Inhibition of biofilm formation and antibacterial potentiation by 2-aminoimidazole compounds evaluated using coagulase negative staphylococci (CNS) isolated from goat mastitis

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Introduction

Biofilm formation is important in the pathogenesis of many bacterial infections of both humans and animals. Coagulase-negative staphylococci (CNS) are significant biofilm-producing pathogens found in human foreign-body infections and ruminant mastitis. We have previously shown differences in biofilm production by representatives of various genotypes of Staphylococcus aureus causing bovine mastitis. We further demonstrated that 2-aminoimidazole compounds have anti-biofilm and antibacterial potentiation properties when tested in vitro using these isolates. The CNS are the bacteria most commonly isolated from goat milk, and they can be important pathogens in the goat mammary gland. Our purpose was to evaluate the biofilm-forming potential of representatives of genotypes of the most common CNS we found in goat mastitis. We hypothesized differences in biofilm-producing potential among goat CNS isolates and that biofilm formation may reflect pathogenic potential. Using the most prolific biofilm-forming CNS isolate, we hypothesized that 2-aminoimidazole compounds would inhibit biofilm production and potentiate antibacterial activity.

Materials and Methods

In a recent study, we isolated 9 different species of CNS from the milks of a sample of North Carolina goats. We tested 15 CNS isolates for their biofilm-producing potential. We then tested a prolific biofilm producer against the 2-aminoimidazole compound. Biofilm formation plates and anti-biofilm plates were inoculated, incubated, and stained. The optical density was measured. A multi-drug resistant goat mastitis CNS isolate was tested *in vitro* with 3 antibiotics and the 2-aminoimidazole compound. The checkerboard MIC plates were incubated and observed for bacterial growth. Data comparing the ability of CNS isolates to produce biofilm were log-10 transformed and analyzed using ANOVA with a mixed model that included trial (3 levels), isolate (15 levels) and random effect of sample (24 levels, nested within trial). Data for evaluating the effect of concentrations of anti-biofilm compound were analyzed using ANOVA considering model factors of trial (3 levels), concentration (9 levels), and the random effect of trial by concentration interaction (27 levels).

Results

There was significant variability in the isolates' ability to form biofilms. ATCC12228, a heavy biofilm-producing isolate, produced more biofilm than all other tested isolates. The isolate we tested, CN118, produced slightly less biofilm than ATCC12228 and CN89, but it was not different than other heavy-producing isolates CN62, 113, 116, and 167. However, CN118 produced significantly more biofilm than isolates CN16, 31, 41, 58, 74, 105, and 165. Anti-biofilm compound at concentrations of 25, 12.5, 6.25 uM significantly ($P<0.01$) reduced biofilm production compared to no drug. Preliminary results indicate that the compound has antibacterial potentiation activity with 3 antibiotics when tested in vitro on a multi-drug resistant goat mastitis CNS isolate.

Significance

The 2-aminoimidazole compounds have potential in the effective treatment of CNS infections.