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Activity of antibiotics against *Mycobacterium* species commonly found in laboratory zebrafish

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Abstract

The zebrafish (*Danio rerio*) is a popular vertebrate model organism used in a wide range of research fields. Importance is placed on zebrafish health and the maintenance of disease-free laboratory fish so that experimental studies are not inadvertently impacted. Mycobacteriosis is a common infection of laboratory zebrafish that is caused by several *Mycobacterium* species. Little is known regarding the potential of antibiotic treatment for zebrafish mycobacteriosis; however, treatment of infected zebrafish may be appropriate to maintain valuable strains. Here, we investigate the antibiotic susceptibility of both rapid and slow growing zebrafish *Mycobacterium* spp. isolates *in vitro*. Antibiotic testing was carried out using a commercially available 96-well microtiter plate format. Results indicate that some but not all antibiotics tested are effective at inhibiting mycobacterial growth and that susceptibility varies among species and strains. Tigecycline, tobramycin, clarithromycin and amikacin were most effective at broad inhibition of rapid-growing mycobacteria; whereas, amikacin, clarithromycin, and rifampin were effective at inhibiting all slow-growing *Mycobacterium marinum* strains tested. Results support the potential for targeted antibiotic treatment of zebrafish infected with mycobacteria, but additional *in vivo* testing should be carried out.

INTRODUCTION

The zebrafish (*Danio rerio*) has become one of the most prominently used vertebrate model organisms (Dahm and Geisler 2006; Phillips and Westerfield 2014). Initially, zebrafish research was conducted in the fields of genetics and development with embryo and larval end points (Schilling and Webb 2007). More recently, adult zebrafish have become popular in the fields of oncology (Feitsma and Cuppen 2008), toxicology (Truong et al. 2011), aging (Gerhard 2003), and behavior (Wong et al. 2010). Laboratory colonies of zebrafish typically consist of both specialized mutant strains and wild-type strains used for breeding (Westerfield 2007). Due to the increased usage of adult zebrafish in biological research and the value placed on maintaining healthy mutant and wild-type stocks, laboratory zebrafish health is of utmost concern. Incidences of background infections are concerning for multiple reasons, for example: infections can result in fish mortality or decreased reproductive output (Kent et al. 2012a). Additionally, research can also be indirectly affected, as subclinical infections may be a source of uncontrolled experimental variance (or non-protocol variation). As such where possible, measures are taken in order to prevent, control and manage these diseases.

A summary report of cases submitted to the Zebrafish International Resource Center's (ZIRC) Diagnostic Services from 2006-2010 indicates that over 40% of facilities submitting specimens had fish diagnosed with mycobacteriosis (Kent et al. 2012b). Mycobacteriosis is caused by *Mycobacterium* spp. and is frequently found in wild and captive fishes (Chinabut 1999), including ornamental marine and freshwater fishes (Noga 2010). There is no single agent of zebrafish mycobacteriosis as at least six species as well as several strains of *Mycobacterium* spp. have been identified (Astrofsky et al. 2000; Kent et al. 2004; Whipps et al. 2008). In particular, *Mycobacterium marinum*, *Mycobacterium chelonae*, *Mycobacterium abscessus*, *Mycobacterium peregrinum*, *Mycobacterium haemophilum* and *Mycobacterium fortuitum* are frequently associated with zebrafish mycobacteriosis (Whipps et al. 2012). Severity of infection varies between species and strains of *Mycobacterium* spp. ranging from high levels of mortality with *M. marinum* and *M. haemophilum*, and little to no observed mortality with *M. abscessus* and *M. chelonae* (Watral and Kent 2007; Whipps et al. 2007a; Whipps et al. 2007b; Whipps et al. 2012). Morbidity due to infection is also variable and includes external signs such as skin lesions, emaciation, raised scales, swollen abdomen, and irregular or lethargic swim behavior (Astrofsky et al. 2000; Kent et al. 2012b). Internally, infection can be observed as granulomas, particularly on the spleen, kidneys and liver (Whipps et al. 2012). Diagnosis may be further complicated because signs of disease are often not observed in subclinical infections (Kent et al. 2004; Whipps et al. 2012).

Recent reviews of zebrafish diseases, including mycobacteriosis, highlight the importance of preventative measures such as quarantine, regular disinfection of eggs and surfaces, UV sterilization of water, and sentinel programs in zebrafish facilities (Kent et al. 2009; Whipps et al. 2012). Once mycobacteriosis is established in a facility control and management of the disease becomes a major challenge and involves invasive measures such as depopulation, facility sterilization and re-derivation of zebrafish populations. Although these measures have been demonstrated to be effective at controlling mycobacteriosis, such intensive measures may not always be feasible if this disease becomes established during an ongoing experiment or in a valuable zebrafish mutant line (Whipps et al. 2012). Alternative methods for controlling and treating zebrafish mycobacteriosis such as targeted use of antibiotics should be considered.

Antibiotic treatment of non-tuberculosis mycobacteriosis in humans is routine (Griffith et al. 2007; Wu et al. 2012), but similar treatments in fish have not been investigated thoroughly. Treatment of fish destined for human consumption with antibiotics is not generally considered feasible as treatments are expensive, long in duration and there are concerns regarding the use of pharmaceuticals in fish for human consumption (Whipps et al. 2012). A limited number of studies investigating antibiotic treatment of fish infected with *M. marinum* have been previously conducted and include treatment of both food fish [yellowtail (*Seriola quinqueradiata*): Kawakami and Kusuda 1990; striped bass (*Morone saxatilis*): Hedrick et al. 1987; sea bass (*Dicentrarchus labrax*): Colorni et al. 1998] and hobby fish [gouramis (*Trichogaster trichopterus*): Santacana et al. 1982; firemouth cichlid (*Cichlasoma meeki*): Boos et al. 1995; Congo tetra (*Phenacogrammus interruptus*): Boos et al. 1995; guppies (*Lebistes reticulatus*): Conroy & Conroy 1999]. The results from these studies are highly variable and range from an observed elimination of infection to no decrease in the

disease; however, these studies all used different treatment methods (i.e., antibiotic added to food, in water bath, in intraperitoneal injection), antibiotic doses, and different experimental end-points to determine the effectiveness of antibiotic treatment. In addition, the susceptibility of fish mycobacteria to antibiotics has not yet been evaluated *in vitro*. Thus, the efficacy of antibiotics as a potential treatment method for zebrafish mycobacteriosis cannot be extrapolated from these previous studies. An evaluation of antibiotic susceptibility of *Mycobacterium* spp. isolated from infected zebrafish is required in order to determine the potential for antibiotic treatment of mycobacteriosis in zebrafish.

Here we investigate the *in vitro* antibiotic susceptibility of rapid and slow growing *Mycobacterium* spp. isolated from infected fish from different zebrafish facilities in the United States. Antibiotic susceptibility will be evaluated through determination of the minimum inhibitory concentration (MIC) of antibiotic required to inhibit bacterial growth in culture. We utilized a commercially available microtiter panel system that is commonly used for drug susceptibility testing of clinical *Mycobacterium* spp. infections. We hypothesized that the *Mycobacterium* species and strains examined in this study display antibiotic MICs consistent with those already identified for human isolates.

METHODS

Bacterial Strains

Isolates maintained in our culture collection are described in Table 1. All organisms have previously been identified based on *hsp65* gene sequencing as described previously (Kent et al. 2004; Poort et al. 2006; Whipps et al. 2007a; Whipps et al. 2007b). In addition to these isolates, reference cultures of rapidly growing *Mycobacterium salmoniphilum* (ATCC13758) and slow growing *M. marinum* (ATCC927) are also included in this study. All isolates were grown on solid-phase Middlebrook 7H10 (MB 7H10) agar (BD Biosciences 262710) supplemented with oleic albumin dextrose catalase (OADC, BD Biosciences 211886) at 28-30°C for seven (rapid-growing) or 14 (slow-growing) days prior to MIC testing.

MIC Testing

MIC testing was performed using the commercially available Sensititre rapid- and slow-growing *Mycobacterium* MIC panels (TREK Diagnostics). For rapid-growing isolates the Sensititre RAPMYCO panel was used according to the instructions of the manufacturer (described in Cavusoglu et al. 2012), with the exception of the use of cation adjusted Mueller Hinton broth (CAMHB, Teknova M5887) without TES buffer as recommended in the CLSI M24-A2 guidelines (CLSI 2011). The inoculated RAPMYCO panel was incubated for three days at 28-30°C. Slow-growing isolates were tested using the Sensititre SLOMYCO panel following the CLSI M24-A2 (CLSI 2011) and the manufacturer's guidelines. An inoculum was prepared by sweeping a confluent portion of growth from the MB 7H10 agar plate and emulsifying it in 5 ml of sterile water. The inoculum density was adjusted to 0.5 McFarland standard using a nephelometer (Sensititre). Fifty µL of the inoculum was diluted in 11 mL CAMHB supplemented with 5% volume of OADC enrichment. The inoculated broth suspension was vortexed and then 100 µL was added to each well of the SLOMYCO MIC panel. The inoculated panel was incubated for seven days

at 28-30°C. Additional positive control plates were prepared by inoculating MB 7H10 agar plates with 1µL of the positive control well contents. Serial dilutions of the inoculated CAMHB were prepared to verify density of 1.0×10^5 - 1.0×10^6 colony forming units (CFU)/ml for the panel inoculum.

Following incubation, the panels were scored daily for three consecutive days to ensure consistent scoring. Growth was scored following the CLSI M24-A2 guidelines for interpretation of broth microdilution MIC end points (CLSI 2011). The MIC for each antibiotic agent tested was determined based on these scores; in the scenario of growth cessation at different antibiotic concentrations between daily readings, the highest MIC was chosen. MIC panels were run in triplicate for each species of *Mycobacterium* included in this study. There are no published breakpoints for MIC values for zebrafish mycobacteria; however, values are available for clinical isolates from humans. These available breakpoints were used to classify MICs as susceptible, intermediate, or resistant.

RESULTS

All species grew successfully in the broth medium and growth endpoints were easily determined. Growth was observed in the positive control well of all panels, as well as on the positive control agar plates. Growth counts of the serial dilution plates confirmed an inoculating density of 1.0×10^5 - 1.0×10^6 CFU/ml.

Rapidly growing *Mycobacterium* spp

A summary of MIC results for six rapid-growing species of *Mycobacterium* spp. tested with 15 different antibiotic treatments is given in Table 2. Species and strain differences in antibiotic susceptibility are observed as well as variation in the effectiveness of different treatments. Using the CLSI M24-A2 MIC break-points for rapidly growing *Mycobacterium* spp., strains were categorized as susceptible, intermediate, or resistant to treatment. Species in the *M. fortuitum* group (*M. fortuitum* and *M. peregrinum*) and *M. salmoniphilum* (ATCC13758) are susceptible to amikacin treatment; however *M. abscessus* and *M. chelonae* strains exhibited intermediate susceptibility. *Mycobacterium chelonae* strains and *M. salmoniphilum* show resistance to cefoxitin. *Mycobacterium abscessus* and *M. fortuitum* show intermediate cefoxitin susceptibility while *M. peregrinum* displays susceptibility. Ciprofloxacin results show resistance or intermediate susceptibility in all strains except the *M. fortuitum* group species which are susceptible to treatment. Susceptibility to clarithromycin is observed in *M. chelonae* species, *M. salmoniphilum* and *M. peregrinum*, but *M. abscessus* and *M. fortuitum* are resistant. Resistance to doxycycline is observed in all strains tested with the exception of one *M. chelonae* strain (ESF35) that was susceptible to treatment. Interestingly, this same strain of *M. chelonae* independently susceptible to doxycycline is the only strain that exhibits resistance to imipenem, while the other strains tested showed intermediate to complete susceptibility. Tobramycin was effective against growth as results show intermediate to full susceptibility among all strains tested. Based on breakpoints listed for sulfamethoxazole all strains tested are resistant to the trimethoprim/sulfamethoxazole treatment.

Of the antibiotic treatments tested without CLSI breakpoints listed, no MIC was observed for treatment with cefepime or ceftriaxone. Amoxicillin/clavulanic acid treatment resulted in no MIC for *M. chelonae* and *M. abscessus* strains and a MIC of 8/4 µg/mL and 16/8 µg/mL for *M. fortuitum* and *M. peregrinum* respectively. No MIC was observed for minocycline treatment except for the previously observed unique *M. chelonae* strain. Moxifloxacin and linezolid treatment resulted in high or no MIC for all strains except *M. fortuitum* and *M. peregrinum*. Tigecycline was effective at inhibiting growth for all species with a MIC range of 0.12-2.0 µg/mL.

Slow growing *Mycobacterium* spp

A summary of MIC results for six slow-growing species of *M. marinum* tested with 13 different antibiotic treatments is given in Table 3. Some strain differences in antibiotic susceptibility are observed as well as variation in the effectiveness of different treatments. Using the CLSI M24-A MIC breakpoint indicating resistance for *M. marinum*, strains were categorized as resistant to treatment. All strains of *M. marinum* tested were susceptible to amikacin and clarithromycin treatments. Resistance to trimethoprim/sulfamethoxazole treatment was observed for all strains tested. Variation in susceptibility between strains was observed for ciprofloxacin, doxycycline, ethambutol, moxifloxacin, rifabutin and rifampin treatments. No CLSI resistance breakpoints are available for ethionamide, isoniazid, linezolid or streptomycin. All strains show MICs for ethionamide at lower test concentrations. Variation in MICs for isoniazid and linezolid were observed across strains, and streptomycin MICs were at the high end of the range of concentrations tested.

DISCUSSION

Overall, the results from this study indicate that there are differences in the effectiveness of each antibiotic tested and that variable antibiotic susceptibilities are observed across different species and strains of mycobacteria. Due to these differences, the type of *Mycobacterium* spp. causing infection should be identified before considering a particular antibiotic treatment.

Antibiotic susceptibilities of different *Mycobacterium* spp

Rapid growers—Infection of *M. chelonae* and *M. abscessus* is common in zebrafish, and infections often present as subclinical with little to no external signs of infection (Watrall and Kent 2007; Whipps et al. 2007a; Whipps et al. 2007b; Whipps et al. 2012). These subclinical infections are extremely concerning as they can be a source of uncontrolled experimental variance (Kent et al. 2004; Whipps et al. 2012). For human clinical *M. chelonae* infections tobramycin is the first recommended treatment, followed by clarithromycin and linezolid as second choices (Cavusoglu et al. 2012; Griffith et al. 2007). Our results show that *M. chelonae* isolates from zebrafish are similarly susceptible to tobramycin and clarithromycin, as well as tigecycline. We also observed resistance to ceftiofur in addition to amoxicillin/clavulanic acid, ciprofloxacin, doxycycline, minocycline, and trimethoprim/sulfamethoxazole. Interestingly, of the *M. chelonae* strains tested, one strain (ESF35) was uniquely susceptible to doxycycline and resistant to imipenem treatments indicating that

there is some variability in drug susceptibility testing amongst different strains of *M. chelonae*.

Results from human clinical research indicate that *M. abscessus* is uniformly resistant to standard antituberculosis agents, and drug-susceptibility testing is highly recommended prior to treatment (Griffith et al. 2007). Recommended antibiotic treatments include clarithromycin, amikacin and cefoxitin; however, clinical strains of *M. abscessus* have been shown to have acquired mutational resistance to clarithromycin and amikacin (Cavusoglu et al. 2012; Griffith et al. 2007). We showed that zebrafish *M. abscessus* is: susceptible to tigecycline; shows intermediate susceptibility to amikacin, cefoxitin and imipenem; and was resistant to all other antibiotic tested. Drug susceptibilities are very similar when comparing *M. chelonae* and *M. abscessus* isolates, which is not surprising as they phylogenetically group within the *M. chelonae* complex (Kent et al. 2004).

Mycobacterium salmoniphilum is a *M. chelonae*-like bacterium isolated from salmon (Ross, 1960; Whipps et al. 2007a). Phylogenetically, it is nested within the *M. chelonae* complex (*M. chelonae* and *M. abscessus*) (Whipps et al. 2007a). Thus, similarities in antibiotic susceptibility to *M. chelonae* are not surprising. One difference is that *M. salmoniphilum* is also susceptible to amikacin whereas *M. chelonae* is not. This pathogen has never been isolated from zebrafish, but has been reported in salmonids, and knowledge of the antibiotic susceptibility of this species might be of interest to that industry.

Infection with *M. fortuitum* and *M. peregrinum*, both members of the *M. fortuitum* complex, are less frequently observed in zebrafish; however, *M. fortuitum* is ubiquitous in water (Galassi et al. 2003). Zoonotic transmission to humans from fish has also been previously reported for *M. fortuitum* (Astrofsky et al., 2000). Clinical research indicates that *M. fortuitum* and *M. peregrinum* are susceptible to many antibiotic treatments, and doxycycline, minocycline, clarithromycin, linezolid and sulfonamides are recommended for treatment in humans (Cavusoglu et al. 2012; Griffith et al. 2007). It should be noted that *M. fortuitum* has previously displayed macrolide (i.e., clarithromycin) resistance. Interestingly, our results show different susceptibilities compared to human isolates; we observed susceptibilities to amikacin, ciprofloxacin, imipenem and tigecycline. *Mycobacterium peregrinum* infections in zebrafish have been reported as low-severe (Kent et al. 2004). In this study, *M. peregrinum* shows similar antibiotic susceptibilities as what we observe for *M. fortuitum* with the addition of cefoxitin and clarithromycin susceptibilities.

Slow growing *M. marinum*—Out of all the *Mycobacterium* spp. included in this study, zebrafish *M. marinum* infections are the most severe (Watrall and Kent 2007; Whipps et al. 2007a; Whipps et al. 2007b; Whipps et al. 2012). Also, zoonotic transmission of *M. marinum* (i.e., fish handlers' disease) is a major concern as treatment is lengthy and could require debridement (Wu et al. 2012). Clinical recommendations for treatment in humans include multiple antibiotics which are commonly used in combination and include: rifampin, rifabutin, ethambutol, clarithromycin, sulfonamides, trimethoprim/sulfamethoxazole, doxycycline and minocycline (Griffith et al. 2007). In addition, clinical testing has shown resistance to isoniazid (Griffith et al. 2007). Previous studies in yellowtail fish infected with *M. marinum* show rifampin, streptomycin and erythromycin to be effective antibiotic

treatments; however, a *Mycobacterium* sp. was re-isolated from surviving fish following treatment in this study suggesting complete elimination of infection was not successful (Kawakami and Kusuda 1990). Our results show that all six strains of *M. marinum* tested were susceptible to amikacin, clarithromycin, and rifampin. Variation in susceptibilities is observed for the other antibiotics tested, and some strains of *M. marinum* are more susceptible to treatment.

Additional considerations for antibiotic treatment of *Mycobacterium* spp

Although *Mycobacterium* spp. isolated from zebrafish responded similarly *in vitro* to different antibiotic treatments compared to human clinical isolates, further *in vivo* testing is required in order to confirm susceptibilities due to the following considerations. First, species of *Mycobacterium* have been reported to have different antibiotic susceptibilities depending on their environment. This difference is thought to be due to differences in gene regulation when mycobacteria are in stationary metabolic phases within biofilms or active phases within a host (reviewed in van Ingen et al., 2012). For example, *M. abscessus* has smooth and rough colony types due to differences in the expression of cell wall glycolipid content (regulated by *mtrAB* expression) that confers natural resistance to antibiotics through glycolipid-rich cell walls in rough colonies (Cangelosi et al. 1999; Cangelosi et al. 2006). In addition to this, the route of drug administrations should be taken into account. Common antibiotic administration techniques used for fish are medicated feed, bath, and injection (Toutain et al. 2010; Yanong 2013). Of these, medicated feed is the most common. When antibiotics are administered through feed they are absorbed into the gut epithelium and enter systemic circulation following a hepatic first-pass (Toutain et al. 2010). Loss of drug commonly occurs during this first-pass and depends on the amount of catabolism occurring in the liver. Although this metabolic pathway is similar to humans, drug bioavailability has been reported lower in fish compared to humans (Martinsen and Horsberg 1995). Similar concerns arise with bath treatments, where drugs enter through the gills and undergo a renal-pass (Toutain et al. 2010). Water-quality issues also arise for topical bath treatments (Yanong 2013). Treatment dose and duration also require further investigation as details regarding pharmacokinetics remain to be elucidated in fish (Yanong 2013). Additionally, zebrafish susceptibility to antibiotic treatment may be strain specific and different genetic lines of fish may respond differently to antibiotic treatment, as variation in strain susceptibility to mycobacterial infections has been previously observed.

Results from this study also indicate that zebrafish mycobacteria are less resistant to antibiotics compared to clinical isolates. This is likely due to less frequent exposure of these zebrafish isolates to antibiotics and therefore weaker selection for resistance. However, antibiotic resistance is common in bacteria (including mycobacteria) isolated from ornamental fish, an industry where antibiotic use is common (Rose et al. 2013). These ornamental fish bacteria have been shown to possess plasmids that may carry resistance genes and acquisition of antibiotic resistance has been shown in to be transferable in fish isolates of *Pseudomonas* spp. and *Aeromonas* spp. between resistant and susceptible strains (Rose et al. 2013). Additionally, acquisition of antibiotic resistance has been shown in to be transferable in fish isolates of *Pseudomonas* spp. and *Aeromonas* spp. between resistant and susceptible strains (Rose et al. 2013). Thus, high potential for zebrafish mycobacteria to

acquire additional antibacterial resistance exists and diligence should surround the usage of antibiotics in laboratory zebrafish. Additionally, because some species of zebrafish mycobacteria are zoonotic and treatment of these infections in humans can be long in duration and require multiple antibiotics (Wu et al. 2012) the potential consequence of antibiotic resistance for fish handlers is of great concern. Considering these risks, it is important to emphasize that antibiotic use in zebrafish be used judiciously. A specific example would be to treat a valuable or rare line of brood fish prior to breeding. We emphasize that infection would need to be diagnosed and the *Mycobacterium* species identified so that the appropriate antibiotic could be used. It would not likely be practical or wise to treat large groups of fish.

In conclusion, *Mycobacterium* spp. isolated from laboratory zebrafish do exhibit susceptibility to some, but not all antibiotics tested. Antibiotic susceptibilities show variation that coincides with phylogenetic groupings of *Mycobacterium* spp. highlighting the importance of species identification to determine the most effective antibiotic treatment for inhibiting mycobacterial growth. Similarities in drug susceptibilities are observed between zebrafish and human isolates; however, zebrafish isolates were resistant to fewer antibiotics, most likely due to the low usage of antibiotics currently in zebrafish compared to humans. More research is required in order to test antibiotic susceptibility of these isolates *in vivo* due to environmentally regulated changes in bacterial gene expression as well as the influence pharmacokinetics has on antibiotic availability.

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TABLE 1

Isolate list for all cultures used for antibiotic susceptibility testing.

Isolate ID	Species	Location	Host	Reference (source)
ESF35	<i>M. chelonae</i>	Pennsylvania, USA	Zebrafish	This study
ZF-48	<i>M. chelonae</i>	Oregon, USA	Zebrafish	Whipps et al. (2008)
ZF-55	<i>M. chelonae</i>	Oregon, USA	Zebrafish	Whipps et al. (2008)
H11-27-1	<i>M. chelonae</i>	New York, USA	Zebrafish	This study
H11-05	<i>M. chelonae</i>	North Carolina, USA	Zebrafish	Whipps et al. (2014)
JAN1	<i>Mycobacterium</i> sp. ^a	Oregon, USA	Zebrafish	Kent et al. (2004)
KCI	<i>M. abscessus</i>	Pennsylvania, USA	Zebrafish	Kent et al. (2004)
ATCC13758	<i>M. salmoniphilum</i>	Washington, USA	Chinook salmon	ATCC13758
ESF36	<i>M. fortuitum</i>	Pennsylvania, USA	Zebrafish	This study
SM4	<i>M. peregrinum</i>	Washington DC, USA	Zebrafish	Kent et al. (2004)
MA-1	<i>M. marinum</i>	Massachusetts, USA	Zebrafish	This study
AR103K	<i>M. marinum</i>	Arkansas, USA	Zebrafish	This study
OR932	<i>M. marinum</i>	Oregon, USA	Zebrafish	This study
ATCC927	<i>M. marinum</i>	Pennsylvania, USA	Salt water fishes ^b	ATCC927
TGI9	<i>M. marinum</i>	Oregon, USA	Zebrafish	This study
OSU214	<i>M. marinum</i>	Oregon, USA	Zebrafish	Ostland et al. (2007)

^a Originally characterized as *M. chelonae* by Kent et al. (2004), subsequently recognized as a distinct entity, but member of the *M. chelonae* complex by Whipps et al. (2007a)

^b First isolated and identified by Aronson (1926) from three saltwater fish species: sergeant major (*Abudefduf mauritii*); croakers (*Micropogon undulatus*); sea bass (*Centropristis striatus*).

TABLE 2

Minimum inhibitory concentrations (ug/ml) for rapidly growing mycobacterial strains in the microdilution broth system. Each isolate culture was tested in triplicate; a single MIC is shown when all replicates resulted in the same MIC. Reference susceptibility (S), intermediate (I) and resistance (R) breakpoints for antibiotics are indicated. Bolded results indicate MICs indicating antibiotic susceptibility.

Antibiotic Agent (S:I:R)	Mycobacterium sp.			M. chelonae			M. abscessus			M. salmoniphilum			M. fortuitum		M. peregrinum	
	JAN1	ESF35	ZF-48	ZF-55	H11-27-1	H11-05	KC1	ATCC13758	ESF36	SM4						
Amikacin (16; 32; 64)	64	32	32, 64, 64	16, 32, 32	32, 32, 64	32, 64, 64	16, 32, 64	16, 16, 32	<1, 8, 16	<1						
Amoxicillin ^a / clavulanic acid ^a	64/32, >64/32, 64/32	>64/32	>64/32	>64/32	>64/32	>64/32	>64/32	64/32, >64/32, >64/32	8/4, 8/4, 16/8	16/8						
Cefepime ^a	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32						
Cefoxitin (16; 32-64; 128)	>128	>128	>128	>128	>128	>128	<32	>128	<16, <32, <32	<16						
Ceftriaxone ^a	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64						
Ciprofloxacin (1; 2; 4)	>4	4	>4	4, >4, >4	>4	>4	>4	1, 2, 4	<0.12	<0.12						
Clarithromycin (2; 4; 8)	2	0.5, 0.5, 1	1, 1, 2	2, 4, 4	1	2	4, >16, >16	1, 1, 2	0.5, >16, >16	0.25, 0.25, 0.5						
Doxycycline (1; 2-8; 16)	>16	1, 1, 2	>16	>16	>16	>16	>16	>16	>16	>16						
Imipenem (4; 8; 16)	4, 8, 8	32	8, 16, 16	8, 16, 16	8, 16, 16	8, 16, 16	4, 8, 8	8, 8, 8	<2	<2						
Linezolid ^a	32	16, 32, 32	<2, >32, >32	32, 32, >32	16, 32, 32	32, 32, >32	>32	16	2, 8, 8	2						
Minocycline ^a	>8	<1	>8	>8	>8	>8	>8	>8	>8	2, 4, >8						
Moxifloxacin ^a	>8	<4, <8, <8	>8	<8, >8, >8	>8	>8	>8	<2, <2, <4	<0.25	<0.25						
Tigecycline ^a	0.25	0.12, 0.12, 0.24	0.25, 0.25, 0.5	0.25, 0.25, 1	0.12, 0.25, 0.25	0.5	0.5, 2, 2	0.25	<0.015, 0.06, 0.12	0.03, 0.5, 2						
Tobramycin (4; 8; 16)	8, 8, 16	4, 4, 8	4, 4, 8	4	4, 8, 8	4, 4, 8	16, 16, >16	4, 4, 8	8, 16, 16	8						
Trimethoprim ^a / sulfamethoxazole (32; - ; 64)	>8/152	8/152	>8/152	>8/152	>8/152	>8/152	>8/152	2/38, 4/76, 8/152	>8/152	8/152, >8/152, >8/152						

^aNo breakpoints indicating susceptibility, intermediate or resistance to antibiotic agent available for this antibiotic in the CLSI M24-A2 standard (CSLI, 2011)

TABLE 3

MIC (ug/ml) for slow growing *M. marinum* strains in the microdilution broth system. Each isolate culture was tested in triplicate; a single MIC is shown when all replicates resulted in the same MIC. Reference resistance (R) breakpoints for antibiotics are indicated. Bolded results indicate MICs indicating antibiotic susceptibility.

Antibiotic Agent (R)	MIC (µg/ml) for SGM isolates					
	MA-1	ARI03K	OR932	ATCC927	TG19	OSU214
Amikacin (>32)	<1, 2, 2	<1, 2, 2	2, 2, 4	2	2, 2, 4	2
Ciprofloxacin (>2)	1, 4, 4	4, 2, 8	4, 4, 8	4, 4, 8	8, 8, 8	2, 2, 4
Clarithromycin (>16)	2, 4, 4	2, 2, 4	4	2	2, 4, 4	2, 4, 4
Doxycycline (>4)	2, 4, 4	4, 4, >16	4, 4, 8	2	2, 4, 4	8
Ethambutol (>4)	1, 8, >16	4, 8, 16	4, 4, >16	2, 2, 4	4, >16, >16	8
Ethionamide ^a	<0.3	<0.3	0.6, 0.6, 1.2	0.6, 0.6, 2.5	0.6	0.6, 0.6, 1.2
Isoniazid (>16)	0.5, 2, 1	1	1, 2, 2	4, 4, >8	4	8
Linezolid ^a	1, 8, 1	8, 8, 32	<1, <1, <1	<1, <1, 2	8, 8, 8	<1, <1, <2
Moxifloxacin (>2)	1, 4, >8	1, 1, >8	2, 4, >8	4, 2, 2	2, >8, >8	4, 2, 2
Rifabutin (>2)	2, 4, 4	4, 8, >8	4, 8, 8	4, 4, 8	8	4, 4, 8
Rifampin (>1)	1, >8, >8	2, >8, >8	2, 4, >8	4, >8, >8	2, >8, >8	4
Streptomycin ^a	8, 16, 16	8	16	16, 16, 8	16	8
Trimethoprim/ sulfamethoxazole (>2/38)	0.5/9.5, >8/152, >8/152	8/152, 8/152, >8/152	8/152, 8/152, >8/152	>8/152	8/152, >8/152, >8/152	>8/152

^aNo breakpoints indicating resistance to antibiotic agent available for this antibiotic in the CLSI M24-A2 standard (CLSI, 2011)