Methicillin resistant *S. aureus* in market hogs, retail pork, and swine veterinarians in the USA

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Abstract

Cross-sectional studies were conducted to obtain preliminary data on the prevalence of MRSA in swine veterinarians and market hogs, and *S. aureus* in retail pork in the USA. Convenience sampling was employed, but samples were broadly sourced across the country. Nasal swabs were collected from 111 swine veterinarians at a national swine veterinary meeting, and from 539 market hogs slaughtered at large US packing plants. Fresh pork products (chops or ground pork) were obtained from retail stores in 15 states. Samples were cultured using double enrichment methods and selective plating. Methicillin resistance was confirmed by PCR testing for the meca gene, and isolates were characterized by spa typing. MRSA prevalence was 6% in swine veterinarians and 30% in market hogs. *S. aureus* was detected in 80% of pork samples. Diverse MRSA spa types were detected in all three subprojects, but spa type 539 (Ridom l034) was the most common spa type isolated from both market hogs and swine veterinarians. This is the predominant spa type reported in pigs and pork producers in Canada, and one of the spa types corresponding to ST398 strains of MRSA common in pigs in Europe. Only 3 isolates of spa type 539 MRSA were found in retail pork samples. Spa type 2 was the second most frequent spa type in market hogs and was also found in retail pork samples and a swine veterinarian.

Introduction

Until fairly recently, methicillin resistant *Staphylococcus aureus* (MRSA) was viewed as a problem limited to the human medical arena. Animal reservoirs were not thought to have any significance in the epidemiology of the organism. Furthermore, clinical MRSA infections of people were predominantly confined to hospitals (i.e. 'hospital acquired'), and MRSA infections occurring in the general community ('community acquired' MRSA, or CA-MRSA) were relatively uncommon events. The rapid emergence of CA-MRSA globally in recent years has been described as a 'quantum change in the biology and epidemiology of a major human pathogen'. Whether animal reservoirs of MRSA have made more than an incidental contribution to this 'quantum change' is still not known, and until now is probably unlikely. However, a spate of reports of MRSA in animals, supported by events of apparent animal-to-human transmission of MRSA, have led to another quantum shift in the perceptions about the role of animal reservoirs of MRSA. At the pessimistic extreme, it has been speculated that the risk posed by MRSA in livestock animals constitutes an 'epidemic waiting to happen'. The pig has been 'front and center' of this discussion, and studies in several countries have consistently detected MRSA colonization of commercial swine, and also pork industry workers. Prevalences of nasal colonization of pig farm workers have greatly exceeded those reported for broader human populations. The vast majority of reports are from Europe, where remarkably only one 'strain' of MRSA (MLST type 398; PFGE untypable; several spa types) has been found in pigs. A Canadian study yielded prevalence data (25% in pigs; 20% in farm workers) similar to those seen in Holland and more recently in Germany. Three-quarters of the Canadian isolates had spa types consistent with MLST 398, but more diversity of isolates was seen than in Europe studies. The sole published study in the USA, limited to two production systems in Iowa, confirmed the presence of ST398 in pigs and people in one system, while the second system was negative. The objectives of this study were to obtain a broader perspective on the prevalence of MRSA in market hogs, swine veterinarians, and retail pork products in the USA, and to characterize the isolates obtained using spa typing.
**Materials and Methods**

**Swine veterinarians**
Our aim was to sample 100 US swine veterinarians by recruiting volunteers attending national swine veterinary meetings in the USA. The sample size was adequate to detect a prevalence of 3% with 95% confidence, lower than prevalences previously reported in veterinarians. All sampling was performed at the 2008 meeting of the American Association of Swine Veterinarians in San Diego, CA. The meeting typically attracts approximately 800 attendees from the USA and approximately 30 other countries, and participants also include veterinary students and others not actively engaged in swine veterinary practice. Volunteers (n = 150) were required to sign statements of informed consent, complete a questionnaire, and submit to a nasal swab. Trained personnel used a single swab (BBL Culture Swab 220099, Becton, Dickinson and Company, MD) to sample both nares of each volunteer. Swabs were coded alphanumerically and transported on ice to the laboratory at the University of Minnesota.

**Nasal swabs of slaughtered pigs**
The target population for the survey was the population of commercial market hogs in the USA. A multi-stage sampling approach was used to approximate a representative sample of market hogs. The structure of the survey was similar to that of de Neeling et al. (2007) used to survey the Dutch swine industry. Differences included 1) the purposive selection of a small number of the largest plants in the USA to obtain samples that were more nationally representative; and 2) use of a within-group sample size of 12 pigs (compared to 10 used in Holland) to increase the likelihood of detecting MRSA in an infected group. Sampling at each plant was conducted on a single day. In most plants, plant personnel were able to arrange groups of pigs (i.e., sourced from a single farm) from different ZIP codes, further ensuring geographical diversity in the study. A total of 539 pigs were sampled in 45 groups sourced from 42 ZIP codes across 10 states (IA, IL, IN, KS, MN, NC, OH, OK, PA, TX). Distal nasal swabs were taken from 12 pigs in each lot immediately after stunning and before entering the scald tanks, and transported on ice to the laboratory.

**Retail pork samples**
The objective of this study was to evaluate the detection of *Staphylococcus aureus* on raw pork chops using two different sampling techniques, and to characterize the spa types for comparison with *Staphylococcus aureus* isolates associated with swine. Fresh pork chops were obtained from retail stores across the U.S. A total of 91 fresh pork products were sampled from 15 states. Purchasers were asked to visit 2 retail stores in their state and purchase meat with different brand names. Two methods for culturing chops were compared: culturing a 1 in.3 piece and culturing a whole chop in enrichment broth. The culture broth was streaked for isolation onto CNA and then individual colonies were streaked for isolation to a second CNA plate. All broths and plates were incubated for 22 h at 37°C. Presumptive *S. aureus* colonies were tested for coagulation by a tube coagulate test with rabbit plasma. DNA extraction was performed by using QIAamp DNA Mini Kit (Qiagen). Confirmation of *S. aureus* species was determined by PCR and sequencing of 16s ribosomal RNA. PCR was performed using HotStar Taq Master Mix Kit (Qiagen).

**Bacteriology**
Nasal swabs were transferred into 5 mL of Mueller Hinton broth containing 6.5% NaCl (Becton, Dickinson and Company, Sparks, MD) and incubated for 18 ± 2 hours at 35 ± 1°C. One mL of broth was transferred into 9 mL of selective enrichment medium (PMB+, Becton, Dickinson and Company, Sparks, MD) and incubated for 18 ± 2 hours at 35 ± 1°C. Samples in which a color change from red to yellow was observed were streaked onto MRSA selective plates (MRSASelect, Bio-Rad Laboratories) and incubated for 18 ± 2 hours at 35 ± 1°C. Suspect colonies (light purple/pink, round, with a slight convex surface) were restreaked onto blood agar plates as necessary to obtain isolates in pure culture. The identity of pure cultures was confirmed as MRSA using PCR methods based on published primers for 16S rRNA (27) and mecA gene (28) that confers methicillin resistance. Spa typing was conducted following published methods for sequencing a single PCR amplicon of the staphylococcal protein A gene. eGenomics software
(http://tools.egenomics.com) was used to analyze sequences for spa typing. Ridom spa types were
determined via the Ridom SpaServer website (http://www.spaserver.ridom.de).

Results

Swine veterinarians
The prevalence of MRSA was 6.3% (7 of 111) and 5.9% (5 of 85) in swine veterinarians and US swine
veterinarians, respectively. Five of the 7 MRSA isolates from swine veterinarians were spa type 539, with
the other spa types being 2, and 963. Increased frequency of pig contact and admission to the hospital in
the past 90 days were associated with MRSA colonization. One of 39 non-veterinary participants (a
student working 20 hours per week with pigs) was positive (spa type 7).

Market hogs
MRSA was detected in 165 (30.6%) of the 539 market hogs sampled. The predominant spa types were
539 and spa type 2, which together accounted for just over half of the isolates. Other spa types recovered
were 97, 142, and 302, but some 28% of isolates were ‘new’ spa types.

Retail pork
S. aureus was detected in a 114 of 143 (80%) samples (Table 1). Recovery was significantly lower when
1” cube samples were taken from pork chops, compared with sampling the remainder of the chop (P <
0.001). The prevalence of S. aureus detected in ground pork samples was lower than pork chops
(P<0.001). For almost all samples, very few suspect colonies were present, despite the multiple
enrichment steps, suggesting a likely low level of contamination. A total of 83 different spa types
constituted the 186 S. aureus isolates cultured from the raw pork samples, and spa types varied greatly
among regions. The most common spa type (n = 19), and the only isolate identified in all three regions,
was 426 (Ridom: 1273). Among spa types common in market hogs, only 3 isolates of spa type 539 MRSA
and 4 of spa type 2 were detected in retail pork.

Discussion

Our results confirm the presence of MRSA, and particularly spa type 539, in swine veterinarians, market
hogs, and retail pork samples in the USA. As sampling was done by convenience, the samples cannot be
claimed to be a representative sample of the target populations. However, for all three target populations,
efforts were made to obtain samples from geographically diverse locations and the study was arguably
national in scope. However, no sampling was conducted on swine farms, thus one must be cognizant that
exposure may have occurred from sources other than pigs (particularly for retail pork samples). The
prevalence (30%) of MRSA observed in market hogs was less than reported in Holland (49%) but higher
than observed in Canada (25%). It is important to note that the Canadian study was conducted on farms
and not at slaughter. Studies with market hogs have inherent risks that animals may become exposed
during transport and lairage, and more comprehensive studies on swine farms are indicated. The finding of
elevated prevalence of colonization of swine veterinarians is consistent with several studies of MRSA
veterinary personnel. However, there is still no evidence of elevated risk of infection of veterinarians with
MRSA. The predominance of spa type 539 in both swine veterinarians and market hogs, supports the
initial report of ST398 MRSA in Iowa, and suggests that such strains may also be common in the US
swine industry. While in Europe MRSA isolates from pigs almost exclusively belong to the ST398 group,
the greater diversity we observed corresponds more closely with data from Canada. A similar contrast
was seen in results for recent studies of meat samples in which livestock associated strains of MRSA
predominated in Europe, but not in a US study from Louisiana. We similarly detected a high prevalence
of S. aureus in pork samples, but only 3 livestock associated isolates (spa type 539).

Conclusion
MRSA isolates consistent with the 'livestock associated' strains common in Europe were found in national surveys of swine veterinarians, market hogs and retail pork in the USA. Isolates obtained were much more diverse than reported from Europe, and more similar to published results from Canada. Further studies are needed to better understand the ecology of S. aureus in pigs and the potential for occupational risks for farm workers.

Acknowledgments
This study was funded by the National Pork Checkoff project 07-196.

References