RESEARCHREPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Prevalence and characterization of Methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs and farm workers on conventional and antibiotic-free swine farms in the USA. **NPB #: 08-178, 08-179, 08-180**

FINAL

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Industry Summary:

This report combines the results of collaborative projects between the University of Minnesota (Dr. Peter Davies), University of Iowa (Dr. Tara Smith) and the Ohio State University (Dr. Wondwossen Gebreyes) that have been reported individually (NPB 08-178, 08-179 and 08-180). Since 2004, reports of MRSA in animals worldwide and apparent animal-to-human transmission have raised concerns about the role of animal populations, and particularly pigs and cattle, as potential reservoirs of zoonotic MRSA infections. The aim of the study was to estimate the prevalence and molecular types of methicillin-resistant *Staphylococcus aureus* (MRSA) on swine farms in major swine producing states in the USA. We collected nasal swabs from pigs and farm workers at 45 swine herds (21 antibiotic-free herds; 24 conventional herds) in Illinois, Iowa, Minnesota, North Carolina and Ohio. MRSA was isolated from 50 of 1085 pigs (4.6%) and 31 of 148 (20.9%) of farm workers. MRSA-positive pigs and people were clustered in four conventional swine farms in Iowa and Illinois. This confirms the presence of MRSA in pigs and swine farm workers in the USA, but the prevalence found is relatively low compared with European studies. Although MRSA may present an occupational hazard to swine workers in confinement buildings on some farms, there is little evidence to date suggesting a significant human or animal health impact of MRSA associated with livestock.

Keywords: MRSA, prevalence, pigs, farm workers, antibiotics

Scientific Abstract: Much uncertainty remains about the origin and public health implications of livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA). This study aimed to investigate the occurrence and prevalence of MRSA in general and LA-MRSA in particular in pigs and farm workers in five states. We collected nasal swabs from pigs and farm workers at 45 swine herds (21 antibiotic-free herds; 24 conventional herds) in Illinois, Iowa, Minnesota, North Carolina and Ohio. MRSA was isolated from 50 of 1085 pigs (4.6%) and 31 of 148 (20.9%) of farm workers. MRSA-positive pigs and people were clustered in four conventional swine farms in Iowa and Illinois. Based on genotyping, spa type t034, a common livestock associated variant, was predominant among both human and swine isolates. These results confirm the presence of LA-MRSA in pigs and swine farm workers in the USA, but the prevalence found is relatively low compared with European studies.

Introduction:

Staphylococcus aureus is a common commensal bacterium in many homeothermic species and an opportunistic pathogen of major concern in human medicine. The emergence of antimicrobial resistant *S. aureus*, and specifically methicillin resistant strains (MRSA) has been a particular problem in hospitals for many decades. From the mid-1990s, the epidemiology of MRSA changed as an increasing incidence of clinical infections was observed in many countries in the general community outside the hospital environment. Furthermore, the strains of MRSA involved were distinct from those predominating in hospital acquired infections. Since 2004, different variants of MRSA have been identified in livestock species, particularly in pigs and cattle, in Europe.[1-2] Studies conducted in North America have also confirmed the occurrence of these 'livestock associated' MRSA (LA-MRSA) both Canada and the USA.[4,5]

The apparently recent emergence of LA-MRSA, and the fact that they are generally resistant to tetracyclines, has promoted considerable discussion of the role that antimicrobial use in livestock may play in the epidemiology of these organisms. The purpose of this study was to obtain a broader perspective on the prevalence of MRSA in pigs and farm workers in the USA, and to estimate prevalence in both conventional swine farms and farms not using antibiotics.

Objectives:

1. To estimate the prevalence of MRSA in pigs on conventional and niche-market (antimicrobial-free) swine farms in several regions of the USA
2. To estimate the prevalence of MRSA in swine farm workers on these same farms
3. To characterize MRSA isolates from these populations using genotypic subtyping methods (PFGE and spa typing)
4. To compare the genotypic and phenotypic characteristics of MRSA isolates from pig and farm workers with isolates from people not involved in swine production, and with isolates reported from other countries and host species

Materials & Methods:

Between April 2008 and July 2010, we collected samples from weaned pigs between 6 and 18 weeks of age (24 per farm), and all workers willing to participate, on 45 farms in five major swine producing states in the USA (IA, IL, MN, NC, OH). Sample size was adequate to detect a positive herd with more than 99% confidence at an expected prevalence of 20% assuming a population size of 1000 animals. The study was designed so that half of the 18 farms to be sampled by each collaborating group would be conventional confinement farms and half would be farms raising pigs without use of antibiotics (antibiotic-free, "ABF"). Due to difficulty in recruiting eligible farms, ultimately 45 farms were included in the study: 18 farms in Minnesota (9 conventional, 9 ABF); 18 farms in Iowa-Illinois (9 conventional, 9 ABF); and 9 farms in Ohio-North Carolina (6 conventional; 3 ABF). All swine farm workers at each site were invited to participate by completing a demographic questionnaire and permitting nasal/oropharyngeal swabs to be taken to determine MRSA colonization status. The survey recorded data on potential risk factors for MRSA colonization, including information about contact with swine and use of personal protective equipment.

Swabs were transported in Stuart's medium at 4°C to the respective laboratories. Samples were inoculated into 2 mL enrichment broth containing 10 g tryptone/L, 75 g NaCl/L, 10 g mannitol/L and 2.5 g yeast extract/L. After 24 h incubation at 35°C, a loopful of broth was inoculated onto selective MRSA agar plates (BBL CHROMagar MRSA, Becton, Dickinson and Company). These plates were incubated 24–48 hours at 35°C and examined for MRSA. Isolates were confirmed to be S. aureus by examining their appearance on Gram stain, by catalase test, tube coagulase test and a S. aureus latex agglutination assay (Pastorex Staph-plus, Bio-Rad). Presumptive Methicillin resistant strains were identified by testing for the presence of penicillin binding protein 2 (PBP2′) (MRSA latex agglutination test, Oxoid Ltd., Hants, UK). MRSA isolates were stored at −80°C. All MRSA isolates were typed by partial sequencing of the Staphylococcus protein A (spa) gene [14,15]. PCR amplicons of the spa gene were obtained using SpAF (5'-GAACAACGTAACGGCTTCATCC-3') and 1517R (5'-GCTTTTGCAATGTCATTTACTG-3')."). Spa types were assigned using both the eGenomics, Inc (www.egenomics.com) and Ridom (http://spaserver.ridom.de) spa servers.

Risk factors were compared for MRSA-positive and MRSA-negative workers on farms with MRSA-positive herds using Fisher's exact test for categorical variables and the Wilcoxon rank sum test for continuous variables. Swine MRSA-positive prevalence rates and associated 95% confidence intervals were adjusted for clustering within herds using generalized linear mixed models in PROC GLIMMIX. To identify risk indicators for MRSA carriage among swine workers, a multivariable logistic regression model was developed using generalized estimating equations in PROC GENMOD with an exchangeable correlation matrix to adjust for correlation within farms. Specifically, we evaluated the effect of length of swine contact (i.e., daily hours of contact) on MRSA carriage along with potential confounders

(including age, race, and gender). All analyses were conducted using SAS version 9.3 (Cary, NC).

Results:

1. *Prevalence of MRSA in pigs on conventional and niche-market (antimicrobial-free) swine farms in several regions of the USA*

   Fifty of the 1085 pigs sampled were culture positive for MRSA (4.6%; adjusted for clustering: 4.1%, 95% CI 1.2% -- 14.3%), with culture positive pigs clustered on 4 (9%) conventional farms in Iowa and Illinois. No positive herds were detected among the 27 farms sampled in Minnesota, North Carolina, or Ohio, and all pigs sampled on the 21 ABF herds were negative for MRSA. The MRSA prevalence among pigs on conventional farms overall was 8.5% (50/588; adjusted for clustering: 7.9%, 95% CI 2.2% -- 28.6%).

2. *Prevalence of MRSA in swine farm workers on these same farms*
   Thirty one of 148 farm workers (20.9%) were culture positive for MRSA. Of these, 27 (87%) worked on farms where MRSA was detected among the sampled swine. The vast majority of positive samples (24/31; 77%) were from 40 workers sampled on the two farms with the highest prevalence of MRSA in pigs.

3. *Characterization of MRSA isolates from pigs and farm workers*
   Eleven different spa types were detected from 68 isolates tested. t034, a common spa type among within the ST398 lineage of livestock associated MRSA, was the predominant spa type (74%) detected among humans and swine. The second most common spa type was spa type t002, with 6 of 7 isolates from single farm.

4. *Comparison of MRSA isolates from pig and farm workers with isolates from people not involved in swine production, and with isolates reported from other countries and host species*.

   The predominant spa type (t034) is among the most common isolates of livestock associated MRSA detected previously in North America and Europe. The second most common spa type (t002), may be more common in North America than elsewhere, but more extensive work is indicted to confirm this. Reports from several countries have also identified other lineages of MRSA other than ST398 (e.g., ST9, ST30), indicating that the diversity of MRSA in livestock around the world is broader than previously thought.

Discussion:

LA-MRSA was first detected in Europe in 2004 after several decades of widespread use of tetracyclines and other antimicrobials in food animals, but paradoxically in the aftermath of increased restrictions on in-feed antimicrobial use in several EU

countries. LA-MRSA isolated from animals have been almost uniformly resistant to tetracyclines which has focused attention of the potential role of antimicrobial use, and particularly tetracyclines, in the emergence of these organisms. We purposively included both ABF and conventional herds to obtain preliminary data on MRSA prevalence in these different groups of farms in the USA. However, the two groups of farms differed in many respects other than use of antimicrobials, including herd size, sources of genetic stock, and general housing systems.

The prevalences found for both farms and pigs in the current study was lower than anticipated from European studies, but are very similar to recent data from Canada on market age hogs sampled on farms. Weese et al (2011) found MRSA in 4.6% of pigs on 5 of 46 (11%) of farms.[6] Both studies suggest that the prevalence of MRSA in North American pigs may be toward the lower end of the range reported across European countries. Important caveats to this study, like preceding studies, are the limited scope and use of convenience sampling rather than formal random sampling to select herds. For this reason, considerable uncertainty remains about the prevalence of LA-MRSA in the US swine industry. The current study was also deliberately biased by the inclusion of a relatively large proportion of antibiotic-free herds (21/45 herds, 46.7% of the tested farms), and a larger herd level study of pigs on farms across the USA may be warranted.

The relatively low prevalence of MRSA we observed among conventional herds confirms that routine antimicrobial use in pigs is not a sufficient cause for emergence of LA-MRSA. Also, the high prevalence of MRSA reported on an ABF herd in Canada suggests that exposure to antimicrobials is also not a necessary condition for the occurrence of LA-MRSA in pigs.[6]

References

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