Abstract

It has been suggested that the emergence of livestock associated MRSA in swine may be a consequence of feeding antibiotics and/or zinc to control swine diseases or promote growth. However, the dynamics and health implications of interspecies transmission of MRSA from pigs to humans is still incompletely understood. To address these questions, better understanding is required of the broader ecology of S. aureus in pigs, including MRSA. To obtain more representative data on the prevalence and characterization of S. aureus, including MRSA, we collected nasal samples from growing pigs on 36 swine farms across 11 states in the USA. All isolates were spa typed, and MLST typing was conducted on selected isolates of each spa type. Antimicrobial and zinc susceptibility testing was performed on a subset of 130 isolates selected purposively to maximize the diversity of isolates by farm and spa type. S. aureus was detected on 35 of the 36 farms, and from 76% of pigs sampled, but no MRSA were found apart from one farm of known status that was included as a MRSA positive control. A total of 33 spa types were detected, with the most prevalent being t337 (ST9), t034 (ST398) and t002 (ST5). Antimicrobial resistance testing for 18 antibiotics showed that resistance was most common to spectinomycin (100%), tetracycline (94%), clindamycin (75%) and penicillin (72%), and a majority of isolates were resistant to 5 or more antibiotics (multidrug resistant S. aureus, MDRSA). Only ST398 MRSA isolates from the positive control farm were positive for the czrC gene, although phenotypic resistance to zinc was observed in some MSSA isolates. These results support earlier studies indicating a relatively low prevalence of MRSA in pigs in the USA, but a high prevalence of MDRSA. The data also concur with the hypothesis that zinc resistance may be more strongly associated with MRSA in swine reservoirs than is resistance of antibiotics such as tetracyclines.

Introduction

Prior to the recognition that pigs and other livestock species can be reservoirs of methicillin resistant Staphylococcus aureus (MRSA), S. aureus was considered a relatively unimportant organism in animals. It is now more than a decade since ST398 MRSA was found to be prevalent in pigs and other livestock species in the Netherlands and other countries,(Voss et al., 2005; Smith and Pearson, 2011), yet many questions remain unanswered about the epidemiology and public health impact of S. aureus in livestock reservoirs. 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 detected( Smith et al., 2009; Molla et al., 2012; Frana et al., 2013; Smith et al., 2013). Remarkably, given the potential importance of this issue, research on the ecology of methicillin sensitive S. aureus has been largely ignored, but is arguably an important foundation for understanding the origin and ecology of MRSA variants. In a pilot study of 2 systems in the USA, we observed that S. aureus are readily detectable in many anatomical sites, and that multiple spa types and
MLST types occurred on individual farms and even within the same pigs (Linhares et al., 2015). The goal of the current study was to obtain more geographically representative information about the prevalence and diversity, including antimicrobial resistance phenotypes, of *S. aureus* and MRSA in growing pigs on commercial farms in the USA.

**Material and Methods**

A cross sectional study was conducted on 36 farms located in 11 states in the USA. Each farm was recruited via swine veterinarians who had participated in a previous study. Each veterinarian sampled only one herd from their client base. An additional herd known to be positive for MRSA was included as a positive control herd. Nasal swabs were collected by the veterinarians from 20 pigs in each herd. Swabs were enriched in Muller Hinton broth and cultured selectively on chromogenic agar plate to detect MRSA. DNA extraction was performed for spa typing, multilocus sequencing typing (MLST) and PCR detection of the czrC gene as an indicator for zinc resistance. Out of 1070 isolates obtained, 130 isolates were selected for antibiotic resistance testing and phenotypic zinc resistant test. Selection was purposive to maximize the diversity of isolates included (at least one isolate from each spa type detected on each farm).

For antibiotic susceptibility testing, a panel of 18 antibiotics were used as conducted routinely for gram-positive organisms at the University of Minnesota Veterinary Diagnostic Laboratory (spectinomycin, ampicillin, penicillin, chlorotetracycline, oxytetracycline, clindamycin, tilmicosin, danofloxacin, sulphadimethoxine, florfenicol, novomycin, tiamulin, gentamycin, enrofloxacin, trimethoprim/sulphamethoxazole, tulathromycin, ceftiofur, troleloid). In addition, zinc susceptibility was evaluated using the agar dilution method at concentrations of 0.5, 1, 2, 4, 8, 16, and 32mM.

### Results

Thirty-six farms from 11 states in the USA were sampled, in addition to the positive control farm. Overall, 739 pig nasal swabs were collected, of which 558 (76%) were culture positive for *S. aureus* from 35 of the 36 farms (97%). Except the positive control farm, on which all 20 pigs tested MRSA positive, no MRSA were detected in any of the pigs. Among the 35 *S. aureus* positive farms there was considerable diversity found with 33 spa types detected within 4 MLST sequence types. The most prevalent spa types (sequence type) were t337 (ST9), t034 (ST398) and t002 (ST5) which together accounted for 59% (627 of 1070). Antimicrobial resistance testing showed resistance was most common to spectinomycin (100%), tetracycline (94%), clindamycin (75%) and penicillin (72%), and 89% (116/130) of isolates were resistant to 5 or more antibiotics (multidrug resistance SA, MDRSA). ST398 (t034) MRSA isolates from the positive control farm were positive for the czrC gene, but no other isolates tested were positive. However, 14% of SA (18/130) tested were phenotypically zinc resistant based on a break point of 4mM zinc.

### Discussion and conclusions

The most striking finding of the study was that none of the 36 study farms were positive for MRSA, and exact binomial confidence intervals show the 95% upper limit for herd prevalence to be 9.7%. Although much higher prevalence (up to 30%) has been previously reported in smaller, and more geographically limited, studies in the USA, (Molla et al., 2012; Frana et al., 2013), relatively low herd prevalence was also reported in larger and geographically diverse studies in the USA and Canada (Weese et al., 2011; Smith et al., 2013). Several studies of occupationally exposed people in the USA are also consistent with the possibility that prevalence of MRSA may be substantially lower in the US swine industry than in many European countries (Rinsky et al., 2013; Warden et al., 2015; Sun et al., 2015). The apparently low MRSA prevalence in pigs in the USA, where antimicrobial growth promotants will be available until 2017, raises further questions about whether growth promotant usage had any material influence on the emergence of livestock associated MRSA globally. Our observations on the czrC gene are also aligned with other studies indicating that zinc and other selective factors (e.g., disinfectants and therapeutic use of cephalosporins) are likely to have had a much greater role in the emergence of these organisms than growth promotant usage (Aarestrup et al., 2010; Cavaco et al., 2011; Moodley et al., 2011; Dorado-Garcia et al., 2015; Sliiferz et al., 2015) Our observations that multiple antimicrobial resistant is prevalent in *S. aureus* isolates from pigs is both unsurprising and consistent with other studies. This phenomenon is probably not new, and the total absence of awareness or concern (in the pre-ST398 era) about *S. aureus* in terms of as an occupational risk suggests that human health risks of MDRSA in livestock are less than intimidating. Regardless, in the USA it is increasingly likely that in a relative vacuum of MRSA in pigs that the phenomenon of multiple drug resistant MRSA will attract more attention from industry critics and may require efforts in both risk assessment and risk communication to quell the concerns raised to the general public.

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In the framework of the National Zoonoses Monitoring in Germany, E. coli from pigs and pork as well as from wild boar meat have been tested for their resistance to a panel of 14 antimicrobials using the broth microdilution method according to CLSI. The determined minimum inhibitory concentrations were evaluated based on the epidemiological cut-off values as provided by EUCAST.

859 isolates from pig herds and 52 isolates out of 568 samples from pork were tested in 2011. More isolates from pork than from pigs were susceptible to all 14 substances (55.8 vs. 23.3 %). Resistance rates to the following substances were higher in isolates from pigs than from pork: tetracycline (62.5 % in pigs / 30.8 % in pork), streptomycin (59.4 / 28.8 %), sulfamethoxazole (47.6 / 25.0 %), ampicillin (44.7 / 25.0 %) and trimethoprim (38.2 / 23.1 %). Resistance to cefotaxim (1.9 %) and ciprofloxacin (5.9 %) was similar in isolates from pig and pork respectively.

AMR in isolates was infrequent. 93.5 % of the 186 isolates from wild boar meat were susceptible to all substances. One isolate was resistant to cefotaxime and 3 isolates were resistant to colistin, while no isolates were resistant to ciprofloxacin.

The results underline that antimicrobial resistance is an issue in pig farming. Although still high, AMR in isolates from pork was substantially lower than in those from pigs. In wild boar meat, E. coli was more frequently detected than in pork, however, AMR was very low. This indicates that resistant bacteria are substantially less frequent in wild boars than in fattening pigs. Exposure of wild boar to antimicrobials or resistant bacteria from farm animals via the environment is too limited to lead to high resistant rates in E. coli from this food chain.

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