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Prevalence, molecular identification and determination of antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* in raw meat

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ABSTRACT

Objective: The aim of this study was to determine the prevalence, molecular identification, and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) in raw meats of retail sale in Balikesir, Türkiye.

Materials and Methods: A total of 250 raw meat samples (beef n=100, chicken n=100, and turkey n=50) were collected from various supermarkets. Mueller-Hinton Broth medium containing 6.5% NaCl was used for pre-enrichment and Baird Parker Agar (BPA) was used as a selective medium. Polymerase Chain Reaction technique was used to confirm the suspected colonies with the *nuc* gene for *S. aureus* and the *mec*A gene for MRSA. Kirby-Bauer standard disc diffusion method was applied for antibiotic susceptibility of MRSA.

Results: Of the 250 investigated raw meat samples, 21.2% were positive for *S. aureus*, which comprised 31% beef, 14% chicken, and 16 %turkey samples. Of the 250 investigated raw meat samples, 4% were positive for MRSA, which comprised 6% beef, 3% chicken, and 2% turkey samples. All MRSA isolates were found to be resistant to penicillin, sulfamethoxazole, trimethoprim, cefoxitin, and oxacillin, but they were susceptible to vancomycin.

Conclusion: In recent years, MRSA has been called a zoonotic pathogen that poses a serious risk for food safety and public health. Therefore, we believe that this study will shed light on new studies on the prevalence of MRSA in various animal-originated foods.

Keywords: *Antibiotic susceptibility, MRSA, Prevalence, Raw meat*

INTRODUCTION

Staphylococcus aureus, which is in the *Staphylococcaceae* family, is a bacterium that causes foodborne intoxications with hospital-acquired bacteremia (Tiemersma et al., 2004). Methicillin has been put into use with the resistance of penicillin to *S. aureus* species, most of which have beta-lactamase activity. However, it was reported that it was isolated from MRSA strains after a short time (Robinson and Enright, 2003). The *mec*A gene-encoded with penicillin-binding protein 2a (PBP2a) is mediated against methicillin resistance in staphylococci, penicillin-like methicillin, oxacillin, and all other beta-lactam antibiotics (Yasuda et al., 2000) and cause serious infections.

MRSA infections are grouped mainly as hospital-acquired MRSA (HA-MRSA), community-acquired MRSA (CA-MRSA), and livestock-acquired (LA-MRSA). The first report from livestock was published in 1975 with MRSA isolation from mastitis cows (Devriese and Hommez, 1975). However, reports on this subject have been more frequently started to be published since 2000. It was reported in 2007 that animals are MRSA reservoirs (Smith and Pearson, 2011) and there is mutual MRSA (spa-type T127 ST1) contamination between

cattle and humans (Juhasz-Kaszanyitzky et al., 2007). In two different cases in Denmark, MRSA isolates, isolated from cows and sheep, and from whole-genome sequences similar to humans on two different farms, revealed that this may be related to animal husbandry (Harrison et al., 2013; Petersen et al., 2013). In the USA, there are approximately 94.000 invasive infections and an estimated 18,650 deaths from MRSA annually (Klevens et al., 2007). On the other hand, in the European Union in 2010, it has been reported to cause illness more than 150.000 people (Köck et al., 2010).

In recent years, with the definition of MRSA in animals used in food production, the presence of MRSA in foods of animal origin has come to the agenda (Febler et al., 2011). Although direct contact with animals appears to be the most likely route of infection, it is reported that the role of MRSA as a food pathogen needs further investigation (Verkade and Kluytmans, 2014). The aim of this study was to investigate MRSA prevalence, molecular characterization, and antibiotic sensitivity in beef, chicken, and turkey meat.

MATERIALS and METHODS

Collection of samples

In this study, a total of 250 raw meat samples (100 beef, 100 chicken meat, and 50 turkey meat) were collected from the markets in Balikesir, Türkiye. The samples were transported to the laboratory under the cold chain (+4°C) and analyzed on the same day.

S. aureus and MRSA isolation and identification

Twenty-five g of each meat sample was weighed, and 225 mL of Mueller-Hinton Broth (Oxoid CM0405) containing 6.5% NaCl was added and homogenized in the stomacher (IUL) for 2 min. The homogenate was incubated at 35±2°C for 16-20 h (EFSA, 2009). At the end of the incubation, 0.5 ml was taken from the pre-enrichment medium and spread to Baird Parker Agar (Merck, Germany) medium and incubated at 35±2°C for 48h (TS 6582-1 EN ISO 6888-1: 2001). Then, Gram staining and coagulase test (Staphytect Plus; Oxoid-DR0850) was performed according to the manufacturer's instructions to the black suspect colonies that grow in petri dishes. As phenotypically Gram (+) and coagulase-positive isolates were incubated in Brain Heart Infusion Broth (BHI; Merck, Germany) medium at 35±2°C for 24 h. After, all isolates were stored at -80°C in a BHI broth medium containing 20% glycerol (20 w/v) until genotypic was identified.

Molecular characterization

DNA extraction was performed using a commercial kit (MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche, Germany) according to the manufacturer's instructions. The process is initial denaturation (94°C for 10 min) and 23 cycles in total; denaturation (94°C for 1 min), binding (51°C for 1 min), extension (72°C for 2 min) and final extension (72°C for 5 min). Primarily for the *nuc* gene; *nuc* 1 (5'-GCGATTGATGGTGATACGGTT-3'); *nuc* 2 (5'-AGCCAAGCCTTGACGAACTAAA GC-3') and for the mecA gene; *mec*A1 (5'-AAAATCGATGGTAAAGGTTGGC-3'); *mec*A2 (5'-AGTTCTGCAGTACCGGATTTGC-3') sequence was used (Maes et al., 2002). Electrophoresis was performed for 75 minutes at 90 V in agarose prepared in 1.5%. Samples of 279 bp lengths were considered positive for *S. aureus*, while 533 bp samples were considered MRSA positive.

Antibiotic susceptibility test

According to Kirby-Bauer's standard disc diffusion method (Baur et al., 1961), all isolates identified as MRSA were incubated in Trypticase Soy Agar (TSA; Biomerieux 43011) medium for 35±1°C 24 h. The antibiotic discs (Thermo Scientific Oxoid) used in the test were placed on the medium at 24 mm intervals. Antibiotics contained in discs; penicillin G 10 µg, gentamicin 10 µg, erythromycin 5 µg, ampicillin 10 µg, sulfamethoxazole-trimethoprim 25 µg, ciprofloxacin 5 µg, tetracycline 30 µg, chloramphenicol 30 µg, cefoxitin 30 µg, and oxacillin. Results were interpreted according to CLSI instructions (2012; 2014).

Reference Strains

In this study, reference strains of *S. aureus* (ATCC 25923) and MRSA (ATCC 43300 *mec*A positive and ATCC 33592 *mecA* negative standard strains) were obtained from Microbiologics Inc. (Saint Cloud, USA).

RESULTS

In this study, *S. aureus* was detected in 53 (21.2%) of 250 raw meat samples. These isolates were isolated from 31 beef, 14 chickens, and 8 turkey meat, respectively (Table 1). On the other hand, 10 isolates from 53 *S. aureus* isolates were evaluated as MRSA. Of these, 6 isolate beef, 3 isolate chickens and 1 isolate turkey meat were detected in raw meat samples (Table 1, Figure 1). In this study, 10 isolates

detected as MRSA were tested for sensitivity to 11 different antibiotics according to Kirby-Bauer's standard disc diffusion method. Antibiotic resistance levels of the isolates were evaluated according to CLSI (2012; 2014). As a result of antibiotic resistance tests, 1 isolate was found to be resistant to gentamicin, 2 isolates of erythromycin, 2 isolates of tetracycline, 1 isolates of ciprofloxacin and 2 isolates of chloramphenicol, all of them were resistant to ampicillin, penicillin, sulfamethoxazole-trimethoprim, cefoxitin and oxacillin. One of the isolates was found to be moderately sensitive to erythromycin and 1 isolate of found to be moderately sensitive to ciprofloxacin.

Table 1. Distribution of *S. aureus* and MRSA isolates in beef, chicken, and turkey meat

Samples	(n)	Positive samples of *S. aureus* (%)	Positive samples of MRSA (%)
Beef	100	31 (31)	6 (6)
Chicken	100	14 (14)	3 (3)
Turkey meat	50	8 (16)	1 (2)
Total	250	53 (21.2)	10 (4)

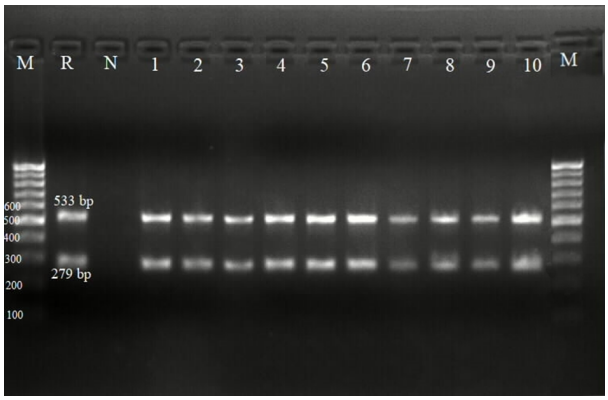

Figure 1. PCR image of strain and reference strains confirmed as MRSA according to the *mecA* gene (M: Marker, ATCC 43300: Positive Control Strain, 1: *mec*A positive 533 bp)

DISCUSSION

Staphylococcal intoxications, mainly caused by *S. aureus*, have an important place worldwide in foodborne intoxications. The fact that MRSA strains, which are thought to be mostly from hospital, have been detected in foods of animal origin in recent years brought serious studies on this issue. In this study, the prevalence, molecular characterization, and antibiotic susceptibility of MRSA were investigated in the samples of beef, chicken, and turkey meat collected from different markets. It was detected in 21.2% (53/250) of *S. aureus* and 4% (10/250) of MRSA in 250 meat samples. When looking at the distribution of meat types, *S. aureus* was found in 31% of the beef samples and MRSA in 6% (Table 1). Looking at the results of studies on raw beef in some countries; In the USA, 3.3% of 30 samples (Pu et al., 2009), in the Netherlands, 10.6% of 395 samples (de Boer et al., 2009), in Nigeria, 14.3% of 77 samples (Nanchi et al. 2014), 0.5% of 847 samples in Korea (Lim et al., 2010); They reported that in 23.3% of 30 samples (Costa et al., 2014) in Brazil, 380 beef samples detected 4.4% (Boost et al., 2013) MRSA. Pu et al. (2009) reported that *S. aureus*, which produces heat-resistant enterotoxins, plays an important role in food-borne intoxications and that the presence of MRSA in meats poses a potential infection threat to those working in the food preparation process. On the other hand, it has been reported that carrier people may play a role in the contamination of meats with MRSA. Bhargava et al. (2011) explained the low prevalence of *S. aureus* and MRSA in raw meat (beef, chicken, and turkey meat) samples in the United States by not using pork in the study. Pork is shown as the largest reservoir of MRSA. Pork was not used as a material in this study. On the other hand, it is reported that differences in percentage rates in a study are directly related to sample size (Jackson et al., 2013).

In this study, the prevalence of MRSA in chicken meat samples was 3% (Table 1). Kwon et al. (2006) detected MRSA in 0.2% of 340 chicken meat samples. Wang et al. (2014) detected MRSA in 2.3% of 264 samples. The results of this study are similar to those obtained by Kwon et al. (2006) and Wang et al. (2014). On the other hand, the results of the study on MRSA prevalence in chicken meat are as follows; Feßler et al. (2011), in 25 of 24 fresh chicken meat samples, in 21.1% of 19 chicken meat products; Karmi et al. (2013), 44% of 25 chicken carcass samples, 52% of 25 chicken piece meat; in 40% of 25 cooked chicken meal (luncheon) samples, 24% of 25 chicken sausage samples and 44% of 25 chicken burger samples, de Boer et al. (2009) 16% of 520 chicken meat samples; Boost et al. (2013) in 6.8% of 455 chicken meat samples and Costa et al. (2014) in 23.3% of 30 chicken meat samples. Considering the results of some similar studies given above, it is seen that our study is much higher than the results. The high prevalence of MRSA in some processed foods (hamburgers and sandwiches) reported that

beef and chicken meat used as raw materials may not be sufficiently cooked or may be due to cross contamination after cooking (Contreras et al., 2015). On the other hand, Kwon et al. (2006) reported that MRSA strains transmitted to humans through chicken meat may cause infections.

In this study, the prevalence of MRSA in turkey meat samples was 2% (Table 1). Feßler et al. (2011) found MRSA in 35.3% of 116 turkey meat samples and 50.0% of 22 fresh turkey meat samples and 52.4% of 21 turkey meat products. Monecke et al. (2013) reported that they detected MRSA in 21.2% of 80 turkeys clinically. Also, contaminated foods can also pose a health risk to food processors. Carcasses obtained from animals colonized with MRSA can be contaminated during slaughter (Lozano et al., 2009). It is reported that the differences between the results obtained, and the previous studies may result from differences in sampling plans and MRSA detection procedures (Feßler et al., 2011). It is seen that the number of studies on the presence of MRSA in turkey meat is limited.

In this study, 4 of the 10 MRSA isolates resisted at least three antibiotics. The transfer of antibiotic resistance from animals to humans can occur by removing antibiotic residues in foods or resistant food-borne pathogens (Pesavento et al., 2007). Accordingly, the detection of MRSA in animals is considered as one of the most important zoonotic origin pathogens in the recently published reports. For eradication of infections caused by MRSA, it is important to identify the vectors causing the contamination and the origin of the agent and their spread throughout the farm and food chain. Again, determining the potential impact of MRSA strains on public health is of great importance. This study does not represent a country-wide prevalence since it was conducted in a province of our country. However, it is important in terms of shedding light on more comprehensive studies on this subject.

CONCLUSION

As a result, in order to reduce and prevent the prevalence of MRSA in meats, the meats to be used in production must first be obtained from healthy animals, and the necessary hygiene rules must be followed during slaughtering, transport and cooling stages. Food Safety Management Systems such as HACCP (Hazard Analysis and Critical Control Points), GMP (Good Manufactured Practice), and GHP (Good Hygiene Practice) should be fully implemented in meat and meat products with the principle of "farm to fork". Controlled and conscious antibiotics should be used in animals used in meat production. On the other hand, this study is the first study on the prevalence of MRSA in raw meats in our country, according to the literature surveys we conducted. Therefore, we believe that this study will shed light on new studies on MRSA prevalence and antibiotic resistance in various animal origin foods in different regions of our country.

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