

Longitudinal study of *Staphylococcus aureus* and MRSA colonization of US swine veterinarians

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Abstract

Patterns of detection of *S. aureus* are being evaluated in a longitudinal study of a cohort of 67 swine veterinarians in the USA. This report presents interim data from the initial period of the study. Overall, approximately 70% of sampling events yielded *S. aureus* in nasal swabs from veterinarians, and 8% yielded MRSA isolates. The majority of isolates belonged to ST398, ST9 and ST5 which together comprise the sequence types that have predominated among MRSA isolates from pigs in Europe, Asia and North America. Detection of *S. aureus* was negatively associated with time since pig contact. Patterns of colonization varied greatly among participants, with approximately one-fifth showing evidence of long term colonization, while transient colonization was suggested for most participants

Introduction

Recognition of the common occurrence of livestock associated *Staphylococcus aureus* (LA-MRSA) in food animals has raised concerns about the potential occupational and public health risks associated with these organisms. Since the initial detection of sequence type (ST) 398 MRSA in pigs in Europe, (Voss et al. 2005) more extensive research has revealed greater genetic diversity among MRSA found in pigs. In particular, isolates of ST9 have been predominant in studies of swine in Asia, (Neela et al. 2009; Lo et al. 2012) while ST5 appear to be common in North America. (Khanna et al. 2008; Molla et al. 2012; Smith et al. 2013; Frana et al. 2013) Although nasal colonization with LA-MRSA is common in livestock workers, questions remain about whether workers are permanently or transiently colonized, and whether this represents any substantial risks to their health.

Numerous studies have documented that individuals with occupational exposure to livestock are at elevated risk of nasal colonization with MRSA, and that the variants of MRSA detected are predominantly those found in animals with which they have contact. Most studies have been cross-sectional and therefore give little insight into the dynamics of colonization in humans, or whether the culture positive nasal swabs represent transient contamination of the nasal epithelium from contaminated environments rather than genuine persistent colonization. There are several studies indicating that many short term exposures result in only transient contamination. (Graveland et al. 2011; van Cleef et al. 2011; Frana et al. 2013) However, these studies do not necessarily represent the situation of workers with prolonged daily contact with livestock. Longitudinal studies in farm workers are problematic as these groups generally have almost daily exposure to barn environments, and mostly to the same groups of animals. Therefore persistent culture positive results in farm workers provide limited insight into patterns of LA-MRSA colonization vs. contamination, as they could simply reflect repeated recent contamination.

Swine veterinarians represent a unique population to investigate this question. They are known to be at elevated risk of MRSA colonization and have regular exposure to pigs. However, unlike farm workers, in the USA they are mostly exposed to diverse swine populations (i.e. multiple client farms). Another shortcoming of most research to date has been an almost myopic focus on MRSA, with minimal information available to date on the general ecology of *S. aureus* (MSSA and MRSA) in animals. The goal of this prospective longitudinal study of US swine veterinarians was to describe patterns of MSSA and MRSA detection in monthly nasal swabs in relation to the frequency and diversity of animal contact and the incidence of associated health events.

Materials and methods

A cohort of swine veterinarians was recruited at the 2012 meeting of the American Association of Swine Veterinarians. To be eligible for the study, volunteers had to be active swine veterinarians having regular (2 per week or more on average) contact with pigs. The target population range for the study was 50 – 70 veterinarians. After giving informed consent, participants were mailed materials for sample collection and transport, and shown a video to standardize the swabbing procedure. Commencing in July 2012, participants were contacted monthly by email to remind them to collect a nasal swab, and to complete an on-line survey. Survey questions related to time since pig exposure and use of personal protective equipment; recent (1 week) history of exposure to pigs including average daily contact and number of herds visited; contact with other animal species; incidence occupationally related injuries; incidence of skin or soft tissue injuries; occurrence on

any staphylococcal infections; and the date of swab collection. For logistic reasons, no attempt was made to standardize the interval from pig contact to nasal swabbing.

Nasal swabs were submitted to a double enrichment procedure using Mueller Hinton enrichment broth containing 6.5% NaCl, then Phenol red mannitol (PRM) broth with and without oxacillin (4mg/ml). Samples showing color change in PRM broths were and cultured on CNA agar and MRSA-selective plates. Putative *S. aureus* isolates were confirmed using the catalase and tube coagulase tests. Putative MRSA isolates were confirmed using PCR for *mecA* gene. DNA from each isolate was extracted with 10mM Tris-HCl and Lysostaphin and stored at -20°C. All confirmed *S. aureus* isolates were sub-typed using spa typing, and randomly selected isolates of all spa types were characterized using MLST following published methods. Spa types were determined using both the Ridom and egeonomics systems, and MLST types were obtained from the website <http://saureus.beta.mlst.net>.

Results

A total of 72 veterinarians were recruited to the study. Four withdrew before sample collection began due to concerns about frequency of pig contact, and 2 commenced sampling at the second (August 2012) sampling. One participant withdrew after 4 months due to migration from the USA. A cohort of 67 veterinarians is continuing to participate in the 18 month study. Sample submission and survey completion rates exceed 95%. The veterinarians reside in 15 major swine producing states of the USA and typically interact with diverse swine populations in their work.

Over the first 7 months of sampling, the prevalence of *S. aureus* among all samples was 70.7% (325/460), and varied among months 54% to 84%. MRSA prevalence varied from 4.4 to 10.6% among months, and MRSA isolates accounted for 7% of all isolates (32/460). Colonization patterns varied greatly among veterinarians, with 12 (21%) participants consistently colonized with the same spa type. Others participants showed considerable variability in spa types detected over time, while only two participants were consistently culture negative. The probability of culture positivity was associated with time since pig exposure, but delay in sample processing did not significantly affect the probability of a positive culture. The most prevalent isolates were t034-ST398 and t002-ST5, which comprised 74.2% (241/325) of all isolates. Several ST9 spa types (t337, t3446, t2498) were also common among MSSA isolates.

Discussion

The study described is ongoing and will be completed after 18 consecutive months of sampling. These interim results are of interest in relation to those from a recently published longitudinal study of swine veterinarians in Holland.¹⁰ Prevalence of *S. aureus* was similar (around 70%) in both studies, but the prevalence of MRSA was markedly higher (44%) in Dutch veterinarians than in our study in the USA (8%).(Verkade et al. 2013)

This observation adds to evidence suggesting that MRSA prevalence in the swine industry in the USA may be lower than seen in many European countries.(Smith et al. 2013) Our data also indicated that ST398, ST9 and ST5 variants are predominant among isolates from swine veterinarians, and likely indicate that most isolates were acquired from occupational exposure to animals. Finally, variable patterns of colonization were observed among veterinarians, with about 20% of veterinarians being consistently colonized with the same spa type. Verkade et al (2013) similarly found 14% of their study subjects appeared to be 'colonized' whereas different isolates were obtained from most individuals over the course of their study (5 samplings over 2 years). One could speculate that individual host determinants are likely to influence likelihood of genuine colonization of people with *S. aureus* of livestock origin, and that permanent colonization is not likely for a majority of people exposed to livestock.

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