



Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa

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Summary Several recent studies have indicated a high prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in retail-available meat. However, few studies have investigated MRSA in meat in the United States. The aim of this study was to determine the presence of *Staphylococcus aureus* (*S. aureus*) on meat samples available at retail stores. Samples of fresh raw pork, chicken, beef, and turkey were purchased from 22 food stores throughout Iowa. *S. aureus* strains were isolated from 27 of 165 samples, giving an overall prevalence of 16.4%. Turkey, pork, chicken, and beef had individual *S. aureus* prevalence rates of 19.4%, 18.2%, 17.8%, and 6.9%, respectively. Two isolates of MRSA were isolated from pork, giving an overall prevalence of 1.2%. One MRSA isolate was positive for the PVL gene. Common *spa* types included t034, t337, t008, and t002. These results suggest that MRSA is present on low numbers of retail meat in Iowa.

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Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive, coagulase positive bacterium that is generally found in the natural flora of the human nasal passage. Methicillin-resistant *Staphylococcus aureus* (MRSA) has recently emerged as a health concern and currently causes approximately 94,000

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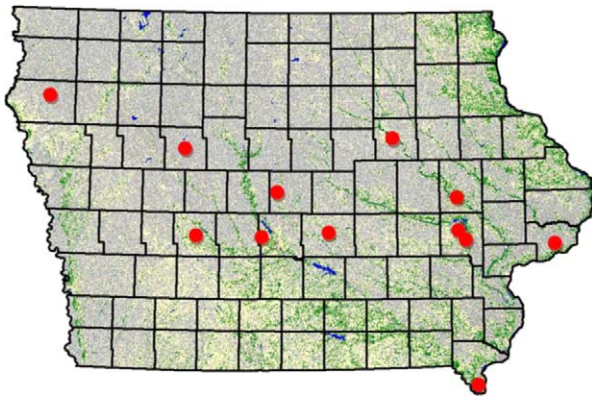


Figure 1 Map of Iowa showing the locations of retail stores sampled. Samples were collected between February and April 2009.

invasive infections yearly in the United States of America, leading to an estimated 18,650 deaths [1].

S. aureus has also come into focus as a foodborne pathogen with an estimated 241,000 domestically acquired infections yearly within the United States [2]. Furthermore, MRSA has been found in several species of meat-producing animals, including pigs [3,4], chickens [5] and cattle [6]. Identification of MRSA in these animals, coupled with the recent emergence of ST398 (non-typable MRSA) in the swine population and the observed potential of this strain to cause severe infections and even death in humans [7], illustrates the importance of investigating meat products as potential vehicles for transmission of MRSA 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 <1 percent in Asia [8–10] up to 11.9 percent in The Netherlands [11], with intermediate prevalence found in other studies [12–16]. While MRSA has been isolated from retail meat in Asia, Canada, Europe, and other regions of the United States, the aim of this study was to establish the prevalence and molecular types of *S. aureus* on raw retail meat samples collected from a rural Midwestern state.

Methods

Sample collection

A convenience sample of fresh raw retail meat was collected between February and April 2009 from 22 grocery stores located across the state of Iowa (Fig. 1). 165 samples were collected from 12 towns. Samples collected included 55 pork (chops

and ground pork), 45 chicken (breast and drumsticks), 29 beef (ground beef and sirloin strips), and 36 turkey samples (drumsticks and ground turkey). Stores were chosen to reflect both urban and rural areas across Iowa with 9 stores from rural areas, and 13 stores from urban areas. Stores sampled included independent stores as well as common chain stores in the state. Stores were visited only once, and meat was chosen mainly from national brand products, where available. Samples were transported on blue ice packs to the laboratory, and processed within 6 h of purchase.

Bacterial culture

Samples were collected using sterile swabs through surface swabbing. The entire external surface of the sample was rubbed with a cotton-tipped swab pre-moistened with enrichment broth containing 10 g tryptone/L, 75 g NaCl/L, 10 g mannitol/L and 2.5 g yeast extract/L. After 24 h incubation at 35 °C in 5 mL enrichment broth, 5 µL of broth was inoculated onto selective MRSA agar plates (BBL CHROM agar MRSA, Becton, Dickinson and Company, MD) and Columbia colistin and nalidixic acid (CNA) plate (Remel, Lenexa, KS). These plates were incubated 24–48 h at 35 °C and examined for *S. aureus*. Isolates were confirmed to be *S. aureus* by examining their appearance on Gram stain, and by doing the catalase test, slide and tube coagulase tests and the *S. aureus* latex agglutination assay (Pastorex Staphplus, Bio-Rad). Methicillin resistance was assessed by testing for the presence of penicillin binding protein 2 (PBP2') (MRSA latex agglutination test, Oxoid Ltd., Hants, UK) and the presence of the *mecA* gene (described below).

Molecular testing

Genomic DNA was extracted using the Wizard Genomic DNA preparation kit (Promega). *pvl* Polymerase Chain Reaction (PCR) was performed on all isolates and *SCCmec* typing (which is dependent on the presence of the *mecA* gene) was performed on MRSA isolates. Multilocus sequence typing (MLST) was performed on all MRSA isolates as previously described [17]. The multiplex *SCCmec* PCR was modified from previously described methods [18,19]. Organisms were grown overnight at 37 °C on blood agar plates. Two or three colonies were taken from the plate and suspended into 200 µL of distilled water. Amplification was performed using 10× Biolase buffer, 200 µM deoxynucleoside triphosphate; 1.5 mM MgCl₂; 200 nM concentration of primers KDP F1/R1, RIF4 F3/R9; 400 nM concentrations of primers CIF2 F2/R2, MECI P2/P3,

RIF5 F10/R13; 800 nM concentration of primers DCS F2/R1, MECA P4/P7; 1.25U BiolaseTaq Polymerase; and 3 μ L of cells for a final volume of 50 μ L. The PCR conditions were 94 °C for 10 min; 30 cycles of the following: 94 °C for 30 s, 53 °C for 30 s, 72 °C for 1 min, and repeat; 72 °C for 4 min. Amplification of the *Staphylococcus* protein A (*spa*) gene was performed through PCR as previously described [20], using primers [21] validated for use with Ridom-StaphType software (Ridom GmbH, Germany). The presence of *pvl* was determined by an additional PCR [22]. All molecular procedures utilized known positive and negative controls.

Antimicrobial susceptibility testing

All isolates were tested for antimicrobial susceptibility by the broth dilution method described by the Clinical and Laboratory Standards Institute [23]. Isolates were tested for susceptibility to penicillin, oxacillin, tetracycline, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, quinupristin/dalfopristin, gentamicin, levofloxacin, moxifloxacin, linezolid, daptomycin, vancomycin, and rifampin.

Statistical analysis

Fisher's exact test was used to assess significance in observed prevalence rates of methicillin susceptible *Staphylococcus aureus* (MSSA) between meat types. Risk factors assessed were type of meat, and geographic location of the store (urban vs. rural). A significance level of 0.05 was used during analysis and two-sided *p*-values were assessed. SAS software version 9.2 (SAS Institute Inc., Cary, NC) was used to perform analyses.

Results

Prevalence of *S. aureus* on retail meat samples and risk factors for colonization

The overall prevalence of *S. aureus* on the commercially available meat samples in Iowa was found to be 16.4% (27/165). Of the 27 *S. aureus* isolates, two (7.4%) were found to be methicillin-resistant, therefore the overall MRSA prevalence was 2/165 (1.2%).

S. aureus was most common in turkey (7/36, 19.4%), pork (10/55 samples, 18.2%) and chicken (8/45 samples, 17.8%). A much lower prevalence was found in beef (2/29 samples, 6.9%). MRSA was found only in pork (2/55, 3.6% of pork samples).

Type of meat, and geographic location of stores were not predictive of *S. aureus* colonization on meat (see Table 2).

Molecular typing

Isolates were characterized using *spa* typing and the RidomStaphType software. Thirteen different *spa* types were found. Of the 27 different isolates, seven (25.9%) were *spa* type t034, which has been associated with ST398 (Tables 1 and 2) [4,24]. Four isolates (14.8%) were t337, previously associated with ST9, another "swine-associated" type [25,26]. An additional 3 isolates (11.1%) were t002, and 2 isolates (7.4%) were t008, which are common human types; another two isolates (7.4%) were identified as t526. The remaining singletons

Table 1 Meat type, *spa* type, and antibiotic resistance profile for isolated *S. aureus*. Antibiotic displayed signifies resistance to the respective antibiotic.

Isolate	Meat type	<i>spa</i> type	MIC antibiotic resistance profiles
B1	Beef	t548	P
B2	Beef	t714	—
C1	Chicken	t008	—
C2	Chicken	t444	P,C
C3	Chicken	t002	—
C4	Chicken	t012	P
C5	Chicken	§	E ⁱ
C6	Chicken	t1684	P
C7	Chicken	t002	T
C8	Chicken	t1491	—
P1	Pork	t337	P,T
P2	Pork	t034	P,O,T,C
P3	Pork	t337	P,T,E
P4	Pork	t3446	P,T,E,C
P5	Pork	t526	P,T
P6	Pork	t337	P,T
P7	Pork	t742	P,T,E,C
P8	Pork	t002	P,T,E,C, Q/D ⁱ
P9 ^{***}	Pork	t008	P,O,T,C
P10	Pork	t526	P,T
T1	Turkey	t337	P,T
T2	Turkey	t034	P,T
T3	Turkey	t034	P,T
T4	Turkey	t034	P,T
T5	Turkey	t034	P,T
T6	Turkey	t034	P,T
T7	Turkey	t034	P,T

P = penicillin; O = oxacillin; T = tetracycline; E = erythromycin; C = clindamycin; Q/D = Quinupristin-Dalfopristin; — = susceptible; ⁱ = intermediately resistant.

^{***} PVL positive.

§ Non-typeable.

Table 2 Statistical characteristics of *S. aureus* on retail available meat in Iowa. Two-sided *p*-values are presented.

	Relative risk	<i>p</i> -Value	95% confidence interval
Meat type			
Chicken	1.164	0.183	(0.975,1.389)
Turkey	1.156	0.172	(0.957,1.396)
Pork	1.181	0.121	(0.994,1.403)
Beef	Ref	—	—
Location			
Urban	0.802	0.670	(0.335,1.915)
Rural	Ref	—	—

are: t012; t444; t548; t714; t742; t1491; t1684; and t3446. Both oxacillin resistant isolates were tested for *SCCmec* type. The MRSA strains were t008/ST8 and t034/ST398, with the t008 isolate determined to be *SCCmec* type IV and the t034 isolate determined to be *SCCmec* type V. The t008 MRSA isolate was additionally found to carry the *pvl* gene. All other *S. aureus* isolates were negative for *pvl*.

Antibiotic susceptibility testing

Five of 27 isolates (18.5%) were susceptible to all antibiotics tested. 21 of 27 isolates (77.7%) were resistant to penicillin; 18/27(66.7%) were resistant to tetracycline. Six (22.2%) were resistant to clindamycin, 4 (14.8%) were resistant to erythromycin, and 2 (7.4%) were resistant to oxacillin. All isolates were susceptible to trimethoprim-sulfamethoxazole, gentamicin, levofloxacin, moxifloxacin, linezolid, daptomycin, vancomycin, and rifampin (see Table 1).

Statistical analysis

Statistical analysis using Fischer's exact test resulted in no significant differences observed. Type of meat was not significant using beef as the reference strain, with turkey, chicken, and pork yielding *p*-values of 0.183, 0.172, and 0.121 respectively. Comparison of geographic location using rural as a reference was also non-significant with a *p*-value of 0.670.

Discussion

The prevalence of MRSA in our retail meat samples (1.2%) was lower than a number of prior studies [11,12,14]. Several reasons could account for this difference. We used surface swabbing rather than destructive methods [11] or external surface rinsing [14], and only a single enrichment step with broth lacking antibiotics (in order to isolate both

methicillin susceptible *S. aureus* (MSSA) and MRSA). Therefore, it is possible we missed isolates which may have been amplified via a double enrichment step [11,12]. However, as previously noted [16], due to a lack in consensus culture methodologies in the current literature, attempts to compare prevalence rates between different studies is difficult, and identification of *S. aureus* on retail available meat should only indicate that it can be found frequently in different regions around the globe. Single enrichment may also act as a better representation of an individual's actual exposure to *S. aureus* during the handling of raw meat than some of the more destructive methodologies.

Similar to de Boer et al., we found a high prevalence of *S. aureus* in chicken and turkey, with a lower prevalence in beef [11]. However, in our study almost all isolates were sensitive to methicillin, and a lower percentage were ST398-associated *spa* types than were found in The Netherlands, yet higher than observed in Canada [15,16]. This suggests that additional studies should examine poultry samples in addition to pork, even though swine have been the primary animal investigated to date on farms. However, like Pu et al. [14], pork was the sole source of MRSA in our study. Whether this reflects differences in processing procedures, differing levels of MRSA prevalence on-farm, or other factors remains to be determined. It is important to note that MLST types for MSSA isolates were extrapolated from their respective *spa* types. Strommenger et al. have previously demonstrated high concordance between MLST and *spa* typing [27].

Interestingly, although ST398 has been primarily associated with swine, both this and a previous study [11] found ST398-associated strains in a high proportion of turkey samples. In addition to t034-ST398, we found a second "swine-associated" strain, t337-ST9 [28,29] in our turkey samples. ST398 prevalence in swine has been shown to be 49%, 24.9% and 12.8% in the US [30], Canada [3] and The Netherlands [31] respectively. ST398 has also

previously been identified in chickens [7] but to our knowledge, no studies have systematically examined colonization with *S. aureus* in live turkeys. Furthermore, ST398 has also been reported in a clinical isolate from a turkey [32] in addition to samples of turkey meat. This suggests that turkeys, in addition to pigs, are a possible reservoir for both the ST398 and ST9 strains in the United States.

ST398 is also commonly associated with tetracycline resistance, which is supported by the observation of 100% of t034 isolates displaying the tetracycline-resistant phenotype. Furthermore, 100% of t337 isolates were also observed to be tetracycline-resistant. While little is known about the origin of these isolates, tetracycline use on farms may select for these resistant isolates.

The finding of t002 and t008 in multiple food isolates is noteworthy. Both of these *spa* types are typically associated with humans, and could potentially have been introduced to the meat during meat processing or packing, subsequent to slaughter. t008/USA300 isolates have also been found previously in meat samples in the US and the Netherlands [12,14]; however, our sample was *pvl* positive, which has only been observed once prior to this study, by Pu et al.

t002 strains have previously been found in live animals [3]. A recent study demonstrated that humans transmitted ST5 (a type which includes t002) to poultry several decades ago, where it subsequently spread and is now endemic [33]. Therefore, post-slaughter human contamination of meat may not necessarily be responsible for the presence of these strains in food or animals. An alternative hypothesis suggests that these strains are endemic in various animal populations and the isolated sample persisted from the farm. Additional genomic research should assist in determining the origin of these isolates.

Conclusion

While the role of retail available meat as a vehicle for *S. aureus* and MRSA infections is still undetermined, the presence of potentially virulent strains of MRSA such as *pvl* positive t008 indicate that this mode of transmission needs further evaluation and cannot be discarded.

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Competing interests

The authors of this article have no conflicts of interest to disclose.

Ethical approval

None required.

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