RESEARCH REPORT
PORK SAFETY

Title: Methicillin Resistant *Staphylococcus aureus* in pigs, pork products and swine veterinarians
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Industry Summary:

Staphylococcus aureus is a bacteria found commonly on the skin and mucous membranes of mammals that can occasionally cause superficial or severe infections. Strains resistant to methicillin (MRSA) are a serious public health problem in hospitals worldwide, but animals were considered to have no role as MRSA reservoirs. Recent reports from Europe and elsewhere found pigs and pig farmers can have a high prevalence of colonization with particular MRSA strains (ST398). Although the public health risk from ST398 MRSA appears to be very limited thus far, the issue of 'livestock associated MRSA' has attracted considerable scientific and media attention, including predictions of a future epidemic and concerns about food safety. The sole published study in the USA, limited to two production systems in Iowa, found a high prevalence ST398 MRSA in pigs and farm staff in one system. The objectives of our study were to obtain a broader perspective on the prevalence of MRSA in market hogs, swine veterinarians, and retail pork products in the USA. 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. MRSA prevalence was 6% in swine veterinarians and 30% in market hogs. *S. aureus* was detected in 80% of pork samples. Diverse spa types were detected in all three subprojects, but spa type 539, corresponding with the 'livestock associated' ST398 strains in Europe, was the most common spa type in both market hogs and swine veterinarians. Only 3 isolates of spa type 539 MRSA were found in retail pork samples. In summary, MRSA isolates consistent with the 'livestock associated' MRSA strains common in Europe were found in US swine veterinarians, market hogs and retail pork. Isolates obtained were much more diverse than reported from Europe, and more similar to published data from Canada. Further studies are needed to better understand the ecology of *S. aureus* in pigs and the potential occupational risks for farm workers. European authorities have concluded that foodborne risks from MRSA in meat are negligible.

Scientific 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 (45 groups sourced from 42 zipcodes, across 10 states). Fresh pork products (pork chops or ground pork) were obtained from retail stores in 15 states. Samples were cultured using double enrichment methods and selective plating as conducted in previous studies in Europe. PCR methods were use to determine the identity of isolates and methicillin resistance (16S rRNA and *mecA* genes respectively). Spa typing was conducted following published methods for sequencing the staphylococcal protein A gene, and spa types were determined using both the eGenomics software and Ridom SpaServer website. MRSA prevalence was 6% in swine veterinarians and 30% in market hogs. *S. aureus* was detected in 80% of pork samples. Diverse spa types were detected in all three subprojects, but spa type 539 (Ridom t034) 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 MRSA. Only 3 isolates of spa type 539 MRSA were found in retail pork samples. Spa type 2, the second most frequent spa type in market hogs (and also detected in retail pork and one veterinarian), was likewise the second most common spa type in pigs in Canada. In Europe MRSA isolates from pigs almost exclusively belong to the ST398 group. However, our results indicated more diversity and were similar to those reported from Canada. The same contrast exists for results for recent studies of meat samples, in which livestock associated strains of MRSA predominated in Europe, but not in a study in Louisiana. In summary, MRSA isolates consistent with the 'livestock associated' strains common in Europe were found in swine veterinarians, market hogs and retail pork in the USA. The MRSA isolates obtained were more diverse than reported from Europe, and more similar to published data from Canada. Further studies are needed to better understand the ecology of *S. aureus* in pigs and the potential occupational risks for people working with pigs.

Introduction:

MRSA infection presents one of the most serious antimicrobial resistance challenges in human clinical medicine. Until recently, accepted medical wisdom held that the epidemiology of transmission and antimicrobial resistance of MRSA were confined to the human arena, and that animal reservoirs (and associated antimicrobial use) were of negligible importance. However, a series of reports of MRSA in animals, and of animal-to-human transmission, has undermined this view. Studies from the Netherlands documented the occurrence of a specific MRSA strain (sequence type [ST]398) in a high percentage (49%) of Dutch pigs, and the same strain was isolated from people with occupational exposure to pigs. The prevalence of MRSA colonization of Dutch pig farmers was estimated to be 760 times higher than in the general population. Dutch health authorities now categorize people exposed to pigs and other livestock as high risk for MRSA, and when hospitalized, these people are held in isolation until MRSA colonization has been excluded. Given the current prominence of MRSA as a human health problem, and the increasing attention given to the Dutch findings in the media, this study was undertaken to obtain some baseline information essential for science-based communication of the potential risks associated with MRSA in pigs and pork.

Objectives:

- To estimate the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in market hogs, fresh pork products, and swine veterinarians in the USA.
- To characterize the antimicrobial resistance profiles and molecular sub-types of MRSA isolates obtained in the prevalence study.

Materials & 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)[1] used to survey the Dutch swine industry. Differences included 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., pigs 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 group immediately after stunning and before entering the scald tanks, and were transported on ice to the laboratory.

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 ± 1ºC. One mL of broth was transferred into 9 mL of selective enrichment medium (PMB+, Becton, Dickinson and Company, Sparks, MD) containing oxacillin and incubated for 18 ± 2 hours at 35 ± 1ºC. 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 ± 1ºC. 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 isolates and methicillin resistance were confirmed using PCR methods (16S rRNA and mecA genes respectively). Spa typing was conducted following published methods for sequencing a single PCR amplicon of the staphylococcal protein A gene, and spa types were determined using both the eGenomics software and Ridom SpaServer website.

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^{o}C. Presumptive *S. aureus* colonies were tested for coagulation by a tube coagulase 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).

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 ± 1ºC. 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 ± 1ºC. 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 ± 1ºC. 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. PCR methods were use to establish the identity of isolate and methicillin resistance (16S rRNA and *mecA* genes respectively).

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 (3 from the USA) 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 weeks with pigs) was positive (spa type 7). Increased frequency of pig contact and admission to the hospital in the past 90 days were associated with MRSA colonization. The prevalence observed was significantly higher than the 1.5% figure estimated for the general US population, indicating that swine veterinarians may be at an increased risk of colonization with MRSA.

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. 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 the186 *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: t273). 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%)[1]but higher than observed in Canada (25%).[2] 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,[3] 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.[2] A similar contrast was seen in results for recent studies of meat samples in which livestock associated strains of MRSA predominated in Europe,[4] but not in a US study from Louisiana.[5] We similarly detected a high prevalence of *S. aureus* in pork samples, but only 3 livestock associated strains. In summary, MRSA spa type 539, consistent with the livestock associated strains common in Europe, was found in swine veterinarians, market hogs and retail pork in the USA. However, the isolates were much more diverse than reported from Europe, and more similar to published data from Canada. Further studies are needed to better understand the ecology of *S. aureus* in pigs and the potential occupational risks for farm workers.

References:

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