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MRSA IN ANIMALS AND THE RISK OF INFECTION IN HUMANS

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INTRODUCTION

Antimicrobial resistance is an escalating problem in human and veterinary medicine. Of particular concern is the increasing prevalence of antibiotic resistance among *Staphylococcus sp.*, bacteria that are Gram-positive, facultative anaerobes (Rich, 2005). These bacteria can produce a number of virulence factors, extracellular toxins and enzymes. *Staphylococcus aureus* is the most common species in humans and it can cause food poisoning, pneumonia, postoperative wound infections and dermatitis. It is one of the most common causes of nosocomial infections (Lee, 2003), including infection at the site of surgical implants and indwelling catheters. (Hillier & Shulaw, 2008). Methicillin-resistant *Staphylococcus aureus* (MRSA) contains the mecA gene that codes for an altered protein, called penicillin binding protein (PBP2a). This altered binding site results in antibiotic resistance (Leonard & Markey, 2008). Currently there are only a few antibiotics that are effective in treating MRSA infections and resistance continues to develop against them also (Hillier & Shulaw, 2008).

Staphylococcus sp. can infect many species of animals including dogs, cats, pigs, horses, cattle, goats, chickens, and sheep (Rich, 2005). *Staphylococcus intermedius* is the most common species seen in dogs and cats, although the presence of *S. aureus*, particularity MRSA, has been increasing in all animals (Rich, 2005). In one study at a university veterinary hospital in Berlin, 44.3% of *S. aureus* isolates in small and exotic animals were methicillin-resistant. These isolates were found mainly in dogs and cats. However, MRSA infections were also found in a guinea pig, a rabbit, a turtle, a bat and a parrot (Walther, et al., 2008). *Staphylococcus sp.* in animals can cause diseases including dermatitis, deep pyoderma, otitis, mastitis, arthritis, and urinary tract infections (Lee, 2003) (Hillier & Shulaw, 2008).

MRSA infection is a major concern in animals and, as in humans, animals can also become carriers. It is suspected that most animals acquire MRSA from humans (reverse zoonosis) (Heot & Hough, 2008). In 1959, Mann was one of the first to culture the nostrils of cats and dogs for *Staphylococcus sp.* and he "proposed that the common house pet can serve as an important reservoir or carrier of staphylococci infective for man" (Mann, 1959). In the 1970's MRSA strains were isolated in milk samples in Belgium (Devriese, Van Damme, & Fameree, 1972) (Devriese & Hommez, 1975). Humans can be carriers for weeks to years with no symptoms. Colonized animals can also harbor the bacteria, yet show no clinical signs of infection. Animals that are carriers of MRSA can be detected by culture and sensitivity testing on nasal, ear and rectal swabs. Dogs and cats have not demonstrated any long term colonization as of yet, however, pigs and horses can become long term carriers (Heot & Hough, 2008). At this time the toxin that causes "flesh eating" staphylococci infections in humans has not been seen in animals, but information about MRSA is changing rapidly (Hillier & Shulaw, 2008)

The prevalence of human MRSA infection in the US was 94,360 in 2005 (Klevens, et al., 2007). By 2007, the number of deaths (18,650) from MRSA infection surpassed the number of deaths from AIDS (Heot & Hough, 2008). Although hospital acquired (HA-MRSA) infections are still very common there has been an increase in community acquired (CA-MRSA) infections over the past 20 years. CA-MRSA can be acquired through direct contact in the community with aerosols and inanimate objects. With the increase in CA-MRSA there are more concerns about animals, including domestic pets, acting as reservoirs and transmitting MRSA back to humans (Duquette & Nuttall, 2004).

Literature and information about MRSA is changing weekly and we still have an incredible amount to learn about this disease and the risk factors associated with animals. Occupational risks to veterinarians, farmers, animal caretakers and owners are a concern, as are risks to animals, who may acquire MRSA from humans (reverse zoonosis) and act as carriers (Hillier & Shulaw, 2008). It is important to explore the risks to humans from animals and look at the effects of protective measures such as hand washing.

The next section will review analytic epidemiologic studies and risks associated with acquiring MRSA from contact with animals. Different aspects of these studies will be looked at individually and general comparisons of the studies will be made. Then the paper will conclude with more information about future needs for addressing this problem.

REVIEW OF STUDIES

"Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans" (van Loo, et al., 2007)

Since 2003 there has been a new strain of MRSA that has emerged that could not be typed with pulse field gel electrophoresis (PFGE). This strain is called non-typable (NT-MRSA). The case-control study presented here is from the Netherlands. The authors looked at data from the National Institute for Public Health and the Environment (RIVM), which is the national reference center for MRSA in the Netherlands. All first isolates of new MRSA carriers in the Netherlands are sent here. The RIVM offered a simple way to select cases and controls based on non-typable MRSA (cases) and typable MRSA (controls) to see if there was any association to animal exposure between these two groups and to further investigate and characterize this new strain.

There were 35 cases selected and 76 controls that came from 24 different referring laboratories. Questionnaires were sent to the laboratories to collect information about the patients, including: birth date, sex, postal code, presence or absence of infection, hospital admission dates, profession, profession of partners, profession of parents, and contact with animals (pigs, cows, horses, chickens, cats or dogs). The researchers were blinded to the status of the subjects while collecting this data. This helped eliminate any potential observation bias.

As with many of the other studies reviewed in this section, standard tests, including polymerase chain reaction (PCR) to identify the mecA gene and typing by use of pulse-field gel electrophoresis (PFGE), were used to confirm the identification of resistant *Staphylococcus sp.* Most studies also mentioned that antimicrobial susceptibility testing was conducted according to the Clinical Laboratory Standards Institute (CLSI) guidelines. By using these standard tests any differences in diagnosis of resistance and typing should be lessened. This will ensure more accurate classification of subjects in these studies.

Many of the studies specifically define how the culture swabs were obtained and from which sites. However, in this study the swabs were done by individual health care professionals and there was no specific data about how swabs were collected or from which sites. It is also not stated if the swabs were taken at likely carrier sites or were from active infection sites. There could be bias associated with this, since some physicians might have more aggressively looked for carriers and might have done more cultures than others. It has also become common in some countries, like the Netherlands, that screening cultures are done whenever anyone is admitted to the hospital.

Comparison showed that controls (typable MRSA) were more likely to have had contact with health care facilities, although there was no odds ratio (OR) calculated. The cases (non-typable MRSA) were more likely to live in rural areas OR 7.7 (CI 2.6-22.7) $p<0.01$ and more likely to have had animal contact. These findings were statistically significant for an increased risk with pig and cattle contact. For pig contact the OR was 9.4 (CI 1.8-47.7) $p<0.01$ and the OR for cattle contact was 13.5 (CI 1.0-179.3) $p<0.01$.

This study seems to be well done and was interesting to read. They addressed a possible issue with observation bias by blinding the researchers collecting the patient information and using the national laboratory helped to standardize the case definitions, even though there was no standard for collecting the samples. There was no mention about any problems with lack of data, which could be suspected to be a problem since the information was gathered from the laboratory records and not directly from the subjects.

"Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners" (Guardabassi, Loeber, & Jacobson, 2004)

This case-control study was conducted at The Royal Veterinary and Agricultural University in Copenhagen, Denmark from September to October, 2002. The cases consisted of 13 dog-owner pairs, in which the dogs had deep pyoderma. There could be a problem with case definition here, since the authors do not state what the definition of deep pyoderma was for these dogs, other than saying that they had been on prolonged antibiotics and were more likely to have resistant strains of bacteria. The controls were volunteers without daily contact with dogs or other pets; however, we know nothing else about this group. There could have been significant selection bias and with such a small sample size we cannot be sure that the controls would represent the general population of those without animal exposure, which could affect the external validity of this study.

Cultures were obtained with sterile swabs from oral and nasal cavities of the dogs and humans and also from the pyoderma lesions in the dogs. Cultures were positive for *S. intermedius* in 12 out of 13 dogs and 7 out of 13 owners, but in only 1of the 13 control humans. This represented a significantly higher risk in the dog owners group than in the controls, $p = 0.03$. While no determination about the direction of the transmission can be made from this study, *S. intermedius* is a normal inhabitant of dog skin and is rare in humans. So, it is likely that the humans were infected by the dogs, since the results showed that owners carried identical types of *S. intermedius* as their dogs. Owners filled out questionnaires about interactions with their dogs (sleeping on the owners beds and letting them lick the owners), but this study was not able to identify any increased risk associated with these behaviors.

While there were statistically significant results regarding the increased risk of resistant S. intermedius in owners of dogs with deep pyoderma, this study might have been enhanced by also including a group of dog owners whose dogs had normal skin. Again the small sample size is a problem with this study and there is no mention of the power of the study. With more information about the power and about the control group we might learn more about the internal validity of this study. However, even with these limitations, the fact that the cultures in humans and dogs were identical is an intriguing finding. More studies should be done with larger sample sizes to investigate the similarities in resistant cultures in humans and the animals with which they are in contact.

"Increase in a Dutch hospital of methicillin-resistant *Staphylococcus aureus* related to animal farming" (van Rijen, van Keulen, & Kluytmans, 2008)

This prospective prevalence survey was conducted from 2002-2006 at Amphia Hospital in the Netherlands. Cases were selected from patients and healthcare workers that were colonized for the first time with MRSA. There were 95 cases of MRSA carriage found, of which 72 were newly identified cases and 22 secondary cases. Twenty-three of the new cases were non-typable. The objective of the study was to "determine (1) the epidemiology of non-typable MRSA in a hospital located in an area with a relatively high density of pig farming and (2) the MRSA carriage rate in patients with exposure to pigs or calves".

Frequency of infection reported in this study was significantly higher with typable MRSA, with an OR of 4.83 (CI 1.34-17.09) and until 2006 there were more cases of typable MRSA. However, in 2006, they demonstrated a "sudden and strong increase in MRSA carriage in the Amphia hospital that was caused entirely by the emergence of non-typable MRSA. This strain was almost completely related to exposure to pigs and calves". They reported a carriage rate of 32% in this new risk group. The risk from exposure to pigs was higher than that from exposure to calves, but was not statistically significant with an OR of 1.6 (CI 0.7-4.3).

The cases in this study consisted of patients and health care workers identified by records at the hospital, but there is no specific mention of how the risk information was obtained. Most likely hospital records were also used and as in the previous study there might have been a lack of information for some patients, including the duration or extent of animal exposure for these cases. Since there was only one hospital used to locate cases and controls, and the fact that health care workers were included in the cases, there could be a problem with this studies external validity. Using more than one hospital through a multi centric study would be a possible solution.

"Community-associated methicillin-resistant *Staphylococcus aureus* in horses and humans who work with horses" (Weese, Rousseau, Traub-Dargatz, Willey, McGeer, & Low, 2005)

Equine farms in Ontario, Canada and New York State were the locations for this prospective prevalence study that evaluated 972 horses and 107 farm personnel, including laborers, veterinarians, horse owners and farm managers. The farms were classified as targeted, those where MRSA colonization or infection had been identified in the previous year, or non-targeted, those without a previous MRSA diagnosis. It was not stated if those non-targeted farms were tested and were negative or had never been tested. This could have introduced bias since farms not tested could have been positive.

Specimen collection was with a single nasal swab specimen from each horse and human. In the horses they specified that the swab was taken from 10 cm inside the nostril, in contact with the nasal mucosa, and for the humans, both anterior nares were swabbed. This study states that direct and enrichment culture techniques were used. MRSA was isolated from 46 of 972 horses on non-targeted farms and 46 of 391 from targeted farms. MRSA was cultured from 14 of the 107 humans, with 12 of 66 on targeted farms and 2 of 41 on non-targeted farms. One farm in New York had the highest prevalence rate of 45%. There was no mention of any factors related to this high rate, except that it was one of the target farms. None of the colonized humans reported hospitalization of themselves or their families within the 30 days prior to their cultures.

Colonized humans had isolates that were indistinguishable from the colonized horses they were in contact with. As with the second study reviewed this is again an intriguing finding and there is a much larger sample size in this study. But as with some of the other prevalence studies there was no specific control group used to determine what the risk is for humans with animal contact versus humans with no animal contact. However, most of these studies are determining prevalence and also looking at specific risk factors that can still be important when considering preventive and protective measures.

The authors state that many variables were evaluated but, they do not mention how information was obtained or what some of the other variables were. Multivariate analysis was used to evaluate the risks for MRSA carriage in humans and animals. In horses increased risk of MRSA colonization was associated with horses being on farms with greater than 20 horses $p<0.001$. In humans the only significant risk factor was regular contact with more than 20 horses $p = 0.018$. It was not stated why 20 horses was a key factor in both of these analysis and no information is given about exposure to fewer than 20 horses, by either humans or horses. There was no other mention made about the types of exposure or differences in people on farms with more or fewer than 20 horses.

One interesting point brought up in this study is related to contact with the health care system. In humans there is an increased risk of MRSA colonization associated with either direct or indirect contact with the human health care system, while this study found that in horses contact with the veterinary health care system was not a risk factor.

"Evaluation of prevalence and risk factors for methicillin-resistant *Staphylococcus aureus* colonization in veterinary personnel attending an international equine veterinary conference" (Anderson, Lefebvre, & Weese, 2007)

The objective of this study was "to evaluate the prevalence of MRSA colonization in veterinary personnel attending an international equine veterinary conference, and to identify risk factors for MRSA colonization in this group". The conference was the 2006 convention of the American Association of Equine Practitioners, held in San Antonio, Texas.

There were 257 participants in this study, of which 26 (10.1%), tested positive for MRSA colonization. Even though this was an international meeting, the largest majority of attendees were from the United States (US) and 75% of those tested were from the US. Of the 26 positives, 23 were from the US, 2 from Ireland and 1 from Canada. Again this represents a problem with selection bias weighted toward North Americans.

The authors specifically mention that the questionnaire used to determine information about potential risks was 3 pages long, and the swab used for culture was taken from only one of the participant's anterior nares, by the participant, under the direction of study personnel. Potential for bias here includes recall bias of information gathered on the questionnaire, especially since participants knew the study was related to MRSA and specific questions were asked about prior exposure to MRSA cases and the human health care system. There is also a concern with the uniformity of the swab samples that were taken. Hopefully veterinarians and veterinary personnel could follow the instructions of the study personnel and properly take their own swabs. However, there could have been variation, with some taking deeper and more thorough swabs than others.

This is a cross-sectional study of prevalence and the authors admit that using no control group of non-equine practitioners limited their interpretation of whether equine veterinarians have a higher risk of MRSA than the general population, even though the prevalence found in this study was higher than that typically found in the general population (10.1% versus 0.2%- 3.5 % respectively). This study and the next one all used convenience samples of veterinarians and veterinary personnel at different meetings for their sample groups. While there is a great advantage to obtaining samples this way, this could also be a source of selection bias.

The participants were volunteers who willingly chose to visit booths set up at these meetings. The external validity of the study could be affected by this type of sample. There could be fundamental differences between the veterinarian and veterinary personnel attending these meetings and the general population of veterinarians. First, different subpopulations of veterinarians will attend these meetings based on location of the meetings and individual interests. Second, certain personality types may be more likely to visit the booth and participate in the study, while others will pass by and not take time to get involved. Third, the participants may have been different from the general population of veterinarians in that they had prior exposure to cases of MRSA. They might have been more knowledgeable about the potential for transmission between animals and humans. However, even this knowledge could work in an opposite way and those with more concern about being a potential carrier may avoid participating. The label of being MRSA colonized could have negative consequences, stigma and potential insurance problems (the individuals health insurance and their practice liability insurance), but ultimately the potential for spread to animal patients, should have prompted them to participate, if only to help protect their own patients.

The most useful and interesting finding in this study concerns the protective effects of hand hygiene. Multivariate analysis showed protective factors for hand washing between farms, OR .23 (CI 0.09-0.59) and hand washing between infectious cases, OR 0.21 (CI 0.08-0.55). The authors note that "this is the first study to demonstrate a statistically significant association between hand hygiene practices and a measurable clinical outcome in veterinary medicine".

While it is noted that alcohol-based hand sanitizers have previously been found more effective at reducing bacterial counts on hands after examining horses, this study did not have a large enough group of participants that stated they routinely used hand sanitizers to demonstrate a significant protective factor for them. Future studies should expand on the use of hand sanitizers to better evaluate their potential protective factors.

"Methicillin-resistant *Staphylococcus aureus* colonization in Veterinary Personnel" (Hanselman, et al., 2006)

This study was conducted at the annual American College of Veterinary Internal Medicine Forum, in Baltimore, in 2005. This is an international conference, that primarily draws Americans, but of the 417 people sampled in this cross sectional, prevalence study 19 countries were represented. A single nasal swab sample was collected by the participants themselves. Once again this represents a potential problem with consistency in the samples and could affect the case definitions. As with other prevalence studies there was no control group used. This again leaves us with no way to look at the increased risk of animal exposure, other than comparing the prevalence in these veterinarians to the prevalence in the general population.

Again even with the limitations of this type of study and no control group, the results showed a prevalence of 6.5% (27/417), which is higher than that of the general population. There were 23 positive veterinarians out of 345 screened, 4 out of 34 technicians and 0 of the remaining 38 were classified as other. However, the more interesting results are seen when we look at the type of practice of these veterinary personnel. There were 12 of 271 small animal personnel and 15 of 96 large animal personnel that were positive, which does show a significant risk for large animal personnel compared to small animal personnel ($p < 0.01$). Further comparison showed no positives in those with no animal patient contact, such as industry and research veterinarians, 0 of 50. Comparing this to the total of the small and large animal personnel positives 27 of 367 there is again a significant difference ($p<0.01$).

These authors include a section about the limitations which include the use of a convenience sample and potential sample bias, use of a cross sectional study with no control group, the need to determine causality between risk factors and colonization, and sampling variation since participants collected their own samples. Again even with limitations I feel that studies like these help define concerns about MRSA and human-animal connections that need to be looked at more closely in the future.

"Surveillance of healthy cats and cats with inflammatory skin disease for colonization of the skin by methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi ssp. schleiferi*" (Abraham, Morris, Griffeth, Shofer, & SC, 2007)

I wanted to include this study, even though it is an animal epidemiology study, to specifically discuss a potential limitation related to sample collection. In this study swab samples were obtained from 5 locations on each cat: buccal and gingival mucosa, mucosa of each distal nares, forehead, groin and external anus. In the last three locations both skin and hair were swabbed. This study used more locations than any of the other studies, yet these authors were concerned that these might not be the best locations. They also specifically state that they used sterile swabs moistened with sterile saline. No other studies reviewed here, mentioned whether swabs were dry or moist.

The authors also discussed problems with the length of time of the swabs. They did a 15 second swab on each site, except the nose (2-5 seconds), due to the cats "lack of tolerance". None of the human studies mentioned the length of time for the swabs, or the tolerance of the humans during the swabs, whether done by the participant or by the researcher. It will be important in the future to make sure that sample acquisition is more standardized and that participants are not allowed to take their own swabs. Also researchers should take the swabs without knowing whether participants are in the animal exposure group or a control group so that no bias in sample collection is introduced.

CONCLUSION

As shown by these studies, there is still much to be understood about MRSA and animal associated risks. More case-control studies should be undertaken, with better selection of cases and controls that will result in better validity. A standardized method should be developed for acquiring samples, which includes length of swab time, determination of best sites and the minimum number of sites to swab. Groups with the largest animal exposures are easiest to find at veterinary and animal health meetings, however, these convenience samples may not represent the best sample population. There are individual differences in people who attend these meetings and in those that might be willing to be screened at such a meeting. Those with known exposure could be less likely to be screened due to concerns about liability in relation to their own patients if they are found to be carriers and concerns about insurance coverage if found to be carriers. There are other groups with animal exposure that I feel it would be interesting to look at also. These include zoo and aquarium workers. I did not find any mention of potential zoo or marine mammal infections or studies of risks to personnel at these locations. I feel this is an obvious next step that needs to be studied.

Further general risks dealing with the type of interactions among humans and animals need to be looked. Only the dog and owner study (Guardabassi, Loeber, & Jacobson, 2004) looked at risks related to behaviors and they found no significant increased risk based on these behaviors. However, as stated earlier that study had a very low sample size and others have proposed that our behaviors do put us at increased risk (Heot & Hough, 2008). Behaviors that seem innocent and caring, such as kissing a pet dog or cat on the head and nuzzling up to a horse are the types that need to be further investigated as potential risks (Heot & Hough, 2008). While hand washing was found to be protective (Anderson, Lefebvre, & Weese, 2007), large animal veterinarians and farm workers may not wash hands between patients due to lack of access to soap and running water. When on a farm the patients may not be near the barn and to go back and wash then return to look at another patient might not realistically take place. However, with further studies and increased assessment of alcohol based hand sanitizers, we can hopefully make recommendations for other options to help protect this group.

Once more data is gathered about risks and protective measures, the next step will be to increase our education efforts about changes in behaviors that can be protective and ways to eliminate risks. This will be especially important since there is a risk of reverse zoonosis and we should strive to not only protect ourselves from potential animal strains of resistant bacteria, but also protect our animal patients and pets, that can become carriers.

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