worry more about dog bites than MRSA. Bites themselves are a major problem, even if MRSA is not involved. Trauma can be serious, and a variety of germs can cause infections, not just MRSA.
• Take measures to reduce the risk of being bitten by handling and training your dog with care.
• If you are bitten, immediately clean the wound as thoroughly as possible. See your doctor if a dog or other animal bites you.
• Talk to your doctor if you have concerns about your health, particularly if you or someone in the household has a weak immune system.

Please Remember:

• Your animal doctor is your best person to get information about the health of your animals/pet(s).
• Information provided here is accurate to the best of our knowledge. These sheets are for general information purposes only.

Adapted from: http://www.wormsandgermsblog.com