Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers

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Abstract

Methicillin resistant *Staphylococcus aureus* (MRSA) colonization has recently been identified in pigs and people that work with pigs, raising concerns about the role of pigs as reservoirs of MRSA for human infection. The objectives of this study were to evaluate the prevalence of MRSA colonization in pigs and pig farmers in Ontario, Canada and to characterize MRSA strains. Nasal and rectal swabs were collected from 285 pigs from three different age groups from 20 pig farms. Nasal swabs were collected from farm personnel and a brief questionnaire was also administered. The prevalence of MRSA colonization in farms was 45% (9/20) whereas the prevalence in pigs was 24.9% (71/285). There was no difference in MRSA colonization between age groups. The prevalence of MRSA colonization in pig farmers was 20% (5/25). There was a correlation between the presence of MRSA in pigs and humans on farms (P value = 0.001). The results of spa typing revealed the predominant strain in pigs and humans was eGenomics *spa* type 539 (Ridom t034, clonal complex 398) which accounted for 59.2% of isolates and has been reported in pigs in Europe. A common human epidemic clone, CMRSA-2 (USA100, clonal complex 5) was also found in both pigs and pig personnel. Indistinguishable strains were found in pigs and pig personnel on all five farms with a colonized human. This study demonstrates that MRSA is common in pigs in Ontario, Canada, and provides further support to concerns about transmission of MRSA between pigs and humans.
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Keywords: Zoonoses; Antimicrobial resistance; *Staphylococcus aureus*

Methicillin resistant *Staphylococcus aureus* (MRSA) is a multidrug resistant Gram-positive bacterium that is a critically important pathogen in human medicine. It is a leading cause of hospital-associated infections (Haddadin et al., 2002; Johnson et al., 2005) and has emerged as important cause of disease in people in the community (Vandenesch et al., 2003; Faria et al., 2005). MRSA strains are resistant to all beta-lactam antimicrobials through a penicillin binding protein (PBP2a) that has a low affinity for all beta-lactams (Berger-Bächi and Rohrer, 2002). This is encoded by the *mecA* gene, which resides on a mobile genetic element called a staphylococcal chromosomal cassette (SCC*mec*). Additionally, MRSA strains are often resistant to a wide range of other antimicrobials.

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MRSA colonization in pigs was first reported in the Netherlands (Voss et al., 2005) where pigs were implicated as a source of human MRSA infection. Currently MRSA has been identified in pigs in France, the Netherlands, Denmark and Singapore (Voss et al., 2005; Guardabassi et al., 2007; de Neeling et al., 2007; Sergio et al., 2007) and pig exposure has been identified as a significant risk factor for MRSA colonization in humans (Vandenbroucke-Grauls and Beaujean, 2006; Rijen et al., 2007). Recent reports have documented apparent transmission of MRSA between pigs and pig farmers and their family members (Voss et al., 2005; Huijsdens et al., 2006). Of particular note was a study from the Netherlands that reported pig farmers were 760 times more likely to be colonized with MRSA than the general population (Voss et al., 2005). Interestingly, a distinctive characteristic of these strains is that they are not typeable by pulsed field gel electrophoresis (PFGE) because of the presence of a novel DNA methylation enzyme (Bens et al., 2006). For that reason, *spa* typing, which involves sequencing of the X region of the protein A gene, is commonly used. A variety of *spa* types have been isolated from pigs and their human contacts, however the majority of those *spa* types are classified as sequence type (ST) 398 by multilocus sequence typing (MLST), suggesting that ST398 strains are somehow more adept at colonizing pigs and can be transmitted between pigs and their human contacts.

There has been no investigation of pigs in North America, despite recognition of MRSA in other animals in North America such as horses, dogs and cats (Rankin et al., 2005; Weese et al., 2005, 2006). The objectives of this study were to determine the prevalence of MRSA colonization in pigs and people that work with pigs on South-western Ontario pig farms, and to investigate risk factors for MRSA colonization in pig personnel.

1. Materials and methods

1.1. Collection of samples

A cross-sectional prevalence study was conducted, based on a convenience sample of 20 pig farms from south-western Ontario, Canada. Nasal and rectal swabs were collected from pigs of three different age groups (suckling pigs, weanling pigs and grower-finisher hogs). Whenever possible, five pigs from each age group were selected using a convenience sampling scheme that avoided sampling more than one pig in co-mingled groups. If all three age groups were not present on the farm, pigs from the available groups were sampled. At the same time as pigs were sampled, nasal swabs were collected from all consenting individuals working on the farm. A brief questionnaire regarding possible risk factors for MRSA colonization was also administered to participating farm personnel. Risk factors that were evaluated included the number of years they had worked with pigs, how long they had worked on that particular farm, number of hours spent working with pigs per week, contact with other animal species, history of recent hospitalization of themselves or any member of household, treatment with antimicrobials in the past 90 days, participation in team sports, visiting a correctional facility, or whether they or members of their family had suffered from skin or soft tissue infection or any MRSA infection in the last 3 months. All sampling was done with cotton-tipped swabs that were placed in liquid Stuart's medium. Swabs were stored at 4 °C and transported directly to the laboratory. This study was approved by the University of Guelph Research Ethics Board and Animal Care Committee.

1.2. Isolation and identification of bacteria

Swabs were inoculated into 2 mL of enrichment broth containing 10 g tryptone/L, 75 g sodium chloride/L, 10 g mannitol/L and 2.5 g of yeast extract/L. Following 24 h incubation, at 35 °C, a loop full of vortexed broth was inoculated onto selective MRSA agar plates (BBL CHROMagar MRSA, Becton, Dickinson and Company, Sparks, MD). The plates were then incubated aerobically at 35 °C for 24–48 h. MRSA colonies were preliminarily identified as characteristic mauve—colored, round colonies. Isolates were confirmed as *S. aureus* by Gram stain appearance, catalase test, tube coagulase test and *S. aureus* latex agglutination assay (Pastorex Staph-plus, Bio-Rad, France). Methicillin resistance was confirmed by testing for the presence of penicillin binding protein (PBP2) (MRSA latex agglutination test, Oxoid

Ltd., Hants, UK). All samples were stored at −80 °C for further testing.

1.3. Pulsed field gel electrophoresis

Isolates were typed by PFGE after SmaI digestion as has been described (Mulvey et al., 2001), with the exception that lysostaphin was added to buffer instead of plugs.

1.4. spa typing

spa typing was performed as has been described (Shopsin et al., 1999). Sequences were analyzed using the eGenomics software (http://tools.egenomics.com). Ridom database equivalents were identified using the Ridom Spaserver website (www.spaserver.ridom.de). eGenomics *spa* types are reported using a numerical system (i.e. *spa* type 539) while Ridom *spa* types are reported using a numerical system preceded by a 't' (i.e. *spa* t034).

1.5. Statistical analysis

The prevalence among age groups was compared with a χ^2-test. The results from nasal and rectal samples were compared using McNemar's test. The associations between putative risk factors measured on the questionnaire and prevalence in humans were tested by univariate logistic regression. Associations were deemed significant at $P < 0.05$. The association between the presence of colonized pigs and colonized farm personnel on the farms was evaluated using Spearman correlation. The prevalence of each *spa* type was determined for farms and pigs and humans on the farms. Statistical analyses were performed using SAS (SAS 9.1 Institute Inc., Cary, NC).

2. Results

Nasal and rectal swabs were collected from 285 pigs: 85 (30%) suckling pigs; 95 (33%) weanlings; 105 (37%) grower-finishers. MRSA was isolated from one or more pigs on 45% (9/20) of farms. The overall prevalence of MRSA colonization in pigs was found to be 25% (71/285); 20% (17/85) in suckling pigs; 28% (27/95) in weanlings; 26% (27/105) in grower-

finishers. There was not a significant difference in prevalence between the different age groups ($P = 0.41$). Of farms where at least one pig was colonized, the prevalence ranged from 6.6 to 100% (mean = 58.8% and median = 70%). Results from nasal and rectal samples were found to be significantly different ($P = 0.0001$), with 16% of pigs positive only on nasal swabs, 7.4% of pigs positive at both nasal and rectal samples, and only 1.4% positive at the rectal but not nasal swabs. All of those that tested positive only on rectal sites were weanlings.

The prevalence of MRSA colonization in humans was 20% (5/25), with colonized individuals being present on 5/9 (56%) of farms where MRSA was present in pigs. No humans tested positive on farms where MRSA was not detected in pigs. The Spearman correlation coefficient between farms with positive pigs and positive humans on the farm was 0.63 ($P = 0.0025$), indicating a significant association between the presence of colonized pigs and colonized humans on a farm. None of the risk factors identified in the questionnaire were found to have a significant association with human MRSA colonization. Of the five humans who were colonized, all had contact with one or more animal species, one had participated in team sports and none had a history of antimicrobial use, hospitalization or other putative risk factors. Of the 20 people who tested negative, all were in contact with one or more animal species, none had a history of recent hospitalization, two had been treated with antimicrobials, four participated in team sports, two had been diagnosed with soft tissue infections and three had family members who had recently been admitted to hospital.

Ten different *spa* types were identified (Table 1). Between 1 and 4 different *spa* types were identified on individual farms, with a mean of two types per farm and a median of one. The most common strain in both pigs and humans was *spa* type 539 (Ridom t034). The second most common strain was a similar *spa* type, with the difference being a deletion of a series of repeats in the less common strain. One other strain (*spa* type 109/Ridom t571) differed from *spa* type 539 by deletion of only one repeat. Combined, these closely related strains accounted for 75% (53/71) of pig isolates and 80% (4/5) of human isolates. The second most common group consisted of *spa* type 2 or related strains (*spa* type 2/Ridom t002, *spa* type 268/

Table 1
Spa typing results of MRSA isolates from pigs and humans on 20 pig farms

Spa type	Ridom	Repeat pattern	Pig prevalence	Farm prevalence	Human prevalence
539	t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	42/71 (59%)	7/9 (78%)	3/5 (60%)
New	t1255	X1-K1-------------------B1-Q1-O1	10/71 (14%)	1/9 (11%)	1/5 (20%)
2	t002	T1-J1-M1-B1-M1-D1-M1-G1-M1-K1	7/71 (9.8%)	3/9 (33%)	1/5 (20%)
268	t067	T1-J1-M1-B1-M1-D1-M1-G1-M1	2/71 (2.8%)	1/9 (11%)	–
387	t653	T1-----M1------------------G1-M1- K1	1/71 (1.4%)	1/9 (11%)	–
109	t571	X1-K1-A1-O1-A1-O1-B1------O1	1/71 (1.4%)	1/9 (11%)	–
New	New	U1-K1-J1-J1-D1-G1-J1-A1-B1	5/71 (7.0%)	2/9 (22%)	–
New	New	I2-Z2-G1-M1-J1-H2-M1-M1-M1-M1-M1-M1-J1-Q1	1/71 (1.4%)	1/9 (11%)	–
New	New	I2-Z2-G1-M1-J1-H2-M1-M1-M1-M1-M1-M1-J1-Q1	1/71 (1.4%)	1/9 (11%)	–
New	New	I2-H2-M1-M1-M1-M1-M1-M1-J1-Q1	1/71 (1.4%)	1/9 (11%)	–

Ridom t067, *spa* type 387/Ridom t653) which accounted for a further 14% (10/71) of pig isolates and the remaining human isolate. Four other *spa* types were identified, with three being similar. On all farms with colonized humans, one or more pigs were identified as carrying the same strain as the colonized human. On three of the four farms with colonized pigs but no colonized humans the prevalence in pigs was low, with only 1–3 colonized pigs identified. The prevalence of colonization in pigs on the remaining farm was 47%.

All *spa* type 539/Ridom t034 and related strains were untypeable by PFGE. Spa type 2 and related strains were classified as Canadian epidemic MRSA-2 (CMRSA-2), also known as USA100 (Christianson et al., 2007). The remaining spa types had unrelated PFGE patterns that were not consistent with recognized Canadian epidemic clones.

3. Discussion

This is the first study to identify MRSA colonization in pigs and people that work with pigs in North America. While the study based on convenience sampling may not represent the true population prevalence, it clearly indicates that MRSA is present in pigs and pig farmers in at least one region of North America and raises many issues. The high prevalence of colonization both at the farm level and the individual pig level on most farms where MRSA was present, was striking. On many farms, most or all tested pigs were colonized, however on some farms only a small number of pigs were colonized. It is unclear whether there are differences in management on farms associated with this variation. It would be interesting to re-test low prevalence farms to determine whether the prevalence of MRSA colonization has increased, as it is possible that the low prevalence could indicate recent introduction of MRSA.

This study sampled pigs of different age groups on farms. The sampling method contrasts with a recent study from the Netherlands (de Neeling et al., 2007) in which MRSA in pigs was detected from slaughterhouse sampled pigs. In the latter study, 10 consecutive pigs in the slaughterline were sampled from each group representing different farms in the nine slaughterhouses. As pigs from each farm were held in turn in the lairage in a slaughterhouse, transmission of MRSA between pigs from different farms was possible prior to sampling and it is unclear whether slaughterhouse sampling truly represents the population prevalence for that age group. Furthermore, slaughterhouse sampled pigs represent only one age group of pigs whereas in the present study, the MRSA colonization in three age groups of pigs was compared. Therefore, farm level sampling should provide more precise information about the epidemiology of MRSA in the pig population, while slaughterhouse screening may be more useful for preliminary studies. It was interesting that there was

no difference in prevalence between age groups in this study. A longitudinal study of MRSA acquisition by pigs on farms is needed.

Typing and nomenclature can be somewhat confusing for MRSA because of the variety of different typing methods, the presence of two different *spa* typing systems and lack of standardized nomenclature. PFGE and *spa* typing were performed because they are widely used internationally and can provide complementary information. MLST was not performed in this study but the three related PFGE non-typeable spa types found here have previously been reported as being clonal complex (CC) 398 (Witte et al., 2007). The predominance of this clone was somewhat unexpected and its origin is unclear. There is no way to determine how long this strain has been in pigs in Canada. In Canada most typing involves PFGE and PFGE non-typeable strains are extremely uncommon (Mulvey, personal communication) so it is probable that this strain has not yet been significantly associated with disease in humans in this country. However, as the epidemiology of MRSA may be changing, ongoing surveillance for this strain in humans is warranted, with investigation of pig contact in situations where PFGE non-typeable strains are found. Unlike recent European reports, CMRSA-2 (USA 100, EMRSA-3, CC5), a common human epidemic clone, was also relatively common in pigs in this study. CMRSA-2 has been the most common community-associated MRSA strains in humans in Canada (Simor et al., 1999; Mulvey et al., 2005) and has also been identified in dogs, cats and horses in Canada (Weese et al., 2005). It is likely that CMRSA-2 colonization in pigs originated from colonized humans, as independent emergence of this MRSA clone in both pig and human populations is extremely unlikely.

This study provides further support to the hypothesis that MRSA can be transmitted between humans and pigs. Although direct comparison cannot be made because a control group was not used in the assessment of human colonization, the prevalence of colonization in pig personnel (20%) was quite high and is much greater than has been reported in the general population in North America. For example, a study using data from 2001 to 2002 National Health and Nutrition Examination Survey (NHANES) in the United States estimated a population colonization rate of only 0.84% (Mainous et al., 2006). Further, a study of science teachers performed in Ontario, Canada at the same time as this study only identified MRSA colonization in 2.7% of individuals (Hanselman, Kruth and Weese, unpublished data). Therefore, it is likely that personnel working with pigs are at higher risk for MRSA colonization compared to the general Canadian population, as has been reported in the Netherlands (Voss et al., 2005).

In addition to public health risks to pig personnel, a recent report implicating MRSA in exudative dermatitis in pigs (Van Duijkeren et al., 2007) raises potential pig health concerns.

Because pigs are food-producing animals, there are inherent concerns about contamination of food. Staphylococcal food poisoning caused by MRSA has been reported (Jones et al., 2002). Recently, MRSA was identified in food products intended for human consumption (Normanno et al., 2007) but none were pig-in-origin. Further study regarding the potential for food borne disease is warranted, but the risks are likely low.

The reasons for the high prevalence of MRSA colonization in pigs both in Canada and in other countries remain uncertain. The presence of similar strains in pigs and their human contacts, the high prevalence of colonization of pig farmers and the possible emergence of ST398 strains as a cause of clinical infections in humans (Ekkelenkamp et al., 2006; Witte et al., 2007) indicates that MRSA in pigs may pose a public health risk to human contacts. Further information is required to identify and implement control measures to reduce the impact of this emerging pathogen of public health concern.

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