

## Sensitivity of *Acanthamoeba castellanii*-Grown *Francisella* to Three Different Disinfectants

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**Abstract :** *Francisella tularensis* is a highly infectious, gram-negative intracellular bacterium and the causative agent of tularemia. The bacterium has been isolated from more than 250 wild species, including protozoa cells. Since *Francisella* is very virulent and persists in the environment for years, the aim of this study was to investigate whether *Acanthamoeba castellanii*-grown *F. novicida* exhibits an alteration in the resistance to disinfectants. It has been shown by other intracellular pathogens, including *Legionella pneumophila* that bacteria grown in amoeba exhibit more resistance to disinfectants. However, there is no data showing *Francisella* viability behaviour after intracellular life cycle in *A. castellanii*. In this study, the bacterial suspensions of *A. castellanii*-grown or in vitro-grown *Francisella* were treated with three different disinfectants, and the bacterial viability after disinfection treatment was determined by a colony-forming unit (CFU) counting method, transmission electron microscopy (TEM), fluorescence microscopy as well as the leakage of intracellular fluid. Our results have shown that didecyldimethylammonium chloride (DDAC) combined with isopropyl alcohol was the most effective in bacterial killing; all in vitro-grown and *A. castellanii*-grown *F. novicida* were killed after only 10s. Surprisingly, in comparison to in vitro-grown bacteria, *A. castellanii*-grown *F. novicida* was more sensitive to decontamination by the benzalkonium chloride combined with DDAC and formic acid and the polyhexamethylene biguanide (PHMB). We can conclude that the tested disinfectants exhibit antimicrobial activity by causing a loss of structural organization and integrity of the *Francisella* cell wall and membrane and the subsequent leakage of the intracellular contents. Finally, the results of this study clearly demonstrate that *Francisella* grown in *A. castellanii* had become more susceptible to many disinfectants.

**Keywords :** *Acanthamoeba*, disinfectant, *Francisella*, sensitivity

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