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Screening of *Staphylococcus aureus* isolates for *mecA* and *mecC* genes carriage

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Summary: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen which causes hospital infections and different systemic infections. MRSA is clarified by the acquisition of the *mecA* gene, which is located on the staphylococcal cassette chromosome *mec* (SCC*mec*). *MecC* gene, is a *mecA* gene homologue showing ~ 69 DNA similarities to the *mecA* gene. *mecC* gene positive *S. aureus* was firstly detected in livestock. Because of that, livestock constitute a reservoir of *S. aureus* harboring *mecC* gene in terms of spread to human. The aim of this study was to determine the *mecC* gene in 177 *S. aureus* isolates collected from two distinct veterinary laboratories (Burdur and Aydin) between 2011 and 2013. Microorganisms were firstly defined by colony morphology, Gram stain, catalase test, mannitol fermentation and coagulase tests. After that, further identification process was performed by MALDI-TOF MS (Bruker Daltonics, Billerica, MA, USA). Antibiotic susceptibility testing by Kirby-Bauer disk diffusion method with oxacillin (1 µg) and cefoxitin (30 µg) was performed according to the CLSI standards. Inhibition zone diameters for cefoxitin ≤19 mm and oxacillin ≤10 mm were considered as resistant to methicillin strains. The investigation of *mecA* and *mecC* genes were performed by conventional PCR method. Forty five (25.4%) *S. aureus* isolates were resistant to oxacillin and cefoxitin by disk diffusion method and also *mecA* genes were detected in all 45 MRSA isolates by conventional PCR method. *MecC* gene was not detected with conventional PCR in any of the 177 *S. aureus* isolates. Although a *mecC* gene positive *S. aureus* isolate has not been detected in our study, it is important to continue the surveillance studies to follow the changes of *mecC* gene in livestock over time.

Keywords: Livestock animals, *mecC* gene, *Staphylococcus aureus.*

Staphylococcus aureus izolatlarının *mecA* ve *mecC* genleri taşıyıcılığı açısından taranması

Özet: Metisilin-dirençli *Staphylococcus aureus* (MRSA) hastane enfeksiyonları ve farklı sistemik enfeksiyonlara yol açan önemli bir patojendir. MRSA oluşumu stafilokok kaset kromozom *mec* (SCC*mec*) üzerinde yer alan *mecA* geni edinimi ile oluşur. *MecC* geni, *mecA* genine ~69 DNA benzerlik gösteren bir *mecA* geni homoloğudur. *MecC* geni pozitif *S. aureus* ilk olarak çiftlik hayvanlarında tespit edilmiştir. Bu nedenle çiftlik hayvanları *mecC* geni barındıran *S. aureus*'un insanlara yayılması açısından rezervuar konumunda bulunmaktadır. Bu çalışmanın amacı, 2011 ve 2013 yılları arasında iki ayrı veteriner laboratuvarından (Burdur ve Aydın) toplanmış olan 177 *S. aureus* izolatında *mecC* geni varlığını saptamaktır. Mikroorganizmalar öncelikle koloni morfolojisi, Gram boyama, katalaz testi, mannitol fermantasyonu ve koagülaz testleri ile tanımlandı. Daha sonra ileri tanımlama işlemi MALDI-TOF MS (Bruker Daltonics, Billerica, MA, ABD) ile yapıldı. Antibiyotik duyarlılık testi oksasilin (1 µg) ve sefoksitin (30 µg) için Kirby-Bauer disk difüzyon yöntemi ile CLSI standartlarına göre yapıldı. İnhibisyon zon çapı sefoksitin için ≤19 mm ve oksasilin için ≤10 mm olan suşlar metisiline dirençli olarak değerlendirildi. *MecA* ve *mecC* geni araştırması konvansiyonel PCR yöntemi ile yapıldı. Kırk beş (%25.4) *S. aureus* izolatı disk difüzyon yöntemi ile oksasilin ve sefoksitine dirençli idi ve *mecA* geni 45 MRSA izolatının hepsinde konvansiyonel PCR yöntemiyle tespit edildi. Konvansiyonel PCR ile 177 *S. aureus* izolatının hiçbirinde *mecC* geni tespit edilmedi. Çalışmamızda *mecC* geni pozitif *S. aureus* izolatı tespit edilmemiş olmasına rağmen çitlik hayvanlarında zamanla oluşabilecek *mecC* geni değişikliklerini takip ederek sürveyans çalışmalarına devam etmek önemlidir.

Anahtar sözcükler: Çiftlik hayvanları, *mecC* geni, *Staphylococcus aureus.*

Introduction

Staphylococcus aureus causes skin and soft tissue infections, pneumonia, meningitis, endocarditis, and osteomyelitis. The occurrence of methicillin-resistant *S. aureus* (MRSA) is clarified by the acquisition of the *mecA* gene, which is located on the staphylococcal cassette chromosome *mec* (SCC*mec*) (1). Since 2006, MRSA was firstly detected in livestock like chickens,

horses, sheep, goats, calves and dairy cattle, livestock constitute a reservoir of MRSA in terms of spread to human accordingly (7).

In 2007, a new *S. aureus* strain harboring *mecA* gene homologue, *mecC* (formerly $mecA_{LGA251}$), was found as an isolate from a bulk tank milk sample in Southwest England which was phenotypically MRSA (i.e., resistant to oxacillin and cefoxitin) (4). Subsequently, MRSA carrying *mecC* gene have been isolated from humans, ruminants, pets, and other animals such as rats, seals, and guinea pigs (8).

The objective of the current study was to investigate the presence of *mecC*-containing *S. aureus* strains isolated from livestock samples that were claimed to be a zoonotic reservoir and a source of transmission to human.

Materials and Methods

In this study, a total of 177 *S. aureus* isolates were collected from two distinct veterinary laboratories (Burdur and Aydin) in Turkey between 2011 and 2013. Bacterial strains were identified firstly by colony morphology, Gram stain, catalase test, mannitol fermentation and coagulase test. After that, further identification process was performed by MALDI-TOF MS (Bruker Daltonics, Billerica, MA, USA). In vitro determination of methicillin resistance in strains was performed by using 1 µg oxacillin and 30 µg cefoxitin disks in accordance with Clinical and Laboratory Standards Institute standards (CLSI) (2).

Bacterial DNAs were prepared by boiling method. Suspension prepared with bacteria boiled in 300 µL of dH2O for 5 min and subsequently centrifuged for 5 min at 13 000 rpm. The investigation of *mecA* and *mecC* genes were performed by conventional PCR method using *mecA* and *mecC* genes primers (*mecA*-P1-5'-TCCAGATTACAACTTCACCAGG-3' and *mecA*-P2-5'-CCACTTCATATCTTGTAACG-3' [162 pb]; *mecC*-P1-5'-GAAAAAAAGGCTTAGAACGCCTC-3' and *mecC*-P2-5'-GAAGATCTTTTCCGTTTTCAGC-3' [138 bp]) in the investigation of Stegger et al. (10). Amplification was performed with the following program: 15 min at 94°C, followed by 30 cycles of 30 s at 94°C, 1 min at 59°C, and 1 min at 72°C, with a final 10 min elongation step at 72°C. 5 µl of PCR product was conducted in gel electrophoresis (%1.5 agarose, 1x TBE, 100V) with using molecular standard (K180-250 UL, Amresco, USA). The gel was stained with ethidium bromide and visualized with UV (Gel Doc 2000, BIO-RAD, USA). *S. aureus* NCTC 10442 (*mecA* gene positive), *S. aureus* N315 (*mecA* gene positive), *S. aureus* ATCC 29213 (*mecA* gene negative), and *mecC* gene positive *S. aureus* (kindly provided by Prof. Anders Rhod Larsen [Statens Serum Institut, Denmark]) were used in the study as reference strains.

Results

All 177 isolates included the study were isolated from mastitis samples in bovine (n=94), sheep (n=45), and goat (n=38). Forty five (25.4%) *S. aureus* isolate were resistant to oxacillin and cefoxitin by disk diffusion method and also *mecA* gene were detected all 45 MRSA isolates by conventional PCR method. All isolates were tested for presence of *mecC* gene by conventional PCR analysis, but *mecC* gene was not found in the *S. aureus* isolates.

Discussion and Conclusion

Methicillin-resistant *S. aureus* is a major health problem in both hospital and community. Diagnosis and detection of MRSA in clinical microbiology laboratory is essential for the choice of proper treatment for patients. In many laboratory detection of MRSA was maintained by the disk diffusion method using oxacillin and/or cefoxitin. Demonstration of *mecA* gene by PCR is known to be as gold standard method (4, 5). But *mecC* gene harboring *S. aureus* cannot be detected by *mecA* gene specific PCR (6). Due to inadequacies of phenotypic methods to the detection *mecC* gene, DNA-based methods are used to determine the *mecC* gene (1).

The origin of the *mecC* gene is not understood sufficiently. The links between humans and livestock have been supported, strongly suggesting the occurrence of cross-transmission of *mecC* isolates between these two populations (3). Since *mecC* was first described from bulk tank milk sample in southwest England, *mecC* gene have been identified in various samples at 13 European countries in 14 different domestic and wild animal species (5). *MecC* gene positive *S. aureus* isolates have not only been detected in animal species but also have been detected in humans as less frequently. Peterson et al. reported that *mecC* constituted 1.5%, with an increasing frequency reaching 1.9% and 2.8% in 2010 and 2011, respectively in Denmark (7). In Germany, 1604 (collected in 2004 to 2005) and 1603 (collected in 2010 to 2011) MRSA isolates were analyzed and found one isolate from each sampling period harbored *mecC* gene (9). In Switzerland, the presence of the *mecC* gene was investigated in 80 MRSA isolate. None was positive for *mecC* gene, suggesting that it was rare in the patient population of that region (1).

To the best of our knowledge, we present the first study investigation of *mecC* gene in *S. aureus* isolates collected from livestock in Turkey. A *mecC* gene positive *S. aureus* isolate has not been detected in our study, but *mecC* gene positive isolates represent a

potential public health problem, and highlight the need for surveillance program and monitoring of animal and environmental reservoirs for the presence and evaluation of *mecC* gene carrying *S. aureus* strains.

References

1. **Basset P, Prod'hom G, Senn L, et al.** (2013): *Very low prevalence of meticillin-resistant Staphylococcus aureus carrying the mecC gene in western Switzerland*. J Hosp Infect, **83**, 257-259.
2. **Clinical and Laboratory Standards Institute** (2012): *Performance standards for antimicrobial susceptibility testing; 22nd informational supplement*. CLSI M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA.
3. **Figueiredo AM, Ferreira FA** (2014): *The multifaceted resources and microevolution of the successful human and animal pathogen methicillin-resistant Staphylococcus aureus*. Mem Inst Oswaldo Cruz, **109**, 265-278.
4. **García-Álvarez L, Holden MT, Lindsay H, et al.** (2011): *Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study*. Lancet Infect Dis, **11**, 595-603.
5. **Paterson GK, Harrison EM, Holmes MA** (2014): *The emergence of mecC methicillin-resistant Staphylococcus aureus*. Trends Microbiol, **22**, 42-47.
6. **Paterson GK, Morgan FJ, Harrison EM, et al.** (2014): *Prevalence and characterization of human mecC methicillin-resistant Staphylococcus aureus isolates in England*. J Antimicrob Chemother, **69**, 907-910.
7. **Petersen A, Stegger M, Heltberg O, et al.** (2013): *Epidemiology of methicillin-resistant Staphylococcus aureus carrying the novel mecC gene in Denmark corroborates a zoonotic reservoir with transmission to humans.* Clin Microbiol Infect, **19**, 16-22.
8. **Porrero MC, Valverde A, Fernández-Llario P, et al.** (2014): *Staphylococcus aureus* carrying *mecC gene in animals and urban wastewater*. *Spain*. Emerg Infect Dis, **20**, 899-901.
9. **Schaumburg F, Köck R, Mellmann A, et al.** (2012): *Population dynamics among methicillin-resistant Staphylococcus aureus isolates in Germany during a 6-year period.* J Clin Microbiol, **50**, 3186-3192.
10. **Stegger M, Andersen PS, Kearns A, et al.** (2012): *Rapid detection, differentiation and typing of methicillin-resistant Staphylococcus aureus harbouring either mecA or the new mecA homologue mec*A_{LGA251}. Clin Microbiol Infect, **18**, 395-400.

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