Abstract

Aquaculture is a rapidly growing sector in global food production, primarily centered on the cultivation of marine and freshwater fish, as well as shellfish species, contributing significantly to increased annual fish production and holding a prominent position on a global industrial scale. However, this industry faces significant challenges, including diseases that often lead to fish mortality. This study explores the combined impact of antibiotics and probiotics on multi-species biofilm in *Labeo rohita* and *Oreochromis niloticus*. Gram-negative bacteria are known to develop biofilms and antibiotic resistance, with resistance levels ranging from 100 to 1000 times. Multi-species biofilm research is relatively scarce. The study investigates the antibiofilm and biofilm dispersal potential of antibiotics and probiotics, both individually and in combination. Seven multi-species groups were created using pathogenic bacterial isolates from both fish species, denoted as A1, A2, A3, A4, A5, A6, and A7, respectively. All multi-species strains were identified up to the species level through 16S rRNA gene sequencing. Biofilm-forming abilities of these multi-species groups were assessed after 24 hours of incubation. A2, A3, and A4 exhibited the highest biofilm formation on day three, while others peaked on day seven. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of levofloxacin and ampicillin were evaluated, ranging from 0.5 µl/ml to 20 µl/ml and 20 µl/ml to 35 µl/ml, respectively, against multi-species groups. Antibiofilm activity was observed at different antibiotic concentrations, with the highest activity at double the MIC. Antibiofilm activity was also demonstrated by four probiotics isolated from the fish's gut: R1, R2, O1, and O2. All probiotics displayed significant antibiofilm activity. Dispersal activity was assessed against different concentrations of levofloxacin and ampicillin, with the maximum dispersal activity observed at double the MIC. Probiotics also exhibited substantial dispersal effects within the biofilm. When antibiotics and probiotics were combined, synergistic effects were observed, resulting in significant antibiofilm and dispersal activity. These findings highlight the potential of antibiotics and probiotics to combat biofilm formation in antibiotic-resistant species. Together, they offer a promising approach to controlling pathogenic diseases in fish.