Effect of age and anatomic site on likelihood of detecting S. aureus in pigs

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Abstract

Intensive sampling of two swine farms in Minnesota was conducted to obtain basic information about the ecology and epidemiology of S. aureus in modern multiple site swine production. The farms were selected by convenience, and two cohorts of animals were sampled in each system. Samples were collected from the nose, tonsils, feces, and skin of suckling pigs, weaned pigs, and finishing pigs in each of two birth cohorts raised in each system, and from the same sites plus the vagina of sows nursing the selected piglets. Highest prevalence of S. aureus was in nasal, tonsil and skin samples (59 – 65%), and lowest prevalence (27%) was in vaginal samples from sows. No MRSA were detected on the farms. Diverse spa types of S. aureus were found on both farms and all age groups. The predominant spa types found belonged to MLST sequence type ST398, ST9, and ST5. S. aureus was also frequently detected in environmental samples, including air, and in people working on the farms. The 3 predominant spa types encountered (t034/ST398; t337/ST9; t002/ST5) have previously been identified as MRSA isolates of pigs in various international studies. The use of the term ‘livestock associated MRSA’ should be broadened to include certain variants belonging to the ST9 and ST5 sequence types (and likely others) rather than be synonymous with ST398 MRSA.

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. Although there has now been extensive research into the occurrence of MRSA in many countries, there are remarkably few studies of generic S. aureus. We contend that understanding of the ecology and epidemiology of LA-MRSA cannot be achieved in a void of information about the parent organism, regardless of antimicrobial resistance status. That is, the ability to understand the origins and implications of LA-MRSA in pigs is limited by lack of basic information about the ecology of Staphylococcus aureus in pork production.

Published studies in pigs have almost universally used nasal swabbing, which is the standard method (with or without pharyngeal swabbing) for detecting S. aureus in humans. Environmental samples (such as dust) are effective for determining herd status for MRSA, but are of limited value for studying the ecology of an organism that is deemed to be normal flora in swine. There are reports of S. aureus being detected in various anatomical sites including sites of pigs including skin, tonsils feces, nose, arthritic joints and internal organs. However, there has been no systematic study comparing sampling protocols for detecting S. aureus in pigs. We conducted a pilot study of two production systems to obtain basic information about S. aureus prevalence in pigs, and the relative sensitivity of detection of S. aureus by anatomical site. Specific aims were to (a) compare the effect of sample site on detection of S. aureus in pigs and (b) compare S. aureus prevalence and spa types in sows, suckling pigs, weaned pigs and finishing pigs.

Materials and Methods

A longitudinal study was conducted in 2 independent pig production systems in Minnesota. Eligibility of farms for the study was limited only by production type, as we wanted to study farms using multiple site systems (breeding herd and growing pigs reared on separate sites) that predominate in the US industry. The farms were selected purposively based on willingness of the producers to participate. The core assumption in design of the project was that S. aureus is part of the normal flora of pigs, and expected prevalence in preferential sites of carriage should exceed 50%. In the absence of prior information, and given resource limitations, we chose to conduct detailed sampling (4 – 5 anatomical sites per animal) in a relatively small number of pigs per sampling event. Samples were collected from recently farrowed sows (n=12 spread through a room) and one piglet from each respective litter (n=12). Subsequent sampling of the same birth cohorts of pigs occurred 4 and 20 weeks later in the “nursery” (n=12) and “market-age” (n=12) phases of pig production, respectively. Two pig cohorts were sampled in each flow, with at least 6 months between cohorts. Swab samples were collected from the nose, tonsil, feces, and skin (axilla) of each pig (plus vagina in sows).

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 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 iso-
late was extracted with 10mM Tris-HCl and Lysostaphan 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 egenomics systems, and MLST types were obtained from the website [http://saureus.beta.mlst.net](http://saureus.beta.mlst.net).

**Results**
A total of 542 *S. aureus* isolates were obtained in the study, none of which were MRSA. Prevalence differed among anatomical sites (p <0.001), and was higher in nose (68%), skin (62%) and tonsil (62%) samples than in fecal (42%) and vaginal (27%) samples. Prevalence of positive pigs (at least one site positive) was similar (from 88% to 96%) in all age-groups (p = 0.55). No MRSA were detected, but multiple spa types were detected in each system. Multiple spa types were also detected in many individual pigs, but there was no apparent association between spa type and anatomical location. Spa types t034 (ST398), t1377 (ST9) and t002 (ST5) were most common. Spa type distributions were very similar between sows and their associated piglets, suggesting transmission from sow to offspring occurs commonly early after birth. Spa type patterns in growing pigs tended to vary between cohorts and farms.

**Discussion**
This study confirms that *S. aureus* can indeed be considered part of the normal flora of pigs, and that the upper respiratory tract and skin are the sites where the bacteria can be most readily isolated. Vaginal colonization was common in sows, and colonization of piglets occurs early in life. Within farms, cohorts and even pigs, considerable diversity of spa types and sequence types was found. Interestingly, the most predominant sequence types (ST398, ST9, ST5) of the MSSA isolates detected on these two farms correspond with those reported most commonly in studies of MRSA in pigs. Since the initial detection of sequence type (ST) 398 MRSA in pigs in Europe,(Voss et al. 2005) research in other regions has revealed considerable 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) The occurrence of these same sequence types and spa types among MSSA in this small pilot study suggest that these lineages of *S. aureus* are likely to have a long evolutionary relationship with pig. We also suggest that the term livestock association MRSA should not be restricted to the ST398 lineage, and that some variants of ST9 and ST5 *S. aureus* should be included under this ‘umbrella’ term.

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**References**