

Isolation and Characterization of Non-tuberculous Mycobacteria (NTM) from Freshwater Ornamental Fish

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Piscine mycobacteriosis is a chronic, systemic bacterial disease of fish caused by nontuberculous mycobacteria (NTM). To investigate the occurrence of different species of mycobacteria in freshwater ornamental fish cultured in Sri Lanka, apparently healthy ornamental fish (family *Poeciliidae* and *Cyprinidae*) were collected from selected farms located in the Western, Central, North Central and North Western provinces during the period from January 2016 to February 2018. Visceral tissue (kidney, liver, spleen separately from large fish, as a pooled sample from small fish) and intestinal samples from each fish were subjected to acid-fast stain to detect acid-fast bacteria (AFB) and cultured on Ogawa egg medium for isolation of Mycobacteria. Presumptive mycobacterial cultures were subjected to genetic identification by PCR amplification of 16S rRNA, 16S-23S ITS, *rpoB* and *hsp65* genes. The identity of selected isolates was confirmed by sequence analysis of 16SrRNA gene. Of 442 fish examined from 59 fresh water ornamental fish farms, 99 fish were AFB positive while 343 were AFB negative. A total of 138 fish (31.2%) belonging to seven different species yielded acid-fast positive cultures. Of 187 genetically confirmed *Mycobacterium* isolates recovered from 138 fish (including multiple isolates from some fish), 96 were identified by 16SrRNA sequencing. The most frequently recovered species was *Mycobacterium fortuitum* (71%, n = 68/96). Other mycobacteria recovered included species of *M. fortuitum* group (n=21), *M. chelonae-abscessus* complex (n=3), *M. marinum* (n= 2), *M. arupense* (n=1) and *M. houstonense* (n=1). Isolation of mycobacteria was significantly high from intestine samples (17.6%: 78/442) than pooled tissues ($P<0.05$, Chi-square test). It is evident that *Mycobacterium* is widely prevalent in apparently healthy ornamental fish sampled in this study. Potential risks associated with handling ornamental fish carrying NTM on human health should not be overlooked. Our findings highlight the importance of screening mycobacteriosis in ornamental fish stocks to understand the true prevalence of NTM.

Keywords: Nontuberculous mycobacteria, Ornamental fish, *Mycobacterium fortuitum*

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