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Isolation of Methicillin Resistant (MR) Staphylococci from Chicken Meat Samples

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Abstract: The aim of this study was to determine the presence of methicillin resistant staphylococci (MRS) in retail raw chicken meat samples sold in Hatay. The antimicrobial susceptibility of the isolates was tested for 13 different antimicrobials by disc diffusion method and investigated for resistance genes encoding methicillin (*mec*A), tetracycline (*tet*M, *tet*K), penicillin (*bla*Z), macrolide (*erm*A, *erm*C), lincosamide (*lnu*A) and aminoglycoside [*aac(6')/aph(2'')*, *aph(3')-IIIa*, *ant(4')-Ia*] resistance via the use of polymerase chain reaction (PCR). In addition, the presence of staphylococcal enterotoxin (SE) genes was also searched by PCR. Out of 50 collected chicken meat samples, 11 (22%) MRS was isolated and the following species were determined: *S. sciuri* (72.7%, 8/11) and *S. epidermidis* (27.3%, 3/11). While all isolates were resistant to oxacillin, penicillin and ampicillin, various rates of resistance were observed for tetracycline (8, 72.7%), clindamycin (3, 27.3%), trimethoprim-sulfamethoxazole (2, 18.2%), erythromycin (2, 18.2%) and rifampicin (1, 9.1%). All MRS harbored *mec*A gene together with *bla*Z. The *tet*M gene responsible for ribosomal protection was detected in all phenotypically tetracycline resistant isolates. *erm*C gene in erythromycin resistant isolates and *lnu*A gene in clindamycin resistant isolates were detected. None of the isolates was found to be positive for SE genes. The results of this study indicated that contamination of retail raw chicken meat samples with MRS poses a risk to public health due to transmission of these bacteria to humans. Additionally, this study also highlights the importance of monitoring antimicrobial resistant bacteria in animal originated foods.
Key words: *Chicken meat, Methicillin resistantce, Staphylococci.*

Tavuk Eti Örneklerinden Metisiline Dirençli (MR) Stafilokok İzolasyonu

Özet: Bu çalışmanın amacı Hatay'da satılan 50 perakende çiğ tavuk eti örneğinde metisiline dirençli stafilokok (MRS) varlığının araştırılması idi. İzolatların antimikrobiyal duyarlılıkları disk difüzyon yöntemiyle 13 farklı antimikrobiyal için test edildi ve metisilin (*mec*A), tetrasiklin (*tet*M, *tet*K), penisilin (*bla*Z), makrolid (*erm*A, *erm*C), linkozamid (*lnu*A) ve aminoglikozid [*aac(6')/aph(2''), aph(3')-IIIa, ant(4')-Ia*] direncine aracılık eden genler yönünden polimeraz zincir reaksiyonu (PCR) ile incelendi. Ayrıca, stafilokokal enterotoksin (SE) genlerinin varlığı da PCR ile araştırıldı. İncelenen 50 perakende çiğ tavuk eti örneğinin 11'inden (%22) MRS izole edildi ve bu izolatların 8'i (%72.7) *S. sciuri* ve 3'ü (%27.3) de *S. epidermidis* olarak identifiye edildi. Tüm izolatlar oksasilin, penisilin ve ampisiline dirençli iken tetrasiklin (8, %72.7), klindamisin (3, %27.3), trimetoprim-sülfametoksazol (2, %18.2), eritromisin (2, %18.2) ve rifampisine (1, %9.1) farklı direnç oranları gözlendi. Tüm MRS izolatları *bla*Z ile birlikte *mec*A genini taşıdı. Fenotipik olarak tetrasiklin dirençli izolatlarda ribozomal korumadan sorumlu *tet*M geni tespit edildi. Eritromisine dirençli izolatlarda *erm*C geni ve klindamisine dirençli izolatlarda *lnu*A geni saptandı. İzolatlar SE genlerinin varlığı açısından negatif bulundu. Bu çalışmanın sonuçları, perakende çiğ tavuk örneklerinde MRS'nin varlığının, antibiyotik direncinin insanlara olası yayılması nedeniyle halk sağlığı açısından önemli olabileceğini göstermiştir. Ayrıca bu çalışma, hayvansal kaynaklı gıdalarda antimikrobiyal dirençli bakterilerin izlenmesinin önemini de vurgulamaktadır.
Anahtar Kelimeler: *Tavuk eti, Metisilin direnci, Stafilokok.*

Introduction

To date, 73 species and 30 subspecies have been described within the *Staphylococcus* genus (https://www.bacterio.net/genus/staphylococcus). Staphylococci are ubiquitous agents that reside on the skin and mucous membranes of humans and animals (Tong et al., 2015). Based on coagulase activity, the staphylococci are classified into 2 major groups as coagulase-positive staphylococci (CoPS) and coagulase-negative staphylococci (CoNS) (França et al., 2021). Although CoPS, especially *S. aureus*, are recognized as important pathogens in humans and animals, CoNS have increasingly gained importance due to the involvement of these agents in a wide spectrum of opportunistic infections in humans and animals (Becker et al., 2014). Over two decades, the emergence and spread of antimicrobial resistance have become one of the biggest threats to public health due to making treatment of bacterial infections very limited (Prestinaci et al. 2015). The members of the genus

Staphylococcus are able to acquire and transfer resistance genes through mobile genetic elements (MGEs) (Bitrus et al., 2018). The methicillin resistance in staphylococci is mostly related to *mec*A gene carried by a MGE named the staphylococcal cassette chromosome *mec* (SCC*mec*) (Rolo et al., 2017). The methicillin-resistance among CoNS has been proposed to be an important issue due to being a potential reservoir of SCC*mec* elements for *S. aureus* (Hanssen and Ericson Sollid, 2006).

It has been demonstrated that raw chicken meat is important food vehicles of foodborne pathogens and antimicrobial resistant bacteria (Martins et al., 2013; Pehlivanlar Önen et al., 2015; Yılmaz et al., 2016; Özdemir and Keyvan, 2016; Can et al., 2017; Dekker et al., 2019). It has been reported that the mechanisms responsible for antimicrobial resistance observed in CoNS strains are the same as those observed in *S. aureus* strains (Osman et al., 2017). On the other hand, the increasing rates of methicillin resistance observed among CoNS strains have led to re-evaluation of these agents for food safety perspective (Chajęcka-Wierzchowska et al., 2015). Therefore, it was aimed to evaluate the occurence of MRS in the chicken retail meat marketed in Hatay.

Materials and Method

Collection of samples: A total of 50 retail chicken meat samples were randomly collected from various butcher shops and markets in Hatay province and its towns in one visit, between May and June in 2021. The samples were transported to the laboratory in well-sealed containers at +4°C immediately after collection.

Isolation and identification of staphylococci: The samples were enriched in Tryptic Soy Broth (TSB) supplemented with 10% NaCl at 37 °C for 24 h (Bhargava and Zhang, 2014). Then, 10 µl of inoculum was plated on Mannitol Salt agar (MSA) supplemented with cefoxitin (4 µg/ml) and incubated aerobically at 37 °C for 24 h (Smith and Kahlmeter, 2005). The colonies that were indicative of staphylocci from each sample were taken and passaged to Blood Agar that was supplemented with 5% defibrinated sheep blood for further biochemical tests. The isolates were identified as previously described phenotypic (Gram staining, catalase, coagulase test) (Koneman et al., 1997), and genotypic methods via PCR amplification using primers targeting 16S rRNA (Staphylococcus genus specific), *nuc* (*S. aureus* species specific), *sod*A (*S. chromogenes*, *S. haemolyticus* species specific), *gap* (*S. arlettae*, *S. sciuri*, *S. saprophyticus*, *S. simulans* species specific) and *rpo*B (*S. epidermidis*, *S. xylosus*, *S.fluerettii* species specific) (Kim et al., 2001; Strommenger et al., 2003; Preethirani et al., 2015).

Antimicrobial susceptibility testing: The MR-CoNS were tested for susceptibility to penicillin (10 U), oxacillin (1 µg), tetracycline (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), rifampicin (5 µg), clindamycin (2 µg), sulfamethoxazole-trimethoprim (23.75/1.25 µg) and erythromycin (15 µg), using disk diffusion method according to Clinical and Laboratory Standards Institute criteria (CLSI, 2021). *S. aureus* ATCC 29213 was used as quality control.

Detection of antimicrobial resistance genes: Antimicrobial resistance genes related to penicillin (*blaZ*), methicillin (*mec*A), aminoglycoside [*aac(6')-Ie-aph(2")-Ia*, *ant(4')-Ia* and *aph(3')-IIIa*], tetracycline (*tet*M, *tet*K), macrolide (*erm*A, *ermC*) and clindamycin (*lnu*A) were investigated as previously reported by Olsen et al. (2006), Choi et al. (2003), Strommenger et al. (2003), and Lina et al. (1999) using PCR assay.

SCC*mec* typing: A multiplex PCR strategy for SCC*mec* types was used as previouly described by Kondo et al. (2007).

Detection of SE genes: Presence of five classical (*sea*, *seb*, *sec*, *sed* and *see*) and 12 newly (*seg*, *seh*, *sei*, *selj*, *selk*, *sell*, *selm*, *seln*, *selo*, *selp*, *selq* and *selr*) identified SE genes in the isolates were investigated using a series of mPCR (Omoe et al. 2013).

Results

Isolation and identification results: Out of 50 chicken meat samples, 11 (22%) MRCoNS were isolated. Two species was identified in 11 isolates including eight *S. sciuri* and 3 *S. epidermidis* (Figure 1).

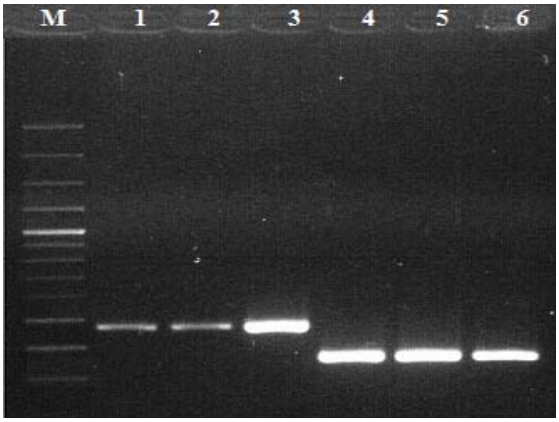

Figure 1. Agarose gel electrophoresis of *S. sciuri* and *S. epidermidis* isolates. Lane M: 100 bp molecular marker. Lane 1-3: *S. sciuri* (354 bp), Lane 4-6: *S. epidermidis* (466 bp).

Antimicrobial susceptibility testing: Zone diameter breakpoints for MR-CoNS and distribution

Table 1. Percentage of susceptibility, intermediacy or resistance for MR-CoNS isolates (n=11).

Antimicrobials	Disc content	Interpretation of zone diameters (mm)			No of MR-CoNS isolates		
		S≥	I	R≤	S (%)	I (%)	R (%)
Penicillin	10 U	29≥	-	≤28	-	-	11 (100)
Oxacillin	1 µg	18≥	-	≤17	-	-	11 (100)
Tetracycline	30 µg	19≥	15-18	≤14	8 (72.7)	-	3 (27.3)
Chloramphenicol	30 µg	18≥	13-17	≤12	11 (100)	-	-
Ciprofloxacin	5 µg	21≥	16-20	≤15	11 (100)	-	-
Gentamicin	10 µg	15≥	13-14	≤12	9 (81.8)	-	2 (18.2)
Rifampicin	5 µg	20≥	17-19	≤16	9 (81.8)	1 (9.1)	1 (9.1)
Clindamycin	2 µg	21≥	15-20	≤14	5 (45.5)	2 (18.2)	4 (36.4)
Sulfamethoxazole-trimethoprim	23.75/1.25 µg	16≥	11-15	≤10	8 (72.7)	-	3 (27.3)
Erythromycin	15 µg	23≥	14-22	≤13	9 (81.8)	-	2 (18.2)

Table 2. Antimicrobial resistance phenotype, genotype and SCC*mec* type determined among MR-CoNS.

Species ID	SCC*mec* type	Resistance phenotype**	Resistance genotype
S. epidermidis 2	IV	OXA, P, TE	*mec*A, *bla*Z, *tet*M
S. epidermidis 24	NT*	OXA, P, TE, DA, RA	*mec*A, *bla*Z, *tet*M, *lnu*A
S. epidermidis 26	NT	OXA, P, TE, DA	*mec*A, *bla*Z, *tet*M, *lnu*A
S. sciuri 8	NT	OXA, P, TE, DA, SXT	*mec*A, *bla*Z, *tet*M, *lnu*A
S. sciuri 23	NT	OXA, P, TE, E, SXT	*mec*A, *bla*Z, *tet*M, *erm*C
S. sciuri 25	III	OXA, P	*mec*A, *bla*Z, *tet*M
S. sciuri 28	NT	OXA, P, TE	*mec*A, *bla*Z, *tet*M
S. sciuri 31	V	OXA, P, TE	*mec*A, *bla*Z, *tet*M
S. sciuri 37	NT	OXA, P	*mec*A, *bla*Z
S. sciuri 48	V	OXA, P, TE, E	*mec*A, *bla*Z, *tet*M
S. sciuri 49	NT	OXA, P	*mec*A, *bla*Z

*Non-typeable, **OXA: oxacillin, P: penicillin, AM: ampicillin, TE: tetracycline, DA: clindamycin, RA: rifampycin, E: erythromycin, SXT: sulfamethoxazole/trimethoprim.

of zone diameters were given in Table 1. As seen in Table 2, all isolates showed resistance to penicillin, oxacillin and ampicillin. Various rates of resistance to tetracycline (8, 72.7%), clindamycin (3, 27.3%), trimethoprim-sulfamethoxazole (2, 18.2%), erythromycin (2, 18.2%) and rifampicin (1, 9.1%) were observed.

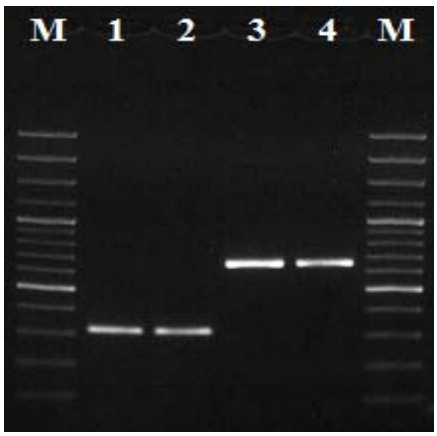

Figure 2. Agarose gel electrophoresis of *bla*Z and *mec*A genes. Lane M: 100 bp molecular marker. Lane 1-2: *mec*A (314 bp) positive isolates, Lane 3-4: *bla*Z positive isolates (675 bp).

PCR detection of antimicrobial resistance and SE genes: *tet*M, *bla*Z and *lnu*A were detected in all tetracycline resistant-, penicillin resistant- and clindamycin resistant-isolates, respectively (Figure 2). Among erythromycin isolates, only one isolate had *erm*C gene (Table 1). None of the isolates had SE genes.

SCC*mec* typing: SCC*mec* types were determined in only four isolates (Table 1). Seven isolates were not assigned to any SCC*mec* type. These seven isolates consisted of two *S. epidermidis* and five *S. hyicus*. While three of the untypeable isolates carried only one of the *mec* complex genes, two of them carried at least one *ccr* complex gene; no amplification was observed in two isolates.

Discussion

Contaminated chicken meat is an important source of transmission of both zoonotic and antimicrobial resistant bacteria (Abebe et al., 2020; EFSA, 2021). The clinical and epidemiologic importance of CoNS, especially MR-CoNS have been increasingly documented in recent years, and therefore, role as potential foodborne pathogens, should not be overlooked due to public health perspective (Chajecka-Wierzchowsk et al., 2014; Bhargava and Zhang, 2014; Osman et al., 2016; Lee et al., 2020). Of the 50 samples inspected, 11 MR-CoNS belonging to two species were isolated (Table 1), of which *S. sciuri* (8/11) was the most dominant species. Huber et al. (2011) detected MR-CoNS in 48.2% of chicken carcasses and found *S. lentus* as

the predominant species. Bhargava and Zhang (2014) isolated MR-CoNS from 7.9% (6/79) of chicken meat samples and identified these isolates as *S. fleuretti* (3), *S. sciuri* (2) and *S. vitilinus* (1). Igbinosa et al. (2016) examined 30 raw chicken meat samples and isolated 10 *Staphylococcus* spp., consisting of 6 *S. aureus*, 3 *S. epidermidis* and 1 *S. xylosus*, found that all isolates were positive for *mec*A gene. Zogg et al (2016) investigated the occurence of MRSA in 80 raw chicken samples (36 from Swiss and 44 imported from Germany), and isolated MRSA from only six (7.5%) of imported raw chicken meat samples. Possible contamination source of chicken meat with *S. sciuri* or *S. epidermidis* could have originated from broilers (Aslantaş, 2020) and humans (Vanderhaeghen et al., 2012). In previous studies conducted in Turkey, the presence of *S. aureus* was searched in raw chicken meat samples, but found that none of the isolates carried the *mec*A gene (Özdemir and Keyvan, 2016; Can et al. 2017).

Many researchers have emphasized that the widespread dissemination of *mec*A gene among CoNS species posed to emergence of novel methicillin-resistant staphylococcal lineages through acquisition and insertion of the SCC*mec* element into the chromosome of susceptible strains (Zhang et al., 2009; Tsubakishita et al., 2010; Zong et al., 2011; Otto, 2013). Although horizontal transfer of *mec*A gene is well understood, the transfer mechanism has not been fully elucidated (Tsubakishita et al., 2010). On the other hand, Hanssen and Ericson Sollid (2006) suggested that CoNSs combine the *mec* and *ccr* genes acquired from an unknown source and then transferred these genes to *S. aureus* (Hanssen and Ericson Sollid, 2006). While SCC*mec* type I, II, and III are predominantly found in hospital-acquired MRSA (HA-MRSA), SCC*mec* type IV and V are primarily detected in community acquired MRSA (Ünal, 2006). On the other hand, while SCC*mec* I, II, and III are rarely reported in MR-CoNS, SCC*mec* type IV and V are mostly detected in MR-CoNS. In previous studies, dominance of SCCmec type IV and V in MR-CoNS was reported, as observed in this study (Zhang et al., 2009; Fessler et al., 2010; Bhargava and Zhang, 2014; Silva et al., 2014). In this study, only one of the *S. epidermidis* isolates carried SCC*mec* type IV. It was particularly reported that *S. epidermidis* might be a reservoir of SCC*mec* type IV, and play an important role in horizontal transfer of SCC*mec* IVa to *S. aureus* (Wisplinghoff et al., 2003). Morever, Barbier et al. (2010) and Jamaluddin et al. (2008) stressed that SCC*mec* IVa was prevalent in *S. aureus* and *S. epidermidis* isolates, and these two species carried SCC*mec* elements with a nucleotide identity of 98–99% to each other. In this study, most of the isolates had untypeable SCC*mec*. Similar observation have also been reported by many researchers (Aslantaş, 2020; Nemeghaire et al. 2014; Bhargava and Zhang 2012), and regarded as indication of diversity of SCC*mec* types in MR-CoNS. This has been attributed to the development and use of currently available SCC*mec* typing schemes based on *S. aureus*. When these SCC*mec* typing schemes are applied, MR-CoNS couldn't have been typed due to two reason: (i) amplification failure of genetic variants of *ccr* and *mec* complex genes, and (ii) un-elucidated machanisms and/or vectors other than SCC that allow the transfer of MR among staphylococci (Hanssen et al., 2004; Zhang et al., 2009).

Multi-drug resistance (MDR) among MR-CoNS has been well-documented in other studies (Nemeghaire et al., 2014; Chajecka-Wierzchowska et al., 2015; Aslantaş, 2020). In this study, 45.5% (5/11) of the isolates exhibited the MDR phenotype defined as resistant to antimicrobials belonging to three or more classes. In a recent study, Aslantaş (2020) reported high prevalence of MDR methicillin resistant *S. sciuri* isolates among broiler flocks. The isolates revealed high level of resistance to tetracycline (72.7%) in concordance with previous studies (Chajecka-Bhargava and Zhang, 2012; Wierzchowska et al., 2015; Aslantaş, 2020). Tetracycline resistance is usually mediated by genes encoding efflux proteins (*tet*K, *tet*L) or ribosomal protection proteins (*tet*M, *tet*O and *tet*S) (Huys et al., 2004). Of these genes, only *tet*M gene was detected in all tetracycline resistant isolates. This result is in agreement with the findings of Bhargava and Zhang (2012) and Chajecka-Wierzchowska et al (2015). Bhargava and Zhang (2012) detected *tet*M in 64.3% of the investigated strains, Chajecka-Wierzchowska et al. (2015) found *tet*M as the most frequent tetracycline resistance gene.

None of the isolates carried any SE genes. The absence or low prevalence of SE genes were previously reported among MR-CoNS. Nemeghaire et al. (2014) detected the *sed* gene in only one of the 71 MR-*S. sciuri* isolates. On the other hand, Aslantaş (2020) did not detect any SE genes among 63 MR-*S. sciuri* isolates.

To sum up, the results of the study showed that (i) retail raw chicken meat samples were contaminated with relatively high levels of MR-CoNS, (ii) MR *S. sciuri* and *S. epidermidis* isolates had a high rate of tetracycline resistance, associated with *tet*M gene and (iii) none of the isolates carried SE genes. Therefore, continuous surveillance is needed to monitor antimicrobial resistant bacteria and necessary precautions should be implemented to control transmission of antimicrobial resistant bacteria to humans.

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