

Determination of Methicillin, Vancomycin, Erythromycin and Clindamycin Susceptibilities in *Staphylococcus aureus* Strains Isolated from Cats' Oral and Nasal Swab Samples

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ABSTRACT

The current study aims to investigate the prevalence and antibiotic susceptibilities including methicillin resistance of *S. aureus* in cats' oral and nasal swabs and to assess their potential role in the epidemiology for antibiotic resistance. *Staphylococcus aureus* is a common commensal bacterium found in the skin, nasal microbiota, mucose membranes and respiratory tracts of humans and animals especially dogs and cats, respectively. This trend of increasing pet ownership has raised concerns about the potential for companion animals to spread zoonotic infections, including *S. aureus*. A total of 12 (%25) *S. aureus* isolates were obtained and identified as bright zoned black colonies on RPF-BP agar from 48 nasal and oral swabs collected from cats. According to the evaluation of methicillin susceptibility using the disk diffusion test following the EUCAST method, 2 *S. aureus* isolates were found to be methicillin-resistant. All *S. aureus* strains isolated were determined to be susceptible to vancomycin. In the double-disk diffusion test conducted to determine susceptibility to erythromycin and clindamycin, one (1) of the methicillin-resistant *S. aureus* isolates also exhibited resistance to both erythromycin and clindamycin. In the PCR test, among the 2 methicillin-resistant *S. aureus* isolates, 2 contained the *mecC* gene, while *mecA* gene was not found. Considering that these animals are pets, it suggests that they could transmit MRSA bacteria to their owners or other individuals through various routes such as biting, licking, and close contact. These results are of significant importance from a public health perspective, especially One Health perspective.

INTRODUCTION

Staphylococcus aureus is a prevalent commensal bacterium found on the skin and within the nasal microbiota, mucous membranes, and respiratory tracts of both humans and animals, particularly dogs and cats (Mourabit et al., 2020; Abdullahi et al., 2022; Afhsar et al., 2023; Das et al., 2023). Although these bacteria typically coexist without causing harm, they can become opportunistic pathogens, especially in individuals with compromised immune systems or when they breach other body sites. This capacity to shift from harmless commensals to pathogenic forms underscores the necessity of monitoring *S. aureus* in both human and veterinary health contexts due to its potential as a zoonotic agent (Abraham et al., 2007; Loeffler and Lloyd, 2010;

Algammal et al., 2020; Abdullahi et al., 2022; Das et al., 2023). Its resilience on hands and surfaces further establishes it as a significant opportunistic pathogen, particularly in immunocompromised populations. Infections associated with this bacterium range from food poisoning and skin infections to respiratory tract infections and unique clotting disorders (Abraham et al., 2007; Abdullahi et al., 2022; Das et al., 2023). Its ability to endure outside the host enhances its risk of causing infections in vulnerable groups (Abdullahi et al., 2022; Das et al., 2023).

Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) present critical challenges in both clinical and community environments. Community-acquired MRSA (CA-MRSA)

has emerged as a prominent public health concern, leading to infections that require immediate attention (Abdel-Moein and Samir, 2012). MRSA is particularly recognized as a leading antibiotic-resistant pathogen, resulting in severe and often difficult-to-treat infections. The rise of these resistant strains emphasizes the urgent need for effective infection control measures and the development of new therapeutic strategies in healthcare settings. VRSA, in particular, poses a serious threat, as vancomycin is typically considered a last-resort antibiotic for MRSA infections. The emergence of VRSA complicates treatment options and raises alarms regarding potential widespread resistance. This scenario underscores the necessity for ongoing surveillance, judicious antibiotic usage, and the pursuit of alternative treatment options to manage infections caused by these resistant strains (Das et al., 2023).

Companion animals, such as dogs and cats, play a significant role in enhancing the emotional and social well-being of their owners. In Türkiye, pet ownership has grown increasingly common, particularly in larger cities, where having pets-especially cats and dogs-has become more socially accepted and is viewed as a status symbol (Abdullahi et al., 2022; Das et al., 2023). This rising trend in pet ownership raises concerns about the potential transmission of zoonotic infections, including *S. aureus*. MRSA has emerged as a notable issue within veterinary medicine, as pets can carry MRSA strains from humans, leading to a marked increase in reported cases in veterinary hospitals over the past decade. Research indicates that MRSA clones in pets often mirror those found in humans, especially strains associated with hospital settings. Furthermore, MRSA can be transmitted between pets and their owners, and the close interactions in domestic environments facilitate the spread of these antibiotic-resistant bacteria. While MRSA and VRSA have been extensively studied in human populations, research focusing on the prevalence of these pathogens in domestic cats and their human counterparts remains limited, particularly in Bangladesh, where detailed investigations into zoonotic strains of *S. aureus* and their resistance genes have not yet been conducted (Abraham et al., 2007; Loeffler and Lloyd, 2010; Algammal et al., 2020; Abdullahi et al., 2022; Das et al., 2023).

Clindamycin, part of the lincosamide class of antimicrobial agents, along with macrolides and streptogramin B, is often used to treat various bacterial infections. These antibiotics inhibit bacterial protein synthesis and can exhibit resistance through two main mechanisms: active efflux, mediated by the *msrA* gene, which pumps out the antibiotic, and modification of the ribosomal target site, encoded by *erm* genes. Resistance can manifest as either constitutive, permanently expressed against all MLSB antibiotics, or inducible, expressed only in the presence of an inducing agent, such as erythromycin (Rich et al., 2005). Understanding these resistance mechanisms is vital for developing effective treatment approaches and addressing antibiotic resistance in clinical settings.

In veterinary medicine, clindamycin is commonly employed to treat various infections, including those affecting the skin, respiratory tract, and oral cavity, as well as infections caused by anaerobic bacteria. It is particularly valuable for treating staphylococcal infections and is often the preferred treatment option when MRSA is identified. Its efficacy against resistant strains makes it a crucial

choice in managing bacterial infections in animals (Rich et al., 2005).

However, some staphylococcal strains may exhibit an inducible form of resistance to clindamycin. These strains may appear susceptible in standard antimicrobial susceptibility tests, yet resistance can develop during treatment, potentially leading to therapeutic failure. This phenomenon highlights the importance of careful monitoring and consideration of resistance patterns when prescribing clindamycin for staphylococcal infections (Faires et al., 2009).

The current study aims to investigate the prevalence and antibiotic susceptibilities, including methicillin resistance of *S. aureus* in cats' oral and nasal swabs, as well as to evaluate their potential role in the epidemiology of antibiotic resistance.

MATERIALS AND METHODS

Sampling

A total of 48 oral and nasal swab samples were examined and evaluated from cats, which were collected and sent by veterinary clinic veterinarians from veterinary clinics in Balıkesir and İzmir for microbiological examination. The swabs were sent to the laboratory by veterinary clinic veterinarians under cold chain conditions in transport medium for microbiological examination. Upon arrival at the laboratory, the swabs were immediately processed for isolation analysis.

Isolation ve identification

Nasal and oral swabs of cats were inoculated onto 5% sheep blood agar (Merck, Germany), MacConkey agar (Merck, Germany), and Rabbit Plasma Fibrinogen-Baired-Parker (RPF-BP) agar (Oxoid, UK). The agars were incubated at 37°C for 24 hours. Bright zoned black colonies on the RPF-BP agar were identified as *S. aureus* (Baired-Parker, 1962; Göçmen et al., 2020). Colonies identified as *S. aureus* were preserved in bead bacterial storage tubes at -20°C for antibiotic susceptibility testing.

Antibiotic susceptibility tests

S. aureus isolates preserved in bead bacterial storage tubes at -20°C were inoculated into nutrient broth and incubated at 37°C. The *S. aureus* isolates that grew in the nutrient broth were then subcultured onto RPF-BP agar (Oxoid, Merck) for purity control. After purification, the isolates underwent antibiotic susceptibility testing using the disk diffusion method according to EUCAST standards. (Bauer et al., 1966; EUCAST, 2017a; 2017b).

S. aureus isolates were initially diluted according to the McFarland 0.5 standard. Antibiotic susceptibility testing for methicillin and vancomycin was performed on Mueller-Hinton agar (Merck, Germany). Methicillin and vancomycin resistance profiles of *S. aureus* isolates were investigated and evaluated phenotypically according to EUCAST standard and Hallabjaiy et al., 2014, respectively. Methicillin resistance was investigated by disc diffusion method using cefoxitin (30 µg disk, Oxoid, UK). Vancomycin resistance was investigated by disc diffusion test, 30 µg vancomycin discs according to Hallabjaiy et al., 2014 (Oxoid, UK) (Hallabjaiy et al., 2014; EUCAST, 2017a; 2017b).

S. aureus strains with <22 mm zone diameter was recorded as Methicillin-resistant according to EUCAST procedure and Vancomycin-resistance was evaluated

according to Hallabjaiy et al., 2014 (Hallabjaiy et al., 2014; EUCAST, 2017a; 2017b).

For erythromycin and clindamycin, the double-disc diffusion D-test described by Rich et al., (2005) was performed. This test was performed using a 2 mg clindamycin disc (Oxoid, UK) and a 15 mg erythromycin disc (Oxoid, UK) on both blood agar and Mueller-Hinton agar (Merck, Germany) for comparative analysis (Rich et al.). Plates were incubated aerobically at 37°C for 18 h. After incubation, the presence of a flattened zone (D-shape) between the discs, where both antimicrobials have diffused, suggests that the organism exhibits inducible clindamycin resistance (Rich et al., 2005).

Table 1. Primer sequences, target genes and references for *mecA* and *mecC* genes

Primers	Sequences	Target genes	Base pairs	References
GMECAR-1	5'-ACTGCTATCCACCCTCAAAC-3'	<i>mecA</i>	163	Mehrotra et al. (2000)
GMECAR-2	5'-CTGGTGAAGTTGTAATCTGG-3'			
Primer-F	5' -GAA AAA AAG GCT TAG AAC GCC TC-3'	<i>mecC</i>	138	García-Alvarez et al. (2011)
Primer-R	5' GAA GAT CTT TTC CGT TTT CAG C-3			García-Garrote F. et al. (2014) Doğan et al. (2016)

The PCR mixture for the *mecA* and *mecC* genes was formulated to a total volume of 50 µl for each gene, which included 30 µl of Taq polymerase Master Mix (Ampliqon, Denmark), 0.4 µl of the forward primer, 0.4 µl of the reverse primer, 17.2 µl of PCR-grade water (DNase and RNase free), and 2 µl of DNA (Mehrotra et al., 2000; García-Alvarez et al., 2011; García-Garrote et al., 2014; Doğan et al., 2016).

The amplification protocol for the *mecA* gene commenced with an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles consisting of denaturation at 94°C for 2 minutes, annealing at 57°C for 2 minutes, and extension at 72°C for 1 minute. The procedure concluded with a final extension step at 72°C for 7 minutes (Mehrotra et al., 2000).

For the amplification of the *mecC* gene, the conditions included an initial denaturation at 94°C for 15 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 59°C for 1 minute, and extension at 72°C for 1 minute. A final elongation step was performed at 72°C for 10 minutes (Doğan et al., 2016).

The PCR products were analyzed by electrophoresis on a 1.5% agarose gel (Prona, USA) containing Novel Juice dye (Thermo Scientific, USA) and a DNA molecular weight marker (Gene Ruler 100 bp DNA Ladder Plus, Thermo Scientific, USA). Gel imaging was conducted using the EBOX CX5 TS EDGE system from Vilber.

Methicillin-resistant *S. aureus* NCTC 12493 (*mecA*) and methicillin-resistant *S. aureus* NCTC 13552 (*mecC*) and *E. coli* ATCC 25923 were utilized as reference strains in PCR as positive and negative controls, obtained from the Ministry of Health, General Directorate of Public Health, Microbiology Reference Laboratory.

RESULTS

Isolation ve identification results

A total of 12 (%25) *S. aureus* isolates were obtained and identified as bright zoned black colonies on RPF-BP agar from 48 nasal and oral swabs of cats.

Detection of *mecA* and *mecC* genes by PCR in isolated phenotypic methicillin resistant *S. aureus* strains

To assess methicillin resistance, DNA was extracted from *S. aureus* isolates using the GeneJET Genomic DNA Purification Kit (MAN0012663, protocol for isolating genomic DNA from gram-positive bacteria, Thermo, USA) along with a lysis buffer, following the manufacturer's guidelines. The methicillin resistance genes, *mecA* and *mecC*, were analyzed through PCR using previously established primers and amplification protocols (Table 1) (Mehrotra et al., 2000; García-Alvarez et al., 2011; García-Garrote et al., 2014; Doğan et al., 2016).

Antibiotic susceptibility tests results

According to the evaluation of methicillin susceptibility using the disc diffusion test following the EUCAST method, 2 *S. aureus* isolates were found to be methicillin-resistant.

All *S. aureus* strains isolated were determined to be susceptible to vancomycin. In the double-disk diffusion test conducted to determine susceptibility to erythromycin and clindamycin, one (1) of the methicillin-resistant *S. aureus* isolates also exhibited resistance to both erythromycin and clindamycin.

Results of *mecA* and *mecC* genesby PCR in isolated phenotypic methicillin resistant *S. aureus* strains

In the PCR test, in 2 methicillin-resistant *S. aureus* isolates the *mecC* gene were detected, while *mecA* gene was not found (Figure 1).

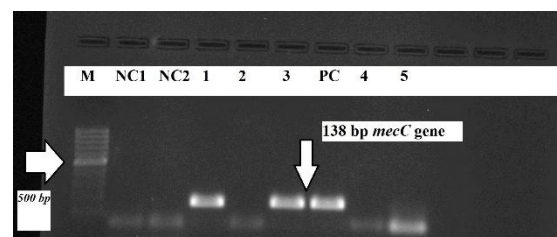


Figure 1. Results of *mecA* and *mecC* genes by PCR in isolated phenotypic methicillin resistant *S. aureus* strains (M: Marker, NC1: *E. coli* ATCC 25923, NC2: *S. aureus* NCTC 12493, PC: Positive control-, *S. aureus* NCTC 13552 (*mecC*) Line 1 and 3: *mecC* positive samples, Line 4-5: Negative samples)

DISCUSSION AND CONCLUSION

The natural oral flora of cats contains a diverse range of bacterial species, including opportunistic pathogens such as *Staphylococcus* spp., which can be transmitted to humans through bites. Among the species of

staphylococci, *S. aureus* and other coagulase-positive staphylococci (CoPS) can act as opportunistic pathogens (Razali et al., 2022).

Globally, dogs and cats account for the majority of animal bites. Infections can occur in 20-80% of cat bites, primarily due to the oral flora of the biting animal (Razali et al., 2022).

Despite this, there have not been sufficient studies investigating the oral and nasal carriage of staphylococci in cats.

Among staphylococci species, *S. aureus* is recognized as the most significant pathogen, responsible for a wide range of infections in both humans and animals. Additionally, *S. aureus* is a common resident of the skin and nostrils in humans and can be transmitted to pets through close contact, such as petting, kissing, or licking the skin. Consequently, *S. aureus* is significantly more prevalent in pets than in stray animals. (Razali et al., 2022). All of the sampled cats were pets (owned cat) in this study. Their regular contact with humans may explain the high isolation rates of *S. aureus* observed in our findings.

According to the results of previous studies, MRSA isolates, which were isolated from cats and dogs, are similar to hospital isolates, indicating that companion animals likely acquire MRSA from humans. It also highlights that both humans and animals are more frequently carriers (colonized) rather than showing symptoms of infection. This means they can serve as reservoirs for MRSA, allowing the bacteria to circulate within the household (Mustapha et al., 2014).

Ma et al., (2020) reported that they isolated 7 *S. aureus* strains from swab samples taken from the noses, oropharynxes, and perineum of cats, and did not detect MRSA. MRSA were found in two *S. aureus* strains in this study. Considering that these animals were pets (owned cat), it suggests that they could transmit MRSA bacteria to their owners or other individuals through various routes such as biting, licking, and close contact. These results are of significant importance from a public health perspective, especially one health perspective.

The risk factors for the carriage of *mecC* MRSA in humans include contact with animals and the presence of underlying health conditions (EFSA and CDC, 2022). According to EFSA and CDC (2022), the presence of the *mecC* gene in the MRSA strains isolated in this study can be considered an important finding from a public health perspective.

Rimbu et al., (2012) reported isolating 49 *S. aureus* strains from materials collected from 135 cats suffering from gingivitis, periodontitis, abscesses, glossitis, tonsillitis, and dental caries. They declared to found that 14.3% of these isolates exhibited resistance to clindamycin, while were not detected resistance to erythromycin.

In this study, resistance to clindamycin and erythromycin was detected in only one methicillin-resistant *S. aureus* isolate. Despite being a single isolate, its detection in MRSA is considered to be epidemiologically significant.

In conclusion, the detection of two MRSA strains in *S. aureus* isolates from the oral and nasal swabs of cats in this study was considered to be epidemiologically significant from a public health and One Health perspective. Furthermore, the detection of clindamycin and erythromycin resistance in one MRSA isolate was considered to be potentially epidemiologically significant. Furthermore, in *S. aureus* strains, including those from

feline cases, resistance to clindamycin and erythromycin is typically attributed to the presence of specific genes, such as *erm* (which confers MLSB resistance) or *mef*. These genes enable bacteria to modify their ribosomal targets, rendering these antibiotics ineffective. Resistance can pose significant challenges, particularly in the treatment of skin and soft tissue infections, where these antibiotics are commonly used (Rich et al., 2005). Therefore, monitoring resistance to methicillin, erythromycin, and clindamycin was considered important for effective treatment planning and public health, in alignment with One Health principles.

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Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: Concept: O.B., Design: O.B., Data Collection or Processing: O.B., Analysis and Interpretation: O.B., Literature Search: O.B., Writing: O.B.

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