

Characterization of *Staphylococcus aureus* colonization in swine veterinarians: an 18-month longitudinal study

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Abstract

Research over 10 years has established the high risk of occupational exposure to MRSA in people working with pigs. However the nature of nasal colonization with livestock associated MRSA (LA-MRSA) and its health implications remain poorly understood. Multiple studies confirmed that detection rates in pigs, farm workers and veterinarians were higher than in people without animal exposure, but elevated risk of infection in these groups has not been clearly shown. We conducted a longitudinal study for 18 months to describe patterns of *S.aureus* (SA) colonization and infection in swine veterinarians. Overall, the monthly prevalence of SA and MRSA from nasal swabs of veterinarians was 58 to 82%, and 6 to 15%, respectively. Predominant spa types were t034 (ST398, 50%), t002 (ST5, 25%) and t337 (ST9, 18%), a distribution similar to that found in a concurrent study in pigs in the USA. Despite this high level of exposure, and frequent occurrence of minor injuries in the cohort, no confirmed cases of infection were reported. Based on detection patterns, veterinarians were classified into three groups: Persistent carrier (PC, 38%), Intermittent carrier (IC, 61%) and Non-carrier (NC, 1%). Based on one-time quantitative testing of nasal swabs without enrichment, PC veterinarians carried significantly higher numbers of SA than IC. Among IC veterinarians, culture positivity was significantly associated with recent contact with pigs. Elevated prevalence of SA and MRSA in US veterinarians appears to be a consequence of exposure to pigs, however MRSA prevalence was much lower than seen in a similar study in Holland. Exposure did not lead to prolonged colonization in most subjects, and the higher numbers of SA in PC subjects suggests that unknown host factors may determine the likelihood of prolonged colonization by SA of livestock origin. Although the period of follow up was limited, the absence of clinical infections despite regular exposure suggests that major health risks due to livestock associated SA are unlikely.

Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) was long perceived as a problem confined to the human medical arena, with animal reservoirs considered to be of negligible epidemiological significance. It is now more than a decade since the discovery of ST398 MRSA in pigs in the Netherlands undermined this paradigm. (Voss et al., 2005) In the interim, substantial research in many countries has consistently confirmed that people working in contact with live pigs (farmers, veterinarians, abattoir workers and their families) are at elevated risk of being culture positive for MRSA, due to exposure to livestock associated variants in the animal reservoir. While ST398 MRSA were initially referred to synonymously as 'livestock associated MRSA', the epidemiology of livestock associated MRSA is now known to be more complex. The predominant genotypes of MRSA in pigs have been found to vary geographically, with ST9 being the predominant lineage in Asia (Chuang and Huang, 2015). Previous studies of MRSA in pigs in North America have variably reported predominance of ST398 or ST5 variants, and ST9 variants have also been isolated. (Smith et al., 2009; Molla et al., 2012; Frana et al., 2013; Smith et al., 2013). Although isolates of the ST398 clonal complex have been predominant in most human studies, this likely reflects the status of the relevant swine populations. Individual studies have reported ST5 and ST9 isolates to be predominant in people exposed to pigs in North America and Asia respectively. (Neela et al., 2009; Frana et al., 2013)

There are multiple reports of severe or fatal systemic infections in people with ST398 *S.aureus* which confirm that organisms of this lineage have the potential to be serious human pathogens.(Ekkelenkamp et al., 2006; Rasigade et al., 2011; Kock et al., 2013) However, there is minimal information about the incidence and severity of clinical infections with ST398 *S.aureus* in livestock workers, and most cases reported of severe and fatal infections with ST398 MRSA have involved elderly and medically compromised patients without known animal contact. To date there is little available information about the actual clinical risk associated with livestock exposures. A recent and large study (over 15,000 person-months) of rural residents in the state of Iowa found MRSA colonization was not elevated in individuals with current or past livestock exposure, and no cases of ST398 MRSA infection were identified. (Wardyn et al., 2015) Furthermore, the nature of the phenomenon of culture positivity of humans with *S.aureus* of livestock origin is incompletely understood. This is due in part to the difficulty in distinguishing long term colonization from serial recontamination in people who are routinely exposed to animals, and also to the paucity of long-term longitudinal studies of at risk individuals. The overall objective of this study was to analyze long term patterns of *S.aureus* [both MRSA and methicillin susceptible *S.aureus* (MSSA)] colonization and infection in a cohort of swine veterinarians in the United States.

Material and Methods

A cohort of 68 swine veterinarians was recruited for the study at a meeting of the American Association of Swine Veterinarians in 2012. The veterinarians were resident in 15 states of the USA, and concentrated in major swine producing states in the Midwest and southeastern regions. After obtaining informed consent, veterinarians were given instructions for self-collection of nasal swabs by video. Collection materials were mailed to the participants who were contacted monthly to collect and mail a nasal swab and to complete an on-line survey to collect information on recent pig exposure (time since last pig contact, hours worked in the previous week, number of farms visited in the previous week), events of physical injury focussing on skin wounds, and select health events (occurrence of skin or soft tissue infections, or confirmed staphylococcal infections).

Culture and identification of nasal swabs was conducted as described previously, and all *S.aureus* isolates were spa typed and tested by PCR for the presence of the *MecA* gene.(Linhares et al., 2015)(Linhares). MLST typing was performed for all spa types identified, but not for all isolates, particularly for spa types of previously established MLST types (e.g., t034 of the ST398 lineage). Quantitative assessment of *S.aureus* populations using direct plating (no enrichment) was conducted on a convenience subset of 41 participants to evaluate the association between frequency of positivity and bacterial load.

Results

Two veterinarians withdrew from the study due to migration and change of work circumstances, leaving a cohort of 66 swine veterinarians who completed the study. Compliance with swab submission and survey completion was over 98%. Overall, the monthly prevalence of SA and MRSA from nasal swabs of veterinarians varied from 58 to 82%, and from 6 to 15%, respectively. Predominant spa types were t034 (ST398, 50%), t002 (ST5, 25%) and t337 (ST9, 18%). Minor injuries were frequently reported throughout the study, but no serious injuries were reported. There were no confirmed cases of *S.aureus* or MRSA infection reported by participants, apart from one positive culture from a case of mild eczema. The frequency of culture positivity of individual veterinarians during the study varied from 0% (1 veterinarian) to 100% of sampling events. The likelihood of culture positivity did not vary with time from sample collection to processing, but did decrease significantly with the interval between sampling and most recent pig contact.

Based on detection patterns, veterinarians were classified into three groups: Persistent carriers who were culture positive on 17 or 18 occasions (PC, 38%) ; Intermittent carriers (IC, 61%) and Non-carriers (NC, 1%). In approximately half of the PC subjects, the same spa type was found at all sampling events, while others showed changes in spa types over time. Based on one-time quantitative testing of nasal swabs without enrichment, PC individuals were significantly more likely to be culture positive, and harbored approximately 2 logs more *S.aureus* per swab than IC individuals.

Discussion

Veterinarians are an interesting group for evaluating *S.aureus* epidemiology as, unlike producers, they are typically exposed to multiple swine herds over time, rather than a single herd. The prevalence of both *S.aureus* and MRSA positivity in the swine veterinarians was elevated compared to estimates for the US population, consistent with earlier studies in several countries. Notably, the prevalence of *S.aureus* was very similar to that reported in a similar longitudinal study of swine veterinarians in the Netherlands.(Verkade et al., 2013), but the prevalence of MRSA in that study (44%) was substantially higher than in US veterinarians. Similarly, a longitudinal study of Dutch swine producers reported an overall MRSA prevalence of 63%, and 38% of producers were persistently positive. The lower prevalence of MRSA in the current study likely reflects much lower prevalence of MRSA in commercial swine population in this country.(Smith et al., 2013; Sun et al., 2015) The predominant spa types and MLST types of *S.aureus* found in the veterinary group also correspond closely with those identified in a multistate companion study of pigs, with most isolates belonging to the ST398, ST5 and ST9 lineages.(Sun et al., 2015) This suggests that the majority *S.aureus* harbored by people working with pigs are likely to be of animal origin.

Our observation that persistent colonization occurs in a substantial minority of exposed individuals is common across the current study and the 2 Dutch studies cited above.(Verkade et al., 2013; van Cleef et al., 2015) The apparently transient nature colonization following most events of exposure to *S.aureus* in animal populations is consistent with observations that culture positivity occurs predominantly among animal workers and their immediate families, but is very uncommon in the general community, even in areas with a high density of pig farming. The health implications of persistent colonization with *S.aureus* of animal versus human origin remains to be determined.

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Seroprevalence of *Yersinia*, Hepatitis E and *Trichinella* among migrant and non-migrant populations in Berlin and Brandenburg (Germany)

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Pork is a potential source of infection for *Yersinia*, Hepatitis E virus and *Trichinella*. In 2014, 2484 cases of yersiniosis, 671 hepatitis E cases and 1 trichinellosis case were reported in Germany. Although migrants represent 27% of Berlin inhabitants, data is lacking on subpopulation-specific exposure risks to zoonotic diseases. We aimed to estimate the seroprevalence and to identify potential risk factors for *Yersinia*, Hepatitis E and *Trichinella* in migrants and non-migrants in Berlin/Brandenburg.

We used a cross-sectional design to determine the seroprevalence (IgG-antibodies) of *Yersinia*, *Trichinella* and Hepatitis E in participants without migration background, and in subpopulations with a Turkish, Russian or Vietnamese background. Using a convenience sample, we included healthy participants, aged ≥ 18 years, residents of Berlin/Brandenburg, presenting to the participating hospitals, health care centers, and migrant associations. Participants were interviewed with a questionnaire asking for demographics, travel history, alimentation, animal contacts and medical history. Multiple logistic regression was used to examine potential risk factors.