Food Control 65 (2016) 132–135

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Short communication

Contamination of post-harvest poultry products with multidrug resistant *Staphylococcus aureus* in Maryland-Washington DC metro area

Hironori Teramoto [a, 1], Serajus Salaheen [a, 1], Debabrata Biswas [a, b, *]

[a] Department of Animal and Avian Sciences, USA
[b] Center for Food Safety and Security Systems, University of Maryland, College Park, MD 201742, USA

ARTICLE INFO

Article history:
Received 12 November 2015
Received in revised form
13 January 2016
Accepted 15 January 2016
Available online 18 January 2016

Keywords:
Staphylococcus aureus
MRSA
Prevalence
Antibiotic resistance

ABSTRACT

We investigated the prevalence of *Staphylococcus aureus* and multidrug including methicillin-resistant *S. aureus* (MRSA) in poultry retail meat samples from the Maryland-Washington DC metro area. A total of 24 *S. aureus* isolates were recovered from 96 whole poultry carcass samples and the prevalence of *S. aureus* were 25.0%, 14.29%, and 33.3% in retail poultry meats collected from farmers markets, organic and conventional retail supermarkets, respectively. Both single and multi-drug resistance isolates were detected in 58.3% (7/12) isolates from conventional retail meat products but none from farmers markets or organic retail meat isolates. Conventional retail meat isolates were found to be resistant to both erythromycin (50.0%) and tetracycline (58.3%). We also detected an MRSA isolate harboring *mecA* gene in conventional retail meat which showed co-resistance towards erythromycin, tetracycline, and vancomycin.

1. Introduction

Staphylococcus aureus has historically been a serious human pathogen, and recent few decades it has become a more serious threatening agent due to acquisition of antibiotic resistance (Novick, 2008; Wilkinson, Muthaiyan, & Jayaswal, 2005). Methicillin-resistant *S. aureus* (MRSA) is so common in the United States (U.S.) and Tseng, Sanchez-Martinez, Arruda, and Liu (2011) reported that MRSA accounts for more than half of all soft-tissue and skin infections. The Centers for Disease Control and Prevention (CDC) estimates that invasive MRSA infections number more than 94,000 a year and caused more deaths in the U.S. than the Human immunodeficiency virus infection and *acquired immune deficiency syndrome* (HIV/*AIDS*) (Bancroft, 2007). In recent years, MRSA has appeared more and more in communities outside of the hospital settings and health care facilities. Several researchers found that Community-associated methicillin-resistant *S. aureus* (CA-MRSA) is a growing concern for emerging issue of a major public health concern worldwide (Gardam, 2000; Kennedy et al. 2008; Pu, Han, & Ge, 2009). Moreover, Staphylococcal Food Poisoning (SFP) is also a major concern in food safety and public health programs and possesses a threat to the food production and processing industry and human populations around the world (Le Loir, Baron, & Gautier, 2003). It has huge economic impacts in terms of medical expenses, food recalls, and reduced human productivity.

In Europe and Asia, it has been found that MRSA colonizes in many food animal species including poultry. According to the surveys in both in The Netherlands and Canada, prevalence of MRSA in farm animals are very high (20–40%) (de Neeling et al. 2007; Khanna, Friendship, Dewey, & Weese, 2008). Transmission of MRSA from farm animals to farmers and their families has also been documented in Europe (Voss, Loeffen, Bakker, Klaassen, & Wulf, 2005). Thus, concerns have been raised that MRSA strains of animal origin is a potential source to cross-contaminate the food and jeopardize the health of individuals handling meats and animal food products (Wulf & Voss, 2008). Recently, many U.S. researchers have also begun testing meat in commercial channels for methicillin sensitive *S. aureus* (MSSA) and MRSA (Abdalrahman, Stanley, Wells, & Fakhr, 2015.) but very few have focused on poultry

* Corresponding author. Department of Animal and Avian Sciences, and Center for Food Safety and Security Systems, University of Maryland, College Park, MD 20742, USA.
E-mail address: dbiswas@umd.edu (D. Biswas).
[1] Authors contributed equally.

http://dx.doi.org/10.1016/j.foodcont.2016.01.024
0956-7135/

meats and none of these studies have investigated the meat products available in the farmers markets or grown in small open access farms and processed in unregulated operations. Moreover, most of these studies were limited in geographical region, survey period, and sample types. In the present study, we aimed to investigate the contamination level and antibiotic resistance pattern of *S. aureus* among industrial, antibiotic-free organic/pasture grown and farmers markets poultry products from the Maryland-Washington DC metro area.

2. Materials and methods

2.1. Sample collection and processing

A total of 96 poultry whole carcass samples including 32 samples from 7 farmers markets, 28 samples from 3 organic retail supermarkets and 36 samples from 3 conventional retail supermarkets from the Maryland-Washington DC metro area were collected over a period of eight months starting from February to September of 2014.

Exterior surface of the packages were sanitized with paper towels soaked with 70% ethanol. The whole chicken carcasses were rinsed thoroughly with equal volume of 0.1% Buffered Peptone Water (Himedia) for 5 min and 10 ml of rinse solution was mixed with the same volume of double-concentrated Trypticase soy broth (TSB, Himedia) with 10% defibrinated sheep blood. After 24 h of incubation at 37 °C, the enrichment broth was streaked in duplicate on Baird–Parker (BP) medium. Following 48 h of incubation, three presumptive *S. aureus* colonies per meat sample (black colonies surrounded by 2- to 5-mm clear zones) were transferred to fresh BP agar plates. These pure cultures were used for identification as well as isolates were stocked in TSB-broth and 20% glycerol for future studies.

2.2. Identification, confirmation and antibiotic resistance genes

For confirmation, isolates were subjected to PCR as previously described (Sasaki et al. 2010) with slight modification. List of PCR primers is shown in Table 1. In brief, 2 μl DNA (DNA extracted using Qiagen DNA extraction kit) was amplified in a final reaction volume of 10 μl containing 0.2 μM of *S. aureus* species-specific primers, 0.2 mM dNTP, 1x reaction buffer (with $MgCl_2$) and 1.25 units of *Taq* DNA polymerase. Thermocycler conditions were 95 °C for 5 min, followed by 30 cycles of 94 °C for 50 s, 55 °C for 30 s, and 72 °C for 30 s, and finally 72 °C for 7 min. PCR products were analyzed by 1.5% agarose gel electrophoresis. For determining the presence of antibiotic resistance genes, the isolates were subjected to multiplex (erythromycin and tetracycline separately) and singleplex (vancomycin and methicillin, separately) PCR as previously described (Clark, Cooksey, Hill, Swenson, & Tenover, 1993; Geha, Uhl, Gustaferro, & Persing, 1994; Strommenger, Kettlitz, Werner, & Witte, 2003).

2.3. Antimicrobial susceptibility testing

All the positive *S. aureus* isolates were tested for resistance against six antimicrobial agents. The antimicrobial agents used in this study are presented in Table 3. Agar dilution method was used according to guidelines established by Clinical and Laboratory Standard Institute (6). Ciprofloxacin and tetracycline were purchased from Alfa Aesar, Ward Hill, MA and the rest of the antibiotics were purchased from Sigma Chemical Co., St. Louis, MO. Agar dilution was carried out in Muller Hinton Agar (Himedia). *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used as reference strains in this study. The MIC breakpoint of each antimicrobial was determined according to the breakpoints suggested by the CLSI established guideline.

2.4. Statistical analysis

Data analysis was carried out using the Statistical Analysis System software (SAS, Institute Inc., Cary, NC, USA). The PROC FREQ statement was used to perform Chi-Square tests and the Cochran-Mantel-Haenszel (CMH) test was used to control for sample type.

3. Results and discussion

3.1. Contamination of poultry meat with S. aureus

A total of 24 *S. aureus* isolates were isolated from 96 chicken carcasses collected from farmers markets, organic and conventional retail supermarkets (Table 2). Among the 32 poultry meat samples from 7 farmers markets, 25.0% (8/32) samples were contaminated

Table 2
Prevalence of *S. aureus* in retail poultry meat from various sources.

Isolates	FM[a]	Org.M[b]	Con.M[c]	p-value[#]
S. aureus	25.0% (8/32)	14.29% (4/28)	33.3% (12/36)	0.22
MRSA/MSSA[d,e]	-/8	-/4	1/11	–

#Statistical analysis for significance of difference among various groups was carried out using Chi-Square test.
[a] Farmers Market Meat.
[b] Organic Retail Meat.
[c] Conventional Retail Meat.
[d] Methicillin Resistant *S. aureus*.
[e] Methicillin Susceptible *S. aureus*.

Table 1
Primer used in the multiplex PCR assay for Campylobacter identification.

Primer	Primer sequence (5′–3′)	Size (bp)	Reference
Sau-F	TCGCTTGCTATGATTGTGG	359	Sasaki et al. (2010)
Sau-R	GCCAATGTTCTACCATAGC		
ermA-F	AAGCGGTAAACCCCTCTG A	190	Strommenger et al. (2003)
ermA-R	TTCGCAAATCCCTTCTCAAC		
ermC-F	AATCGTCAATTCCTGCATGT	299	Strommenger et al. (2003)
ermC-R	TAATCGTGGAATACGGGTTTG		
tetK-F	GTAGCGACAATAGGTAATAGT	360	Strommenger et al. (2003)
tetK-R	GTAGTGACAATAAACCTCCTA		
tetM-F	AGTGGAGCGATTACAGAA	158	Strommenger et al. (2003)
tetM-R	CATATGTCCTGGCGTGTCTA		
vanA-F	CATGAATAGAATAAAAGTTGCAATA	1030	Clark et al. (1993)
vanA-R	CCCCTTTAACGCTAATACGATCAA		
mecA-F	GTAGAAATGACTGAACGTCCGATAA	310	Geha et al. (1994)
mecA-R	CCAATTCCACATTGTTTCGGTCTAA		