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Antibiotic susceptibility of mycobacteria isolated from ornamental fish

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Abstract

Introduction: Nontuberculous mycobacteria (NTM) are increasingly recognised as causative agents of opportunistic infections in humans for which effective treatment is challenging. There is very little information on the prevalence of NTM drug resistance in Poland. This study was aimed to evaluate the susceptibility to antibiotics of NTM, originally isolated from diseased ornamental fish. **Material and Methods:** A total of 99 isolates were studied, 50 of them rapidly growing mycobacteria (RGM) (among which three-quarters were *Mycobacterium chelonae*, *M. peregrinum*, and *M. fortuitum* and the rest *M. neoaurum*, *M. septicum*, *M. abscessus*, *M. mucogenicum*, *M. salmoniphilum*, *M saopaulense*, and *M. senegalense*). The other 49 were slowly growing mycobacteria (SGM) isolates (among which only one was *M. szulgai* and the bulk *M. marinum* and *M. gordonae*). Minimum inhibitory concentrations for amikacin (AMK), kanamycin (KAN), tobramycin (TOB), doxycycline (DOX), ciprofloxacin (CIP), clarithromycin (CLR), sulfamethoxazole (SMX), isoniazid (INH) and rifampicin (RMP) were determined. **Results:** The majority of the isolates were susceptible to KAN (95.95%: RGM 46.46% and SGM 49.49%), AMK (94.94%: RGM 45.45% and SGM 49.49%), CLR (83.83%: RGM 36.36% and SGM 47.47%), SMX (79.79%: RGM 30.30% and SMG 49.49%), CIP (65.65%: RGM 24.24% and SGM 41.41%), and DOX (55.55%: RGM 9.06% and SGM 46.46%). The majority were resistant to INH (98.98%: RGM 50.50% and SGM 48.48%) and RMP (96.96%: RGM 50.50% and SGM 46.46%). **Conclusion:** The drug sensitivity of NTM varies from species to species. KAN, AMK, CLR and SMX were the most active against RGM isolates, and these same four plus DOX and CIP were the best drugs against SGM isolates.

Keywords: *Mycobacterium* spp., antimicrobial susceptibility, minimum inhibitory concentration, fish.

Introduction

Fish mycobacteriosis is a chronic progressive disease caused by several species of the *Mycobacterium* genus. Mycobacterial species are capable of causing serious diseases in most vertebrates, including humans (28), and infect a wide range of tissue and organ types, with pulmonary infections being the most frequent (57). According to the List of Prokaryotic Names with Standing in Nomenclature, there are over 150 recognised species of mycobacteria (42), all of which other than those in the *M. tuberculosis* complex and *M. leprae* are nontuberculous mycobacteria (NTM), also known as environmental mycobacteria, atypical mycobacteria and mycobactaria other than tuberculosis (MOTT). These are generally free-living organisms ubiquitous in the environment and are known to infect a number of aquatic animals, including fish. The host range of this disease is correspondingly broad and includes over 150 species of both marine and freshwater ornamental fish (*e.g. Astronotus ocellatus*, *Carassius auratus*, *Colisa lalia*, *Cyprinus carpio* subsp. *haematopterus*, *Danio rerio*, *Helostoma temminckii*, *Hyphessobrycon serape*, *Labidochromis caeruleus*, *Microgeophagus ramirezi*, *Paracheirodon innesi*, *Poecilia reticulata*, *Symphysodon discus*, *Trichogaster lalius*, *Xiphophorus helleri* and *X. maculatus*) (15, 19, 23, 31, 44, 45). The most common NTM pathogens of fish include *M. marinum*, *M. fortuitum*, *M. peregrinum* and *M. chelonae*. Other species isolated from fish include *M. abscessus*, *M. arupense*, *M. avium*, *M. chesapeaki*, *M. conceptionense*, *M. flavescens*, *M. gordonae*, *M. haemophilum*, *M. kansasii*, *M. montefiorense*, *M. mucogenicum*, *M. neoaurum*, *M. nonchromogenicum*, *M. parascrofulaceum*, *M. porcinum*, *M. pseudoshottsii*, *M. salmoniphilum*, *M. saopaulense*, *M. scrofulaceum*, *M. senegalense*, *M. septicum*, *M. shimoidei*, *M. shottsii*, *M. simiae*, *M. terrae*, *M. szulgai*, *M. triviale*, *M. triplex*, *M. ulcerans*

and *M. xenopi* (18, 23, 34, 41, 43, 44, 45). In recent years, human nontuberculous mycobacterial infections and diseases have significantly increased (32). There are approximately 30 NTM that are pathogenic to humans, who commonly acquire infections if they are aquarium staff and tropical fish breeders.

The clinical signs of fish mycobacteriosis are nonspecific and include dermal ulceration, scale loss, pigmentary changes, abnormal behaviour, spinal defects, and emaciation. Ascites and granulomas may appear in all internal organs, *e.g.* the kidneys, liver and spleen (17). Bacterial species such as *M. fortuitum*, *M. marinum*, *M. smegmatis*, *M. flavescens*, *M. peregrinum* and *M. chelonae*, which are well-known pathogens in fish and humans, have been isolated from apparently healthy fish (23, 50). Mycobacterial infections in fish are a risk factor for the human population; nevertheless, relatively few studies have investigated large collections of ornamental fish for the presence of mycobacteria (26, 31, 34, 44).

Regarding food fish rather than ornamental fish and antimycobacterial therapy rather than mycobacterial presence, Kawakami and Kusuda (29) reported that rifampicin, streptomycin, and erythromycin were effective in reducing mortality associated with *Mycobacterium* spp. in cultured yellowtail (*Seriola quinqueradiata*). However, there are no treatments for mycobacteriosis in cultured food fish approved by the US Food and Drug Administration, nor are there any unapproved products which have been proven effective in application in the field. If antibiotics are to be used in fish mycobacteriosis treatment, their appropriacy must be validated in terms of the benefit against the risk, because the development and spread of antimicrobial resistance has become a global public health problem that is impacted by both human and non-human antimicrobial usage (37, 65). In this study, the drug susceptibility of 99 isolates of *Mycobacterium* spp. isolated from diseased ornamental fish to nine antibiotics was investigated.

Material and Methods

Bacterial strains and growth conditions. The study was conducted on 99 NTM strains originally isolated from diseased ornamental fish between January 2015 and December 2016 in the bacteriology laboratory of the Department of Fish Diseases and Biology, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Poland. The atypical mycobacteria studied were as follows: *M. abscessus* (1 isolate), *M. chelonae* (16 isolates), *M. fortuitum* (10 isolates), *M. gordonae* (15 isolates), *M. marinum* (33 isolates), *M. mucogenicum* (1 isolate), *M. neoaurum* (2 isolates), *M. peregrinum* (12 isolates), *M. salmoniphilum* (1 isolate), *M. saopaulense* (1 isolate), *M. senegalense* (4 isolates), *M. septicum* (2 isolates), and *M. szulgai* (1 isolate). The strains of mycobacteria were identified on the basis of molecular characteristics as described previously (44). After successful identification, the strains were stored at −80°C in Youmans broth supplemented with 20% foetal bovine serum until the minimum inhibitory concentrations (MICs) were determined. *Mycobacterium marinum* ATCC 927 and *M. smegmatis* ATCC 19420 were used as reference strains, and *M. peregrinum* ATCC 700686 was used as a quality control strain in the antimicrobial susceptibility tests. Mycobacteria were grown in Middlebrook 7H9 broth for 3 to 5 days, and the culture suspension was adjusted with additional sterile distilled water to equal a McFarland 1.0 turbidity standard (approximately 10^8 CFU per mL) as described by Aubry *et al.* (3).

Antimicrobial agents and chemicals. Lyophilisates of eight antimicrobial agents, *i.e.* amikacin (AMK), ciprofloxacin (CIP), clarithromycin (CLR), doxycycline (DOX), isoniazid (INH), rifampicin (RMP), sulfamethoxazole (SMX) and tobramycin (TOB), were purchased from Sigma-Aldrich Company (St Louis, MO, USA), while the ninth lyophilisate, kanamycin (KAN), was purchased from A&A Biotechnology (Gdynia, Poland). Cation-adjusted Mueller–Hinton broth (CA-MHB) and albumin, dextrose and catalase (ADC) supplement were supplied by Difco (Detroit, MI). Resazurin was ordered from Sigma-Aldrich (St. Louis, MO, USA). All antibiotic solutions were prepared before the day of the experiment and stored at −70°C.

Determination of minimum inhibitory concentration. The MICs of each antimicrobial were determined by the CA-MHB microdilution method, as recommended by the Chemical and Laboratory Standards Institute (CLSI), using 96-well plates. The suspensions of mycobacteria for a resazurin microtitre assay were prepared from Lowenstein–Jensen subcultures. The inocula were adjusted with sterile distilled water to a turbidity equivalent to that of a 1.0 McFarland standard, and the suspensions were then diluted (1:200) using CA-MHB for rapidly growing mycobacteria (RGM) or CA-MHB + 5% ADC supplement (CA-MHB-S) for slowly growing mycobacteria (SGM). The antibiotics were serially diluted twofold in 100 µL of CA-MHB or CA-MHB-S. Each well of a sterile plate was inoculated with 100 µL of mycobacteria suspension, and 100 µL of serial twofold dilutions of the agent was added to each well. A drug-free growth control and a mycobacteria-free sterility control aliquot of the medium were included in each plate. If the microbial growth in the control sample was sufficient, the MICs were measured on day 3. Otherwise, the incubation period was extended, and the MICs were measured on day 4 or 5. The MICs of all antibiotics except for clarithromycin were determined after 3 days (for RGM) or after 4, 5 or 7 days (for SGM) of incubation at 37°C. Plates with *M. marinum*, *M. chelonae* and *M. salmoniphilum* were incubated at 30°C. Extended incubation of plates for clarithromycin was performed as described by Nash *et al.* (39), with successive readings after 5, 7, 9, and 14 days. After incubation, resazurin at 0.01 g/100 mL was added to each well, and the plates were incubated a second time for 24 h. Each test was performed in triplicate. A colour change from blue

(the oxidised state) to pink (the reduced state) indicated bacterial growth. The MIC was defined as the lowest concentration of the drug that prevented this change in colour. The MIC_{90} and MIC_{50} values were defined as concentrations that inhibited 90% and 50% of the isolates, respectively. Susceptibility was evaluated according to the CLSI (12, 13) and WHO (66) breakpoint recommendations (Table 1).

Table 1. MIC breakpoints used for categorisation of susceptibility of RGM and SGM to nine antimicrobial agents

Antimicrobial agent	Range (μg/mL)	MIC breakpoints (μg/mL)					
		RGM			SGM		
		S	I	R	S	I	R
AMK	1–128	≤16	32	≥64	-	-	>32
KAN	1–64	≤16	32	≥64	-	-	>32
TOB	1–64	≤1	2–4	≥8	-	-	>4
DOX	0.25–32	≤2	4	≥8	≤2	4	≥8
CLR	0.25–32	≤2	4	≥8	≤8	16	≥32
CIP	0.125–16	≤1	2	≥4	-	-	>2
SMX	1–64	≤32	-	≥64	≤32	-	≥64
RMP	0.25–64	-	-	>1	-	-	>1
INH	1–64	-	-	≥5	-	-	≥5

MIC – minimum inhibitory concentration; RGM – rapidly growing mycobacteria; SGM – slowly growing mycobacteria; S – susceptible; I – intermediate; R – resistant; AMK – amikacin; KAN – kanamycin; TOB – tobramycin; DOX – doxycycline; CLR – clarithromycin; CIP – ciprofloxacin; SMX – sulfamethoxazole; RMP – rifampicin; INH – isoniazid

Table 2. Antimicrobial resistance or susceptibility determined by microdilution

NTM species (number of isolates tested)	Resistant (R) or susceptible (S)	Percentage of strains resistant or susceptible for each antimicrobial agent								
		Amikacin	Kanamycin	Tobramycin	Doxycycline	Clarithromycin	Ciprofloxacin	Sulfamethoxazole	Rifampicin	Isoniazid
M. abscessus (n = 1)	R			100.00	100.00			100.00	100.00	100.00
	I						100.00			
	S	100.00	100.00			100.00				
M. chelonae (n = 16)	R	6.25		43.75	100.00	6.25	100.00	100.00	100.00	100.00
	I	12.50	12.50	50.00						
	S	81.25	87.50	6.25		93.75				
M. fortuitum (n = 10)	R	10.00		100.00	90.00	50.00	10.00	10.00	100.00	100.00
	I		10.00			40.00				
	S	90.00	90.00		10.00	10.00	90.00	90.00		
M. gordonae (n = 15)	R			20.00		6.67	20.00		93.33	100.00
	I			46.67						
	S	100.00	100.00	33.33	100.00	93.33	80.00	100.00	6.67	
M. marinum (n = 33)	R			3.03	9.09		12.12		93.94	100.00
	I			87.88		3.03				
	S	100.00	100.00	9.09	90.91	96.97	87.88	100.00	6.06	
M. mucogenicum (n = 1)	R			100.00	100.00		100.00		100.00	100.00
	I					100.00				
	S	100.00	100.00					100.00		
M. neoaurum (n = 2)	R								100.00	100.00
	I									
	S	100.00	100.00	100.00	100.00	100.00	100.00	100.00		
M. peregrinum (n = 12)	R	8.33	8.33	8.33	66.67	8.33	8.33		100.00	100.00
	I			33.33	8.33	8.33				
	S	91.67	91.67	58.33	25.00	83.34	91.67	100.00		
M. salmoniphilum (n = 1)	R				100.00				100.00	100.00
	I									
	S	100.00	100.00	100.00		100.00	100.00	100.00		
M. saopaulense (n = 1)	R				100.00			100.00	100.00	100.00
	I			100.00			100.00			
	S	100.00	100.00			100.00				
M. senegalense (n = 4)	R				25.00	25.00	75.00	25.00	100.00	100.00
	I			25.00			25.00			
	S	100.00	100.00	75.00	75.00	75.00		75.00		
M. septicum (n = 2)	R				50.00				100.00	100.00
	I				50.00		50.00			
	S	100.00	100.00	100.00		100.00	50.00	100.00		
M. szulgai (n = 1)	R			100.00			100.00		100.00	
	I									
	S	100.00	100.00		100.00	100.00		100.00		100.00

NTM – nontuberculous mycobacteria; S – susceptible; I – intermediate; R – resistant

Table 3. Antibiotic susceptibility profiles of RGM and SGM isolated from diseased fish

Antimicrobial agent	Susceptibility					
	RGM (n = 50)			SGM (n = 49)		
	S	I	R	S	I	R
AMK	90.00	4.00	6.00	100	0.00	0.00
KAN	92.00	6.00	2.00	100	0.00	0.00
TOB	32.00	28.00	40.00	16.33	73.47	10.20
DOX	18.00	4.00	78.00	93.88	0.00	6.12
CLR	72.00	12.00	16.00	95.92	2.04	2.04
CIP	48.00	8.00	44.00	83.67	0.00	16.33
SMX	60.00	0.00	40.00	100.00	0.00	0.00
RMP	0.00	0.00	100.00	6.12	0.00	93.88
INH	0.00	0.00	100.00	2.04	0.00	97.96

RGM – rapidly growing mycobacteria; SGM – slowly growing mycobacteria; S – susceptible; I – intermediate; R – resistant; AMK – amikacin; KAN – kanamycin; TOB – tobramycin; DOX – doxycycline; CLR – clarithromycin; CIP – ciprofloxacin; SMX – sulfamethoxazole; RMP – rifampicin; INH – isoniazid

Results

The results of the antimicrobial drug susceptibility tests are shown in Table 2 and are presented in terms of resistance, intermediate resistance and susceptibility.

The majority of the isolates were susceptible to KAN (95.95%: RGM 46.46% and SGM 49.49%), AMK (94.94%: RGM 45.45% and SGM 49.49%), CLR (83.83%: RGM 36.36% and SGM 47.47%), SMX (79.79%: RGM 30.30% and SMG 49.49%), CIP (65.65%: RGM 24.24% and SGM 41.41%), and DOX (55.55%: RGM 9.06% and SGM 46.46%). Most of the strains were moderately susceptible to TOB (50.50%: RGM 14.14% and SGM 36.36%) (data not shown). While KAN (92.00%), AMK (90.00%), CLR (72.00%) and SMX (60.00%) were the most active against RGM isolates, AMK (100%), KAN (100%), SMX (100.00%), CLR (95.92%), DOX (93.88%) and CIP (83.67%) were the most effective drugs against SGM isolates (Table 3).

Almost all of the isolates were resistant to INH (98.98%: RGM 50.50% and SGM 48.48%) and RMP (96.96%: RGM 50.50% and SGM 46.46%) (data not shown). RGM isolates were resistant to RMP (100%), INH (100%), and the greater part of them were resistant to DOX (78.00%), and SGM isolates were resistant to INH (97.96%) and RMP (93.88%) (Table 3).

Discussion

Nontuberculous mycobacteria are known to be ubiquitous in the environment. *Mycobacterium marinum*, *M. chelonae*, *M. peregrinum*, *M. gordonae*, *M. fortuitum*, *M. abscessus*, *M. mucogenicum*, *M. neoaurum*, *M. septicum*, *M. senegalense* and *M. szulgai* have been isolated from diseased fish (1, 11, 16, 44, 45, 63, 64) and have been shown to be the causative agents of pulmonary, skin, soft tissue and disseminated diseases in humans (1, 10, 11, 16, 36, 46, 54, 55). As in other countries, in Poland also Kwiatkowska *et al.* (32) observed an increased frequency of NTM isolated from clinical samples. Several isolates in the present study, namely *M. abscessus*, *M. chelonae*, *M. fortuitum*, *M. gordonae*, *M. marinum*, *M. mucogenicum*, *M. neoaurum*, *M. peregrinum* and *M. szulgai*, were also identified by Kwiatkowska *et al.* (32) in human clinical samples.

At present, standard therapeutic strategies for treating NTM infections are yet to be laid down. In this study, nine antimicrobial agents were tested against 99 NTM pathogens isolated from diseased ornamental fish (44). The growth of most NTM isolates was inhibited by AMK and KAN, only a few isolates showing a multidrug resistance profile which rendered these antimicrobials ineffective. Similarly, Yakrus *et al*. (67) also found that only 1 of 75 strains of *M. abscessus* and *M. chelonae* was resistant to AMK, and Swenson *et al.* (53) observed AMK to be most active against *M. fortuitum*. In the present study, the *M. abscessus*, *M. chelonae*, *M. fortuitum*, *M. mucogenicum*, *M. saopaulense* and *M. szulgai* isolates showed a multidrug resistance profile to at least three different classes of antimicrobials.

Mycobacterium marinum is intrinsically resistant to pyrazinamide and INH. Antibiotic agents that have been shown to be active against *M. marinum* include CLR, RMP, SMX, ethambutol, tetracyclines, some of the quinolones, and those used in combination therapy, *i.e.* ethambutol and RMP (27). The MICs of CIP, trimethoprim, azithromycin, telithromycin, quinupristin/dalfopristin, gemifloxacin, ofloxacin, and levofloxacin are above the concentrations usually obtained *in vivo*, and consequently, *M. marinum* may be considered resistant to them (5, 7, 47, 60). Chang and Whipps (11) showed that six strains of *M. marinum* isolated from diseased zebrafish were susceptible to AMK, CLR and RMP. In the present study, the majority of the *M. marinum* isolates were susceptible to AMK, CLR, KAN, DOX, CIP and SMX, whereas most isolates were resistant to RMP and INH.

There is currently no effective and definitive treatment for *M. gordonae* infection. Ethambutol, rifabutin, linezolid, CLR and new quinolones are active *in vitro* as antibiotics, but *in vivo* data are still insufficient (22, 56). Treatment with RMP, INH, pyrazinamide, and ethambutol was successfully used by Tsankova *et al.* (56). In a study by Goswami *et al.* (21), most of the *M. gordonae* isolates were sensitive to CLR and AMK and resistant to the first-line antitubercular drugs INH, RMP, ethambutol and streptomycin. In the

present study, the majority of *M. gordonae* isolates were susceptible to AMK, KAN, DOX, CLR and CIP and SMX, but resistant to INH and RMP.

Mycobacterium abscessus, *M. chelonae*, *M. salmoniphilum* and *M. saopaulense* are members of the *M. chelonae-abscessus* complex (35, 40, 53). Natural susceptibility to AMK, cefoxitin and imipenem and resistance to many other chemotherapeutic agents are characteristics of *M. abscessus* (35). Current treatment recommendations for *M. abscessus* pulmonary infections include therapy combining two or more intravenous drugs (AMK, tigecycline, imipenem and cefoxitin) with one or two oral antimicrobials, including macrolides, linezolid, clofazimine and, occasionally, a quinolone (38). Almost all *M. abscessus* strains tested by Shen *et al.* (51) were found to be resistant to SMX, vancomycin, oxacillin, clindamycin, and all fluoroquinolones, and more than 50% of the isolates were resistant to tetracyclines, carbapenems, and aminoglycosides, except for amikacin. The lowest resistance rates to cefoxitin (10%), azithromycin (10%), AMK (10%), and CLR (20%) (51) were demonstrated by *M. abscessus*. In this study, *M. abscessus* was susceptible to AMK, KAN and CLR, and resistant to TOB, DOX, SMX, RMP and INH. These findings are comparable to those described in other studies (14, 35, 43).

Regimens for the treatment of *M. chelonae* infections may include TOB, CLR, CIP, DOX and AMK. Hatakeyama *et al.* (25) showed that *M. chelonae* was susceptible to AMK, TOB, CLR, SMX, imipenem, linezolid and tigecycline. In this study, the majority of the *M. chelonae* isolates were susceptible to AMK, CLR and KAN, but resistant to DOX, CIP, SMX, RMP and INH.

The antimicrobial pattern of *M. saopaulense* is characterised by susceptibility to CLR and resistance to DOX, TOB and cefoxitin. Variable results, intermediate or resistant, were obtained with AMK, CIP, minocycline and moxifloxacin (40). In this research, the *M. saopaulense* isolate was susceptible to AMK, KAN and CLR, and resistant to DOX, SMX, RMP and INH.

Nogueira *et al.* (40) found that *M. salmoniphilum* was susceptible to AMK, CLR, and CIP and resistant to DOX, which is consistent with our results finding the test strain to be susceptible to AMK, KAN, TOB, CLR, CIP and SMX, and resistant to DOX, RMP and INH.

Mycobacterium fortuitum, *M. peregrinum*, *M. septicum* and *M. senegalense* are members of the *M. fortuitum* complex. *M. fortuitum* isolates are usually susceptible to multiple antimicrobial agents, including AMK, CIP, CLR, DOX, sulphonamides, cefoxitin, and imipenem (6, 53, 61). Hatakeyama *et al.* (25) showed that *M. fortuitum* was susceptible to AMK, CIP, moxifloxacin, imipenem, linezolid, meropenem and tigecycline. In our research, most of the *M. fortuitum* isolates were found to be susceptible to AMK, KAN, CIP and SMX, and resistant to TOB, DOX, RMP and INH. The results of this study correlate well with those of other investigators (33), differing only from those reported by Lee *et al.* (33), who found strains susceptible to CLR (93%) and DOX (84%).

At present, little information is available on antibiotic activity against *M. peregrinum*. In a study by Guz *et al.* (23), test strains of *M. peregrinum* were found to be susceptible to AMK, ofloxacin and capreomycin, and resistant to RMP, INH, streptomycin, ethambutol, sulfamethoxazole/trimethoprim, clofazimine and erythromycin cyclocarbonate. Santos *et al.* (48) showed that the new fluoroquinolones with the C8-methoxy group, especially moxifloxacin, exhibit greater activity against this species. In the present study, most strains were susceptible to AMK, KAN, CIP, SMX, CLR and TOB, which correlates well with the results reported by Hatakeyama *et al.* (25).

Most of the *M. septicum* strains tested by Lian *et al.* (36) were found to be susceptible to AMK, CIP, SMX, KAN, ofloxacin and levofloxacin. The strains of *M. septicum* described by Schinsky *et al.* (49) were susceptible to AMK, CIP, DOX, SMX, TOB, KAN, amoxicillin-clavulanate, erythromycin, imipenem, minocycline, trimethoprim-sulfamethoxazole, vancomycin, gentamicin and neomycin but resistant to ampicillin, cefamandole, cefotaxime, ceftriaxone and streptomycin. Go *et al.* (20) found *M. septicum* isolates to be susceptible to AMK, CIP, imipenem, linezolid, moxifloxacin, and trimethoprim-sulfamethoxazole but universally resistant to CLR and DOX. In our research, the *M. septicum* isolates were susceptible to AMK, KAN, TOB, CLR and SMX, but resistant to RMP and INH.

Talavilikar *et al.* (54) showed that *M. senegalense* was susceptible to AMK, CLR, CIP, DOX, cefoxitin, imipenem and trimethoprim/sulfamethoxazole, which is consistent with previously published results (2, 62). In the present study, most *M. senegalense* isolates were susceptible to AMK, KAN, TOB, DOX, CLR and SMX and resistant to RMP and INH.

The majority of *M. neoaurum* isolates tested by Brown-Elliott *et al.* (9) were susceptible to AMK, TOB, CIP, DOX, SMX, cefoxitin, gatifloxacin, imipenem, linezolid, moxifloxacin, tigecycline and trimethoprim/sulfamethoxazole. In our research, the *M. neoaurum* isolates were susceptible to AMK, KAN, TOB, DOX, CLR, CIP, SMX and INH and resistant to RMP and INH.

Rapidly growing mycobacteria are usually resistant to standard antimicrobial therapy, but *M. mucogenicum* is generally more susceptible to antimicrobials. Isolates of this species are susceptible to most antibacterial agents, *i.e.* AMK, KAN, CLR, CIP, imipenem, cefoxitin, linezolid, cephalothin, polymyxin B, and fluoroquinolones, but like other RGM, they are resistant to first-line antitubercular agents, *i.e.* RMP, INH, and pyrazinamide (8, 24, 52). Han *et al.* (24) reported that 100% (25/25) of *M. mucogenicum* isolates were susceptible to AMK, CLR, cefoxitin, imipenem and trimethoprim-sulfamethoxazole. In addition, 88% of isolates were susceptible to CIP, and 67% were susceptible to DOX, whereas 45% of strains were resistant to minocycline. Furthermore, Van Ingen *et al.* (58)

tested 15 *M. mucogenicum* strains against a panel of 11 antibiotics and found the majority to be susceptible to AMK, CLR, CIP and rifabutin. The current NTM practice guidelines do not explicitly state a specific treatment protocol for *M. mucogenicum* but do state that most isolates are susceptible to multiple antimicrobial agents, including AMK, CLR, KAN, DOX, quinolones and imipenem (4, 24). In our research, the *M. mucogenicum* isolate was susceptible to AMK, KAN and SMX but resistant to CIP, RMP and INH.

Isolates of *M. szulgai* are often susceptible *in vitro* to most antitubercular agents. The most common regimen includes RMP, ethambutol and macrolides and/or quinolones (59). Lung disease induced by *M. szulgai* was successfully treated with RMP, CLR and ethambutol by Kempisty *et al.* (30). In the present study, the *M. szulgai* isolate was susceptible to AMK, KAN, DOX, CLR, SMX and INH and resistant to RMP, CIP and TOB.

In summary, this study determined the antibiotic susceptibility of ornamental fish mycobacteria. The antimicrobial resistance rate of *Mycobacterium* spp. isolated from ornamental fish is high and thus needs to be monitored. Tested in this investigation for effect against isolates from diseased fish, KAN, AMK, CLR and SMX were the most inhibitory of rapidly growing mycobacteria, while AMK, KAN, SMX, CLR, DOX and CIP were the most efficacious against slowly growing mycobacteria. Our results confirm that antibiotic-resistant bacteria can be found in fish, which has potential consequences for public health. Consequently, continued monitoring of *Mycobacterium* spp. for antibiotic resistance should be performed in ornamental fish to help to establish strategies for the treatment of mycobacteriosis.

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