

Occurrence of multi-drug resistant *Pseudomonas* species in freshwater ornamental fish and associated aquatic environment in Sri Lanka

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Introduction and Objectives: The genus *Pseudomonas* is a highly diverse and complex bacterial group considered as opportunistic pathogens in fish, livestock, and humans. The extensive use of antimicrobials in freshwater aquaculture has resulted in the emergence of antimicrobial resistance among bacteria in farmed fish and their associated environment. The present study aimed to characterize pseudomonads from freshwater ornamental fish and the farming environment in Sri Lanka and to determine their antimicrobial susceptibility.

Methods: A total of 16 *Pseudomonas* isolates recovered from the skin mucus of apparently healthy ornamental fish (n=8), effluent water (n=1), pond sediment (n=1) and biofilms (n=6) from five ornamental fish farms were used in the study. Upon culturing on tryptic soy agar and glutamate starch phenol red agar, isolates were identified by their morphological characteristics and conventional biochemical tests; oxidase, catalase, oxidation-fermentation, triple sugar ion, citrate, urease, and indole. They were genetically confirmed by PCR using *Pseudomonas* genus specific primers targeting the 16S-rRNA gene and partial sequence analysis of the same gene. Antimicrobial susceptibility against ten antimicrobials was determined by the Kirby-Bauer method according to the CLSI guidelines.

Results: *Pseudomonas mosselii* was the predominant species (n=5) followed by *Pseudomonas aeruginosa* (n=3), *Pseudomonas putida* (n=3), *Pseudomonas resinovorans* (n=2), *Pseudomonas fluorescens* (n=1), *Pseudomonas alcaligenes* (n=1) and *Pseudomonas entomophila* (n=1). All isolates were resistant to amoxicillin and nitrofurantoin. Isolates showed a remarkable resistance to erythromycin (87.5%), cefotaxime (87.5%), trimethoprim-sulfamethoxazole (81.3%), enrofloxacin (75%) and chloramphenicol (62.5%). Comparatively low resistance was observed against tetracycline (25%) and neomycin (12.5%) while all isolates were sensitive to rifampicin. All were multidrug resistant (MDR) (75% were resistant to more than five antimicrobial classes) and showed a multiple antibiotic resistance index higher than 0.2. This study revealed the occurrence of diverse MDR *Pseudomonas* species in apparently healthy tropical ornamental fish and an associated aquatic environment in Sri Lanka.

Conclusions: These resistant bacteria may disseminate from aquatic to terrestrial animals and humans leading to difficulties in treating their infections. Our findings highlight the potential role of the ornamental fish farming environment as a reservoir of MDR *Pseudomonas* spp. This is the first report on the existence and emergence of MDR pseudomonads in the Sri Lankan ornamental fish farming environment.

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