

Research on the biofilm formation of *Staphylococcus pseudintermedius*
clinical isolates from dogs and cats

Abstract of Doctoral Thesis

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In veterinary medicine, *Staphylococcus pseudintermedius* is isolated as a causative organism of various infections and is also known as biofilm producer. However, since biofilm recognition in the veterinary field is low, sufficient measures against *S. pseudintermedius* infection for biofilm have not been taken. We investigated clinically isolated *S. pseudintermedius* derived from dogs and cats about the ability of biofilm formation, their source of isolation, antibiotic resistance and the difference of inflammatory responses depending on the potency of biofilm organization.

Firstly, 250 isolates of clinical *S. pseudintermedius* from dogs and cats were intended for isolation and evaluation of the ability of biofilm formation. All isolates were classified as strong (24.8 %), moderate (52.0 %), and weak (23.2 %) biofilm producers. The risk of the biofilm related infections of *S. pseudintermedius* was appeared to be no difference regarding hosts, infection sites, and medical facilities.

The positive correlation was found between minimum inhibitory concentration and biofilm organization potency in 9 antibiotics in the examination about the association between antibiotic resistance and biofilm organization potency. Moreover, in ampicillin-resistant isolates of methicillin-susceptible *S. pseudintermedius* (MSSP), the strong biofilm producers were more resistant than the weak biofilm producers. It was suggested that biofilm formation was associated with antibiotic resistance of the MSSP.

To investigate the pathogenicity of *S. pseudintermedius* biofilm, we compared the expression of inflammatory cytokines in RAW264.7 cells induced by biofilm-conditioned medium (BCM) preparing with filter sterilized culture medium of different of biofilm organization potency. As the results, a significant increase in expression of IL-1 β and TNF- α was observed in

RAW264.7 cells cultured with BCM of strong biofilm producer than with BCM of weak biofilm producer ($P < 0.01$). We examined what was the inflammatory attractant and how inflammatory response was occurred in BCM of strong biofilm producer, and revealed that the heat-resistant secreted proteins were induced inflammatory responses via the toll-like receptor (TLR) signaling pathway. Also, the strong biofilm producers had specific banding patterns and peaks when we analyzed by SDS-PAGE and MALDI TOF-MS, respectively. Therefore, it was recognized that a difference in secreted proteins relays on the biofilm organization potency.