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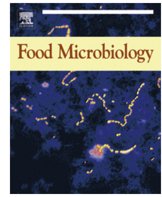

Prevalence and molecular characterization of *Staphylococcus aureus* in commercially available meat over a one-year period in Iowa, USA

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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 *mec*A 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 *mec*A. 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 10 g meat ranged from 2 to 517 (median: 8 CFU per 10 g 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.

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1. Introduction

Staphylococcus aureus, a commensal and opportunistic pathogen, can cause a wide spectrum of diseases, from skin and soft tissue infections (SSTIs) to severe life-threatening invasive disease such as infective endocarditis (Smith et al., 2013; Furuya and Lowy, 2006). 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) (Graham et al., 2006; Wertheim et al., 2005). It is estimated that 1.5% of the U.S. population is colonized with methicillin−resistant *S. aureus* (MRSA) (Graham et al., 2006). These colonized persons are carriers of *S. aureus*, and colonization is a risk factor for developing subsequent symptomatic infections (Furuya and Lowy, 2006; Graham et al., 2006). *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, including those resistant to the antibiotic oxacillin (Lowy, 1998; Wisplinghoff et al., 2004; Jackson et al., 2013).

Since their first recognition among Dutch and French swine

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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 (Smith et al., 2009; Khanna et al., 2008; Huijsdens et al., 2006). Antibiotic-resistant *S. aureus* has been found in several species of meat-producing animals, including pigs (Smith et al., 2009; Khanna et al., 2008; Huijsdens et al., 2006), chickens (Persoons et al., 2009), and cattle (Fessler et al., 2010). Therefore, meat products have the potential to act as vehicles 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 (Kitai et al., 2005; Kwon et al., 2006) to 11.9% in the Netherlands (de Boer et al., 2009), with intermediate prevalence found in other studies (Pu et al., 2009; van Loo et al., 2007). 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 (de Boer et al., 2009; Hanson et al., 2011; Waters et al., 2011; Bhargava et al., 2011), turkey (de Boer et al., 2009; Waters et al., 2011; Bhargava et al., 2011), pork (Jackson et al., 2013; Pu et al., 2009; Hanson et al., 2011; Waters et al., 2011; O'Brien et al., 2012; Weese et al., 2010; Buyukcangaz et al., 2013; Molla et al., 2012a), and beef (Jackson et al., 2013; de Boer et al., 2009; Pu et al., 2009; Bhargava et al., 2011).

Interestingly, CC398 strains typically lack known enterotoxin genes (Kadlec and Schwarz, 2009; Monecke et al., 2007); 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 (Alam et al., 2015). 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 (Bhat et al., 2009; Krziwanek et al., 2009); 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*.

2. Methods

2.1. 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. ABF 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.

2.2. 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 s. A 50 mL aliquot from the peptone wash was then added to 50 mL of Baird Parker broth (2× concentration) with tellurite enrichment (Sigma products-Sigma-Aldrich, St. Louis, MO) in a 250 mL sterile screw cap jar. After 24 h of incubation at 35 °C, 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 h at 35 °C 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 −80 °C.

2.3. 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 (CLSI, 2012). 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 (Magiorakos et al., 2012).

2.4. 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 (Bosgelmez-Tinaz et al., 2006; Lina et al., 1999). Using previously described methods (Shopsin et al., 1999; Koreen et al., 2004) the Staphylococcus protein A (*spa*) gene was amplified using SpaF (5′-GAACAA-CGTAACGGCTT-CATCC-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

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