Antimicrobial Susceptibility and Methicillin Resistance in *Staphylococcus pseudointermedius* and *Staphylococcus schleiferi* subsp. *coagulans* Isolated from Dogs with Pyoderma in Japan

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ABSTRACT. To understand species distribution, trends of antimicrobial susceptibility and prevalence of methicillin resistance in canine *staphylococci* in Japan, 190 coagulase-positive staphylococci (CoPS) were isolated from dogs with pyoderma in 2 Japanese veterinary referral hospitals. Using a multiplex polymerase chain reaction (M-PCR) method, two CoPS species were identified: 170 *Staphylococcus pseudointermedius* (89.5%) and 20 *S. schleiferi* subsp. *coagulans* isolates (10.5%). In these isolates, susceptibility to 7 antimicrobial agents was determined. Overall, the levels of susceptibility to cefalexin (CEX), amoxicillin/clavulanic acid (CVA/AMPC), minocycline (MINO), ofloxacin (OFLX), norfloxacin (NFLX), lincomycin (LCM) and clindamycin (CLDM) in *S. pseudointermedius* isolates were 38.2, 52.4, 34.7, 31.2, 34.1, 1.2 and 11.2%, respectively. In *S. schleiferi* subsp. *coagulans* isolates, 55% demonstrated susceptibility to CEX, 80% to CVA/AMPC, 70% to MINO, 45% to OFLX or NFLX and 30% to CLDM. None of *S. schleiferi* subsp. *coagulans* isolates was susceptible to LCM. To determine the prevalence of methicillin-resistant strains, we used a PCR method, which enabled detection of the fragment of *mecA* gene in 66.5% (113 of 170) in *S. pseudointermedius* and 30.0% (6 of 20) in *S. schleiferi* subsp. *coagulans* isolates. The frequencies of susceptibility to CEX, CVA/AMPC, OFLX, NFLX and CLDM were significantly lower in methicillin-resistant CoPS than in methicillin-susceptible CoPS isolates. These data suggest a high level of methicillin resistance in *staphylococci* isolated from dogs with pyoderma in Japan.

KEY WORDS: antimicrobial susceptibility, canine pyoderma, methicillin-resistant staphylococci, minocycline, *Staphylococcus* species.
nutrient agar plate (Nissui Pharmaceutical Co., Ltd.). Gram-positive cocci were tested with 3% hydrogen peroxide (Kanto Chemical Co., Inc., Tokyo, Japan) for catalase activity. Catalase-positive cocci were mixed with rabbit plasma (Kohjin Bio Co., Ltd., Saitama, Japan) and incubated overnight to test for coagulase production. Coagulase-positive isolates were stored in Microbank® (Pro-Lab Diagnostics Inc., Richmond Hill, ON, Canada) at –80°C until use.

Antimicrobial susceptibility testing: Antimicrobial susceptibility tests were performed using seven antibiotics commonly selected in Japanese veterinary dermatological practice: cefalexin (CEX), amoxicillin/clavulanic acid (CVA/AMPC), minocycline (MINO), ofloxacin (OFLX), norfloxacin (NFLX), lincomycin (LCM) and clindamycin (CLDM). All specimens were tested by the disc diffusion method, and classified as susceptible, intermediate or resistant.

**RESULTS**

Isolation and species identification of coagulase-positive staphylococci: One hundred and ninety CoPS were isolated from lesional skin of 186 dogs with pyoderma. Two CoPS species were identified by M-PCR in this study: 170 of S. pseudintermedius (89.5%) and 20 of S. schleiferi subsp. coagulans (10.5%). Other CoPS species such as S. intermedius and S. aureus were not isolated.

Antimicrobial susceptibility tests: The sensitivities of S. pseudintermedius and S. schleiferi subsp. coagulans isolates to individual antimicrobials are shown in Table 2. Less than 50% of S. pseudintermedius strains were susceptible to CEX, MINO, OFLX, NFLX, LCM and CLDM (1.2–38.2%). Sensitivity to CVA/AMPC was the highest (52.4%). The frequencies of susceptibility to CEX, CVA/AMPC and MINO among the S. schleiferi subsp. coagulans strains were 55, 80 and 70%, while 45, 45 and 30% of strains showed sensitivity to OFLX, NFLX and LCM, respectively. None of the strains was found to be susceptible to LCM.

Prevalence of methicillin-resistant (MR) strains: The levels of prevalence of MR S. pseudintermedius (MRSP) and MR S. schleiferi subsp. coagulans (MRSS) were 66.5% (113/170) and 30.0% (6/20), respectively.

Difference in susceptibility between methicillin-resistant (MR) and methicillin-susceptible (MS) staphylococcal isolates: The proportions of strains susceptible to individual antimicrobials between MR and MSCoPS strains are listed in Table 3. The frequencies of susceptibility to CEX, CVA/AMPC, OFLX, NFLX and LCM in MRCoPS strains were significantly lower than those of MSCoPS strains (P<0.01). One of the 71 MSCoPS strains and one of the 119 MRCoPS strains were susceptible to LCM, and there was no significant difference in the susceptibility rates to MINO and LCM between MR and MSCoPS strains.

**DISCUSSION**

In the present study, *Staphylococcus pseudintermedius* was identified in canine pyoderma in 89.5% of total isolates. Since first reported [8], *S. pseudintermedius* has been thought to be a staphylococcal species of commensal flora and a major cause of skin infection including pyoderma [1, 31]. The results of this study support the assertion that *S. pseudintermedius* may be a major pathogen of canine pyoderma.
gen in canine pyoderma after also suggest that this organism is the most important patho-isolated from dogs with pyoderma [3, 12, 18]. Our results [19], recent studies reported that the organism has also been from external auditory meatus with canine otitis externa present study. Although the organism was first isolated quency of 10.5% from the lesional skin of dogs in the study. These results demonstrated a high prevalence of MR strains in humans similar to that we identified in dogs.

The frequency of methicillin-resistant S. pseudintermedius (MRSP) isolation in this study was markedly higher than that previously observed by other researchers who reported the existence of MRSP isolated from the skin of healthy and affected dogs [16, 29, 30]. All bacterial specimens in this study were collected from dogs in a veterinary teaching hospital and a veterinary dermatology clinic. Therefore, many of the samples were possibly isolated from cases of recurrent pyoderma. Furthermore, some of these cases may have received antimicrobial treatment in the past, although the details remain unclear. Consequently, owing to selective pressure, staphylococcal isolates in this study may have shown a higher than usual rate of methicillin resistance. The prevalence of MR S. aureus (MRSA) strains in human hospitals was found to be 66.8% in Japan [2]. These results demonstrated a high prevalence of MR strains in humans similar to that we identified in dogs.

The rates of antimicrobial susceptibility to CEX, CVA/ AMPC, OFLX and NFLX in MRCoPS isolates in this study were significantly lower than those in MSCoPS isolates. MR staphylococci produce an altered penicillin-binding protein (PBP 2' or PBP 2a) coded by the mecA gene, that has extremely lower affinity for binding β-lactams [17, 37]. Therefore, the PBP 2’ makes bacteria resistant to all β-lactam antibiotics. In contrast, quinolone resistance in staphylococci is caused by mutations in the DNA gyrase and topoisomerase, that are essential for bacterial DNA synthesis [20, 35]. There are some reports that MRSA isolates obtained from human hosts had mutations in these genes and these isolates were resistant against old generation quinolones such asNFLX or ciprofloxacin [6, 26, 33]. Similarly, Descloux et al. reported that all MRSP isolates from diseased dogs also had mutations in these genes together with resistance to enrofloxacin [7]. These reports and our results suggest that it is important to confirm the susceptibility to quinolones for appropriate use in treatment of MRCoPS infections in dogs.

In this study, there was no significant difference in the susceptibility to MINO between MR and MSCoPS isolates. Two mechanisms of resistance to tetracyclines have been identified in staphylococci: active drug efflux mechanism resulting from the acquisition of tetK and tetL genes, and ribosomal protections mediated by tetM or tetO genes [11, 21, 28]. In addition, it was also reported that tetM- or tetK-

S. schleiferi subsp. coagulans were isolated with a frequency of 10.5% from the lesional skin of dogs in the present study. Although the organism was first isolated from external auditory meatus with canine otitis externa [19], recent studies reported that the organism has also been isolated from dogs with pyoderma [3, 12, 18]. Our results also suggest that this organism is the most important pathogen in canine pyoderma after S. pseudintermedius.

The frequency of methicillin-resistant S. pseudintermedius (MRSP) isolation in this study was markedly higher than that previously observed by other researchers who reported the existence of MRSP isolated from the skin of healthy and affected dogs [16, 29, 30]. All bacterial specimens in this study were collected from dogs in a veterinary teaching hospital and a veterinary dermatology clinic. Therefore, many of the samples were possibly isolated from cases of recurrent pyoderma. Furthermore, some of these cases may have received antimicrobial treatment in the past, although the details remain unclear. Consequently, owing to selective pressure, staphylococcal isolates in this study may have showed a higher than usual rate of methicillin resistance. The prevalence of MR S. aureus (MRSA) strains in human hospitals was found to be 66.8% in Japan [2]. These results demonstrated a high prevalence of MR strains in humans similar to that we identified in dogs.

The rates of antimicrobial susceptibility to CEX, CVA/
and tetM-positive MRSA isolates had high levels of resistance to MINO [36]. However, several recent publications have reported that in vitro susceptibility of MINO against MRSA isolates from human hosts was significantly high as compared to other antimicrobials such as CLDM, CVA/AMPC or levofloxacin [9, 34, 40]. In fact, our data also indicate that the proportion of MRCoPS strains resistant to MINO was the lowest among those for seven antimicrobial agents (29.4% of all MRCoPS isolates, data not shown). In addition, the proportion of MRCoPS isolates that were classified with intermediate susceptibility to MINO accounted for 35.3% (42/119) of the total CoPS isolates in our study. The efficacy of MINO against MRCoPS may be revealed by determination of their minimum inhibitory concentrations and identification of the tet genes in addition to our results.

The prevalence of susceptibility to LCM and CLDM was extremely low in both MR and MSCoPS isolates in our study. Similarly, Futagawa-Saito et al. indicated comparably low levels of susceptibility to LCM in staphylococcal isolates from healthy (8/44) and diseased (36/44) dogs in Japan [13]. However, the incidence of susceptibility to LCM and CLDM reported by Vanni et al. was quite high in Staphylococcus spp., which were isolated from healthy (255/310) and diseased (55/310) dogs in Italy between 2006 and 2007 [39]. These investigations and our results suggest that the levels of susceptibility to LCM in staphylococcal isolates from diseased dogs are possibly lower than those from healthy dogs and, at least in Japan, there is a low frequency of susceptibility to lincomamides such as LCM and CLDM in staphylococcal isolates from diseased dogs, and Japanese veterinary clinicians should not expect therapeutic efficacy of lincomamides against canine pyoderma.

In conclusion, our results indicate a high level of resistance of CoPS isolates to the antimicrobials usually used for treatment against staphylococcal infections. Moreover, a high level of MRCoPS strains seems to be increasingly common in cases of canine pyoderma. However, there are significant differences in antimicrobial susceptibilities between staphylococcal species, and between MSCoPS and MRCoPS strains, especially for \( \beta \)-lactams and quinolones. This suggests the importance of performing antimicrobial susceptibility testing and confirming the presence of methicillin resistance among staphylococcal isolates from canine pyoderma.

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REFERENCES


