In vitro antibiotic resistance of Staphylococci isolated from different animal species

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Abstract: The purpose of this study is to investigate resistance to antibiotics of Staphylococcus species isolated from various samples belonging to different animal species. Among 48 Staphylococcus spp. strains, Staphylococcus intermedius was the most common species, followed by S. aureus, S. epidermidis, S. hyicus, S. saprophyticus. In a total of 48 Staphylococcus strains, the highest antibiotic resistance was observed to oxacillin (79.17%), tetracycline (39.58%), and ampicillin and cefoxitin (31.25%). Of 48 Staphylococcus strains, 42 showed resistance to at least one antimicrobial agent, while 23 of the strains had multidrug resistance. Antimicrobial resistance to tetracycline and ampicillin, erythromycin, streptomycin was detected frequently in S. aureus, S. intermedius, and S. epidermidis, respectively. Resistance rates for ampicillin, cefoxitin, and enrofloxacin were determined as 66.67% in S. hyicus strains. S. saprophyticus was determined to show resistance to 13 antibiotics other than meropenem. The highest antibiotic resistance was determined in S. aureus, S. intermedius, S. epidermidis, and in 48 Staphylococcus strains to oxacillin. Consequently, this study revealed resistance to various antibiotics in Staphylococcus species. Additionally, the presence of high oxacillin resistance and multidrug resistance in the Staphylococcus strains revealed the importance of determination of antimicrobial susceptibility before treatment and for rational use of antibiotics.

Key words: Staphylococcus spp., domestic animals, antimicrobial resistance, oxacillin

1. Introduction

Staphylococci are a part of the normal bacterial flora of the urogenital and digestive system mucous membranes and skin of several mammalian animals and poultry [1,2,3]. Most of the 44 Staphylococcus species defined so far are present in animals [2,4]. Staphylococcus aureus (S. aureus) is accepted as the most prevalent pathogen species in both humans and animals, while other significant pathogen species in veterinary medicine were reported as S. hyicus and S. intermedius (reclassified as S. pseudointermedius) [4,5,6]. As it is difficult to phenotypically distinguish S. pseudointermedius, which was recently defined from S. delphini, it is believed that it would be better to use the term “S. intermedius group” for the species S. intermedius, S. delphini, and S. pseudointermedius [4,5,6,7,8]. Based on the coagulase test, Staphylococci used to be defined as coagulase-positive S. aureus and negative staphylococci. However, while S. intermedius, S. pseudointermedius, and S. delphini are positive in terms of coagulase and S. hyicus shows a variety, coagulase-negative staphylococci are also associated with various infections in humans and animals [6]. S. aureus may lead to suppurative infections such as mastitis, dermatitis, and botryomycosis in cows, sheep, goats, horses, pigs, cats, and dogs. S. intermedius causes several different suppurative infections such as endometritis and pyoderma in cats and dogs [1,2,9]. S. hyicus causes exudative epidermitis in pigs and cutaneous infections in horses and cows [3]. Due to reports that S. intermedius can be transmitted from animals to humans (especially from pets to owners), like S. aureus (zoonotic significance), S. intermedius also poses a serious public health risk [10,11,12].

Several different antibiotic drugs are used in the treatment of Staphylococcus spp. infections. However, usage of these drugs for shorter or longer than normal duration, and usage without antimicrobial susceptibility tests or microbiological analyses, had led to the emergence of antibiotic-resistant staphylococcus strains. Increased resistance to antibiotics in recent years, including multidrug resistance (MDR), will lead to untreatable Staphylococcus infections [13]. Some studies reveal antibiotic resistance in Staphylococcus species isolated from various animal species and humans [10,14,15,16,17,18,19]. It is known that especially the increase in methicillin-resistant Staphylococci creates a risk for animal health and public health [20,21,22].
Staphylococcal Cassette Chromosome mec (SCCmec) code the penicillin-binding protein 2a and lead to methicillin resistance by reducing the susceptibility of staphylococci to all β-lactam antibiotics [23,24,25]. In addition to the infections they cause in animals, methicillin-resistant staphylococci have become a significant risk due to their potential to be transmitted to people who are in close contact with animals, such as pet owners and veterinary clinic staff [20,26,27].

The purpose of this study is to determine resistance to antibiotics of Staphylococcus species isolated from samples belonging to different animal species brought to the Clinics of the Faculty of Veterinary Medicine at Ankara University with various complaints.

2. Materials and methods

2.1. Bacterial strains

Staphylococcus spp. strains were obtained from various samples of different animal species submitted to the Clinics of the Faculty of Veterinary Medicine at Ankara University. A total of 48 Staphylococcal strains, of which 15 strains were from dogs (31.25%), 12 from cats (25%), nine from cows (18.75%), four from horses (8.33%), three from chickens (6.25%), two from goats (4.17%), and one each from a calf, pigeon, and parrot (2.08%) were used in this study (Table 1).

2.2. Identification of Staphylococcus spp. strains

Staphylococcus spp. strains were identified based on colony characteristics, catalase production, Gram’s stain, coagulase reaction, pigment production, and Deoxyribonuclease (DNase) reaction on DNase agar, etc. [2,9].

2.3. Antimicrobial susceptibility testing

Antibiotic resistance of staphylococci was tested with the Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) (2008) [28]. The following antibiotic discs (Oxoid, Basingstoke, UK) were used: ampicillin (10µg), enrofloxacin (5µg), ciprofloxacin (5µg), meropenem (10µg), chloramphenicol (30µg), streptomycin (10µg), mupirocin (200µg), erythromycin (15µg), rifampicin (5µg), tetracycline (30µg), gentamicin (10µg), tobramycin (10µg), and cefoxitin (30 µg). For oxacillin (1µg) resistance, Mueller Hinton agar (Oxoid, CM0337, UK) onto which 2% NaCl was added was used. A Staphylococcus aureus ATCC® 25923 strain was used as the positive control. The inhibition zone diameters were assessed based on CLSI [28]. Among the tested antibiotics, strains that showed resistance to ≥3 antimicrobial agent classes were defined as multidrug-resistant (MDR) strains [29,30].

3. Results

3.1. Bacteriological identification

Staphylococcus spp. strains were isolated from samples belonging to different animal species, distributed among S. intermedius 21 (43.75%), S. aureus 15 (31.25%), S. epidermidis 8 (16.67%), S. hyicus 3 (6.25%), and S. saprophyticus 1 (12.08%) (Table 1).

3.2. Antimicrobial susceptibility testing

In a total of 48 Staphylococcus spp. strains, the highest antibiotic resistance was determined to oxacillin 38 (79.17%), tetracycline 19 (39.58%), and ampicillin and cefoxitin 15 (31.25%). Regarding the resistance rates (Table 2), 42 (87.5%) strains were resistant to at least one drug, and 47.92% of strains were multidrug-resistant. Resistance rates in S. aureus, S. intermedius, and S. epidermidis were variable, with 40% of S. aureus strains exhibiting resistance to cefoxitin and ampicillin, 20% of strains being resistant to erythromycin and enrofloxacin, and tetracycline and tobramycin; with 38.10% of S. intermedius strains being resistant to erythromycin, 19.05% of strains exhibiting resistance to ampicillin, tobramycin, gentamicin, and chloramphenicol; with 37.5% of S. epidermidis strains exhibiting resistance to streptomycin, 25% of strains being resistant to ampicillin and tetracycline, 12.5% being resistant to gentamicin, cefoxitin, chloramphenicol, erythromycin, mupirocin, and rifampicin. Resistance was not observed to rifampicin, ciprofloxacin, mupirocin, and meropenem in S. aureus strains, to mupirocin in S. intermedius, and to meropenem, tobramycin, ciprofloxacin, and enrofloxacin in S. epidermidis. Resistance rates of S. hyicus strains were determined to be 66.67% to ampicillin, cefoxitin, and enrofloxacin; 33.33% to tetracycline, erythromycin ciprofloxacin, and mupirocin. Resistance was not noted to meropenem, tobramycin, gentamicin, streptomycin, rifampicin, and chloramphenicol in S. hyicus strains. S. saprophyticus was determined to show resistance to 13 antibiotics other than meropenem. Also, antimicrobial resistance rates to oxacillin were noted in S. aureus, S. intermedius, S. epidermidis, S. hyicus, and S. saprophyticus (93.33%, 76.19%, 62.5%, 66.67%, and 100%, respectively).

4. Discussion

This study investigated the antibiotic resistance of Staphylococcus species isolated from samples belonging to different animal species with various clinical symptoms and the presence of methicillin-resistant Staphylococcus species with zoonotic potential. A large proportion of cat and dog samples were obtained from the skin and ear, whereas all parrot and horse samples were taken from the skin. In our study, S. intermedius was identified as the most prevalent species from samples of the skin and ear. This could be related to the number of samples collected from the skin and ear. The most prevalent species were reported as S. intermedius and S. aureusin dogs with otitis externa and pyoderma [15,31,32]. S. aureus was isolated from cow milk samples in our study. Some researchers detected the most prevalent species as S. aureus and S. epidermidis,
Table 1. Distribution of the *Staphylococcus* spp. strains based on the animal species and samples they were isolated from [n (%)].

<table>
<thead>
<tr>
<th>Animal Species</th>
<th>Dog</th>
<th>Cat</th>
<th>Cow</th>
<th>Horse</th>
<th>Chicken</th>
<th>Goat</th>
<th>Lamb</th>
<th>Pigeon</th>
<th>Parrot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>skin swab</td>
<td>ear swab</td>
<td>joint swab</td>
<td>nail wound swab</td>
<td>vaginal swab</td>
<td>skin swab</td>
<td>ear swab</td>
<td>nose swab</td>
<td>oral swab</td>
</tr>
<tr>
<td><em>S. intermedius</em></td>
<td>5 (23.81)</td>
<td>3 (14.28)</td>
<td>1 (4.76)</td>
<td>1 (4.76)</td>
<td>1 (4.76)</td>
<td>4 (19.05)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>1 (6.67)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (33.33)</td>
<td>5 (16.67)</td>
<td>2 (13.33)</td>
<td>1 (6.67)</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>-</td>
<td>3 (37.5)</td>
<td>-</td>
<td>-</td>
<td>3 (37.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>S. hyicus</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (33.33)</td>
</tr>
<tr>
<td><em>S. saprophyticus</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2. Antibiotic resistance in *Staphylococcus* species with different animal species origins [n (%)].

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>S. aureus (15)</th>
<th>S. intermedius (21)</th>
<th>S. epidermidis (8)</th>
<th>S. hyicus (3)</th>
<th>S. saprophyticus (1)</th>
<th>Total (48)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>OX</td>
<td>1 (6.67)</td>
<td>0</td>
<td>14 (93.33)</td>
<td>3 (14.28)</td>
<td>2 (9.52)</td>
<td>16 (76.19)</td>
</tr>
<tr>
<td>CFX</td>
<td>9 (60)</td>
<td>0</td>
<td>6 (40)</td>
<td>16 (76.19)</td>
<td>0</td>
<td>5 (23.81)</td>
</tr>
<tr>
<td>AMP</td>
<td>9 (60)</td>
<td>0</td>
<td>6 (40)</td>
<td>17 (80.95)</td>
<td>0</td>
<td>4 (19.05)</td>
</tr>
<tr>
<td>MER</td>
<td>15 (100)</td>
<td>0</td>
<td>0</td>
<td>19 (90.48)</td>
<td>0</td>
<td>2 (9.52)</td>
</tr>
<tr>
<td>TOB</td>
<td>11 (73.33)</td>
<td>2 (13.33)</td>
<td>2 (13.33)</td>
<td>17 (80.95)</td>
<td>0</td>
<td>4 (19.05)</td>
</tr>
<tr>
<td>CN</td>
<td>10 (66.67)</td>
<td>0</td>
<td>5 (33.33)</td>
<td>16 (76.19)</td>
<td>1 (4.76)</td>
<td>4 (19.05)</td>
</tr>
<tr>
<td>S</td>
<td>13 (86.67)</td>
<td>1 (6.67)</td>
<td>1 (6.67)</td>
<td>13 (61.90)</td>
<td>1 (4.76)</td>
<td>7 (33.34)</td>
</tr>
<tr>
<td>TET</td>
<td>7 (46.67)</td>
<td>0</td>
<td>8 (53.33)</td>
<td>14 (66.67)</td>
<td>0</td>
<td>7 (33.34)</td>
</tr>
<tr>
<td>E</td>
<td>11 (73.33)</td>
<td>1 (6.67)</td>
<td>3 (20)</td>
<td>13 (61.90)</td>
<td>0</td>
<td>8 (38.10)</td>
</tr>
<tr>
<td>CL</td>
<td>13 (86.67)</td>
<td>0</td>
<td>2 (13.33)</td>
<td>17 (80.95)</td>
<td>0</td>
<td>4 (19.05)</td>
</tr>
<tr>
<td>MUP</td>
<td>15 (100)</td>
<td>0</td>
<td>0</td>
<td>21 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ENR</td>
<td>12 (80)</td>
<td>0</td>
<td>3 (20)</td>
<td>16 (76.19)</td>
<td>2 (9.52)</td>
<td>3 (14.29)</td>
</tr>
<tr>
<td>CIP</td>
<td>13 (86.67)</td>
<td>2 (13.33)</td>
<td>0</td>
<td>19 (90.48)</td>
<td>0</td>
<td>2 (9.52)</td>
</tr>
<tr>
<td>RIF</td>
<td>15 (100)</td>
<td>0</td>
<td>0</td>
<td>19 (90.48)</td>
<td>0</td>
<td>2 (9.52)</td>
</tr>
</tbody>
</table>

"S. aureus, S. agalactiae, and S. hyicus from cow milk with bovine mastitis in Turkey and Poland, respectively [33,34]. S. intermedius (dog), S. aureus (cow), and S. epidermidis (goat) were isolated from the samples collected from wounds under the nails. Vanni et al. [15] also isolated S. intermedius (30%) from samples collected from under the nails of diseased and healthy dogs. S. saprophyticus was isolated from a cat urine sample, while it was determined to be susceptible to only meropenem among the antibiotics tested in our study. Some researchers have reported that S. pseudointermedius (20.1%), S. saprophyticus (2.9%), and S. aureus (2.5%) were isolated from urine samples of cats and dogs diagnosed with urinary system infection [22,35]."

"In the treatment of Staphylococcus spp. infections, long-term usage or repeated usage of both broad-spectrum and narrow-spectrum antibiotics may lead to the emergence of antimicrobial resistance, especially multidrug resistance. Considering the antibiotic resistance of all Staphylococcus spp. strains that we analyzed in our study, the resistance we determined to tobramycin (14.58%), streptomycin (25%), tetracycline (39.58%), and erythromycin (29.17%) were found to be higher than those reported by other researchers [14,33,36]. The resistance to ampicillin (31.25%), gentamicin (22.92%), rifampicin (8.33%), mupirocin (6.25%), chloramphenicol (16.67%), enrofloxacin (18.75%), and ciprofloxacin (8.33%) were lower [14,32,33,36]. It was determined that a large proportion of Staphylococcus spp. strains (87.5%) analyzed in the study showed resistance to at least one antimicrobial agent, while almost half of the strains (47.92%) had multidrug resistance. Some researchers have reported 36.4% and 35% multidrug resistance in Staphylococci isolated from different animal species [14,36]. Penna et al. [32,35] determined the ratio of strains resistant to at least one antimicrobial agent as 77.1% and 89% among the Staphylococcus strains they isolated from dogs with urinary system infection and otitis externa.

In the S. intermedius strains analyzed, we observed resistance to erythromycin (38.1%), streptomycin and tetracycline (33.34%), ampicillin (19.05%) and ciprofloxacin (9.52%) were similar to those in other studies [31,37,38,39,40]. However, the same strains’ resistance to gentamicin and chloramphenicol (19.05%), enrofloxacin (14.29%), and rifampicin (9.52%) were determined to be lower than those reported by other researchers [15,32,35,38]. Additionally, all of the S. intermedius strains susceptible to mupirocin concurred with the results of Penna et al. [32].

Resistance of S. aureus strains to ampicillin (40%), enrofloxacin (20%), chloramphenicol (13.33%), and streptomycin (6.67%) were lower than those reported by other researchers, however; higher resistance to tetracycline (53.33%), gentamicin (33.33%), erythromycin (20%). and tobramycin (13.33%) were observed [14,16,32,34,35,36,40,41]. All of the S. aureus strains susceptible to ciprofloxacin, rifampicin, mupirocin, and meropenem concurred with the results of some researchers [14,33,36,40]."

"In S. epidermidis strains, resistance to ampicillin (25%), erythromycin, mupirocin, chloramphenicol, and gentamicin (12.5%) were determined to be lower than those reported by some researchers [25,32,34,35,39]. In terms of resistance to streptomycin (37.5%), tetracycline (25%) and rifampicin (12.5%), our results were determined to be higher [14,16,25]. Similar to the results presented in this study, Kirkan et al. [33], no ciprofloxacin-resistant strain was determined among S. epidermidis strains. On the other hand, the finding that all analyzed S. epidermidis strains were susceptible to enrofloxacin and tobramycin was different from the results of some researchers [25,32,35]."

"No study was found of resistance to meropenem in S. intermedius, S. aureus, S. epidermidis, and all Staphylococcus spp. strains, and resistance to tobramycin S. intermedius strains. Therefore, the comparison of resistance to meropenem and tobramycin Staphylococcus spp. strains with other studies could not be made. However, analyzing resistance to meropenem and tobramycin in Staphylococcus strains is believed to provide a significant contribution to literature. Also, owing to a few strains of S. hyicus and S. saprophyticus, the antimicrobial resistance results of the strains have not been compared with other studies.

Several methods are being used in revealing methicillin resistance in staphylococci [17,42,43,44,45]. However, there is confusion in the determination of methicillin resistance in staphylococci due to heterogeneous resistance in coagulase-negative staphylococci and studying different Staphylococcus species in different geographical regions [17,45,46,47]. In the CLSI report in 2008, it was stated that using cefoxitin is more suitable in determining methicillin resistance [28]. Considering the comparison of resistance to the two antimicrobials, the resistance determined to oxacillin and cefoxitin was observed to agree in the S. hyicus (66.67%) and S. saprophyticus (100%) strains, whereas it showed differences in the S. aureus (93.33% / 40%), S. intermedius (76.19% / 23.81%), S. epidermidis (62.5% / 12.5%), and all Staphylococcus strains (79.17% / 31.25%). High oxacillin resistance in the S. intermedius, S. aureus, S. epidermidis, and all Staphylococcus strains was in agreement with the results of other researchers [25,33,38,48]. However, some researchers reported oxacillin resistance to be low in S. aureus strains [14,32,35,36,49]. Low cefoxitin resistance in the analyzed S. intermedius, S. epidermidis, and all Staphylococcus spp. strains was similar to the results in some studies [16,36,39]. Cefoxitin resistance observed
in approximately half (40%) of the S. aureus strains was in agreement with the findings of Couto et al. [36], whereas Kot et al. [16] reported encountering no cefoxitin-resistant S. aureus strains. A literature review did not reveal any study of cefoxitin resistance in S. saprophyticus strains, and this study can be considered as the first to determine cefoxitin resistance in a S. saprophyticus strain.

Consequently, this study indicated that Staphylococcus strains and Staphylococcus species originating from different animal species have high oxacillin resistance, but all Staphylococcus strains have high levels of meropenem resistance a common feature. It has also shown that almost half of the Staphylococcus strains have MDR. It was demonstrated that determining antimicrobial susceptibility and effective treatment based on this, especially in infections caused by Staphylococcus species with MDR, carries great significance in terms of both animal health and reduction of the risk of resistance to antibiotics. Additionally, this study also revealed the necessity of taking the necessary health precautions by keeping in mind the probability of transmission of MRGs with zoonotic potential to pet owners and healthcare employees in close contact with animals and the formation of control programs regarding the carriage of the factor.

Conflict of interest
The authors declare no conflict of interest.

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