Prevalence and Molecular Characterization of *Staphylococcus aureus* in Commercially Available Meat over a One-Year Period in Iowa, USA

Dipendra Thapaliya^{1,2,3}, Brett M. Forshey^{1,2}, Jhalka Kadariya³, Megan K Quick^{1,2}, Sarah Farina^{1,2}, Ashley O' Brien^{1,2}, Rajeshwari Nair^{1,2}, Amos Nworie^{1,2}, Blake Hanson^{1,2}, Ashley Kates^{1,2}, Shylo Wardyn^{1,2}, and Tara C Smith^{1,2,3}

¹Department of Epidemiology, University of Iowa College of Public Health
145 N. Riverside Drive, Iowa City, IA, 52242, United States of America

²Center for Emerging Infectious Diseases, University of Iowa College of Public Health
2501 Crosspark Rd, Coralville, IA, 52241, United States of America

³Current affiliation: Kent State University, College of Public Health
Department of Biostatistics, Environmental Health Sciences and Epidemiology
750 Hilltop Drive, Kent, OH, 44242, United States of America

Corresponding author at:

Tara C. Smith, PhD
Kent State University, College of Public Health
Department of Biostatistics, Environmental Health Sciences and Epidemiology
750 Hilltop Drive, Kent, Ohio, 44242
Phone: (330) 672-3946
Fax: (330) 672 - 6505
Email: tsmit176@kent.edu

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of infectious disease morbidity and mortality. Previous studies have confirmed the presence of *S. aureus*, including MRSA, on raw meat products. We investigated the prevalence and molecular epidemiology of *S. aureus* and MRSA in commercially-distributed antibiotic-free and conventional raw meat products (n= 3290) purchased in 8 Iowa retail stores weekly for a period of one year. Isolates were characterized using *spa* typing, and PCR was used to detect the presence of the Panton-Valentine leukocidin (PVL) and *mecA* genes. Quantitation of *S. aureus* on meat products was carried out one week per month. The prevalence of *S. aureus* on meat samples was 27.8% (913/3290). Compared to antibiotic-free meat samples, higher prevalence of both MRSA and methicillin-susceptible *S. aureus* (MSSA) were found in conventional meat samples. Among the *S. aureus* isolates, 18 were PVL-positive (1.9%) and 41 (4.5%) carried *mecA*. Phenotypic oxacillin resistance was observed for 17.1% (41/239) of the isolates tested, while 23% (55/239) were multi-drug resistant. A total of 132 *spa* types were detected from 913 contaminated meat samples. Overall, t002 was the most common *spa* type identified (137; 15.0%). The number of colony-forming units (CFU) per 10g meat ranged from 2 to 517 (median: 8 CFU per 10g of meat; mean: 28) with the highest bacterial load observed on turkey samples. These data reinforce the need to consider meat products as potential vehicles of *S. aureus* transmission from farm into human households, and the potential need for public health intervention programs pre and post-slaughter in meat processing facilities.

Keywords: *Staphylococcus aureus*, MRSA, Meat, Antibiotic resistance, Genotype

Introduction

Staphylococcus aureus, a commensal and opportunistic pathogen, can cause a wide spectrum of diseases, from soft tissue infections (STIs) to severe life-threatening invasive disease such as infective endocarditis [1, 2]. Approximately 30% of the U.S. population is colonized with methicillin-susceptible *S. aureus* (MSSA), with the most common site for colonization being the anterior nares (nostrils) [3, 4]. It is estimated that 1.5% of the U.S. population is colonized with methicillin-resistant *S. aureus* (MRSA) [1]. These colonized persons are carriers of *S. aureus*, and colonization is a risk factor for developing subsequent symptomatic infections [2, 3]. *S. aureus* accounts for nearly 20% of bloodstream infections in the hospital setting, and treatment of infections caused by this versatile and dangerous pathogen has become increasingly difficult due to the emergence of multidrug-resistant (MDR) strains-particularly, resistant to the antibiotic oxacillin [5-7].

Since their first recognition among Dutch and French swine farmers in the early 2000s, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) clonal complex 398 (CC398) and closely related sequence types within CC398 have been detected in various animal species and humans, documenting the importance of considering novel routes of exposure when studying MRSA epidemiology [8-10]. Antibiotic-resistant *S. aureus* has been found in several species of meat-producing animals, including pigs [8-10], chickens [11], and cattle [12]. Therefore, meat products have the potential to act as vehicle for transmission of MRSA and other antibiotic-resistant strains from the farm into the general human population. Several studies have documented the presence of MRSA on raw retail meat products, with prevalence ranging from less than 1% in Asia [13, 14] to 11.9% in the Netherlands [15], with intermediate prevalence found in other studies [16, 17]. Isolates found on these samples have included both LA-MRSA as

well as “human” MRSA strains, including ST8 (“community-associated MRSA”) and ST5 (“hospital-associated MRSA”). These have been isolated from raw chicken [15, 18-20], turkey [15, 19, 20], pork [7, 16, 18, 19, 21-24], and beef [7, 15, 16, 20].

Interestingly, CC398 strains typically lack known enterotoxin genes [25, 26]; as such, these isolates may be less likely to cause “classic” food-borne outbreaks due to ingestion of pre-formed toxin, and therefore may be overlooked as a food-borne risk. However, these strains and other molecular types of *S. aureus* may put consumers at risk via a different mechanism: food handling and subsequent self-inoculation and colonization with meat-origin *S. aureus* that may subsequently contaminate the household, where *S. aureus* may then become resident and spread to others [27]. A number of studies have suggested the possibility that this type of acquisition may have occurred, reporting CC398 colonization or infection in individuals with no contact with livestock [28, 29]; as such, meat has been one route whereby these individuals may have contracted CC398.

This study aimed to investigate the prevalence, antibiotic susceptibility profiles, and genotypes of *S. aureus* among U.S. meat and poultry samples (n=3290) from two geographical locations within Iowa. Our yearlong sampling strategy also allowed us to begin to examine seasonal differences in *S. aureus* prevalence on meat products. This is part of a larger study examining the epidemiology of *S. aureus* in the community, looking at raw meat products as one source of exposure to *S. aureus*.

Methods

Sample collection

Eight stores in two geographical locations in Iowa were sampled to reflect both urban and rural areas. Four stores were sampled from the urban area (Iowa City) and 4 stores from rural area (Keokuk County). One store was discontinued in the rural area after 8 weeks due to a limited variety of available samples. A total of 3290 raw meat product samples were collected on a weekly basis for 52 weeks from January 2012 through December 2012. These stores were a mix of national and regional supermarkets and retail chains as well as local independent food markets and cooperative grocery stores. Of the 3290 samples, 530 were antibiotic-free (ABF) (antibiotics were not used in husbandry) and 2760 were conventional meat products. AFB meat samples were collected from stores 1, 2, and 8 from Iowa City area and store 6 from Keokuk County. Meat samples collected from stores 2 and 3 from Iowa City area, and 5 from Keokuk County were unwrapped product displayed on bulk, whereas meat samples collected from rest of the stores were pre-wrapped. Meat samples were double-bagged upon purchase to avoid cross-contamination. Samples were transported on blue ice packs to the laboratory, and were refrigerated until processing on the same day of purchase.

Bacterial isolation and identification

Upon arrival at the laboratory, packaging was removed using a sterile razor blade and a portion of the meat samples (approximately 100 g; minimum 19 g; maximum 388 g; median 117.5 g) was transferred into a Whirl-Pak™ bag (Nasco, Fort Atkinson, WI) with sterile tongs. Fifty mL of sterile 0.1% peptone broth was added to the samples via a sterile graduated cylinder. The sample was then vigorously massaged by hand for 30 seconds. A 50 mL aliquot from the peptone wash was then added to 50mL of Baird Parker broth (2X concentration) with tellurite enrichment (Sigma products-Sigma-Aldrich, St. Louis, MO) in a 250 mL sterile screw cap jar. After 24 hours of incubation at 35⁰C, a loopful of broth was inoculated onto Baird Parker agar

(BPA) with EY tellurite enrichment (BD) and selective MRSA agar plates (BBL CHROMagar MRSA, Becton, Dickinson and Company) and incubated 24-48 hours at 35⁰C and examined for bacterial growth. Presumptive *S. aureus* (black colonies with clear halos on BPA) and presumptive MRSA (mauve colonies on CHROMagar) were confirmed by doing the catalase test, the slide coagulase test, and the *S. aureus* latex agglutination assay (Pastorex Staph-plus, Bio-Rad). *S. aureus* isolates were stored at -80⁰C.

Antimicrobial susceptibility testing (AST)

Two hundred and thirty-nine (n=198, MSSA and n=41, MRSA) isolates were tested for antibiotic susceptibility by the broth dilution method described by the Clinical and laboratory Standards Institute [30]. As we were unable to test all isolates, we tested all isolates collected one week per month and all MRSA isolates. Isolates were tested for susceptibility to oxacillin, tetracycline, erythromycin, clindamycin, trimethoprim-sulfamethoxazole (TMP-SMX), quinupristin/dalfopristin, gentamicin, levofloxacin, linezolid, daptomycin, vancomycin, and rifampin. MRSA isolates were tested for additional antibiotics benzylpenicillin, ciprofloxacin, moxifloxacin, minocycline, and nitrofurantoin. Isolates showing intermediate levels of susceptibility were classified as resistant. Isolates that were resistant to three or more classes of antimicrobials or that were resistant to oxacillin were considered MDR [31].

Molecular testing

Genomic DNA was extracted using the Wizard Genomic DNA preparation kit (Promega, Madison, WI). Polymerase Chain Reaction (PCR) was performed on all isolates. The presence of *mecA* and PVL genes (*lukS*, *lukF*) were determined by PCR [32, 33]. Using previously described methods [34, 35] the *Staphylococcus* protein A (*spa*) gene was amplified using SpaF (5'-

GAACAA-CGTAACGGCTTCATCC-3') and 1514R (5' –CAGCAGTAGTGCCGTTTGCCT-3'). *spa* types were assigned using Ridom (<http://spaserver.ridom.de>) *spa* servers. The Based upon Repeat Pattern (BURP) algorithm was applied to *spa* types to group *S. aureus* isolates into genetic clusters, according to relatedness (i.e. within 6 steps) to more common *spa* types, such as t002 and t008 [36]. Where possible, placement into a particular cluster complex was evaluated based on typical *spa* type-multilocus sequence type (MLST) associations reported in the literature [37] and the Ridom website (<http://spa.ridom.de/spatypes.shtml>; eg, t045 and t002 are typically associated with CC5). Previous publications have shown excellent concordance between *spa* and MLST results [38]. *spa* types associated with CC398 and CC9 were considered LA-MRSA. MLST was performed on selected *S. aureus* isolates as described by Enright et al. [39]. The isolates for MLST were selected based on their *spa* type frequency greater or equal to 1%. Sequence Types (STs) were assigned using organism specific MLST database (<http://saureus.mlst.net/>). Positive (USA 300) and negative controls (reaction mixture without DNA template) were used for *mecA*, PVL and *spa* PCR.

Quantitation

One thousand microliters of homogenized meat and peptone water was transferred onto the center of the 3M™ Petrifilm™ Staph Express Count Plate from the 250mL screw cap jar. A disk spreader was used to spread the liquid uniformly over the entire plate. The 3M™ Petrifilm™ Staph Express Count Plate was then incubated at 35⁰C for 22 hours. At 22 hours, the 3M™ Petrifilm™ Staph Express Disk was placed on each 3M™ Petrifilm™ Staph Express Count Plate, and was incubated for 1-2 hours. At 23-24 hours, the numbers of colonies present on the 3M™ Petrifilm™ Staph Express Count Plate was enumerated.

Statistical analysis

Fisher's exact test and Pearson's chi-square test with Yate's correction were used to evaluate heterogeneity among variables with regards to outcome (e.g. *S. aureus* positivity and antibiotic resistance). Statistical significance was assessed at $\alpha = 0.05$. All analyses were conducted using R v3.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Prevalence of *S. aureus*

A total of 3290 meat samples (530 ABF and 2760 conventional) were purchased from 8 stores in the Iowa City area and Keokuk County. The overall prevalence of *S. aureus* was 27.8% (913/3290; 95% CI 23.9% - 32.3%), with a prevalence of 26.5% (872/3290; 95% CI 24.9% - 28.0%) and 1.2% (41/3290; 95% CI 0.9% - 1.6%) for MSSA and MRSA (based on the presence of the *mecA* gene), respectively. The overall prevalence of *S. aureus* among ABF and conventional meat samples were 25.3 (134/530; 95% CI 21.6% - 28.9%) and 28.2% (779/2760; 95% CI 26.5% - 29.9%). Although no statistical significance was observed (p value, 0.05), the prevalence of MRSA on conventional meat samples (1.4% (39/2760; 95% CI 1.0% - 1.8%)) was higher compared to ABF meat samples (0.4% (2/530; 95% CI 0 - 0.8%)). Similarly, higher prevalence of MSSA was observed on conventional meat samples (26.8%; 740/2760; 95% CI 25.1% - 28.4%) compared to ABF meat samples (24.9%; 132/530; 95% CI 21.2% - 28.5%). However, no statistical significance was observed (p value, 0.39). Among ABF meat samples, the highest prevalence of *S. aureus* was found on antibiotic-free chicken breast samples (66/123; 53.7%), followed by various pork products (Table 1). A lower prevalence of *S. aureus* contamination was found for beef products (13.7%; 20/146), and fish (6.25%; 1/16). None of the turkey samples were contaminated with *S. aureus*. One sample from ground beef (2%; 1/50), and one sample from pork chop (1.9%; 1/52) were contaminated with MRSA. Among

conventional meat samples, the highest prevalence of *S. aureus* was found on pork sausage (42.3%; 124/293) followed by other pork products. A lower prevalence of *S. aureus* contamination was found on ground turkey (22.9%; 52/227), beef (24.4%; 213/874), and Fish (3.3%; 1/30). The highest prevalence of MRSA was found on ground turkey (4.4%; 10/227) followed by pork (1.9%; 20/1049) (Table 1). *S. aureus* contamination in meat and poultry was significantly higher ($p < 0.0001$) in stores selling conventional meat products (29.3%; 845/2883; 95% CI 27.7%-31.0%) compared to the one that sold only antibiotic-free meat and poultry products (16.7%; 68/407; 95% CI 13.2%-20.8%). Similarly, although no statistical significance was observed ($p = 0.22$), MRSA contamination on meat and poultry was also higher in these stores (1.4%; 39/2883; 95% CI 1.0%-1.8%) when compared with one that sold only ABF meat and poultry products (0.5%; 2/407; 95% CI 1% - 1.8%).

Overall, the prevalence of *S. aureus* was similar in the rural stores (425/1459; 29.1%) compared with the urban stores (26.6%; 488/1831). Likewise, the prevalence of MRSA contamination was similar between rural (1.4%; 21/1459) and urban (1.1%; 20/1831) stores. A large difference in *S. aureus* positivity was observed between two regional supermarket chains that were represented in both urban and rural locations ($\chi^2 = 17.3$, $df = 1$, $p < 0.001$). In chain 1, 26.4% (275/1042) of meat samples were positive for *S. aureus* (27.5% [147/534] and 25.2% [128/508]) at the urban and rural locations, respectively) compared with 35.0% (350/1001) at chain 2 (35.1% [177/504] and 34.8% [173/497] at the urban and rural locations, respectively;

The prevalence of *S. aureus* contamination varied significantly by season ($\chi^2 = 19.8$, $df = 3$, $p = 0.0002$), with the highest prevalence in the autumn (269/840; 32.0%) and winter (284/972; 29.2%) compared with summer (222/855; 26.0%) and spring (138/623; 22.2%). For each month from September to January, the prevalence of *S. aureus* contamination exceeded

30% (high of 39.7% in January), whereas from February to August, the prevalence of *S. aureus* contamination was below 30% (low of 19.8% in May).

Characterization of *S. aureus* isolates

All positive *S. aureus* isolates were subjected to *spa* typing. A total of 132 *spa* types were detected from 913 contaminated meat samples. Overall, t002 was the most common *spa* type identified (137; 15.0%), followed by t273 (87; 9.5%), t034 (44; 4.8%), t021 (44; 4.8%), t037 (39; 4.3%), t010 (37; 4.0%), t337 (33; 3.6%), t189 (32; 3.5), and t491 (20; 2.2%) (Figure1). No other *spa* type constituted more than 2% of *S. aureus* isolates. *spa* types were grouped based on genetic proximity to *spa* types typically associated with specific cluster complexes. CC5-related isolates (e.g. t002) were the most common, followed by CC30-related (e.g. t012) isolates (Table 2).

The distribution of *spa* types (and cluster complexes) varied by meat source (i.e. animal). Among chicken samples, CC5 (124/239; 51.9%) was the most common cluster complex, and t002 (85/239; 35.6%) was the most common *spa* type. Among beef samples, CC30 (57/233; 24.5%) and CC5 (47/233; 20.2%) were the most common, represented by t002 (25; 10.7%) and t021 (24; 10.3%). Isolates from turkey samples predominantly belonged to CC398 (33/52; 63.5%), specifically *spa* type t034 (27; 52%). For pork samples, isolates were more evenly distributed across cluster complexes, with no cluster complex exceeding 18% of isolates. t273 (55/387; 14.2%), t337 (28; 7.2%), and t002 (24; 6.2%) were the most common *spa* types associated with pork samples.

The distribution of sequence types was broadly similar between rural and urban locations, with the exception of CC30, CC5, and CC15 strains. For rural stores, CC30-associated *spa* types

represented 14.8% (63/425) of the total, compared with 10.0% (49/488) from urban stores ($\chi^2=4.4$, $df = 1$, $p = 0.036$). Similarly, CC15-associated *spa* types were more common in rural stores (17.2% [73/425] vs. 7.4% [36/488]; $\chi^2=19.8$, $df = 1$, $p < 0.001$), while CC5-associated *spa* types were more common in urban (159/488; 32.6%) than rural stores (83/425; 19.5%; $\chi^2=19.2$, $df = 1$, $p < 0.001$). ST398- and ST9-associated *spa* types were similar between urban and rural locations (7.2% and 6.1% for CC398 in urban and rural sites, respectively, and 9.4% and 7.7% for CC9 in urban and rural sites, respectively).

A total of 21 sequence types (STs) were detected from 47 tested isolates. Overall, ST5 was the most prevalent STs (10; 21.3%) followed by ST15 (7; 14.9%), ST30 (4; 8.5%), ST398 (3; 6.4%), ST1 (2; 4.3%), ST8 (2; 4.3%), ST12 (2; 4.3%), ST45 (2; 4.3%), ST72 (2; 4.3%), ST188 (2; 4.3%). No other STs constituted more than 1% of the tested *S. aureus* isolates (Table 5).

The overall prevalence of the PVL genes among *S. aureus* isolates was 2.0% (18/913; 95% CI 1.0% - 2.9%) (Table 2). Although no statistical significance was observed ($p=0.32$), the prevalence of PVL gene was found higher among antibiotic-free meat samples (2.9%; 4/134; 95% CI 0.1% - 5.8%), compared to conventional meat samples (1.8%; 14/765; 95% CI 0.8% - 2.7%). The highest prevalence of PVL was among *spa* types associated with CC8 (11/67; 16%), including t008 (6/16; 38%) and t051 (5/6; 83%). No other cluster complex exceeded 3% positivity for PVL (Table 2). *mecA* positivity also varied by *spa* type and cluster complex (Table 2). As with PVL, CC8 isolates had the highest proportion of positive samples (39.7%)

Antibiotic Susceptibility Testing

In total, 239 isolates were tested for antibiotic susceptibility (Table 3). Oxacillin resistance was observed for 17.1% (41/239) of the isolates. Eighty isolates (33.5%) were

resistant to tetracycline; 58 (24.3%) were resistant to erythromycin; 32 (13.42%) were resistant to clindamycin; 8 (3.3%) were resistant to quinupristin/dalfopristin; 3 (1.2%) were resistant to gentamicin; 3 (1.2%) were resistant to daptomycin; 2 were resistant to rifampin (0.8%); 13 (5.4) were resistant to levofloxacin, and 2 (0.8%) were resistant to TMP-SMX. None were resistant to linezolid or vancomycin. Fifty-five isolates (23.0%) were MDR-*S. aureus*.(Table 4). Among MRSA isolates (n = 41), all were resistant to benzylpenicillin; 16 (39.0%) were resistant to ciprofloxacin; 1 (2.4%) was resistant to moxifloxacin; 13 (31.7%) were resistant minocycline; and 1 (2.4%) was resistant to nitrofurantoin.

Antibiotic resistance patterns varied by meat source and sequence type (Table 3). Tetracycline resistance was more common for pork (53%) and turkey (13/19; 68.4%) than for beef (7%) or chicken (14%; Fisher's exact test, $p < 0.001$). MDR-*S. aureus* (Table 3) was more common for turkey (52.6%) than for pork (32%), chicken (11.7%), and beef (10.2%; Fisher's exact test, $p < 0.001$). Clindamycin and erythromycin resistance were highest among isolates belonging to CC9 (50% and 54.5%, respectively) followed by CC5 (20.6%) for clindamycin and CC8 (37.5%) for erythromycin. Tetracycline resistance was observed for more than half of tested isolates belonging to CC398 (85.7%), CC9 (68.1%), and CC15 (56.5%).

Quantification

In total, 252 samples were tested to quantify the amount of *S. aureus* colonies present on the meat products. The majority of samples (155; 61.5%) did not have a sufficient number of colonies to be quantified using the Petrifilm system. For the rest, the number of CFU per 10g of meat ranged from 2 to 517 (median, 8 CFU per 10g of meat; mean, 28). Bacterial load was highest on turkey samples (15/18 [83%] detectable; median, 37 CFU per 10g; mean, 108 CFU

per 10g), followed by beef (12/58 [21%] detectable; median, 10; mean 30), chicken (37/71 [52%] detectable; median, 9; mean, 12), and pork (33/104 [32%] detectable; median, 5; mean, 10).

Discussion

In this study, we investigated the prevalence and molecular epidemiology of *S. aureus* in U.S. meat and poultry samples (n=3290; ABF 530 and conventional 2760) for a period of one year comparing samples from a rural county and an urban county. We found that the *S. aureus* contamination in sampled meat and poultry ranged from 22.8% -32.5% with the highest contamination found in pork (32.5%). The overall prevalence of MRSA ranged from 0.5% (chicken) to 4.3% (turkey). The overall prevalence of *S. aureus* and MRSA in our study was 27.8% and 1.2% respectively. Although no statistical difference was observed, the prevalence of both MRSA and MSSA was higher among conventional meat samples compared to ABF meat samples.

The prevalence of *S. aureus* in retail pork (32.5%) and beef products (22.8%) is consistent with the results of other U.S. studies where *S. aureus* prevalence ranged from 18.2% to 64.8% in pork [7, 16, 18, 19, 21, 23] and 6.9% to 37% in beef [16, 18-20]. The results of our current study were lower than those found previously in a smaller study by our group, despite using the same methodology [21]. Similarly, the *S. aureus* contamination in our beef products is almost one third less than previously reported [7]. The prevalence of *S. aureus* in chicken and turkey in the current study was lower than a similar U.S. study, where 41% and 77% of chicken and turkey products were contaminated respectively [19], but higher in chicken than two other previous U.S studies [18, 40] and in turkey than one of those [18] (turkey was not examined in

the second). Nevertheless, the overall prevalence of *S. aureus* in our study is consistent with one prior study conducted in the United States which documented 29% prevalence of *S. aureus* in raw retail meat products [41].

The prevalence of MRSA observed in previous studies of U.S. retail meat products has ranged from 1.3% to 4% in beef [7, 16, 20], and 3% to 7% in pork [7, 16, 18, 21, 23, 24]. One study has documented 3.9% prevalence of MRSA in chicken [20]. The prevalence of MRSA in beef, pork and chicken in our study was lower than previous studies. Like others, the highest prevalence of MRSA was found on turkey [15]. The prevalence of MRSA in our turkey samples (4.4%) was higher than a previous study that documented MRSA contamination of 1.7% of turkey samples collected in the U.S. [20].

Previous studies in the U.S. investigating prevalence of *S. aureus* and/or MRSA in retail meat had small sample sizes [7, 16, 18-20] and/or focused on a single type of meat [21, 40]. The strength of our study is that it represents the largest sampling of raw meat products examined for *S. aureus* presence conducted to date globally. Our study involved weekly meat sampling of both urban and rural areas for a period of one year. We collected wide varieties of both conventional and raised-without-antibiotics meats, as well as a variety of cuts or treatments of meat products (including pork chop, pork sausage, pork loin, bacon, ground beef, New York strip steak, beef sirloin, rib eye, antibiotic-free chicken breast, drumstick, chicken breast, as well as salmon and tilapia).

We also measured bacterial load monthly, though the majority of samples (155; 61.5%) did not have a sufficient number of colonies to be quantified using the Petrifilm system. In future samplings, a more sensitive system of measurement is recommended. The CFU/g of meat in our

quantitation was relatively low (2-517 CFU/10g of meat). Bacteria contaminating these surfaces from raw meat may contaminate food preparer's skin and potentially lead to colonization or further contamination of household surfaces [42]. The transfer of bacteria from surfaces that come in contact with food to the skin have been well documented [42-44]. A study conducted to estimate the percentage of MRSA transferred from inoculated retail pork products to contact surfaces such as cutting boards, knives and pork skin (a model for human skin) indicated the transfer of MRSA from meat products to the contact surfaces. For example, 8.04 log CFU/g MRSA on pork loin in contact with a cutting board, knife, and pork skin transferred 86%, 29% and 81% of MRSA respectively [42].

Furthermore, we carried out convenience sampling in two areas within one state, which limits the generalizability of our results. However, our inclusion of both urban and rural areas as well as collection of samples from national and regional supermarkets and retail chains, specialty food markets, and cooperative grocery stores adds more strength to this study. Despite our efforts to quantify and compare the prevalence of *S. aureus* and MRSA in conventional and antibiotic free meat products, the limited availability of antibiotic-free meats across the stores adds further limitation to this study; especially to compare between two geographic locations as we could not purchase any antibiotic free meats in Keokuk County except antibiotic-free chicken. However, the results provide some insights regarding the prevalence of *S. aureus* and MRSA in antibiotic-free meat and poultry products sold by one store in Iowa City.

The inability to determine the ultimate origin of *S. aureus* isolates (animal or human) is another limitation to this study. We cannot draw firm conclusions about sources or whether these isolates may have originated from pre or post-slaughter contamination, or a combination of the two. While European meat studies have suggested that isolates of ST398 and associated types are

typically from livestock [15], in the U.S., the epidemiology of livestock-associated *S. aureus* is more complex [45], as “human” types of *S. aureus* have frequently been found in live pigs [1, 46-50], and “human-origin” ST398 has been described [51] and found in individuals lacking any livestock association [28, 52-54].

The high prevalence of resistance to tetracycline and erythromycin in our study is consistent with Waters et al. [19]. Similar to their findings our study also found the highest MDR prevalence among turkey. However, compared to that report, the overall prevalence of MDR was lower in our study (64% vs 32% in pork, 79% vs 52.6% in turkey; 35% vs 10.2% in beef; and 26% vs 11.7% in chicken). In Waters et al., 52% of *S. aureus* isolates were MDR, while our study found only 23%. A number of factors including sample size, sampling duration, location, and difference in meat cuts sampled may have contributed to such variations. For example, we did not include turkey cutlets and chicken thigh in our samples, and their samples did not include pork sausage and pork loin, as well as New York steak, beef sirloin and rib eye. Furthermore, our study focused only on samples sold in Iowa, while their study samples were collected from five major U.S. cities.

The highest prevalence of CC5-associated *spa* types in chicken and CC398-associated *spa* types in turkey are consistent with the result found by Waters et al. [19]. We found the highest prevalence of MRSA isolates belonging to *spa* types associated with CC8 (20.9%) and CC5 (6.6%). Twenty-three of our 41 MRSA positive isolates (56%) were *spa* types t002 or t008. The three MRSA isolates in the Waters et al. study also belonged to ST8 and ST5. It is interesting to note that a significant proportion of MRSA isolates (20.9%) were *spa* types associated with CC8 that include t008, a community-associated strain followed by CC5 (6.6%)

that includes *spa* type t002. These isolates are common human types but are also found in live swine [9, 55] and poultry [56].

Almost half of the turkey isolates were t034/ST398 which is primarily associated with swine [8]. Consistent with previous studies [15, 18, 19], the results of this study found ST398-associated strains in a high proportion of turkey samples. Our study also found contamination by ST9 (t337), a second “swine-associated” strain, in one of our chicken samples, suggesting inter-species transmission may have occurred.

Our study design also allowed us to begin to address seasonal differences in presence of *S. aureus* on meat products. In humans, seasonal variation appears to exist in *S. aureus* infections. The significant rise of *S. aureus* infections have been documented during summer and autumn in temperate and tropical regions [57], similar to that often seen with enteric food-borne infections [58]. A review of epidemiological studies evaluating the seasonality in *S. aureus* colonization and infection suggested an association of summer and autumn months with *S. aureus* skin and soft-tissue infections (SSTIs) [59]. A study examining the MRSA infection in the United States noted the increased MRSA infection in second two quarters (July through December) compared to first two quarters (January through June) in pediatric patients [57]. Our finding of the highest prevalence of *S. aureus* contamination of meat products in the autumn and winter is consistent with that of higher MRSA infections in these months. However, further longitudinal study is warranted to draw any decisive conclusions, and to examine causality and any additional risk for *S. aureus* colonization or infection that may be attributable to meat handling or meat-source contamination of the home.

Conflict of Interest: None

Acknowledgements: This project was supported by AFRI food safety grant no. #2011-67005-30337 from the USDA National Institute of Food and Agriculture (TCS). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Smith TC, Gebreyes WA, Abley MJ, Harper AL, Forshey BM, Male MJ, et al. Methicillin-resistant *Staphylococcus aureus* in pigs and farm workers on conventional and antibiotic-free swine farms in the USA. PLoS One. 2013;8(5):e63704. doi: 10.1371/journal.pone.0063704. PubMed PMID: 23667659; PubMed Central PMCID: PMC3646818.
2. Furuya EY, Lowy FD. Antimicrobial-resistant bacteria in the community setting. Nature reviews Microbiology. 2006;4(1):36-45. Epub 2005/12/17. doi: 10.1038/nrmicro1325. PubMed PMID: 16357859.
3. Philip L. Graham III SXL, Elaine L. Larson. A U.S. population - based survey of *Staphylococcus aureus* colonization. Annals of Internal Medicine. 2006;144:318-25.
4. Heiman FL Wertheim DCM, Margreet C Vos, Willem van Leeuwen, Alex van Belkum, Henri A Verbrugh, Jan L Nouwen. The role of nasal carriage in *Staphylococcus aureus* infections. The Lancet Infectious Diseases. 2005;5(12):751-62.
5. Lowy FD. *Staphylococcus aureus* infections. N Engl J Med. 1998;339(8):520-32. Epub 1998/08/26. PubMed PMID: 9709046.
6. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004;39(3):309-17. Epub 2004/08/13. doi: 10.1086/421946. PubMed PMID: 15306996.
7. Jackson CR, Davis JA, Barrett JB. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from retail meat and humans in Georgia. J Clin Microbiol. 2013;51(4):1199-207. doi: 10.1128/JCM.03166-12. PubMed PMID: 23363837; PubMed Central PMCID: PMC3666775.
8. Smith TC, Male MJ, Harper AL, Kroeger JS, Tinkler GP, Moritz ED, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. PLoS ONE. 2009;4(1):e4258. Epub 2009/01/16. doi: 10.1371/journal.pone.0004258. PubMed PMID: 19145257; PubMed Central PMCID: PMC2626282.
9. 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. Epub 2007/11/21. doi: S0378-1135(07)00493-2 [pii] 10.1016/j.vetmic.2007.10.006 [doi]. PubMed PMID: 18023542.
10. Huijsdens XW, van Dijke BJ, Spalburg E, van Santen-Verheuve MG, Heck ME, Pluister GN, et al. Community-acquired MRSA and pig-farming. Ann Clin Microbiol Antimicrob. 2006;5:26. Epub 2006/11/14. doi: 1476-0711-5-26 [pii]

10.1186/1476-0711-5-26 [doi]. PubMed PMID: 17096847.

11. Persoons D, Van Hoorebeke S, Hermans K, Butaye P, de Kruif A, Haesebrouck F, et al. Methicillin-resistant *Staphylococcus aureus* in poultry. *Emerging infectious diseases*. 2009;15(3):452-3. Epub 2009/02/26. doi: 10.3201/eid1503.080696. PubMed PMID: 19239762; PubMed Central PMCID: PMC2666289.

12. Fessler A, Scott C, Kadlec K, Ehrlich R, Monecke S, Schwarz S. Characterization of methicillin-resistant *Staphylococcus aureus* ST398 from cases of bovine mastitis. *The Journal of antimicrobial chemotherapy*. 2010;65(4):619-25. Epub 2010/02/19. doi: 10.1093/jac/dkq021. PubMed PMID: 20164198.

13. Kitai S, Shimizu A, Kawano J, Sato E, Nakano C, Uji T, et al. Characterization of methicillin-resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. *J Vet Med Sci*. 2005;67(1):107-10. Epub 2005/02/09. doi: JST.JSTAGE/jvms/67.107 [pii]. PubMed PMID: 15699605.

14. Kwon NH, Park KT, Jung WK, Youn HY, Lee Y, Kim SH, et al. Characteristics of methicillin resistant *Staphylococcus aureus* isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. *Vet Microbiol*. 2006;117(2-4):304-12. Epub 2006/06/30. doi: S0378-1135(06)00203-3 [pii]

10.1016/j.vetmic.2006.05.006. PubMed PMID: 16806746.

15. de Boer E, Zwartkruis-Nahuis JT, Wit B, Huijsdens XW, de Neeling AJ, Bosch T, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *Int J Food Microbiol*. 2009;134(1-2):52-6. Epub 2009/01/16. doi: S0168-1605(08)00646-6 [pii]

10.1016/j.ijfoodmicro.2008.12.007. PubMed PMID: 19144432.

16. Pu S, Han F, Ge B. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* strains from Louisiana retail meats. *Applied and environmental microbiology*. 2009;75(1):265-7. Epub 2008/11/04. doi: 10.1128/AEM.01110-08. PubMed PMID: 18978079; PubMed Central PMCID: PMC2612222.

17. van Loo I, Huijsdens X, Tiemersma E, de Neeling A, van de Sande-Bruinsma N, Beaujean D, et al. Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. *Emerging infectious diseases*. 2007;13(12):1834-9. Epub 2008/02/09. doi: 10.3201/eid1312.070384. PubMed PMID: 18258032; PubMed Central PMCID: PMC2876750.

18. Hanson BM, Dressler AE, Harper AL, Scheibel RP, Wardyn SE, Roberts LK, et al. Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. *J Infect Public Health*. 2011;4(4):169-74. doi: 10.1016/j.jiph.2011.06.001. PubMed PMID: 22000843.

19. Waters AE, Contente-Cuomo T, Buchhagen J, Liu CM, Watson L, Pearce K, et al. Multidrug-Resistant *Staphylococcus aureus* in US Meat and Poultry. *Clin Infect Dis*. 2011;52(10):1227-30. doi: 10.1093/cid/cir181. PubMed PMID: 21498385; PubMed Central PMCID: PMC3079400.

20. Bhargava K, Wang X, Donabedian S, Zervos M, da Rocha L, Zhang Y. Methicillin-resistant *Staphylococcus aureus* in retail meat, Detroit, Michigan, USA. *Emerging infectious diseases*. 2011;17(6):1135.

21. O'Brien AM, Hanson BM, Farina SA, Wu JY, Simmering JE, Wardyn SE, et al. MRSA in conventional and alternative retail pork products. *PLoS ONE*. 2012;7(1):e30092. Epub 2012/01/26. doi: 10.1371/journal.pone.0030092. PubMed PMID: 22276147; PubMed Central PMCID: PMC3261874.

22. Weese JS, Reid-Smith R, Rousseau J, Avery B. Methicillin-resistant *Staphylococcus aureus* (MRSA) contamination of retail pork. *The Canadian Veterinary Journal*. 2010;51(7):749.

23. Buyukcangaz E, Velasco V, Sherwood JS, Stepan RM, Koslofsky RJ, Logue CM. Molecular typing of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) isolated from animals and retail meat in North Dakota, United States. *Foodborne pathogens and disease*. 2013;10(7):608-17.

24. Molla B, Byrne M, Abley M, Mathews J, Jackson CR, Fedorka-Cray P, et al. Epidemiology and genotypic characteristics of methicillin-resistant *Staphylococcus aureus* strains of porcine origin. *Journal of clinical microbiology*. 2012;50(11):3687-93.

25. Kadlec K, Schwarz S. Novel ABC transporter gene, *vga(C)*, located on a multiresistance plasmid from a porcine methicillin-resistant *Staphylococcus aureus* ST398 strain. *Antimicrob Agents Chemother*. 2009;53(8):3589-91. Epub 2009/05/28. doi: AAC.00570-09 [pii]

10.1128/AAC.00570-09. PubMed PMID: 19470508; PubMed Central PMCID: PMC2715595.

26. Monecke S, Kuhnert P, Hotzel H, Slickers P, Ehricht R. Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. *Veterinary Microbiology*. 2007;125(1-2):128-40. doi: DOI 10.1016/j.vetmic.2007.05.016. PubMed PMID: ISI:000250804200014.

27. Alam MT, Read TD, Petit RA, 3rd, Boyle-Vavra S, Miller LG, Eells SJ, et al. Transmission and microevolution of USA300 MRSA in U.S. households: evidence from whole-genome sequencing. *MBio*. 2015;6(2):e00054. doi: 10.1128/mBio.00054-15. PubMed PMID: 25759497; PubMed Central PMCID: PMC4453535.

28. Bhat M, Dumortier C, Taylor BS, Miller M, Vasquez G, Yunen J, et al. *Staphylococcus aureus* ST398, New York City and Dominican Republic. *Emerg Infect Dis*. 2009;15(2):285-7. Epub 2009/02/06. PubMed PMID: 19193274.

29. Krziwanek K, Metz-Gercek S, Mittermayer H. Methicillin-Resistant *Staphylococcus aureus* ST398 from human patients, upper Austria. *Emerg Infect Dis*. 2009;15(5):766-9. Epub 2009/05/01. PubMed PMID: 19402964; PubMed Central PMCID: PMC2687006.

30. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty - Second Informational Supplement*. Wayne, PA: Clinical and Laboratory Standards Institute. p CLSI document M100-S122; 2012.

31. Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*. 2012;18(3):268-81.

32. Bosgelmez-Tinaz G, Ulusoy S, Aridogan B, Coskun-Ari 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):410-2. Epub 2006/06/13. doi: 10.1007/s10096-006-0153-8. PubMed PMID: 16767493.

33. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, et al. Involvement of Pantone-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis*. 1999;29(5):1128-32. Epub 1999/10/19. doi: CID990251 [pii]. PubMed PMID: 10524952.

34. Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol*. 1999;37(11):3556-63. Epub 1999/10/19. PubMed PMID: 10523551.

35. Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, Kreiswirth BN. *spa* typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. *J Clin Microbiol*. 2004;42(2):792-9. Epub 2004/02/10. PubMed PMID: 14766855; PubMed Central PMCID: PMC344479.

36. Mellmann A, Weniger T, Berssenbrügge C, Rothgänger J, Sammeth M, Stoye J, et al. Based Upon Repeat Pattern (BURP): an algorithm to characterize the long-term evolution of *Staphylococcus aureus* populations based on *spa* polymorphisms. *BMC microbiology*. 2007;7(1):98.

37. Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. *PloS one*. 2011;6(4):e17936.

38. Strommenger B, Kettlitz C, Weniger T, Harmsen D, Friedrich AW, Witte W. Assignment of *Staphylococcus* isolates to groups by *spa* typing, Smal macrorestriction analysis, and multilocus sequence typing. J Clin Microbiol. 2006;44(7):2533-40. doi: 10.1128/JCM.00420-06. PubMed PMID: 16825376; PubMed Central PMCID: PMC1489514.
39. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol. 2000;38(3):1008-15. PubMed PMID: 10698988; PubMed Central PMCID: PMC86325.
40. Hanning I, Gilmore D, Pendleton S, Fleck S, Clement A, Park SH, et al. Characterization of *Staphylococcus aureus* isolates from retail chicken carcasses and pet workers in Northwest Arkansas. Journal of food protection. 2012;75(1):174-8. doi: 10.4315/0362-028X.JFP-11-251. PubMed PMID: 22221374.
41. Kelman A, Soong YA, Dupuy N, Shafer D, Richbourg W, Johnson K, et al. Antimicrobial susceptibility of *Staphylococcus aureus* from retail ground meats. Journal of food protection. 2011;74(10):1625-9. doi: 10.4315/0362-028X.JFP-10-571. PubMed PMID: 22004808.
42. Snyder HL, Niebuhr SE, Dickson JS. Transfer of Methicillin-Resistant *Staphylococcus aureus* from Retail Pork Products onto Food Contact Surfaces and the Potential for Consumer Exposure. Journal of Food Protection®. 2013;76(12):2087-92.
43. da Silva Malheiros P, dos Passos CT, Casarin LS, Serraglio L, Tondo EC. Evaluation of growth and transfer of *Staphylococcus aureus* from poultry meat to surfaces of stainless steel and polyethylene and their disinfection. Food control. 2010;21(3):298-301.
44. Todd EC, Michaels BS, Greig JD, Smith D, Holah J, Bartleson CA. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 7. Barriers to reduce contamination of food by workers. Journal of Food Protection®. 2010;73(8):1552-65.
45. Smith TC. Livestock-Associated *Staphylococcus aureus*: The United States Experience. PLoS Pathog. 2015;11(2):e1004564. doi: 10.1371/journal.ppat.1004564. PubMed PMID: 25654425.
46. 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):234-43. doi: 10.1111/j.1863-2378.2012.01527.x. PubMed PMID: 22883566.
47. Dressler AE, Scheibel RP, Wardyn S, Harper AL, Hanson BM, Kroeger JS, et al. Prevalence, antibiotic resistance and molecular characterisation of *Staphylococcus aureus* in pigs at agricultural fairs in the USA. Vet Rec. 2012;170(19):495. doi: 10.1136/vr.100570. PubMed PMID: 22505242.
48. Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL, Karriker LA, et al. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from pork farms and visiting veterinary students. PLoS One. 2013;8(1):e53738. doi: 10.1371/journal.pone.0053738. PubMed PMID: 23301102; PubMed Central PMCID: PMC3536740.
49. Molla B, Byrne M, Abley M, Mathews J, Jackson CR, Fedorka-Cray P, et al. Epidemiology and genotypic characteristics of methicillin-resistant *Staphylococcus aureus* strains of porcine origin. J Clin Microbiol. 2012;50(11):3687-93. doi: 10.1128/JCM.01971-12. PubMed PMID: 22972820; PubMed Central PMCID: PMC3486229.
50. Gordoncillo MJ, Abdujamilova N, Perri M, Donabedian S, Zervos M, Bartlett P. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in backyard pigs and their owners, Michigan, USA. Zoonoses Public Health. 2012;59(3):212-6. doi: 10.1111/j.1863-2378.2011.01437.x. PubMed PMID: 21914153.
51. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. MBio. 2012;3(1). Epub 2012/02/23. doi: 10.1128/mBio.00305-11. PubMed PMID: 22354957; PubMed Central PMCID: PMC3280451.

52. Mediavilla JR, Chen L, Uhlemann AC, Hanson BM, Rosenthal M, Stanak K, et al. Methicillin-Susceptible *Staphylococcus aureus* ST398, New York and New Jersey, USA. *Emerging Infectious Diseases*. 2012;18(4):700-2. Epub 2012/04/04. doi: 10.3201/eid1804.111419. PubMed PMID: 22469250.
53. David MZ, Siegel J, Lowy FD, Zychowski D, Taylor A, Lee CJ, et al. Asymptomatic carriage of sequence type 398, spa type t571 methicillin-susceptible *Staphylococcus aureus* in an urban jail: a newly emerging, transmissible pathogenic strain. *J Clin Microbiol*. 2013;51(7):2443-7. doi: 10.1128/JCM.01057-13. PubMed PMID: 23658269; PubMed Central PMCID: PMC3697676.
54. Uhlemann AC, Porcella SF, Trivedi S, Sullivan SB, Hafer C, Kennedy AD, et al. Identification of a highly transmissible animal-independent *Staphylococcus aureus* ST398 clone with distinct genomic and cell adhesion properties. *MBio*. 2012;3(2). Epub 2012/03/01. doi: 10.1128/mBio.00027-12. PubMed PMID: 22375071; PubMed Central PMCID: PMC3302565.
55. Sunde M, Tharaldsen H, Marstein L, Haugum M, Norström M, Jacobsen T, et al. Detection of methicillin-resistant *Staphylococcus aureus* sequence type 8 in pigs, production environment, and human beings. *Journal of Veterinary Diagnostic Investigation*. 2011;23(2):348-50.
56. Lowder BV, Guinane CM, Zakour NLB, Weinert LA, Conway-Morris A, Cartwright RA, et al. Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. *Proceedings of the National Academy of Sciences*. 2009;106(46):19545-50.
57. Mermel LA, Machan JT, Parenteau S. Seasonality of MRSA infections. *PloS one*. 2011;6(3):e17925.
58. Lal A, Hales S, French N, Baker MG. Seasonality in human zoonotic enteric diseases: a systematic review. *PLoS One*. 2012;7(4):e31883. doi: 10.1371/journal.pone.0031883. PubMed PMID: 22485127; PubMed Central PMCID: PMC3317665.
59. Leekha S, Diekema D, Perencevich E. Seasonality of staphylococcal infections. *Clinical Microbiology and Infection*. 2012;18(10):927-33.

Table 1. Distribution of MSSA and MRSA in commercially distributed meats

Sample type	ABF (n =530)			Conventional (n = 2760)		
	N	All <i>S. aureus</i> positive n (%)	MRSA positive n (%)	N	All <i>S. aureus</i> positive n (%)	MRSA positive n (%)
Chicken	223	91(40.8)	0	580	148 (25.5)	4 (0.7)
Antibiotic free chicken breast	123	66 (53.7)	0	0	0	0
Drumstick	51	12 (23.5)	0	284	69 (24.3)	2 (0.7)
Chicken breast	49	13 (26.5)	0	296	79 (26.7)	2 (0.7)
Beef	146	20 (13.7)	1 (0.7)	874	213 (24.4)	5 (0.6)
Ground beef	50	4 (8)	1 (2)	304	73 (24.0)	4 (1.3)
New York steak	46	9 (19.6)	0	223	56 (25.1)	0
Beef Sirloin	50	7 (14)	0	300	74 (24.7)	1 (0.33)
Rib eye	0	0	0	47	10 (21.3)	0
Pork	142	22 (15.5)	1 (0.7)	1049	365 (34.8)	20 (1.9)
Pork chop	52	8 (15.4)	1 (1.9)	294	117 (39.8)	2 (0.7)
Pork sausage	44	12 (27.3)	0	293	124 (42.3)	10 (3.4)
Pork loin	3	1 (33.3)	0	211	65 (30.8)	2 (0.9)
Bacon	43	1 (2.3)	0	251	59 (23.5)	6 (2.4)
Turkey (ground)	3	0	0	227	52 (22.9)	10 (4.4)
Fish	16	1(6.25)	0	30	1 (3.3)	0
Salmon	8	0	0	16	1 (6.2)	0
Tilapia	8	1(12.5)	0	14	0	0
Total	530	134 (25.3)	2 (0.4)	2760	779 (28.2)	39 (1.4)

583

584

Table 2: Molecular characteristics of *S. aureus* isolates

Cluster complex (representative <i>spa</i> type)	N (% of total)	Most common <i>spa</i> types (n)	PVL N (%)*	<i>mecA</i> N (%)
CC5 t002	242 (26.5)	t002 (137), t010 (37), t548 (12) t688 (10)	3(1.2)	16 (6.6)
CC30 (t012)	112 (12.3)	t021 (44), t037 (39), t012 (11), t726 (10)	2(1.8)	1 (0.9)
CC15 (t094/t091/t491)	109 (11.9)	t491 (20), t346 (18), t2801(16), t091 (14), t094(13)	0(0.0)	1(0.9)
CC1 (t273)	97 (10.6)	t273 (87), t127 (3), t11931 (3)	1 (1.0)	2 (2.1)
CC9 (t337)	79 (8.7)	t337 (33), t3446 (13), t888 (11)	0 (0.0)	2 (2.5)
CC8 (t008)	67(7.3)	t5323 (17), t008 (16), t5413 (15) t051 (6), t5593 (6)	11(16.2)	14(20.9)
CC398 (t034)	61(6.7)	t034 (44), t571 (4)	0 (0.0)	3 (4.9)
t189	45 (4.9)	t189 (32), t267 (7)	1(2.6)	0 (0.0)
Other	101(11.1)	t4223 (16), t160 (15), t026 (9)	0 (0.0)	2 (2.0)
Total	913 (100.0)		18/913 (2.0)	41/913 (4.5)

585

586

Table 3:Antibiotic susceptibility profile of selected isolates (N=239)
(N=198, MSSA; N=41, MRSA)

Characteristic	N tested	tetR (%)	clindR (%)	eryR (%)	MDR (%)
Sequence type					
CC5 (t002)	68	15 (22.0)	14 (20.6)	23 (33.8)	18 (26.5)
CC30 (t012)	26	1 (3.8)	0 (0.0)	0 (0.0)	1 (3.8)
CC15(t094/t091/t49 1)	23	13 (56.5)	0 (0.0)	2 (8.7)	1 (4.3)
CC1 (t273)	23	11 (47.8)	1 (4.3)	1 (4.3)	2 (8.7)
CC9 (t337)	22	15 (68.1)	11 (50.0)	12 (54.5)	12 (54.5)
CC8 (t008)	24	6 (25.0)	1 (4.1)	9 (37.5)	14 (58.3)
CC398 (t034)	14	12 (85.7)	2 (14.3)	3 (21.4)	3 (21.4)
t189	14	1 (7.1)	0 (0.0)	3 (21.4)	0 (0.0)
Other	25	6 (24.0)	3 (12.0)	5 (20.0)	4 (16.0)
Meat type					
Pork	100	53 (53.0)	19 (19.0)	28 (28.0)	32 (32.0)
Chicken	60	8 (13.3)	8 (13.3)	13 (21.6)	7 (11.7)
Beef	59	5 (8.5)	2 (3.4)	11 (18.6)	6 (10.2)
Turkey	19	13 (68.4)	3 (15.8)	6 (13.6)	10 (52.6)
Fish	1	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Location					
Urban	130	51 (39.2)	19 (14.6)	36 (27.7)	29 (22.3)
Rural	109	29 (26.0)	13 (11.9)	22 (20.2)	26 (23.8)
Total	239	80 (33.5)	32 (13.4)	58 (24.3)	55 (23.0)

587 tetR, tetracycline resistance; clindR, clindamycin resistance; eryR, erythromycin resistance

Table 4: Antibiotic susceptibility pattern among MSSA and MRSA of selected isolates

Antibiotic	MSSA (N=198) N (%)	MRSA(N=41) N (%)	Total (N=239) N (%)
Oxacillin	0 (0.0)	41 (100)	41 (17.1)
Tetracycline	59 (29.8)	21 (51.2)	80 (33.5)
Erythromycin	33 (16.6)	25 (61.0)	58 (24.3)
Clindamycin	17 (8.6)	15 (36.6)	32 (13.4)
Quino/Dalfo	4 (2.0)	4 (9.7)	8 (3.3)
Gentamicin	2 (1.0)	1 (2.4)	3 (1.2)
Daptomycin	2 (1.0)	1 (2.4)	3 (1.2)
Rifampin	0 (0.0)	2 (4.9)	2 (0.8)
TMP-SMX	1 (0.5)	1 (2.4)	2 (0.8)
Levofloxacin	0 (0.0)	13 (31.7)	13 (5.4)
MDR	14 (7.0) (t337,t3446)*	41 (100) (t002)*	55 (23.0)

*Denotes leading MDR types

588

589

590

591

592

593

594

595

596

597

598

599

600

601

602

603

Isolate ID		MLST	<i>spa</i> type	<i>mecA</i>	PVL
UM01GB01		ST-1	t065	-	-
UM03PL49		ST-1	t273	-	-
UM06AC44	*	ST-5	t002	-	-
UM07CD13		ST-5	t3446	-	-
UM06CD17		ST-5	t088	-	-
UM05PL43		ST-5	t037	-	-
UM05GB33		ST-5	t688	-	-
UM03NY37		ST-5	t010	-	-
UM02CX12	*	ST-5	t002	-	-
UM03GB29		ST-5	t688	-	-
UM03GB52		ST-5	t179	-	-
UM02CX49	*	ST-5	t548	-	-
UM03CX50		ST-6	t5413	-	-
UM08BS35		ST-8	t008	-	-
UM05NY41		ST-8	t4223	-	-
UM06PS49		ST-9	t337	-	-
UM05CX22		ST-12	t888	-	-
UM05CD09		ST-12	t160	-	-
UM05PC01		ST-15	t008	-	+
UM07GB14		ST-15	t084	-	-
UM08AC49	*	ST-15	t084	-	-
UM08NY50		ST-15	t084	-	-
UM05PL47		ST-15	t2801	-	-
UM06PL11		ST-15	t491	-	-
UM05BS13		ST-30	t012	-	-
UM01AC10	*	ST-30	t122	-	-
UM03NY13		ST-30	t338	-	-
UM03BA38		ST-30	t021	-	-
UM01CD21		ST-45	t004	-	-
UM03PC09		ST-45	t026	-	-
UM05GB28		ST-59	t216	-	-
UM06PL47		ST-72	t148	-	-
UM08GT44		ST-72	t126	+	-
UM02GB51	*	ST-87	t216	-	-
UM07PC41		ST-188	t189	-	-
UM02CD01	*	ST-188	t189	-	+
UM05NY48		ST-350	t346	-	-
UM07CX40		ST-398	t034	-	-
UM08PS29		ST-398	t034	-	-
UM06BA44		ST-398	t114	+	-
UM08GB48		ST-1159	t091	-	-
UM08PS36		ST-2250	t346	-	-
UM02PC52	*	ST-10002	t012	-	-
UM05BA48		ST-10003	t5323	-	-

UM07PC48	ST-10007	t021	-	-	605
UM05NY38	ST-10008	t209	-	-	606

607 *denotes antibiotic-free meat samples

608

609

610

611

612

613

614

615

616

617

618

619

620

621

622

623

624

625

626

627

628

629