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Efficacy of totarol against *Staphylococcus pseudintermedius* and *Staphylococcus coagulans* in dogs and cats: An *in vitro* study

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Abstract

We investigated the susceptibility of totarol in 20 staphylococci strains, consisting of *Staphylococcus pseudintermedius* (n = 10) and *S. coagulans* (n = 10), from dogs and cats with skin infections. Each species contained 5 methicillin-resistant and 5 methicillin-susceptible strains. The minimum inhibitory concentrations (MICs) of totarol were determined by microbroth dilution method. As a result, all strains had the MIC of 4 μg/mL, except for one methicillin-susceptible *S. coagulans* strain, which had the MIC of 40 μg/mL. These MICs were extremely lower than the totarol concentration in commercially-available topical products. The present findings would support the use of totarol-containing topical products as one of the treatment options for canine and feline skin infections with both methicillin-susceptible and -resistant staphylococci.

Key Words: Skin infection, staphylococci, totarol

Staphylococcal skin infection is a major bacterial disease in dogs[3] and occasionally occurs in cats.[1] *Staphylococcus pseudintermedius* and *S. coagulans* (formerly *S. schleiferi* subsp. *coagulans*) are the species most commonly involved in these skin infections.[3] Systemic antimicrobial treatment was previously highly effective for skin infection, but the current high prevalence of methicillin-resistant staphylococci (MRS) has rendered this mode of treatment less effective.[3] Under the circumstances, topical treatment has been highly recommended prior to or together with systemic treatment because of the several advantages including consistent potency against antimicrobial-resistant staphylococci.[6]

Totarol, a diterpene compound extracted from the tōtara tree (*Podocarpus totara*) which is common in New Zealand, has antibacterial properties against *S. aureus*, including methicillin-resistant isolates.[7,8,10] Currently, several topical products containing totarol are commercially available for veterinary use. The previous clinical trials showed that totarol-containing spray or clay have non-inferior efficacy to chlorhexidine wash

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Table 1. Summary of the strains used in this study and their MICs of totarol

Strain No.	Animals	Isolation year	Bacterial species	*mecA* gene	Totarol MIC (μg/mL)
1	Dog	Jan. 2022	*S. coagulans*	−	4
2	Dog	Jan. 2022	*S. coagulans*	−	4
3	Dog	Jan. 2022	*S. coagulans*	−	4
4	Dog	Jan. 2022	*S. coagulans*	+	4
5	Dog	Jan. 2022	*S. coagulans*	+	4
6	Dog	Jan. 2022	*S. coagulans*	+	4
7	Cat	Mar. 2022	*S. coagulans*	−	4
8	Cat	Apr. 2022	*S. coagulans*	−	40
9	Cat	May 2022	*S. coagulans*	+	4
10	Cat	Mar. 2023	*S. coagulans*	+	4
11	Cat	Aug. 2022	*S. pseudintermedius*	−	4
12	Cat	Oct. 2022	*S. pseudintermedius*	+	4
13	Cat	Oct. 2022	*S. pseudintermedius*	+	4
14	Cat	Oct. 2022	*S. pseudintermedius*	−	4
15	Dog	Oct. 2022	*S. pseudintermedius*	+	4
16	Dog	Oct. 2022	*S. pseudintermedius*	+	4
17	Dog	Oct. 2022	*S. pseudintermedius*	−	4
18	Dog	Oct. 2022	*S. pseudintermedius*	−	4
19	Dog	Oct. 2022	*S. pseudintermedius*	−	4
20	Dog	Oct. 2022	*S. pseudintermedius*	+	4

for canine pyoderma without adverse effects.[12] In this trial, however, most of the cases were caused by methicillin-susceptible *S. pseudintermedius*, and assessed based only on clinical signs. Thus, there is no evidence of *in vitro* efficacy of totarol against staphylococci, including MRS, causing skin diseases in companion animals.

In this study, the *in vitro* susceptibility of wild-type isolates of *S. pseudintermedius* and *S. coagulans* from skin of dogs and cats to totarol was investigated in order to assess the efficacy of topical application of the compound.

A total of 20 strains of *Staphylococcus* spp., including *S. pseudintermedius* (n = 10) and *S. coagulans* (n = 10), were used in this study. These isolates were selected from our collection obtained between 2022 and 2023 in Japan and were obtained from the skin of dogs (n = 12) and cats (n = 8). Further details of the isolates are noted in Table 1. Bacterial species were identified by performing multiplex PCR,[9] followed by a coagulase test (only for *S. coagulans*).[5] The *mecA* gene was screened by the disk diffusion test, and it was detected by PCR, as previously described.[4]

The minimum inhibitory concentrations (MIC) of totarol (Alaron Products Ltd., Nelson, New Zealand) were determined by the microbroth dilution method with slight modifications.[4] Hydrophobic totarol powder was dissolved in dimethyl sulfoxide (DMSO) and diluted 1000-fold in Mueller-Hinton broth (MHB; Eiken, Japan). MHB with concentrations of 0.5, 1, 1.5, 2, 4, 40, and 400 μg/mL totarol were used in the study. Colonies of bacterial isolates were suspended according to the McFarland standard of 0.5 followed by a 10-fold dilution in phosphate buffered saline. These suspensions were added to totarol-containing wells and incubated for 16 h at 37°C. MHB plates with DMSO without totarol were used as positive controls. MIC was defined as the lowest concentration at which visible growth of an isolate was inhibited.

In this study, we firstly assessed the antibacterial effect of totarol against *S. pseudinteremedius* and *S. coagulans*, the major veterinary skin pathogens. The MICs obtained are noted in Table 1. Most of the 20 tested strains exhibited totarol MICs of 4 μg/mL. There have been several reports of totarol bacterial activity against *S. aureus*. Muroi and Kubo[7] reported three methicillin-susceptible *S. aureus* (MSSA) (ATCC 12598, 25923, and 11632) and three methicillin-resistant *S. aureus* (MRSA, ATCC 33591, 33592, and 29247) strains that exhibited MIC of 0.78–1.56 μg/mL. Nicolson et al.[8] reported that the MICs of MSSA ATCC 9144 strain and a MRSA clinical isolate were 3.2 and 2 μg/mL, respectively. Smith et al.[11] found that the MSSA ATCC 25923 strain exhibited an MIC of 2 μg/mL. In addition, Shi et al.[10] demonstrated that MSSA ATCC 29213 strains and 13 food-borne *S. aureus* isolates exhibited MICs of 2–4 μg/mL. As the results, most of our strains exhibited approximately one doubling dilution higher MICs than those previously reported in *S. aureus*. These findings may imply that the *in vitro* efficacy of torarol is slightly different between staphylococci species, although we cannot provide clear reasons. Nevertheless, our data indicate that totarol has high antibacterial efficacy against *S. pseudintermedius* and *S. coagulans*. Notably, the totarol MICs were similar between methicillin-resistant and -susceptible isolates of both species. Therefore, it is likely that totarol is an active compound for these veterinary MRS, in addition to MRSA, indicating that application of totarol can be one of the few treatment options for MRS infections in companion animals.

Only one methicillin-susceptible *S. coagulans* strain (No.8) exhibited a high MIC (40 μg/mL) to totarol. One possible reason is over expression of the NorA efflux pump as noted by Smith et al.,[11] where overexpression of the efflux pump increased the MIC of totarol from 1.25 to 16 μg/mL in one *S. aureus* isolate. However, the participation of such resistance mechanisms in the strain is unclear. Our study also suggests that the strains with higher MIC of totarol are less prevalent (1/20, 5%), although all of the strains used in this study were obtained prior to the domestic distribution of totarol-containing topical products (April, 2023). The concentration of totarol in commercial topical products is 0.2%–0.3% (2000-3000 μg/mL), which is substantially higher than the MIC of totarol-resistant staphylococci. Thus, it is unlikely that the prevalence of these resistant strains would affect the clinical efficacy of topical totarol against staphylococcal skin infection.

Although the antibacterial mechanism of totarol has not been fully elucidated, previous studies have demonstrated that totarol can serve as an inhibitor of oxygen consumption in some reactions of the respiratory chain[2,8] as well as disrupting the cytoplasmic membrane.[10] Such putative action mechanisms are unrelated to methicillin resistance, resulting in the high efficacy of totarol on MRS. Totarol can also be an inhibitor of penicillin binding protein 2a and the multidrug efflux pump in *S. aureus*,[8,11] indicating that it has a synergistic effect with antibiotics. These findings may highlight the usefulness of totarol not only when used alone but also when applied in combination with antimicrobial drugs. However, clinical studies are needed to support this hypothesis.

There were several limitations in this study. Firstly, we used only a small number of staphylococci strains because these strains were selected from a temporal collection of clinical specimens submitted to VDT Co., Ltd. for bacteriological inspections. Secondly, staphylococcal cassette chromosome mec (SCCmec) types were not determined in our strains, although whether or not SCCmec types affect totarol susceptibility was not clarified in the previous studies.[7,8,10]

In conclusion, we demonstrated the high *in vitro* efficacy of totarol against wild-type *S. pseudintermedius* and *S. coagulans* isolates, irrespective of the status of methicillin resistance. We believe that the present findings support the use of totarol-containing topical products as one of the treatment options for skin infections in dogs and cats caused by both methicillin-susceptible and -resistant staphylococci.

Conflict of interest

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References

1) Cavana P, Robino P, Stella MC, Bellato A, Crosaz O, Fiora SR, Nebbia P. Staphylococci isolated from cats in Italy with superficial pyoderma and allergic dermatitis: Characterisation of isolates and their resistance to antimicrobials. Vet Dermatol 34, 14-21, 2023. doi: 10.1111/vde.13127.
2) Haraguchi H, Oike S, Muroi H, Kubo I. Mode of antibacterial action of totarol, a diterpene from Podocarpus nagi. Planta Med 62, 122-125, 1996. doi: 10.1055/s-2006-957832.
3) Hiller A, Lloyd DH, Weese JS, Blondeau JM, Boothe D, Breitschwerdt E, Guardabassi L, Papich MG, Rankin S, Turnidge JD, Sykes JE. Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis (Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases). Vet Dermatol 25, 163-e43, 2014. doi: 10.1111/vde.12118.
4) Hsiao Y-H, Imanishi I, Iyori K. Efficacy of olanexidine gluconate in canine superficial pyoderma: a randomised, single-blinded controlled trial. Vet Dermatol 32, 664-e176, 2021. doi: 10.1111/vde.13038.
5) Kawakami T, Shibata S, Murayama N, Nagata M, Nishifuji K, Iwasaki T, Fukata T. Antimicrobial susceptibility and methicillin resistance in *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi* subsp. *coagulans* isolated from dogs with pyoderma in Japan. J Vet Med Sci 72, 1615-1619, 2010. doi: 10.1292/jvms.10-0172.
6) Loeffler A, Lloyd DH. What has changed in canine pyoderma? A narrative review. Vet J 235, 73-82, 2018. doi: 10.1016/j.tvjl.2018.04.002.
7) Muroi H, Kubo I. Antibacterial activity of anacardic acid and totarol, alone and in combination with methicillin, against methicillin-resistant *Staphylococcus aureus*. J Appl Bacteriol 80, 387-394, 1996. doi: 10.1111/j.1365-2672.1996.tb03233.x.
8) Nicolson K, Evans G, O'Toole PW. Potentiation of methicillin activity against methicillin-resistant *Staphylococcus aureus* by diterpenes. FEMS Microbiol Lett 179, 233-239, 1999. doi: 10.1111/j.1574-6968.1999.tb08733.x.
9) Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki S, Kawakami T, Fukata T, Hiramatsu K. Multiplex-PCR method for species identification of coagulase-positive staphylococci. J Clin Microbiol 48, 765-769, 2010. doi: 10.1128/JCM.01232-09.
10) Shi C, Che M, Zhang X, Liu Z, Meng R, Bu X, Ye H, Guo N. Antibacterial activity and mode of action of totarol against *Staphylococcus aureus* in carrot juice. J Food Sci Technol 55, 924-934, 2018. doi: 10.1007/s13197-017-3000-2.
11) Smith ECJ, Kaatz GW, Seo SM, Wareham N, Williamson EM, Gibbons S. The phenolic diterpene totarol inhibits multidrug efflux pump activity in *Staphylococcus aureus*. Antimicrob Agents Chemother 51, 4480-4483, 2007. doi: 10.1128/AAC.00216-07.
12) Ward-McGrath H, Bell A, Bridges J, Jones B. Some observations on the treatment of superficial pyoderma in dogs with totarol. Companion quarterly 32(4), 35-38, 2021.