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Castillo Neyra, R., Frisancho, J.A., Rinsky, J.L., Resnick, C., Carroll, K.C., et al. (2014). Multidrug-resistant and methicillin-resistant Staphylococcus aureus (MRSA) in hog slaughter and processing plant workers and their community in North Carolina (USA). *Environmental Health Perspectives*, 122(5), 471-477.

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<http://dx.doi.org/10.1289/ehp.1306741>

Received: 2 March 2013

Accepted: 4 February 2014

Advance Publication: 7 February 2014

Multidrug-Resistant and Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Hog Slaughter and Processing Plant Workers and Their Community in North Carolina (USA)

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Running title: Drug resistant *S. aureus* and hog production in NC

Acknowledgments: We acknowledge John McGready for his assistance with the statistical methods. This work was supported by NIOSH grant number 5R21OH009829 and by the Heinz Endowments (615 Liberty Ave. Pittsburgh, PA 15222). During this study Dr. Castillo was supported by a fellowship from the Center for a Livable Future and a Fulbright scholarship, and Ms. Rinsky was supported by a predoctoral fellowship from the National Institute of Environmental Health Sciences (NIEHS; award no. T32ES007018).

Conflict of Interest: No commercial party with a direct financial interest in the results of the research supporting this article has or will confer a benefit upon the authors or upon any organization with which the authors are associated.

Abstract

Background: Use of antimicrobials in industrial food-animal production is associated with the presence of antimicrobial resistant *Staphylococcus aureus* among animals and humans. Hog slaughter/processing plants process large numbers of animals from industrial animal operations, and are environments conducive to the exchange of bacteria between animals and workers.

Objectives: To compare the prevalence of methicillin-resistant *S. aureus* (MRSA) and multidrug resistant *S. aureus* (MDRSA) carriage between processing plant workers, their household members, and community residents.

Methods: We conducted a cross-sectional study of hog slaughter/processing plant workers, their household members, and community residents in North Carolina. Participants responded to a questionnaire and provided a nasal swab. Swabs were tested for *S. aureus*, and isolates tested for antimicrobial susceptibility and subjected to multilocus sequence typing.

Results: The prevalence of *S. aureus* was 21.6%, 30.2%, and 22.5% among 162 workers, 63 household members, and 111 community residents, respectively. The overall prevalence of MRSA and MDRSA tested by disk diffusion was 4.8% and 6.9%, respectively. The adjusted prevalence of MDRSA among workers was 1.96 times (95% CI: 0.71, 5.45) the prevalence in community residents. The adjusted average number of antimicrobial classes to which *S. aureus* isolates from workers were resistant was 2.54 times (95% CI: 1.16, 5.56) the number among isolates from community residents. One MRSA isolate and two MDRSA isolates from workers were identified as sequence type 398, a type associated with exposure to livestock. **Conclusions:** Although the prevalence of *S. aureus* and MRSA was similar in hog slaughter/processing plant workers and their household and community members, *S. aureus*

isolates from workers were resistant to a greater number of antimicrobial classes. These findings may be related to the non-therapeutic use of antimicrobials in food-animal production.

Introduction

Staphylococcus aureus is an important pathogen that can cause serious and life-threatening infections in humans. Clinical problems caused by *S. aureus* range from localized illnesses, such as necrotizing skin infections and folliculitis, to systemic diseases, including toxic shock syndrome (Lowy 1998). *Staphylococcus aureus* infections have become more dangerous and costly to treat over the past 20 years due to increasing prevalence of antimicrobial resistance. Of considerable concern is methicillin-resistant *Staphylococcus aureus* (MRSA), as well as multidrug-resistant *S. aureus* (MDRSA) (Gordon and Lowy 2008). Several studies in hospitals in the United States have reported that MRSA is the most common cause of skin and soft tissue infections (King et al. 2006; Moran et al. 2006; Parchman and Munoz 2009) and MRSA carriage is associated with subsequent infection and increased morbidity and mortality compared to non-carriage (Datta and Huang 2008).

Staphylococcus aureus colonizes skin and can persist in the nares; positive nasal carriage is indicative of exposure and is associated with increased risk of clinical infection in hospitalized populations (Davis et al. 2004; Stevens et al. 2010). Based on risk factors associated with exposure, MRSA strains are often classified as healthcare-associated MRSA (HA-MRSA), or community-associated MRSA (CA-MRSA). Since 2001, the increases in MRSA exposures and infections in the United States are largely due to community-associated strains, such that MRSA can no longer be controlled based solely on measures implemented within health care settings (Como-Sabeti et al. 2009; Stefani et al. 2012).

Within the category of CA-MRSA, studies in several countries have identified specific strains associated with livestock, which have been termed livestock-associated MRSA (LA-MRSA)

(Armand-Lefevre et al. 2005; Bisdorff et al. 2012; DeBoer et al. 2009; Ogata et al. 2012; Smith and Pearson 2011; Waters et al. 2011). Studies have reported increased risks of MRSA carriage among persons working with livestock including swine (Aubry-Damon et al. 2004; Denis et al. 2009; Geenen et al. 2012; Morcillo et al. 2012; Mulders et al. 2010; Nijsten et al. 1996; Voss et al. 2005), among veterinarians treating livestock (Garcia-Graells et al. 2012; Hanselman et al. 2006), and, more recently, among persons without direct livestock contact but residing in areas of high livestock density (Feingold et al. 2012). In addition, several recent studies have reported on prevalence of MDRSA carriage among livestock, farm workers, and slaughterhouse workers (Khanna et al. 2008; Oppliger et al. 2012; Smith and Pearson 2011; VanCleaf et al. 2010).

In comparison to the European Union (EU), relatively fewer studies examining MRSA and MDRSA exposures in hog production have been conducted in the United States (Leedom Larson et al. 2010; Osadebe et al. 2012; Rinsky et al. 2013; Smith et al. 2009) and, to our knowledge, no studies have been published examining the prevalence of MRSA among workers in US hog slaughter and processing plants or the household members of these workers. For this reason we undertook a study of workers in a large hog slaughter and processing plant, their household members, and community residents. The objective of our study was to test the hypothesis that workers have higher prevalence of carriage of non-susceptible strains of *S. aureus*, including MRSA and MDRSA, as compared to residents in the same area who do not work in hog slaughter and processing. We also tested the hypothesis that workers are more likely to carry *S. aureus* isolates that are resistant to more antimicrobials as compared to community residents from the same area. We included household members in this study based on studies of household transmission of *S. aureus* and MRSA which reported transmission rates within households as

high as 43% (Davis et al. 2012). We hypothesized that household members of workers would also have greater exposure to non-susceptible strains of *S. aureus* than community referents.

Methods

Study design and recruitment

We conducted a cross-sectional study between September and November 2011, in Tar Heel, North Carolina, location of the Smithfield plant, the largest hog slaughter and processing plant in the United States. Tar Heel is sparsely populated (117 residents, according to US Census 2011) with most workers and community referents residing in nearby cities and towns in southern North Carolina and northern South Carolina. The workforce at the Tar Heel plant includes approximately 4,500 workers and is unionized, which facilitated enrollment of workers in the study. Study participants were recruited through outreach efforts by our partner, the United Food and Commercial Workers International Union (UFCW) local 1208. Prior to data collection, we met with local and national officials of the UFCW, as well as with shop stewards of the local union (employees who represent the union at each work area within the plant). These individuals informed the union membership about the study. We asked workers to invite up to two members of their community (people who lived nearby, but who did not live with them or work at the plant), and up to two people living with them who did not work at the plant. Through these efforts we enrolled three categories of participants: 1) plant workers; 2) household members of plant workers (up to two per worker); and 3) community residents. All data collection activities were conducted at the union office, located within one mile of the plant. Prior to initiating the study, Smithfield was informed about the study through telephone contact with the Vice President for Environmental Affairs.

Participant enrollment took place between Thursdays and Sundays in three waves. All workers had been at work within the past week and many came directly from work. Prior to enrollment, a verbal screening was conducted to determine eligibility of persons approaching the enrollment sessions: all participants were required to be ≥ 18 years, able to speak and understand either English or Spanish, reside in the local area (for community residents) defined as southern North Carolina and northern South Carolina, and were not working at a healthcare facility. Those who met these inclusion criteria were assigned a unique participant code and were directed to interview stations, where oral informed consent was obtained prior to data collection. No personal identifiers were collected in order to protect confidentiality. The study was reviewed and approved by the Johns Hopkins Bloomberg School of Public Health Committee on Human Research.

Data collection and biological sampling

An extensive interview was conducted using a standardized questionnaire collecting information on demographic data, current and past occupational history, recent health history (including infections and any use of antimicrobials), contact with live animals (livestock and companion animals), and typical diet. Fluent English/Spanish speakers administered the questionnaire in both languages. We pretested the questionnaire in English and Spanish for clarity and consistency on six non-Hispanic and six Hispanic union members.

After completing the questionnaire, trained personnel collected a swab sample (BD Diagnostic Systems dual swab with Amies agar gel) from both nares of each participant. The rayon-tipped swab applicator was then placed into its plastic tube containing transport medium. The transport

tube was labeled with the participant code, and shipped to our laboratory at Johns Hopkins by express courier service.

Microbiological and molecular analyses

Upon arrival at the laboratory, all samples were kept at room temperature until they were processed by the Johns Hopkins Hospital (JHH) Laboratory of Medical Microbiology, within 72 hours of collection. Nasal swabs were cultured on 5% sheep blood agar (SBA, BBL, Sparks, MD) and CHROMAgar Staph aureus (BBL, Sparks, MD) and incubated aerobically at 35° C for up to 48 hours before reading. Any suspected colony (β -hemolytic on 5% SBA or mauve-colored colonies on ChromAgar Staph aureus) was further subjected to Gram staining, the catalase assay and slide agglutination test (ProLab, Richmond Hill, Ontario, Canada). Gram-positive cocci in clusters that were catalase positive and coagulase positive were identified as *S. aureus* (Becker and von Eiff 2011) and subcultured on 5% SBA to isolate pure colonies before being transferred into 30% glycerol and frozen at -80°C.

One isolate from each *S. aureus*-positive culture was then transferred to our laboratory for antimicrobial susceptibility testing using the disk diffusion method (CLSI 2008). Isolates were first regrown in Mueller Hinton broth and then examined for susceptibility to cefoxitin, ciprofloxacin, clindamycin, erythromycin, gentamicin, sulfamethoxazole/trimethoprim, quinupristin/dalfopristin, and tetracycline. We used the zone of growth inhibition around specific-antibiotic disks to assess the minimum inhibitory concentration (MIC). Based on these MICs and according to CLSI standards (CLSI 2008) the isolates were classified as susceptible, intermediate, or resistant to each antimicrobial except for cefoxitin, which was classified as either susceptible or resistant. Cefoxitin-resistant isolates were identified as phenotypic MRSA

since resistance to cefoxitin predicts resistance to methicillin (Fernandes et al. 2005; Magiorakos et al. 2011).

We performed PCR assays targeting *S. aureus nuc* gene and *mecA* gene, using the primers:

nuc-1: 5'-TCAGCAAATGCATCACAAACAG-3';

nuc-2: 5'-CGTAAATGCACTTGCTTCAGG-3';

mecA-1: 5'-GGGATCATAGCGTCATTATTC-3' and

mecA-2: 5'-AACGATTGTGACACGATAGCC-3', and methods previously reported (Poulsen et al. 2003). We defined as genotypic MRSA those specimens positive for the *mecA* gene, and because of variation in *mecA* sequences (Fluit 2011; García-Álvarez et al. 2011; Hanssen et al. 2004) that could lead to false negatives, we examined both phenotypically and genotypically characterized MRSA in our analyses. We performed multilocus sequence typing (MLST) of the seven housekeeping genes to identify *S. aureus* genetic strains as described by Enright et al. (2000).

Statistical analysis

The distributions of demographic, exposure, and outcome variables were examined and compared across the three categories of participants (workers, household members, community residents). As noted above, isolates were classified as either susceptible or resistant to cefoxitin; and as susceptible, intermediate, or resistant to other antimicrobials based on MIC values (CLSI 2008). In addition, isolates were classified as either susceptible or non-susceptible (the latter category including both intermediate and resistant isolates) as proposed by Magiorakos et al. (2011). Consistent with Magiorakos et al. (2011), we classified isolates as MDRSA if they were

non-susceptible to ≥ 3 classes of antimicrobials, or were MRSA (i.e., resistant to ceftazidime). While the susceptible and non-susceptible categories may be more important for epidemiological purposes (Magiorakos et al. 2011) the CLSI definition is reliable in determining therapeutic failure (Kahlmeter et al. 2003). For purposes of comparison to the clinical literature, we examined both classifications.

The prevalence of *S. aureus*, non-susceptible *S. aureus*, MDRSA, and MRSA was determined for each participant group and for the study population as a whole. We also determined the proportions of *S. aureus* isolates that were non-susceptible, MDRSA, and MRSA among participants with positive *S. aureus* swabs. For comparisons of proportions across participant categories, Chi-squared test and Fisher's exact test were used depending on the number of individuals in each category.

We used unadjusted and adjusted Poisson regression to compare the average number of antimicrobials to which *S. aureus* isolates were resistant (based on CLSI definition) between workers, household members, and community residents. We also used unadjusted and adjusted log binomial regression models to compare the prevalence of MDRSA among workers, household members, and community residents. All multivariable models were adjusted for age (in categories), any self-reported use of antimicrobials in the previous six months (yes/no), and any self-reported visit to a medical facility in the previous six months (yes/no). A medical facility was defined as any place where medical care is provided including hospitals, clinics, doctor offices, and nursing homes. The variables included in the adjusted models were selected based on *a priori* assumptions.

Finally, we examined the patterns of antimicrobial resistance found in the *S. aureus* isolates and the distribution of *S. aureus* and genotypic MRSA strains based on MLST analysis. All statistical analyses were performed using Stata 11 (StataCorp, 2009), with a significance level of 0.05.

Results

Study population

We enrolled a total of 336 participants. One hundred sixty two participants were hog slaughter plant workers, 63 were household members who came from 50 different households, and 111 were community residents.

Community residents were more often white non-Hispanic (18%) than workers (3.1%) and their household members (1.6%) ($p < 0.01$) (Table 1). On average, workers were older than household members and community residents (mean ages of 41, 38.6, and 32.3 years, respectively. ANOVA, $F(2,2) = 9.01$, $p < 0.01$). There were more women than men in each group (58.5% overall) but there were no statistically significant differences among groups with regard to sex, visit to a medical facility or using antimicrobials in the last 6 months, having a MRSA diagnosis in the past year, or animal contact at home unrelated to hog slaughter and processing work.

Prevalence of *S. aureus*, non-susceptible *S. aureus*, MDRSA and MRSA

The overall prevalence of *S. aureus* nasal carriage among the study population was 23.5% (79/336) and was elevated among household members (30.2%) than workers (21.6%) or community members (22.5%) ($p = 0.38$) (Table 1). We tested 78 isolates from the 79 *S. aureus*-positive participants for antimicrobial susceptibility (one isolate did not grow). The overall prevalence of non-susceptible *S. aureus* was 19.4%, with similar prevalence between groups. The overall prevalence of MDRSA was 6.9% (23/335) and was 8.0%, 6.5%, and 5.4% among

workers, household members and community residents, respectively. The overall prevalence of phenotypic MRSA was 4.8% (16/335), with 5.6% among workers, 4.8% among household members, and 3.6% among community residents. Nine of the 16 phenotypic MRSA isolates were positive for *mecA* providing an overall prevalence of genotypic MRSA of 2.7% (9/335); with a prevalence among workers of 3.1%, household members of 3.2%, and community residents of 1.8%.

Proportion of non-susceptible *S. aureus*, MDRSA and MRSA in *S. aureus* isolates

The proportion of *S. aureus* isolates (n=78) that were non-susceptible to at least one antimicrobial was elevated in community members (96.0%) than workers (80.0%) or household members (72.2%) (p=0.09) (Table 2). The proportion of MDRSA among all *S. aureus* isolates was elevated in isolates from workers (37.1%) than household members (22.2%) or community residents (24.0%) (p=0.41), and the proportion of phenotypic MRSA also was higher in workers (25.7%) than household members (16.7%) or community residents (16.0%) (p=0.67). The proportion of *mecA*-positive MRSA was 14.3% in workers, 11.1% in household members, and 8% in community residents. The prevalence of MDRSA and MRSA in *S. aureus* isolates was similar between household members and community residents.

Antimicrobial resistance profile of *S. aureus*

We also examined the distribution of susceptible, intermediate, and resistant isolates and found unequal proportions across participant groups (Fisher's exact test, p<0.01). Proportions extracted from Figure 1 show that among participants carrying *S. aureus*, workers had the highest proportion of *S. aureus* resistant to at least one antimicrobial class (48.6%; 17/35) followed by household members (38.9%; 7/18) and community residents (20.0%; 5/25). The highest

proportion of *S. aureus* showing intermediate resistance to at least one antimicrobial class was found in community members (76.0%; 19/25) followed by household members (33.3%; 6/18) and workers (31.4%; 11/35).

Detailed resistance profiles of these isolates (Figure 1) suggest that the numbers of different classes of antimicrobials to which *S. aureus* isolates were resistant varied among the participant groups. Workers carried *S. aureus* that were resistant to more antimicrobials than isolates carried by household members or community residents. Isolates from community residents were more likely to have intermediate resistance than isolates from workers or household members. The patterns of resistance to specific antimicrobials also varied among groups. Erythromycin non-susceptibility (resistant or intermediate) was the most common phenotype observed in all groups. Workers and household members had the highest prevalence of erythromycin resistant *S. aureus* (Figure 1). The most common pattern of multiple resistance in the entire study population was non-susceptibility to erythromycin and ciprofloxacin (14.1%; 11/78); followed by non-susceptibility to erythromycin, cefoxitin, and ciprofloxacin (9%; 7/78); and non-susceptibility to erythromycin and cefoxitin (6.4%; 5/78).

Group differences in *S. aureus* antimicrobial resistance

Compared with isolates from community residents, isolates from workers and household members were on average resistant to 2.54 times (95% CI: 1.16, 5.56) and 1.69 times (95% CI: 0.64, 4.46) more antimicrobial classes, respectively, after adjusting for age, visiting a medical facility, and taking antimicrobials in the last six months (Table 3). Age, visiting a medical facility in the last 6 months, and taking antimicrobials in the last 6 months were not significantly associated with the number of antimicrobial classes to which the isolates were resistant, and did not confound the associations with working in a hog processing facility.

The prevalence of MDRSA carriage in workers was 1.96 times higher (95% CI: 0.71, 5.45) than in community residents after adjusting for other variables ($p = 0.20$) (Table 4). The prevalence of MDRSA in household members was comparable to community residents (PR= 1.04; 95% CI: 0.25, 4.28).

MLST and *S. aureus* strains by group

Nineteen unique sequence types (ST) were identified from 68 *S. aureus* isolates (Figure 2). Sequence types for the 11 remaining isolates could not be determined. *S. aureus* isolates from workers demonstrated greatest sequence type diversity. ST1 and ST5 were found in all three participant groups. ST8 was common among *S. aureus* isolates from workers and household members (21% and 22%, respectively), but absent among isolates from community residents. ST72 was also observed only among isolates from workers ($n = 1$) and household members ($n = 3$). Notably, three isolates, all from workers, were identified as ST398, including one MRSA isolate and two MDRSA isolates. Among MRSA isolates, ST8 was the predominant sequence type (38%), followed by ST1 (19%).

Discussion

To our knowledge, this is the first published study in the United States to examine carriage of *S. aureus*, MRSA and MDRSA in hog slaughter and processing plant workers and their communities. Although the prevalence of *S. aureus* and MRSA was similar among all three participant groups, *S. aureus* isolates from workers were resistant to a greater number of antimicrobial classes than isolates carried by household members or community residents. Workers also had an elevated prevalence of MDRSA as compared to community residents, though the difference was not statistically significant. The overall prevalence of *S. aureus* in our

population was 23.5%, which is lower than the estimated prevalence in US adults (27.4% for people between 20 and 59 years old) based on NHANES data for 2003 - 2004 (Gorwitz 2008), but the prevalence of MRSA in our population (4.8% based on CLSI criteria, 2.7% *mecA* positive) was higher than the NHANES estimate of 1.1%. The prevalence of MRSA carriage in our study was also greater than estimates from two studies of young, healthy, adult military recruits which reported prevalence of MRSA carriage between 0.5 and 2% (Findlay et al. 2010; Zinderman et al. 2004).

PCR using previously reported primers (Poulsen et al. 2003) did not detect *mecA* in seven of the 16 phenotypically characterized MRSA isolates, consistent with the presence of variant *mecA* genes that are not detected by standard probes (García-Álvarez et al. 2011; Petersen et al. 2013). For this reason we reported both phenotypic and genotypic MRSA as suggested by Fluit (2011). We did not conduct further PCR analyses to identify any *mecA* variants. We looked for ST398, a strain variant of the clonal complex (CC) 398 that has been associated with exposure to hogs and other livestock (Armand-Lefevre et al. 2005; Feingold et al. 2012; Smith and Pearson 2011). Three ST398 isolates were identified in workers using MLST, including one that was MRSA, and two that were susceptible to methicillin (cefoxitin) but classified as MDRSA based on resistance to ≥ 3 other antimicrobial classes. Studies in European countries have showed that pigs are a source of MRSA CC398 infections in humans, with the predominant ST being ST398 (Lewis et al. 2008), and that MRSA CC398 is much more prevalent among persons exposed to hogs than their family members and non-exposed community residents (Cuny et al. 2009; Oppliger et al. 2012; VanCleeef et al. 2010). Similar to our results, a Swiss study of antimicrobial resistant *S. aureus* in pigs and pig farmers reported that 22% of all MRSA and methicillin-susceptible *S. aureus* CC398 strains were multidrug resistant (Oppliger et al. 2012).

We observed evidence of greater *S. aureus* genotype diversity in isolates from workers (11 MLST sequence types) than isolates from household members or community residents (7 and 9 sequence types respectively), whereas Oppliger et al. (2012) reported more *S. aureus* genotype diversity in isolates from non-farmers than pig farmers. We identified ST5 in all three participant groups, ST8 in workers and household members, and ST398 in workers only. Similarly, a French study observed *S. aureus* ST5 in both pig farmers and non-farmers, and ST8 and ST398 in pig farmers only (Armand-Lefevre et al. 2005). ST1 was identified in isolates from all three groups in our study, and was the most common isolate identified in pork meat in a US study (Waters et al. 2011). However, ST1 was not prevalent in pigs, pig farmers, or non-farmers in the Swiss study (Oppliger et al. 2012).

The most common *S. aureus* genotypes in hog slaughter and processing plant workers in our study were ST8 (belonging to CC8) and ST5 (belonging to CC5), with the predominant MRSA genotype being ST8 (4/9 isolates). In contrast, studies from other countries reported CC9 and CC398 as the predominant *S. aureus* and MRSA genotypes in pigs and pig farmers (Armand-Lefevre et al. 2005; Oppliger et al. 2012). ST8 and ST5 have been consistently reported to be the most common MRSA strains in isolates from pigs and pork in the United States (Molla et al. 2012; Pu et al. 2009; Waters et al. 2011). We did not identify ST9 (within CC9) among *S. aureus* isolates, although this sequence type was previously found in pigs and pork in the United States (Molla et al. 2012; Waters et al. 2011).

Importantly, we found that, among participants carrying *S. aureus*, workers had the highest proportion of *S. aureus* resistant to at least one antimicrobial class. Moreover, workers had isolates resistant to more antimicrobial classes and also had an elevated prevalence of carriage of

MDRSA as compared to community residents. Multidrug resistance also was more pronounced in isolates from Swiss hog farmers than isolates from non-farmers (Oppliger et al. 2012).

Infections caused by multidrug resistant bacteria are associated with worse health outcomes and higher expenditures (Cardoso et al. 2012; Stone 2009), but few studies have examined the prevalence of MDRSA in human populations in the US. One previous North Carolina study reported a 16% prevalence of MDRSA carriage among industrial livestock operation workers compared with 9% among antibiotic-free livestock operation workers (Rinsky et al. 2013). The greater number of drugs to which isolates from workers in our study were resistant is also noteworthy, and may be associated with the use of multiple antimicrobials in hog feeds (Silbergeld et al. 2008).

Resistance to erythromycin was more prevalent than resistance to any other antimicrobial classes, similar to Oppliger et al. (2012); however, patterns of resistance to other antimicrobials differed between the two studies, possibly reflecting differences in the use of antimicrobials as swine feed additives between Switzerland and the United States.

In this study we observed prevalence of carriage of resistant strains of *S. aureus* greater than the US population in all groups, but did not observe differences between groups for some carriage outcomes. Although differences may have been obscured in part because of small sample sizes within groups, it is also possible that the non-worker groups in our study were exposed through environmental pathways from both farms and slaughter and processing operations. Studies by our group and others support this possibility. For example, *S. aureus* and MDRSA have been measured in air releases from intensive hog farms in the United States (Chapin et al. 2005; Gibbs et al. 2004, 2006), detected at distances of 150 m downwind from swine houses in Germany

(Schulz et al. 2012), found in hogs being transported in open trucks from farms to the slaughter house, and in untreated swine house wastes and other releases (Burkholder et al. 2007). This explanation is also supported by other work by our group on clusters of MRSA infections among persons residing in areas of intensive hog production in the Netherlands and in northern North Carolina (Feingold et al. 2012).

The overall elevated rates of MDRSA and MRSA across participant groups, and higher in the worker group may be explained by the concentration of pig farms over the greater Tar Heel region and the common use of different antimicrobial formulations as growth promoters. The slaughterhouse plant in this study serves as a hub for collecting pigs from these farms. In this way, workers at the plant may be exposed to *S. aureus* resistant to different antimicrobials originated in different farms. In contrast, non-workers, depending on their living location, may be exposed only to a subset of bacteria from pig farms.

Conclusions

Our results raise concerns about the exposure of hog slaughter and processing plant workers to antimicrobial-resistant *S. aureus*. *S. aureus* isolates from workers were, on average, resistant to more classes of antimicrobials than isolates from community residents. In addition, among *S. aureus*-positive participants, a greater proportion of workers carried strains of *S. aureus* resistant to at least one antimicrobial class. Further, the overall prevalence of MRSA carriage identified in our study population in 2011 was higher than the estimate for the US general population based on NHANES data for 2003-2004 (Gorwitz et al. 2008).

The observation of a similar elevated prevalence of MRSA among all groups in our study may be in part related to non-occupational exposures in the region, which has the highest density of

industrial hog farms and hogs in the US (Wing et al. 2000). Further studies will be crucial for the identification of factors associated with non-occupational exposures.

Our results suggest a need for surveillance of antimicrobial-resistant *S. aureus* in populations with direct or indirect exposure to livestock. Finally, our study adds to concerns about the use of antimicrobials for non-therapeutic purposes as part of food-animal production, a practice thought to contribute to selection for antimicrobial-resistant strains of *S. aureus* in the community, especially in the food-production system.

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Table 1. Study population characteristics by participant category

	Total n=336 (%)	Worker n=162 (%)	Household member n=63 (%)	Community resident n=111 (%)	X² test statistic (df)	p
Age categories					48.13 (10)	<0.01
18 - 25	89 (26.5)	24 (14.8)	31 (49.2)	34 (30.6)		
26 - 35	66 (19.6)	32 (19.8)	10 (15.9)	24 (21.6)		
36 - 45	65 (19.3)	40 (24.7)	7 (11.1)	18 (16.2)		
46 - 55	62 (18.5)	43 (26.5)	6 (9.5)	13 (11.7)		
56 - 82	50 (14.8)	23 (14.2)	8 (12.7)	19 (17.1)		
Female	196 (58.5)	88 (54.7)	41 (65.1)	67 (60.4)	2.26 (1)	0.32
Race/ethnicity					31.07 (6)	<0.01
African-American	231 (68.8)	114 (70.4)	46 (73.0)	71 (64.0)		
Hispanic	52 (15.5)	30 (18.5)	13 (20.6)	9 (8.1)		
White non-Hispanic	26 (7.7)	5 (3.1)	1 (1.6)	20 (18.0)		
Native American	18 (5.4)	9 (5.6)	2 (3.2)	7 (6.3)		
Other	9 (2.7)	4 (2.5)	1 (1.6)	4 (3.6)		
Animal contact on home property	161 (47.9)	74 (45.7)	28 (44.4)	59 (53.2)	1.85 (2)	0.42
Visit medical facility in last 6 months	193 (58.0)	89 (54.9)	40 (64.5)	64 (58.7)	1.73 (2)	0.42
MRSA diagnosis in the last year	3 (0.9)	2 (1.2)	1 (1.6)	0 (0.0)	- ^d	0.43
Use of antimicrobials in last 6 months	80 (23.8)	37 (22.8)	17 (27.0)	26 (23.4)	0.44 (2)	0.82
Prevalence						
<i>S. aureus</i>	79 (23.5)	35 (21.6)	19 (30.2)	25 (22.5)	1.94 (2)	0.38
Non-susceptible <i>S. aureus</i>	65 (19.4)	28 (17.3)	13 (21.0)	24 (21.6)	0.88 (2)	0.65
MRSA phenotype ^a	16 (4.8)	9 (5.6)	3 (4.8)	4 (3.6)	0.55 (2)	0.76
MRSA <i>mecA</i> ^b	9 (2.7)	5 (3.1)	2 (3.2)	2 (1.8)	- ^d	0.74
MDRSA ^c	23 (6.9)	13 (8.0)	4 (6.5)	6 (5.4)	0.73 (2)	0.70

^a Phenotypic MRSA defined as *S. aureus* resistant to ceftazidime; ^b MRSA identified by detection of the *mecA* gene, genotypic MRSA is a subset of that detected phenotypically; ^c MDRSA denotes *S. aureus* non-susceptible to 3 or more of the antimicrobials used in this study or resistant to ceftazidime, ^d p-value was calculated with Fisher's exact test.

Table 2. Distribution of non-susceptibility, multidrug-resistance, and MRSA among those positive for *S. aureus*

	Total n=78 (%)	Worker n=35 (%)	Household member n=18 (%)	Community resident n=25 (%)	P^e
Non-susceptible <i>S. aureus</i> ^a	65 (83.3)	28 (80.0)	13 (72.2)	24 (96.0)	0.09
MRSA phenotype ^b	16 (20.5)	9 (25.7)	3 (16.7)	4 (16.0)	0.67
MRSA <i>mecA</i> ^c	9 (11.5)	5 (14.3)	2 (11.1)	2 (8.0)	0.90
MDRSA ^d	23 (29.5)	13 (37.1)	4 (22.2)	6 (24.0)	0.41

^a*S. aureus* intermediate or resistant to any antimicrobial class. ^bPhenotypic MRSA defined as *S. aureus* resistant to cefoxitin. ^cMRSA identified by detection of *mecA* gene, genotypic MRSA is a subset of that detected phenotypically. ^dMDRSA denotes *S. aureus* non-susceptible to 3 or more of the antimicrobials used in this study or resistant to cefoxitin. ^ep-value calculated with Fisher's exact test.

Table 3. Unadjusted and adjusted estimates of the association between exposures and the mean number of antimicrobials classes to which a *S. aureus* isolate was resistant.

	N	Unadjusted Mean Ratio (95% CI)	p	Adjusted Mean Ratio (95% CI)	p
Participant Category:					
Community resident	25	Ref.	--	Ref.	--
Household member	18	1.70 (0.70, 4.10)	0.24	1.69 (0.64, 4.46)	0.29
Worker	35	2.46 (1.17, 5.17)	0.17	2.54 (1.16, 5.56)	0.02
Age in years:					
18 – 25	29	Ref.	--	Ref.	--
26 – 35	17	1.93 (0.97, 3.87)	0.06	1.67 (0.80, 3.46)	0.17
36 – 45	12	1.13 (0.46, 2.77)	0.79	1.10 (0.43, 2.78)	0.85
46 – 55	11	1.05 (0.41, 2.72)	0.91	0.78 (0.28, 2.20)	0.64
56 – 82	8	1.45 (0.56, 3.74)	0.44	1.14 (0.43, 3.08)	0.79
Visit medical facility in last 6 months ^a	39	1.33 (0.75, 2.36)	0.33	1.37 (0.75, 2.48)	0.31
Antimicrobials in last 6 months ^b	19	0.85 (0.44, 1.66)	0.64	0.93 (0.47, 1.85)	0.83

^aReference group are those who did not visit a medical facility in last 6 months. ^bReference group are those who did not take antimicrobials in last 6 months.

Table 4. Unadjusted and adjusted prevalence ratios estimating the association between exposures and carriage of multidrug-resistant *S. aureus*

	N	Unadjusted PR (95% CI)	p	Adjusted PR (95% CI)	p
Participant Category:					
Community resident	111	Ref.	--	Ref.	--
Household member	62	1.19 (0.35, 4.07)	0.78	1.04 (0.25, 4.28)	0.96
Worker	162	1.48 (0.58, 3.79)	0.41	1.96 (0.71, 5.45)	0.20
Age in years:					
18 – 25	88	Ref.		Ref.	--
26 – 35	66	1.33 (0.45, 3.95)	0.60	0.97 (0.30, 3.15)	0.96
36 – 45	65	0.68 (0.18, 2.61)	0.57	0.54 (0.14, 2.17)	0.39
46 – 55	62	0.95 (0.28, 3.21)	0.93	0.55 (0.14, 2.22)	0.40
56 – 82	50	1.17 (0.35, 3.96)	0.80	1.07 (0.31, 3.74)	0.91
Visit medical facility in last 6 months ^a	193	0.96 (0.42, 2.22)	0.92	0.98 (0.41, 2.32)	0.96
Antimicrobials in last 6 months ^b	80	0.89 (0.34, 2.31)	0.80	1.07 (0.40, 2.86)	0.90

PR: Prevalence Ratio.

^aReference group are those who did not visit a medical facility in last 6 months. ^bReference group are those who did not take antimicrobials in last 6 months.

Figure Legends

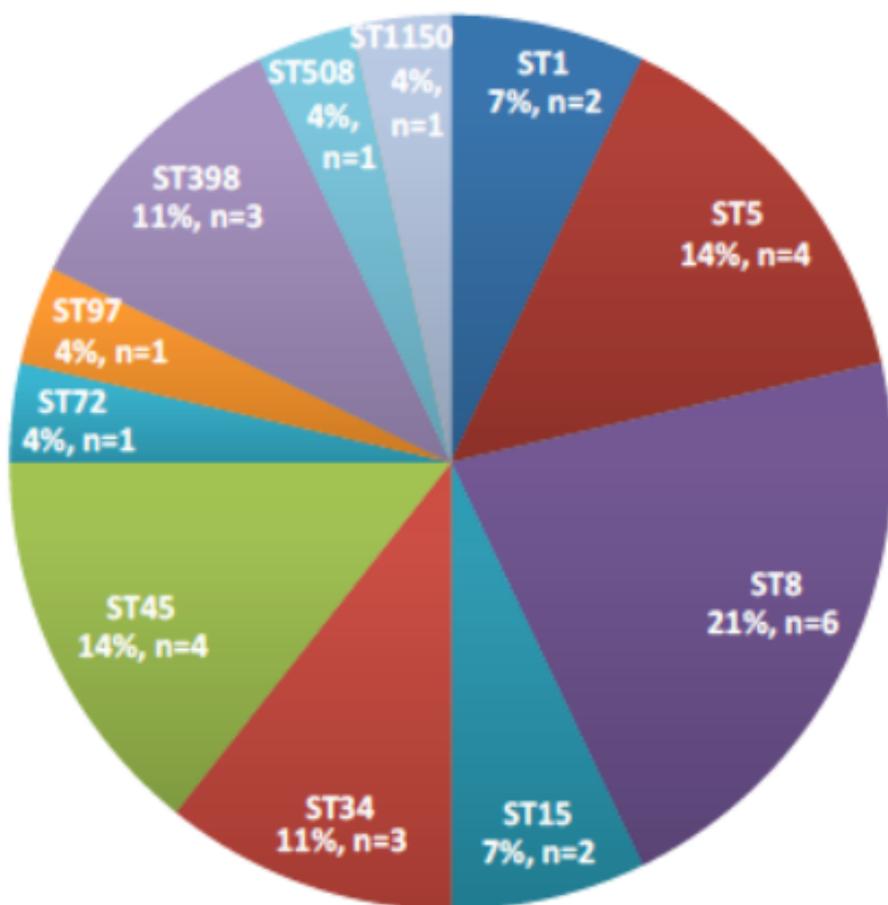
Figure 1. Heat map showing the pattern of antimicrobial resistance of the 78 isolates of *S. aureus*. Antimicrobial resistance was assessed by disk diffusion and cutoffs defined by CLSI guidelines.

Figure 2. *Staphylococcus aureus* sequence type diversity and distribution. Sequence types were based on 7 housekeeping genes that were derived from whole genome sequences of each isolates.

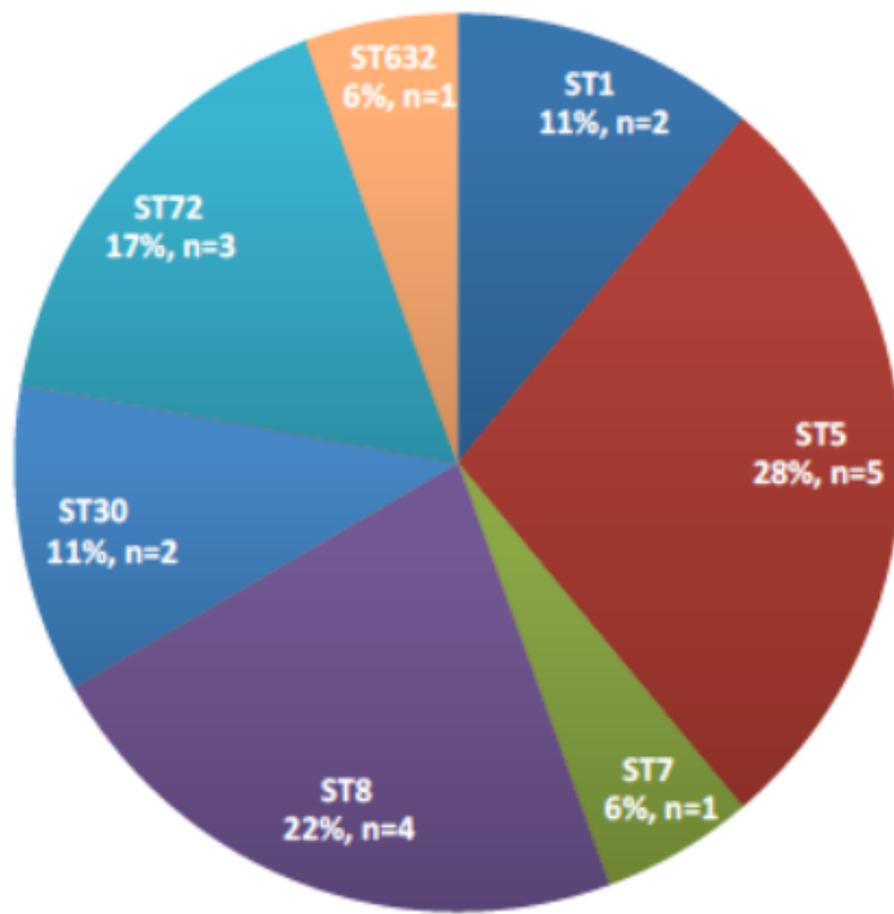


Each row represents 1 isolate tested for susceptibility from a *S. aureus*-positive participant.
 * Resistance to cefoxitin was classified as either susceptible or resistant based on CLSI guidelines.

Worker (n=28)



Household member (n=18)



Community member (n=22)

