HHS Public Access

Author manuscript

J Agromedicine. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

J Agromedicine. 2016 ; 21(2): 149–153. doi:10.1080/1059924X.2016.1142917.

Detection of Airborne Methicillin-Resistant *Staphylococcus Aureus* Inside and Downwind of a swine Building, and in Animal Feed: Potential Occupational, Animal Health, and Environmental Implications

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Abstract

Aerosolized methicillin-resistant *Staphylococcus aureus* (MRSA) was sampled inside and downwind of a swine facility. Animal feed was sampled before and after entry into the swine facility. Aerosolized particles were detected using an optical particle counter for real time measurement and with an Andersen Sampler to detect viable MRSA. Molecular typing and antimicrobial susceptibility testing were performed on samples collected. Viable MRSA organisms isolated inside the swine facility were primarily associated with particles > 5μm, and those isolated downwind from the swine facility were associated with particles <5μm. MRSA isolates included *spa* types t008, t034, and t5706 and were resistant to methicillin, tetracycline, clindamycin, and erythromycin. Animal feed both before and after entry into the swine facility tested positive for viable MRSA. These isolates were of similar *spa* types as the airborne MRSA organisms. Air samples collected after power washing with a biocide inside the swine facility resulted in no viable MRSA organisms detected. Our pilot study showed that the ecology of MRSA is complex. Additional studies are warranted on the maximum distance that viable MRSA can be emitted outside the facility, and the possibility that animal feed may be a source of contamination.

Keywords

MRSA; air sampling; swine; zoonosis; bioaerosol; agriculture; confined animal feeding operation

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a pathogen of public health concern.[-] Recent evidence has demonstrated MRSA carriage among both livestock and the workers caring for these animals, suggesting that MRSA is a zoonotic agent of potential occupational and environmental health significance. MRSA of livestock origin

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("livestock-associated MRSA" [LA-MRSA]) has been recognized as causing more than 20% of the cases of MRSA in the Netherlands. Of special note, a high MRSA carriage rate was found among workers on farms positive for MRSA in pigs or environmental dust samples, suggesting this organism may also be transmitted via the environment. Khanna et al. revealed in a study from Canada that LA-MRSA in pigs correlated with human MRSA carriers (20% prevalence) on the swine farms, and the *spa* type t034/sequence type (ST) 398 was the predominant strain in both swine and swine workers. In a pilot study, Smith et al. reported that almost half of the swine workers and pigs studied on two farming systems in Iowa and Illinois were colonized with MRSA, also found to be ST398.

A study conducted in animal feeding operations detected *S. aureus* as the predominant viable bacterial species present in air samples. This finding, along with the Gibbs et al. detection of antimicrobial resistant bacteria being recovered 150 meters downwind of swine confined animal feeding operations (CAFOs), illustrates the potential of transmission of antimicrobial resistant bacteria from swine feed operations to persons outside of the facility.

In animal production, antibiotics have traditionally been used to treat sick animals, prevent infections at therapeutic levels, and promote animal growth at subtherapeutic levels. The use of antibiotics in animal production has been shown to increase the presence of bacteria with resistant genes in the intestines of swine herds. The use of antibiotics has also been suggested to be a risk factor for the development of LA-MRSA. It has been suggested in a study that the use of aminocyclitol (an aminoglycoside) to treat *Escherichia coli* infections was related to the development of livestock associated MRSA in cattle and swine.

Our pilot study had several objectives. The first objective was to determine the relative size of particles from which MRSA could be isolated. Secondly to determine if viable airborne MRSA could be isolated at a greater distance than 150 meters downwind (note that Gibbs et. al. had previously isolated MRSA only at 150 m). The third objective was to determine if MRSA was present in animal feed, and if it was of similar typing to that isolated from the air. Our final objective was to investigate the effect of power washing with a biocide on the presence of airborne MRSA.

MATERIALS AND METHODS

Sample Site

The study site, located in a Midwest state in the United States, was conveniently selected as typical of all-in/all-out, nursery-grower, swine production facilities in the region. We had previously documented that the workers and swine at the facility were culture positive for MRSA. The socking density of the facility tested was one feeder pig per 4 ft^2.

Air Sampling

The concentration of viable MRSA and total particulates inside the swine feeding facility and 215 meters downwind (determined by wind vane) of the facility was determined by collecting air samples using stages one (> 7μm), two (4.7- 7μm), and five (1.1-2.1μm) of a six stage viable Andersen Sampler (Andersen Sampler In., Atlanta, GA, USA) at a flow rate of 28.3 liters per minute (l/min) and an Optical Particle Counter (GRIMM Technologies Inc.,

Douglas, GA, USA) with 15 channels at a flow rate of 1.5 l/min. Particle size was categorized as >5 μm and <5 μm. The same instruments were used in the study for quality assurance and quality control. After each sampling period the culture plates were sealed with tape, labeled, placed in a Ziploc bag and finally placed (agar face-down) into a cooler with ice packs for transport to the laboratory.

Animal Feed Sampling

Animal feed was collected on the sampling days directly off the feed truck when it arrived at the swine facility and from the feeding trough inside the swine facility. At the laboratory bacterial diagnostics and molecular testing was performed as previously described. Briefly, 25 grams of animal feed was placed in Baird Parker broth and incubated overnight at 35°C. The broth was then plated onto CHROMagar and Columbia Naladixic acid (CNA) plates and incubated another 24-48 hours. Potential MRSA isolates were sub-cultured and diagnostics were performed.

The sampled facility was power washed with detergent and a biocide, Keno X5 (active ingredients hydrogen peroxide 26.5% and peroxyacetic acid 4/9%; CID Lines, Belgium, Europe), between cycling of pigs (46 days) due to the all-in/all-out nature of the site. Air sampling was also conducted after the facility was cleaned using the same method previously described.

Molecular Typing

Additional molecular diagnostics were performed on a selection of isolates. Twelve samples were selected from the plates based on colony morphology that was representative of the plate samples. Molecular tests conducted included antimicrobial susceptibility testing (CLSI 2006 and CLSI 2009), *mec*A polymerase chain reaction (PCR), *spa* typing, and Panton-Valentine leukocidin (PVL) PCR. Positive and negative controls were used for all tests.

RESULTS

A total of 81 CNA and 81 CHROMagar plates were collected. Sampling day one collected 5,665 total colony forming units and 35 MRSA colony forming units. Sampling day two collected 7,621 total colony forming units and 238 MRSA colony forming units. Sampling day three collected 9,454 total colony forming units and 466 MRSA colony forming units.

Table 1 (see supplementary material) shows the median and range for total particles detected with the Optical Particle Counter. The median particle count for particles (> 5um) inside the facility was 1particles/m^3 (p/ m^3) (range = 0.106-3.095 p/ m^3), and for outside downwind of the facility a median of 0.006 p/ m^3 (range=0-6.238 p/ m^3) was measured. The median particle count for particles (<5 um) inside the facility was 2.470 p/ m^3 (range=1.170-1,119.093 p/ m^3), and for downwind the facility a median of 0.363 p/ m^3 (range=0-42.738 p/ m^3) was measured.

Viable sampling using the Andersen Sampler is shown in Table 2 (see supplementary material). The particles (<5um) inside the swine facility ranged from 11.6 × 10^3 cfu/m^3 to 15.9 ×10^3 cfu/m^3 with the mean of 13.8 cfu/m^3. Downwind of the facility the concentration

of viable total particles (<5 um) ranged from 15 cfu/m^3 to 111 cfu/m^3, with the mean of 63 cfu/m^3. MRSA particles (> 5um) inside the swine facility ranged from 547 cfu/m^3 to 1,103 cfu/m^3; the mean concentration was 825 cfu/m^3. MRSA particles (<5 um) inside the swine facility ranged from74 cfu/m^3 to 302 cfu/m^3; the mean concentration was 188 cfu/m^3. Downwind of the facility, MRSA particles (<5 um) were detected with the mean concentration of 5 cfu/m^3.

Table 3 (see supplementary material) shows the results for antimicrobial susceptibility testing and molecular typing. Twelve isolates (100%) were resistant to methicillin; eight of twelve (67%) were resistant to tetracycline and clindamycin; and four of twelve (33%) were resistant to erythromycin. All of the isolates were *mec*A positive. The pit fan exhausted air (air exhausted from the pit below the swine facility) isolate was PVL positive and *spa* type t008. The other *spa* types identified were t034 and t5706. Animal feed from both the truck and inside the swine facility tested were resistant to methicillin (4/4) and erythromycin (4/4). The isolates collected from animal feed were also *mec*A positive and spa type t034. Table 4 (see supplementary material) demonstrates that after the swine building was emptied, power washed, and disinfected, no MRSA colony-forming units was detected compared to when the swine building was occupied.

DISCUSSION

In this pilot study, we were able to detect airborne MRSA inside the swine facility and 215 meters downwind of the swine facility. To our knowledge at this time, this is the first study to detect airborne MRSA greater than 150 meters from a swine CAFO. Green et al. showed that antimicrobial-resistant *S. aureus* can be recovered from the air exhausted from swine CAFOs at distances of 150 meters. Schulz et al. detected airborne MRSA 150 meters downwind from swine barns. The detection of airborne viable MRSA at a greater distance than shown preciously suggests an important consideration of MRSA potentially being transmitted airborne to other swine facilities within 215 meters.

The animal feed tested inside the swine facility and the animal feed tested directly from the truck prior to entering the swine facility tested positive for MRSA. The animal feed having tested positive before entry into the swine CAFO may suggest animal feed as a potential source of MRSA at the facility. MRSA *spa* type t034 was detected in the animal feed, air sampled inside the CAFO, and air sampled downwind of the CAFO. Cavaco et al. showed that metal supplements, such as zinc oxide and copper sulfate in animal feed, may promote selective pressure for MRSA emergence. Our findings suggest that airborne MRSA in these facilities may have animal feed origin.

Our study also found that when the swine CAFO was emptied and disinfected no MRSA particles were detected in the air. This finding demonstrated that disinfecting swine CAFOs can reduce and potentially eliminate MRSA transmission within a facility. With only one observational assessment, additional intervention studies need to be done to determine the effectiveness of power washing and disinfecting swine CAFOs as infection control measures.

This pilot study had limitations. Our study was based on sampling only one swine farm. This prevents generalization of results to other farms, especially in different geographical locations dissimilar from the settings in our study. As this study was a pilot, we did not take into consideration the age and size of the pigs, time of day for sampling, and ventilation rate. Additionally, we only tested over a few days during the fall season.

Donham et. al. characterized the airborne dust particles in swine barns and determined that particles greater than 5 microns were primarily sourced from fecal material and particles less than 5 microns were sourced from fecal material and exfoliated epithelial cells (skin and gastrointestinal origin). MRSA was detected inside the building primarily on particles greater than 5 microns, which suggests feed may be a source. Outside, MRSA was detected primarily on particles less than 5 microns, suggesting that the larger particles do not remain airborne, and/or that smaller, animal-sourced particles may also be important contamination sources. Further studies need to be done to identify other potential sources, other than pigs, of airborne MRSA inside buildings. As the current study resulted in MRSA isolation from animal feed and 215 meters downwind of the swine CAFO, a more complex ecology of MRSA in swine facilities is emerging. To help further define the ecology of this organism, additional studies are needed to further evaluate animal feed as an additional source of MRSA in swine barns.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

Funding: This research was funded by the National Institute for Environmental Health Sciences through the University of Iowa Environmental Health Sciences Research Center, NIEHS/NIH P30 ES005605.

We thank Dr. Patrick O'Shaughnessy at the Environmental Modeling and Exposure Assessment Facility at the University of Iowa for providing the aerosol monitoring equipment and Mr. Ralph Altmaier for his technical assistance. Dr. Tara Smith and the laboratory staff at the Center for Emerging Infectious Disease at the University of Iowa for the laboratory resources provided. We are grateful to Dr. Mike Male for his assistance in the selection of sampling facility.

REFERENCES

1. Barber M. Methicillin-resistant staphylococci. J Clin Pathol. 1961; 14:385–93. [PubMed: 13686776]
2. Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn CTiensasitorn C, Hiramatsu K. Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2001; 45(5):1323–1336. [PubMed: 11302791]
3. Graffunder EM, Venezia RA. Risk factors associated with nosocomial methicillin-resistant Staphylococcus aureus (MRSA) infection including previous use of antimicrobials. J Antimicrob Chemother. 2002; 49(6):999–1005. [PubMed: 12039892]
4. Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998; 339(8):520–532. [PubMed: 9709046]
5. Donham KJ. Community and occupational health concerns in pork production: A review. J Anim Sci. 2010; 88(13 electronic suppl):E102–E11. [PubMed: 20154166]

6. O'Shaughnessy PT, Donham KJ, Peters TM, Taylor C, Altmaier R, Kelly KM. A task-specific assessment of swine worker exposure to airborne dust. J Occup Environ Hyg. 2009; 7(1):7–13. [PubMed: 19904655]
7. Springer B, Orendi U, Much P, Hoger G, Ruppitsch W, Krziwanek K, Metz-Gercek S, Mittermayer H. Methicillin-resistant Staphylococcus aureus: a new zoonotic agent? Wien Klin Wochenschr. 2009; 121(3-4):86–90. [PubMed: 19280131]
8. van Loo I, Huijsdens X, Tiemersma E, de Neeling A, van de Sande-Bruinsma N, Beaujean D, Voss A, Klutymans J. Emergence of methicillin-resistant Staphylococcus aureus of animal origin in humans. Emerg Infect Dis. 2007; 13(12):1834–1839. [PubMed: 18258032]
9. VAN DEN Broek IV, VAN Cleef BA, Haenen A, Broens EM, VAN DER Wolf PJ, Van DEN Broek MJ, Huijsdens XW, Kluytmans JA, VAN DE Giessen AW, Tiemersma EW. Methicillin-resistant Staphylococcus aureus in people living and working in pig farms. Epidemiol Infect. 2009; 137(5): 700–708. [PubMed: 18947444]
10. Khanna T, Friendship R, Dewey C, Weese JS. Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers. Vet Microbiol. 2008; 128(3-4):298–303. [PubMed: 18023542]
11. Smith TC, Male MJ, Harper AL, Kroeger JS, Tinkler GP, Moritz ED, Capuano AW, Herwaldt LA, Diekema DJ. Methicillin-resistant Staphylococcus aureus (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. PLoS One. 2009; 4(1):e4258. [PubMed: 19145257]
12. Gibbs S, Green C, Tarwater P, Scarpino P. Airborne antibiotic resistant and nonresistant bacteria and fungi recovered from two swine herd confined animal feeding operations. J Occup Environ Hyg. 2004; 1(11):699–706. [PubMed: 15673090]
13. Gibbs SG, Green CF, Tarwater PM, Mota LC, Mena KD, Scarpino PV. Isolation of antibiotic-resistant bacteria from the air plume downwind of a swine confined or concentrated animal feeding operation. Environ Health Perspect. 2006; 114(7):1032–1037. [PubMed: 16835055]
14. Looft T, Johnson TA, Allen HK, Bayles DO, Alt DP, Stedtfeld RD, Sul WJ, Stedtfeld TM, Chai B, Cole JR, Hashsham SA, Tiedje JM, Stanton TB. In-feed antibiotic effects on the swine intestinal microbiome. Proc Natl Acad Sci U S A. 2012; 109(5):1691–1696. [PubMed: 22307632]
15. Broens EM, Graat EA, Van der Wolf PJ, Van de Giessen AW, De Jong MC. Prevalence and risk factor analysis of livestock associated MRSA-positive pig herds in The Netherlands. Prev Vet Med. 2011; 102(1):41–49. [PubMed: 21733585]
16. Fessler AT, Kadlec K, Schwarz S. Novel apramycin resistance gene apmA in bovine and porcine methicillin-resistant Staphylococcus aureus ST398 isolates. Antimicrob Agents Chemother. 2011; 55(1):373–375. [PubMed: 20876371]
17. Donham KJ, Scallon LJ, Popendorf W, Treuhaft MW, Roberts RC. Characterization of dusts collected from swine confinement buildings. Am Ind Hyg Assoc J. 1986; 47(7):404–410. [PubMed: 3529905]
18. O'Brien AM, Hanson BM, Farina SA, Wu JY, Simmering JE, Wardyn SE, Forshey BM, Kulick ME, Wallinga DB, Smith TC. MRSA in conventional and alternative retail pork products. PLoS One. 2012; 7(1):e30092. [PubMed: 22276147]
19. Boşgelmez-Tınaz G, Ulusoy S, Arıdoğan B, Coşkun-Arı F. Evaluation of different methods to detect oxacillin resistance in Staphylococcus aureus and their clinical laboratory utility. Eur J Clin Microbiol Infect Dis. 2006; 25(6):410–412. [PubMed: 16767493]
20. Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN. Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. J Clin Microbiol. 1999; 37(11):3556–3563. [PubMed: 10523551]
21. Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter M-O, Gauduchon V, Vandenesch F, Etienne J. Involvement of Panton-Valentine Leukocidin—producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis. 1999; 29(5):1128–1132. [PubMed: 10524952]
22. Green C, Gibbs S, Tarwater P, Mota L, Scarpino P. Bacterial plume emanating from the air surrounding swine confinement operations. J Occup Environ Hyg. 2006; 3(1):9–15. [PubMed: 16482973]

23. Schulz J, Friese A, Klees S, Tenhagen BA, Fetsch A, Rosler U, Hartung J. Longitudinal study of the contamination of air and of soil surfaces in the vicinity of pig barns by livestock-associated methicillin-resistant Staphylococcus aureus. Appl Environ Microbiol. 2012; 78(16):5666–5671. [PubMed: 22685139]

24. Cavaco LM, Hasman H, Aarestrup FM. Zinc resistance of Staphylococcus aureus of animal origin is strongly associated with methicillin resistance. Vet Microbiol. 2011; 150(3-4):344–348. [PubMed: 21411247]

25. Moodley A, Nightingale EC, Stegger M, Nielsen SS, Skov RL, Guardabassi L. High risk for nasal carriage of methicillin-resistant Staphylococcus aureus among Danish veterinary practitioners. Scand J Work Environ Health. 2008; 34(2):151–157. [PubMed: 18470441]

26. Hasman H, Moodley A, Guardabassi L, Stegger M, Skov RL, Aarestrup FM. Spa type distribution in Staphylococcus aureus originating from pigs, cattle and poultry. Vet Microbiol. 2010; 141(3-4): 326–331. [PubMed: 19833458]

27. Wagenaar JA, Yue H, Pritchard J, Broekhuizen-Stins M, Huijsdens X, Mevius DJ, Van Duijkeren E. Unexpected sequence types in livestock associated methicillin-resistant Staphylococcus aureus (MRSA): MRSA ST9 and a single locus variant of ST9 in pig farming in China. Vet Microbiol. 2009; 139(3-4):405–409. [PubMed: 19608357]

28. Osadebe LU, Hanson B, Smith TC, Heimer R. Prevalence and characteristics of Staphylococcus aureus in Connecticut swine and swine farmers. Zoonoses Public Health. 2013; 60(3):234–243. [PubMed: 22883566]