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Mycobacterium marinum infection in fish and man: epidemiology, pathophysiology and management; a review

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ABSTRACT

Mycobacterium marinum is an opportunistic pathogen inducing infection in fresh and marine water fish. This pathogen causes necrotizing granuloma like tuberculosis, morbidity and mortality in fish. The cell wall-associated lipid phthiocerol dimycocerosates, phenolic glycolipids and ESAT-6 secretion system 1 (ESX-1) are the conserved virulence determinant of the organism. Human infections with *Mycobacterium marinum* hypothetically are classified into four clinical categories (type I–type IV) and have been associated with the exposure of damaged skin to polluted water from fish pools or contacting objects contaminated with infected fish. Fish mycobacteriosis is clinically manifested and characterized in man by purple painless nodules, liable to develop into superficial crusting ulceration with scar formation. Early laboratory diagnosis of *M. marinum* including histopathology, culture and PCR is essential and critical as the clinical response to antibiotics requires months to be attained. The pathogenicity and virulence determinants of *M. marinum* need to be thoroughly and comprehensively investigated *and* understood. In spite of accumulating information on this pathogen, the different relevant data should be compared, connected and globally compiled. This article is reviewing the epidemiology, virulence factors, diagnosis and disease management in fish while casting light on the potential associated public health hazards.

1. Introduction

Fish mycobacteriosis is a chronic progressive disease caused by ubiquitous acid-fast bacilli, identified as non-tuberculous mycobacteria (NTM) (Novotny et al. 2004). NTM could be classified into slowly and rapidly growing mycobacteria, where *Mycobacterium marinum* is affiliated to the first class. *Mycobacterium marinum*, *M. fortuitum* and *M. chelonae* are among the most identified NTM mycobacterial species associated with fish mycobacteriosis (Novotny et al. 2004). Piscine mycobacteriosis is a common disease of marine, brackish and freshwater fish (Decostere et al. 2004) infecting more than 200 species of freshwater and marine fish in a vast region extending from the subarctic zone to the tropical one (Zanoni et al. 2008; Gauthier and Rhodes 2009; Jacobs et al. 2009; Ackleh et al. 2015). This disease also infects tropical aquarium fish (Noga 2010) and is considered a major cause of morbidity and mortality in free-living fish (Chang and Whipps 2015).

Mycobacterium marinum is an environmental, aerobic waterborne bacterium, belonging to photochromogenic Group I non-tuberculous *Mycobacteria* according to Runyon's classification. It has a relatively short generation time amounting to 4–6 h as compared to 20 h in the case of *M. tuberculosis*, and optimally grows at 30 °C but hardly at 37 °C (Clark and Shepard 1963). *M. marinum* is one of the most common atypical *Mycobacteria* that cause human opportunistic infection (Rallis and Koumantaki-Mathioudaki 2007). It is considered as the most important fish pathogen, associated with multiple symptoms, e.g. uncoordinated swimming, abdominal swelling, loss of weight, skin ulceration, white nodule formation as granuloma in liver, kidney, spleen in both fresh and marine water fish (Ferguson 2006; El Amrani et al. 2010).

The transmission of *M. marinum* between fishes is poorly understood. The primary route of infection is the oral one via consumption of infected dead fish, contact with affected fish skin or through gills (El Amrani et al. 2010). *M. marinum* outbreaks were reported in striped bass in USA (Gauthier et al. 2008), in sturgeon fish in China (Zhang et al. 2015) and in F1 captive-bred Australian lung fish (Strike et al. 2016). Fish mycobacteriosis has become an important threat to aquatic industry coinciding with the rapid development of sturgeon's culture in China (Li et al. 2009).

Mixed mycobacterial infections have an economic impact in sturgeon in China, being associated with more than 50% mortality during 2009–2010 (Zhang et al. 2015).

Mycobacterium marinum is posing a zoonotic concern, since it produces granulomatous lesions in human skin and deep tissues (Lewis et al. 2003; Petrini 2006). Additionally, it causes cutaneous infections manifested by ulcers, skin nodules and nodular lymphangitis, when exposed to abrasions and lacerations in salt water or injuries associated with fish spines (Jernigan and Farr 2000). Human infection consists of nodular cutaneous lesions that could progress to tenosynovitis, arthritis and osteomyelitis (Aubry et al. 2002; Wongworawat et al. 2003). Humans may contract infection with a fish spine injury occurring at a fishing pond, in the fish processing industry or through contaminated pond water (Tsai et al. 2007).

Since the infection severity of *M. marinum* is very wide, ranging from spontaneous healing to deep long-lasting infection (Aubry et al. 2002), the identification of existent or emerging virulent strains of *M. marinum* is highly recommended for the control of this potential human health hazard. Many versatile genotyping methods are being developed for classifying *M. marinum* such as variable number of tandem repeat (van der Sar et al. 2004; Ucko and Colorni 2005). The ensuing results of such studies on *M. marinum* need to be compared, correlated, connected and globally compiled. In response to this need, this article is reviewing the epidemiology, virulence factors, diagnosis and disease management in fish while casting light on the potential associated public health hazards and focusing on the zoonotic significance of this pathogen and referring to the possible areas of insufficient studies.

2. Etiological agent and ecology

Piscine mycobacteriosis is caused by different NTM mycobacterial species. *M. marinum* was first reported in marine fish by Von Betegh (1910) and first isolated by Aronson (1926). *M. marinum* belongs to the Order Actinomycetales, Suborder Corynebacterineae and Family Mycobacteriaceae. It induces fish tank granuloma in humans, which was first described in 1951, as resulting from '*Mycobacterium balnei*' (Nordén and Linell 1951). Linell and Norden (1954) recognized this pathogen after investigating eighty persons who used the same public swimming pool and were affected by granulomatous lesions in their skin. Later on the causative agent of piscine mycobacteriosis in man was confirmed as *M. marinum* (Swift and Cohen 1962).

This bacterium is pleomorphic, Gram-positive, acid-fast, aerobic, taking the shape of non-motile rods. The bacterium cell wall is specifically rich in mycolic acids, which are the major and specific lipid component in the cell wall envelope. They are 3-hydroxy long chain fatty acid (60–90 carbon atoms) (Gangadharam and Jenkins 1997) which are essential for the survival of members of the genus (Marrakchi et al. 2014). The genus includes approximately 75 species of close relations based on similarities in their sequences of 16S rRNA (Goodfellow and Magee 1998). Additionally, the cell wall envelope of *Mycobacterium* sp. comprises other lipids, e.g. trehalose 6,6-dimycolate (TDM), lipoarabinomannan (LAM), phthiocerol dimycocerosates (PDIMs), phenolic glycolipids (PGLs) (Draper 1991; Daffé and Draper 1997; Smith 2003; Onwueme et al. 2005). These groups of lipids are closely associated with the virulence of *Mycobacterium* (Brennan 2003; Smith 2003; Reed et al. 2004).

M. marinum is an ubiquitous waterborne bacterium with an optimal growth temperature around 30 °C, which causes the infection of bats, fish, mice and amphibians after its inoculation (Clark and Shepard 1963). Experimental infection of mice with *M. marinum* at a temperature below 30 °C developed pulmonary lesions, but not at 34 °C (Clark and Shepard 1963). *M. marinum* is prevalent all over the world in marine water, brackish water, and fresh water and naturally infects more than 150 species of fish, frog, freshwater eels and oyster (Beecham et al. 1991; Zeeli et al. 2003). In Africa, *M. marinum* has been isolated from normal humans' skin and also from soil (Pattyn, 1984). It grows on Lowenstein–Jensen media at 30 °C within duration of 2–3 weeks (Vincent et al. 2003).

M. marinum can invade macrophages, preventing the fusion of phagosome-lysosome and replicating inside (El-Etr et al. 2001). *M. marinum* strains could be classified into two virulence groups based on their genetic diversity. Molecular variations were spotted among *M. marinum* strains isolated from different environments (Ang et al. 2000). Two clusters (I and II) of *M. marinum* could be defined with the use of amplified fragment length polymorphism. Cluster I of *M. marinum* was isolated from humans suffering from fish tank granuloma and with acute infection symptoms, while cluster II strains were originating from poikilothermic species and were predominantly associated with chronic infection (van der Sar et al. 2004). Hence, the detection of some genotypes of *M. marinum* in humans may suggest variable zoonotic potentials.

M. marinum strains infecting humans were isolated from swimming pools, wells, rivers and fish tanks. *Mycobacterium* spp. are opportunistic pathogens that residue in aquaria environment and water supplies (Yanong et al. 2010). This pathogenic organism produces a yellow pigment when subjected to light and uses a similar pathogenic mechanism for survival and replication inside the host macrophages, forming a granuloma during chronic infection (Barker et al. 1997; Tobin and Ramakrishnan 2008).

Clinical isolates of *M. marinum* from human and fish sources isolated from the same country were reported

to have genetic variations (Ucko and Colorni 2005). It was cited that *M. marinum* is very closely related to *M. ulcerans*, and the two species are suggested to be in close relation to *M. tuberculosis* based on 99.3% similarity in 16S rRNA sequences (Roberts, 2001), DNA/DNA hybridization and comparative fatty acid profiles (Tønjum et al. 1998). Strains of *M. marinum* derived from fish reveal considerable differences in phenotypic, genetic and virulence properties (Sechi et al. 2002; Ucko et al. 2002). The comparative analysis of genomic sequences of *M. marinum* strains from fish and man will have beneficial implications, enabling the development of novel molecular markers for detection and facilitating the study of the epidemiology and evolution of the pathogen (Kurokawa et al. 2013).

3. Epidemiology in fish

Mycobacterium sp. have been identified as one of the most important micro-organisms causing morbidity and mortality in cultivated and wild fishes all over the world (Gauthier and Rhodes 2009). It has been recorded in fresh water and marine fish in the tropical and subarctic regions (Frerichs 1993; Diamant et al. 2000; Rhodes et al. 2004). The cultured water fish species including sea bass and striped bass (Hedrick et al. 1987), the *Oreochromis mossambicus* fish (Noga et al. 1990) and freshwater fishes belonging to the families Anabantidae, Characidae and Cyprinidae (Astrofsky et al. 2000) are considered especially susceptible to the infection with *Mycobacterium*.

The infection mostly occurs within 2 weeks after exposure to aquariums or after direct inoculation of the organism either from fish fins or bites. Moreover, the infection rates may have a wide range of 10%–100% (Francis-Floyd and Yanong 2002). Three species of *Mycobacterium* (*M. marinum*, *M. fortuitum* and *M. chelonae*) have been recorded to induce pathogenicity in fish (Novotny et al. 2004; Gauthier and Rhodes 2009). Water and related biofilms are the natural habitats of these three species (Pedley et al. 2004). The first species is slowly growing (Tønjum et al. 1998) while the other two are rapidly growing mycobacterium (Han et al. 2007).

M. marinum is the most common species isolated from various fishes and is recognized as a human pathogen (Gauthier et al. 2003; Jacobs et al. 2009). It causes episodes of infections in the cultured fish (dos Santos et al. 2002; Brocklebank et al. 2003; Aranaz et al. 2008), but it was also reported responsible for sporadic infections in rainbow and brown trout in Italy (Salogni et al. 2007) and was also isolated from shellfish (Beecham et al. 1991).

A case of *Mycobacterium marinum* infection was described in a farm of hybrid striped bass in Italy (Bozzetta et al. 2010). This species was considered as a potential source of infections for humans during sport activities and also a hazard risk for workers in the fish farm. The economic effect of mycobacteriosis in fish industry is still underestimated owing to the long incubation period, chronic nature and difficult diagnosis particularly at the early infection stage (Chinabut 1999). *M. marinum* was the most prevalent species (89.5%) causing sturgeon mycobacteriosis as evidenced by PCR-denaturing gradient gel electrophoresis and library sequencing of rpoB gene (Zhang et al. 2015).

Fish acquire the mycobacteriosis infection through the consumption of contaminated food and polluted water (Gauthier et al. 2003; Nenoff and Uhlemann 2006). Transovarian transmission was also recorded in viviparous fish (Frerichs 1993) and in live bearing fishes (Conroy 1966). The vertical transmission of mycobacteria was previously explained (Chinabut 1999), although this transmission route has not been documented in salmonids (Astrofsky et al. 2000). Zebra fish embryos were infected with *M. marinum* via bath exposure (Davis et al. 2002), while adult zebra fish were infected through the gut rather than gills via gavage and bath exposure (Harriff et al. 2007).

The digestive transmission of *M. marinum* was also suggested when the digestive tract was infected in 16.5% of the horse mackerel (Ortega et al. 2014) in spite of sporadic detection of granuloma in gills. Thereby, effective control measures of mycobacteriosis in fish farm should consider the gut as a natural route of exposure to infection with *M. marinum* (Harriff et al. 2007). Transmission of piscine mycobacteriosis occurs through ingestion of contaminated feed and cannibalism of infected fish (Chinabut et al. 1990; Grady et al. 1992).

In summary, the disease is transmitted through skin injuries and the external bacteria are included in the cutaneous transmission route (Post 1987). Aquatic vertebrates as frogs and turtles are sources for fish infection with mycobacteriosis. Also, snails are believed to be an important reservoir in aquarium fish (Post 1987). Additionally, water fleas were also reported as source of *M. marinum* infection when aquarium fish fed on water fleas harboring this pathogen (Grange et al. 1985). Similarly it has been reported that contaminated water fleas are responsible for producing characteristic granulomata of fish mycobacteriosis in Siamese fighting fish, when they were used as a source of live food for rearing this fish (Sodjit et al. 1993).

Prevalence of fish mycobacteriosis had been reported in previous studies (Gauthier et al. 2011). In a study from the Chesapeake Bay, *M. marinum* was isolated in only 3% (6 out of 192) examined spleen samples from striped bass *Morone saxatilis* (Rhodes et al. 2004). In another study, only one isolate of *M. marinum* was revealed from 106 kidney samples from fighting fish *Betta* spp after subculturing on Lowenstein–Jensen agar, Ziehl Neelsen acid-fast staining and 16 S rRNA sequencing (Najiah et al. 2011).

In a survey over the period 2002–2005 in Italy, the isolation percentages of *M. marinum* from positive ornamental fish were around 4.4% (Zanoni et al. 2008). A much higher percentage of 41.7% was isolated from gills, muscle and intestine of ornamental fish against a medium value of 19.3% from environmental samples of aquaria fish (Slany et al. 2014). This higher spread of *M. marinum* in these ornamental fishes was attributed to high density of fish, optimum water temperature of aquarium (close to 25 °C) and the frequent transfer of fish between the aquaria (Slany et al. 2014), the environmental factors which keep the aquarium most suitable for the spread of fish mycobacteriosis. Alternatively, host factors may play an additional role in the prevalence of piscine mycobacteriosis, based on the variable susceptibility of medaka and zebrafish for *M. marinum* (Broussard and Ennis 2007).

The initial dose of infecting *M. marinum* was advocated to be an influential factor, since low initial doses were associated with chronic disease, while high dose ($>10^7$ CFU/g) were associated with acute and subacute infections (Prouty et al. 2003). The reported substantial genetic variation of *M. marinum* strains between fish and humans (Ucko and Colorni 2005) may also play a role in the nature of established infection. For example, Zebra fish developed acute infection when inoculated with *M. marinum* isolate of human origin, but chronic disease when inoculated with *M. marinum* isolate of fish origin (van der Sar et al. 2004).

4. Clinical signs in fish

M. marinum causes chronic, progressive disease of Zebra fish in a dose-dependent fashion. The induced tuberculosis in the zebra fish is similar to active human tuberculosis, where the predominant lesion at late stages from the infection are the presence of necrotizing granulomas, hallmark lesion of active tuberculosis with abundant bacteria in the necrotic areas (Swaim et al. 2006). Acute infection was characterized by uncontrolled development of the pathogen and death of all fish within 16 days, while chronic infections were marked by the formation of granuloma in different organs and longer survival in the range of 4–8 weeks (van der Sar et al. 2004).

M. marinum-induced tuberculosis in adult zebra fish; most of zebrafish died within 2 weeks of infection with 8970 bacteria, while infection with 5 CFU caused 44% mortality and infection with 60 CFU caused 83% mortality by 16 weeks. The infected fish showed a reduction in feed intake and weakened swimming for 1 week during the period prior to death. The infected fish either remained listless at the bottom or at the surface, constantly opening and closing their mouths to increase the gas exchange. The dying fish were often marked with external red lesions on the trunk ventral to the lateral line, an exophthalmia with ascites in close proximity to internal organs, where the former two signs are indicators for osmoregulatory stress and kidney failure (Swaim et al. 2006).

5. Pathogenesis and virulence determinants

Many virulence determinants and pathogenicity mechanisms are shared between *M. marinum* and *M. tuberculosis complex*. *M. marinum* is an opportunistic human pathogen causing a TB-like infection in the ectotherms, and also, can induce caseation granulomas in Zebra fish, similar to those composed in man (Tobin and Ramakrishnan 2008). The exact mechanism of the host's immune reaction against *M. marinum* still needs to be thoroughly investigated and explored. It has been established that *M. marinum* actively stimulates actin-polymerization within phagosomes by a complicated cellular mechanism enabling the organism to propel itself, by actin-based motility, into adjacent cells. Thus, *M. marinum* can escape into the cytoplasm of the infected macrophages in an RD1-dependent fashion, then spreading again from cell to cell, circumventing the host defense mechanisms and promoting permanent infection (Stamm et al. 2003). Nevertheless, the absence of macrophages during early infection with *M. marinum* was reportedly associated with higher bacterial burden indicating the macrophages control the infection at early stages and are not a niche for optimal growth (Clay et al. 2007).

M. marinum virulence determinants causing granulomas are not fully identified. Many hosts like adult and embryonic zebra fish, animals and protozoa were utilized as models to study *M. marinum* pathogenesis and host-pathogen interactions (Davis et al. 2002). In a study on gold fish, the signature-tagged mutagenesis (STM) was utilized for characterization of *M. marinum* virulence genes needed for its *in-vivo* survival (Ruley et al. 2004), identifying 33 putative virulence genes, only 5 of which are homologues with *M. tuberculosis*. These 5 genes included pks genes, PPE family genes, and a transcriptional regulator with an AraC signature (Ruley et al. 2004).

Several *M. marinum* genes and pathways were found to have almost the same effects in different hosts. These conserved virulence determinants vary from secreted molecules to cell envelope biosynthesis and to cell physiology adaptation. An example of conserved virulence determinant of the organism is the cell wall-associated lipid PDIM, PGLs and ESAT-6 secretion system 1 (ESX-1) which if interrupted, attenuation of *M. marinum* in mammalian, fish, and protozoan cells will occur, referring to their significance for the pathogen in different hosts (Weerdenburg et al. 2015; Huang et al. 2016). In *M. tuberculosis*, PDIM and ESAT-6 have been concluded important for the infection as well (Sassetti and Rubin 2003), indicating that both genes are conserved across mycobacterial species. In addition

to these genes, the mammalian cell entry 4 (mce4) and mce1 gene clusters could also be identified as general virulence factors of both *M. marinum* and *M. tuberculosis* (Sassetti and Rubin 2003; Senaratne et al. 2008), where *mce4* gene encodes a cholesterol import system providing energy to mycobacteria during infection (Pandey and Sassetti 2008).

Many common virulence determinants could be identified, other virulence genes had a very crucial effect in a narrow range of host cells and thus may be utilized as indicators for adaptation of bacterial host (Weerdenburg et al. 2015). An example of these host-related genes are *M. marinum* specific set of gene cluster that are homologues to ESX-1 cluster. Proteins of ESX-1 have a very definite role in host adaptation. Orthologues of these genes were recovered only in *M. marinum* or *M. liflandii* (Weerdenburg et al. 2015).

Many studies have firmly established a role for ESX-1 in virulence, both in *M. marinum* and in *M. tuberculosis*. This type VII secretion system, which is encoded by the *esx-1* gene cluster, is responsible for the secretion of numerous virulence factors (Sechi et al. 2002; Bottai and Brosch 2009; Weerdenburg et al. 2015). In contrast to *M. tuberculosis*, *M. marinum* has almost two identical copies of the ESX-1 substrates esxAB. The extra set appears to be specifically necessary for the infection of the fish cell line CLC, suggesting a host-specific ESX-1 function (Weerdenburg et al. 2015).

One of the features of *M. marinum* pathogenicity is its exclusive ability to access the macrophage cytosol and utilize host actin for motility (Stamm et al. 2003). This ability was not noticed in bacteria lacking ESX-1 system and thereby, growth attenuation was detected in different hosts (Cosma et al. 2004; Stoop et al. 2011; Kennedy et al. 2014). Recently, a novel protein secretion system named as ESX-5 gene, which was proven restricted *to M. tuberculosis* and *M. marinum* (Abdallah et al. 2006; Abdallah et al. 2009). ESX-5 is responsible for the transport of more than 100 proteins of PE and PPE families in *M. marinum* (Daleke et al. 2011). In another study, ESX-5 deficient *M. marinum* was attenuated in embryos of zebra fish indicating the importance of ESX-5 gene for *M. marinum* to establish and persist infections (Weerdenburg et al. 2012).

The pathogenicity of mycobacteria is associated with their capacity to export different virulence factors, for which mycobacteria own many protein secretion systems. SecA2 was identified in the pathogenic mycobacteria as a mycobacterial Sec translocation pathway involved in protein translocation across the inner membrane (Braunstein et al. 2003) to evade the innate and adaptive immune responses (Jensen et al. 2012; Watkins et al. 2012). The association between Sec A2 attenuation and the inability of mycobacteria to block the maturation of phagosome (Sullivan et al. 2012) confirms its essential role in developing pathogenicity. Identifying SecA2 substrates in *M. marinum* confirms

that protein kinase G (PknG) can play an important role as a virulence effecter. Partial regaining of secA2 virulence following the overexpression of PknG confirms the role played by this protein in controlling the mycobacterial virulence and restoring blockage of phagosomal maturation (van der Woude et al. 2014).

6. Hematological changes in fish

The changes in the hematological parameters may indicate fish physiological responses against different environmental stresses and infections. *Tilapia* infected with *M. marinum* manifested an overall decrease in erythrocyte number and haematocrit with hypochromic microcytic anemia associated with a significant increase in circulating immature erythrocytes (Barham et al. 1980).

In another study, the hematological parameters exhibited nonsignificant alterations in the erythrocytic indices of Nile *Tilapia* inoculated with *M. marinum,* for eight days at 30 °C with only individual cases of hypochromic, microcytic anemia and wide variations in thrombocyte count. Leukocytosis occurred in fish after one day of infection as a result of neutrophilia and slight lymphocytosis, being the most characteristic feature of acute infection. After 3 days of infection, chronicity signs began to appear in the form of lymphocytosis and neutropenia, while after 14th and 35th days and up to the 45th day the monocytosis emerged and prevailed. Eosinophilia was only noticed in one fish at the 14th day after infection. Some other morphological abnormalities including immature cells, cytoplasmic vacuolisation in the monocytes, toxic cytoplasmic granulations in neutrophils and intensive cytoplasmic basophilia were recorded in infected fish at different times (Ranzani-Paiva et al. 2004).

7. Histopathological investigation in fish

Histopathological examinations mostly demonstrate a nonspecific inflammatory infiltration of epithelioid cells, lymphocytes and Langhan's giant cells without caseation. The early lesions commonly reveal a collection of polymorphonuclear cells surrounded with histiocytes. The histopathological examination might be significantly consolidated by the rapidly improving possibilities of vivo imaging, such as magnetic resonance spectroscopic/microscopic techniques or even two-photon microscopy/tomography and high-resolution ultrasound (Rallis and Koumantaki-Mathioudaki 2007).

The pathological response to the infection of Zebra fish with *M. marinum* was reported when this fish was inoculated with three different strains of *M. marinum* (Mma7, Mma11 and Mma20) isolated from different sources (van der Sar et al. 2004). Microscopic manifestations of fish infected with Mma20 encompassed

extreme numbers of mycobacteria with loss of structural organization, severe necrosis, invasion of peritoneum and surrounding tissues with numerous inflammatory cells ending in severe peritonitis and early fish death. In case of Mma11 inoculation, organized granulomas were detected in liver, kidney pancreas, intestines and connective tissues. The infection with Mma7 strain was associated with the induction of fewer and less organized granulomas, which may have sometimes an outer layer of epithelial cells and a necrotic center (Rallis and Koumantaki-Mathioudaki 2007).

8. Diagnosis of *M. marinum* infections in fish

Mycobacterial cell wall resists the acid–alcohol decolorisation after being stained with carbolfuchsin. So Mycobacteria are revealed in fish tissue sections by staining with Ziehl–Neelsen acid-fast stain. The related actinomycetes including *Nocardia*, *Tsukamurella*, *Gordonia* and *Rhodococcus* are considered as partially acid-fast (Holt 1994), while *Legionella* spp. occasionally reveal some acid-fastness (Bentz et al. 2000). Acid-fast bacilli are frequently visible in piscine mycobacterial granulomas, although granulomas containing no visible acid-fast bacilli are reported in many experimentally infected species (Watral and Kent, 2007).

Mycobacterium could be effectively cultured on a variety of selective liquid and agar media (Rhodes et al. 2004), for example mycobactin is used to enhance the growth of slowly growing *Mycobacterium* on the standard media. Non-selective media are ineffective for slow growers but appropriate in the fast growers. It is necessary to differentiate between mycobacteria recovered from fishes, and fish-pathogenic mycobacteria. Aseptic isolation of *Mycobacterium* from internal organs confirms that the strains do not contain any contaminants (Rhodes et al. 2004).

The isolation of such bacteria from feces, external surfaces and whole viscera is questionable due to the presence of external contaminants (Rhodes et al. 2004). Tissue samples of fish are homogenized and then plated on appropriate culture media, including Middlebrook 7H10 or Lowenstein–Jensen media, to enhance *Mycobacterium* spp. growth. Being slow growing organisms, several *Mycobacterium* spp. necessities the maintenance of culture plates for a period ranging from 2 to 3 months before considering as negative.

Culture-based detection of *Mycobacterium* spp. from skin or gills of fish is complicated by the existence of background microbiota which compete *Mycobacterium* growth on the standard media. Because of its hydrophobicity, *Mycobacterium* is highly resistant to treatment with both acidic and basic chemicals and other compounds such as benzalkonium chloride and hypochlorite (Brooks et al. 1984; Rhodes et al. 2004). These substances have been utilized to isolate *Mycobacterium* from samples with a high microbial background, although they may also adversely affect the recovery of *Mycobacterium* (Brooks et al. 1984; Rhodes et al. 2004). Therefore, using the most appropriate decontamination procedures needs through evaluation for both minimum concentrations and exposure times.

Various molecular diagnostic tools have been established for detecting and identifying *Mycobacterium* spp. representing public health hazard. The small subunit, 16S rRNA gene is a popular target for accurate diagnosis due in part to the availability of *Mycobacterium* 16S gene sequences in the repositories as the GenBank and the Ribosomal Differentiation Database of Microorganisms. Identification of *M. marinum*, *M. fortuitum*, and *M. chelonae,* using restriction fragment length polymorphism analysis of the 16S rRNA gene (PCR-RFLP) was first explained in 1997 (Talaat et al. 1997). Moreover, DNA probe and gene sequencing have been used to identify *Mycobacterium* spp. up to the species level (Turenne et al. 2001; Chemlal and Portaels 2003).

Direct sequencing from fish tissues can be a useful option when the culture of Mycobacteria fails (Poort et al. 2006). Although numerous isolates of fish shared high homology in *Mycobacterium* genes, they require detection of multiple gene targets (Devulder et al. 2005). Recently, FRET (fluorescence resonance energy transfer) showed high specificity via melting curve analysis, which effectively discriminate and distinguish *M. marinum* from other mycobacteria (Salati et al. 2010). This diagnostic technique is summarized in a primary PCR with SYBR green (at a detection limit of 10^2 *Mycobacterium* DNA copies) and then a secondary amplification using FRET in real-time PCR.

9. Management of *M. marinum* infections in fish

Complete control of piscine mycobacteriosis may need the destruction of all affected stocks and disinfecting the holding tanks and plumbing (Roberts 2001; Noga 2011). Ethanol, lysol and sodium chlorite have been reported efficiently capable of destroying *M. marinum* in aquaria, while potassium peroxymonosulfate is ineffective. Moreover, sodium hypochlorite is considered as a powerful sterilizing agent when the contact time was prolonged to more than 10 min (Mainous and Smith 2005). Nevertheless, researchers had stated that disinfections are not successful for controlling fish mycobacteriosis, due to the resistance of mycobacteria to common disinfectants (Jacobs et al. 2004). Furthermore, the Ag85A DNA vaccine protected striped bass for short term against *M. marinum*, but it could not provide long-term protection (Pasnik and Smith 2005).

10. Transmission to man

M. marinum penetrates the skin via minor traumata arising from resting the elbows on the border of the fish pond. Nowadays, the exposure of swimmers to mycobacteria was reduced due to the chlorination of water in the swimming pool (Ang et al. 2000). The growth of *M. marinum* increased in a Swedish dolphin atrium due to the decrease of water chlorination. It has coincided with numerous animals acquiring cutaneous and subcutaneous *M. marinum* infection. Additionally, infection may be acquired by handling shellfish or fish or following trauma attributable to infected foreign bodies for instance wood splints (Beecham et al. 1991).

However, the disease is not transmittable from person to person (Clark et al. 1990). Currently, most human infection cases happen with the exposure to aquaria (Aubry et al. 2002) as well as skin injuries occurring during the processing or preparations of seafood (Clark et al. 1990; Lawler 1994). The current practices of sanitary chlorination restrict largely the outbreaks of mycobacterial infections. The terms 'fish tank granuloma' and 'fish handler's disease' is often used due to the relation between home aquariums and the water-associated activities including swimming, fishing and boating (Ang et al. 2000).

M. marinum infections may have occupational hazards for pet shop workers. Many infections take place in fish fanciers who maintain the aquarium at home producing what is called fish fanciers 'finger syndrome' (Wheeler and Graham 1989). Some mycobacterial infection may take place through direct injury from fins of fish or bites, but most infections are acquired during aquarium handling, e.g. cleaning or water changing (Bhatty et al. 2000; Jernigan and Farr 2000). The incubation period of *M. marinum* infection in humans ranges between 3 weeks and 9 months (Jernigan and Farr 2000).

The evidence of zoonotic transmission of *M. marinum* had been formerly confirmed through studying the genetic linkages of isolates from both human and fish sources using pulsed field gel electrophoresis (Tsai et al. 2007; Slany et al. 2013) and amplified fragment length polymorphism (Doedens et al. 2008). However, in one human clinical case, *M. marinum* was inoculated through a fish spine injury (Tsai et al. 2007). Zoonotic infection had been ascribed to mycolactone toxin produced by mycobacteria (Chemlal et al. 2002). Although it is assumed that the local aquaculture industry is not the source of human infections in combination with the lack of records of human infections by some strains (*M. marinum* Eilaticum and *M. marinum* Cyprinum), but still only certain strains of *M. marinum* have zoonotic potential (Ucko and Colorni 2005).

11. Clinical presentation in human

Mycobacterial infections may be either painful or painless, but it may be life threatening (Wu et al. 2002).

Infections with *M. marinum* may be hypothetically classified into four clinical categories (type I–type IV) to help guide therapeutic options (Bhatty et al. 2000). Type I *M. marinum* is commonly observed in immunocompetent patients and known as single or limited (1–3) lesions marked by superficial cutaneous infection, appearing in forms of crusted or ulcerated nodules or verrucous plaques. These single lesions develop within weeks or months of contact with infected fish (Aubry et al. 2002).

Type II *M. marinum* is numerous (more than 3) lesions with inflammatory nodules or in a sporotrichoid spreading pattern or with abscesses and granulomas in an immune-suppressed patient. This 'sporotrichoid' infection starts with distal inoculation and can progress to nodular lymphangitis (Bartralot et al. 2005). Type III *M. marinum* appears as deep infections associated with or without skin infection signs including arthritis, tenosynovitis, osteomyelitis and/or bursitis. Type IV *M. marinum* is disseminated infection with lung disease and other systemic manifestations. Bacteraemia is very rare but may occur in severely immuonocompromised patients (Bhatty et al. 2000). Other sites of *M. marinum* infection were also reported. Severe intranasal lesions resulted also from *M. marinum* infection (Asakura et al. 2016), while the disseminated infection comprised the skin, the viscera and the lung (Petrini 2006) and laryngeal lesions (Gould et al. 1968).

12. Treatment of *M. marinum* infections in man

The proper treatment of *M. marinum* infection is practically required for rapid recovery in man, avoiding progress to deeper tissues. Insufficient data about efficacy of many treatment regimens (Gluckman 1995) render the treatment fairly difficult. Moreover, *M. marinum* is a naturally multi-drug resistant organism, and the treatment is primarily dependent on the investigators and their personal experiences (Edelstein 1994). Monotherapy is usually effective in the infection of skin and soft tissue, but this is not sufficient in deeper infections. In the superficial cutaneous infection, monotherapy including clarithromycin, trimethoprim and ciprofloxacin was reportedly considered as an effective treatment, while in case of deeper infections combination therapy of two drugs may be more effective.

In case of a sporotrichoid distribution pattern, a combination of two drugs, namely ethambutol and rifampicin is recommended. Streptomycin, isoniazid and pyrazinamide should be excluded from treatment regimens because *M. marinum* is more resistant. Treatment of infected deeper structure takes longer periods and the acquired resistance has not been recorded in *M. marinum* while clinical awareness is very important to initiate the diagnosis and the treatment (Aubry et al. 2002). Various therapeutic alternatives such as

electrodessication, therapy with X-ray, cryotherapy, local hyperthermic therapy and photodynamic therapy have been recorded and recommended (Rallis and Koumantaki-Mathioudaki 2007). Although phage therapy using the mycobacteriophage D29 proved effective in the treatment of Buruli Ulcer (BU) caused by *M. ulcerans* in the murine footpad model (Trigo et al. 2013), there is no bacteriophage specific for controlling *M. marinum* infections.

13. Conclusions

Piscine mycobacteriosis arising from *M. marinum* infection can have adverse effects on the fish of fresh water, marine and brackish water, constituting zoonotic implications. The data on the pathogenesis and virulence factors of *M. marinum* are still scarce and questionable. Further studies are required to trace the immune defense responses against *M. marinum* infection in fish. Prophylaxis and vaccine development are extremely important to protect different aquaria from *M. marinum* infection. The advent of molecular diagnostic tools and advanced epidemiological studies on fish mycobacteriosis can enhance our understanding of the strategic plans designed for controlling such disease. Due to its communicable public health hazard, the disease should be cautiously avoided by voiding swimming in fish pools and wearing gloves during handling and processing of fish. Future studies should also evaluate the application of phage therapy in the fish aquarium to prevent and control *M. marinum* infection.

Disclosure statement

All authors of this review article have no conflict of interest with any organizations or persons that influence the paper content.

References

Abdallah AM, Verboom T, Hannes F, Safi M, Strong M, Eisenberg D, Musters RJP, Vandenbroucke-Grauls CMJE, Appelmelk BJ, Luirink J, et al. 2006. A specific secretion system mediates PPE41 transport in pathogenic mycobacteria. Mol Microbiol. 62(3):667–679. doi:10.1111/j.1365-2958.2006.05409.x

Abdallah AM, Verboom T, Weerdenburg EM, Gey van Pittius NC, Mahasha PW, Jimenez C, Parra M, Cadieux N, Brennan MJ, Appelmelk BJ, et al. 2009. PPE and PE_PGRS proteins of *Mycobacterium marinum* are transported via the type VII secretion system ESX-5. Mol Microbiol. 73(3):329–340. doi:10.1111/j.1365-2958.2009.06783.x

Ackleh AS, Delcambre ML, Sutton KL. 2015. A second-order high-resolution finite difference scheme for a size-structured model for the spread of *Mycobacterium marinum*. J Biol Dyn. 9(Suppl 1):156–187. doi:10.1080/17513758.2014.962998

Ang P, Rattana-Apiromyakij N, Goh CL. 2000. Retrospective study of *Mycobacterium marinum* skin infections. Int J Dermatol. 39(5):343–347.

Aranaz A, Gibello A, Alvarez J, Mata AI, Rodriguez A, Fallola C, Fernández-Garayzábal JF, Dominguez L. 2008. *Mycobacterium peregrinum* infection in farmed European tench (*Tinca tinca* L.). Vet Microbiol. 131(3–4):393–399. doi:10.1016/j.vetmic.2008.04.017

Aronson JD. 1926. Spontaneous tuberculosis in salt water fish. J Infect Dis. 39:315–320.

Asakura T, Ishii M, Kikuchi T, Kameyama K, Namkoong H, Nakata N, Sugita K, Tasaka S, Shimizu T, et al., Hoshino Y 2016. Disseminated *Mycobacterium marinum* infection with a destructive nasal lesion mimicking extranodal NK/T cell lymphoma: a case report. Med (Baltimore). 95(11): e3131.

Astrofsky KM, Schrenzel MD, Bullis RA, Smolowitz RM, Fox JG. 2000. Diagnosis and management of atypical Mycobacterium spp. infections in established laboratory Zebrafish (*Brachydanio rerio)* facilities. Comp Med. 50(6):666–672.

Aubry A, Chosidow O, Caumes E, Robert J, Cambau E. 2002. Sixty-three cases of *Mycobacterium marinum* infection: clinical features, treatment, and antibiotic susceptibility of causative isolates. Arch Intern Med. 162(15):1746–1752.

Barham W, Smit G, Schoonbee H. 1980. The effect of bacterial infection on erythrocyte fragility and sedimentation rate of rainbow trout, *Salmo gairdneri* Richardson. J Fish Biol. 16(2):177–180.

Barker LP, George KM, Falkow S, Small P. 1997. Differential trafficking of live and dead *Mycobacterium marinum* organisms in macrophages. Infect Immun. 65(4):1497–1504.

Bartralot R, García-Patos V, Sitjas D, Rodríguez-Cano L, Mollet J, Martín-Casabona N, Coll P, Castells A, Pujol RM. 2005. Clinical patterns of cutaneous nontuberculous mycobacterial infections. Br J Dermatol. 152(4):727–734.

Beecham HJ 3rd, Oldfield EC 3rd, Lewis DE, Buker JL. 1991. *Mycobacterium marinum* infection from shucking oysters. Lancet. 337(8755):1487.

Bentz JS, Carroll K, Ward JH, Elstad M, Marshall, CJ. 2000. Acid-fast-positive *Legionella pneumophila*: a possible pitfall in the cytologic diagnosis of mycobacterial infection in pulmonary specimens. Diagn Cytopathol. 22(1):45–48.

Bhatty MA, Turner DP, Chamberlain ST. 2000. *Mycobacterium marinum* hand infection: case reports and review of literature. Br J Plast Surg. 53(2):161–165.

Bottai D, Brosch R. 2009. Mycobacterial PE, PPE and ESX clusters: novel insights into the secretion of these most unusual protein families. Mol Microbiol. 73(3):325–328.

Bozzetta E, Varello K, Giorgi I, Fioravanti ML, Pezzolato M, Zanoni RG, Prearo M. 2010. *Mycobacterium marinum* infection in a hybrid striped bass farm in Italy. J Fish Dis. 33 (9):781–785. doi:10.1111/j.1365-2761.2010.01167.x

Braunstein M, Espinosa BJ, Chan J, Belisle JT, Jacobs WR. (2003). SecA2 functions in the secretion of superoxide

dismutase A and in the virulence of *Mycobacterium tuberculosis*. Mol Microbiol. 48(2):453–464.

Brennan PJ. 2003. Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis*. Tuberculosis. 83 (1):91–97.

Brocklebank J, Raverty S, Robinson J. 2003. Mycobacteriosis in Atlantic salmon farmed in British Columbia. Can Vet J. 44 (6):486–489.

Brooks RW, George KL, Parker BC, Falkinham, JO III. 1984. Recovery and survival of nontuberculous mycobacteria under various growth and decontamination conditions. Can J Microbiol. 30:1112–1117.

Broussard GW, Ennis DG. 2007. *Mycobacterium marinum* produces long-term chronic infections in medaka: a new animal model for studying human tuberculosis. Comp Biochem Physiol Toxicol Pharmacol: CBP. 145(1):45–54. doi:10.1016/j.cbpc.2006.07.012

Chang CT, Whipps CM. 2015. Activity of antibiotics against mycobacterium species commonly found in laboratory Zebrafish. J Aquatic Ani Health. 27(2):88–95. doi:10.1080/08997659.2015.1007176

Chemlal K, Huys G, Laval F, Vincent V, Savage C, Gutierrez C, Laneelle M-A, Swings J, Meyers WM, Daffe M, et al. 2002. Characterization of an unusual Mycobacterium: a possible missing link between *Mycobacterium marinum* and *Mycobacterium ulcerans*. J Clin Microbiol. 40(7):2370–2380.

Chemlal K, Portaels F. 2003. Molecular diagnosis of nontuberculous mycobacteria. Curr OpinInfect Dis. 16(2):77–83. doi:10.1097/01.aco.0000065074.06965.96

Chinabut S. 1999. Mycobacteriosis and nocardiosis. In: Woo PTK, Bruno DW, editors. Fish diseases and disorders. Vol. 3. New York (NY): CABI Publishing; p. 319–340.

Chinabut S, Limsuwan C, Chanratchakool P. 1990. Mycobacteriosis in the snakehead, *Channa striatus (Fowler)*. J Fish Dis. 13(6):531–535.

Clark HF, Shepard CC. 1963. Effect of environmental temperatures on infection with *Mycobacterium marinum* (balnei) of mice and a number of poikilothermic species. J Bacteriol. 86(5):1057–1069.

Clark R, Spector H, Friedman D, Oldrati K, Young C, Nelson S. 1990. Osteomyelitis and synovitis produced by *Mycobacterium marinum* in a fisherman. J Clin Microbiol. 28 (11):2570–2572.

Clay H, Davis JM, Beery D, Huttenlocher A, Lyons SE, Ramakrishnan L. (2007). Dichotomous role of the macrophage in early *Mycobacterium marinum* infection of the Zebrafish. Cell Host Microbe. 2(1):29–39.

Conroy DA.1966. A report on the problem of bacterial fish diseases in the Argentine Republic. Bull Off Int Epizoot. 65 (5):755–768.

Cosma CL, Humbert O, Ramakrishnan L. 2004. Superinfecting mycobacteria home to established tuberculous granulomas. Nat Immunol. 5(8):828–835. doi:10.1038/ni1091

Daffé M, Draper P. 1997. The envelope layers of mycobacteria with reference to their pathogenicity. Adv Microb Physiol. 39:131–203.

Daleke MH, Cascioferro A, de Punder K, Ummels R, Abdallah AM, van der Wel N, Peters PJ, Luirink J, Manganelli R, Bitter W. 2011. Conserved Pro-Glu (PE) and Pro-Pro-Glu (PPE) protein domains target LipY lipases of pathogenic mycobacteria to the cell surface via the ESX-5 pathway. J Biol Chem. 286(21):19024–19034. doi:10.1074/jbc.M110.204966

Davis JM, Clay H, Lewis JL, Ghori N, Herbomel P, Ramakrishnan L. 2002. Real-time visualization of mycobacterium-macrophage interactions leading to initiation of granuloma formation in Zebrafish embryos. Immunity. 17 (6):693–702.

Decostere A, Hermans K, Haesebrouck F. 2004. Piscine mycobacteriosis: a literature review covering the agent and the disease it causes in fish and humans. Vet Microbiol. 99(3-4):159–166. doi:10.1016/j.vetmic.2003.07.011

Devulder G, de Montclos MP, Flandrois J. 2005. A multigene approach to phylogenetic analysis using the genus Mycobacterium as a model. Int J Syst Evol Microbiol. 55(1):293–302.

Diamant A, Banet A, Ucko M, Colorni A, Knibb W, Kvitt H. (2000). Mycobacteriosis in wild rabbitfish *Siganus rivulatus* associated with cage farming in the Gulf of Eilat, Red Sea. Dis Aquat Organ. 39(3):211–219.

Doedens RA, van der Sar AM, Bitter W, Schölvinck EH. 2008. Transmission of *Mycobacterium marinum* from fish to a very young child. Pediatr Infect Dis J. 27(1):81–83.

dos Santos NM, do Vale A, Sousa MJ, Silva, MT. 2002. Mycobacterial infection in farmed turbot *Scophthalmus maximus*. Dis Aquat Organ. 52(1):87–91. doi:10.3354/dao052087

Draper P. 1991. The structure of the mycobacterial cell envelope is not yet understood. Res Microbiol. 142(4):420–422.

Edelstein H. 1994. *Mycobacterium marinum* skin infections: Report of 31 cases and review of the literature. Arch Intern Med. 154(12):1359–1364.

El-Etr SH, Yan L, Cirillo JD. 2001. Fish monocytes as a model for mycobacterial host-pathogen interactions. Infect Immun. 69(12):7310–7317.

El Amrani MH, Adoui M, Patey O, Asselineau A. (2010). Upper extremity *Mycobacterium marinum* infection. Orthop Traumatol Surg Res. 96(6):706–711. doi:10.1016/j.otsr.2010.02.016

Ferguson HW. 2006. Spleen, thymus, Reticulo-Endothelial system, blood. In: Ferguson HW, editor. Systemic pathology of fish: A text and atlas of normal tissues in teleosts and their responses in disease. 2nd ed. London (UK): Scotian Press; p. 199–214.

Francis-Floyd R, Yanong R. 2002. Mycobacteriosis in fish. Florida Cooperative Extension Serivce, Institute of Food and Agricultural Sciences, University of Florida, Ruskin, Fact Sheet VM-96.

Frerichs G. 1993. Mycobacteriosis: Nocardiosis. Bacterial Dis Fish. 1:219–233.

Gangadharam PR, Jenkins PA. 1997. Mycobacteria: I. Basic aspects. Vol 1. Portland, ME: Springer Science & Business Media.

Gauthier DT, Helenthal AM, Rhodes MW, Vogelbein WK, Kator HI. 2011 Characterization of photochromogenic *Mycobacterium spp*. from Chesapeake Bay striped bass *Morone saxatilis*. Dis Aquat Organ. 95(2):113–124. doi:10.3354/dao02350

Gauthier DT, Latour RJ, Heisey DM, Bonzek CF, Gartland J, Burge EJ,

Gauthier DT, Rhodes MW. 2009. Mycobacteriosis in fishes: A review. Vet J. 180(1):33–47. doi:10.1016/j.tvjl.2008.05.012.

Gauthier DT, Rhodes MW, Vogelbein WK, Kator H, Ottinger CA. 2003. Experimental mycobacteriosis in striped bass *Morone saxatilis*. Dis Aquat Organ 54(2):105–117. doi:10.3354/dao054105

Gluckman SJ. 1995. *Mycobacterium marinum*. Clin Dermatol. 13(3):273–276.[CrossRef

Goodfellow M, Magee JG. 1998. Taxonomy of mycobacteria. In: Goodfellow, Jenkins, editors. Mycobacteria: I. Basic aspects. New York (NY): Chapman and Hall; p. 1–71.

Gould WM, McMeekin DR, Bright RD. 1968. *Mycobacterium marinum* (balnei) infection: report of a case with cutaneous and laryngeal lesions. Arch Dermatol. 97(2):159–162.

Grady AW, Wolff A, Besch-Williford C. 1992. Diagnostic exercise: visceral granulomas in a fish. Lab Anim Sci 3:316–317.

Grange JM, Fraga TE, Tarazona RTM. 1985. Enfermedades micobacterianas. Ed. Científica PLM. Honduras, Central America.

Han XY, De I, Jacobson KL. 2007. Rapidly growing mycobacteria: clinical and microbiologic studies of 115 cases. Am J Clin Pathol. 128(4):612–621. doi:10.1309/1kb2gkyt1bueylb5

Harriff MJ, Bermudez LE, Kent ML. 2007. Experimental exposure of Zebrafish, *Danio rerio* (Hamilton), to *Mycobacterium marinum* and *Mycobacterium peregrinum* reveals the gastrointestinal tract as the primary route of infection: a potential model for environmental mycobacterial infection. J Fish Dis. 30(10):587–600. doi:10.1111/j.1365-2761.2007.00839.x

Hedrick RP, McDowell T, Groff J. 1987. Mycobacteriosis in cultured striped bass from California. J Wild Dis. 23(3):391–395.

Holt, J. G. (1994). Bergey's manual of determinative bacteriology. 9th ed. Baltimore (MD): Williams and Wilkins.

Huang X, Wang H, Meng L, Wang Q, Yu J, Gao Q, Wang D. 2016. Role of eosinophils and apoptosis in PDIMs/PGLs deficient mycobacterium elimination in adult Zebrafish. Dev Comp Immunol. 59:199–206.

Jacobs JM, Lazur A, Baya A. 2004. Prevention and disinfection of mycobacterium sp. in aquaculture. Maryland Sea Grant Extension Publication, Publication number UM-SG-SGEP, Finfish Worksheet 9.

Jacobs JM, Stine CB, Baya AM, Kent ML. 2009. A review of mycobacteriosis in marine fish. J Fish Dis. 32(2):119–130. doi:10.1111/j.1365-2761.2008.01016.x

Jensen K, Ranganathan UDK, Van Rompay KK, Canfield DR, Khan I, Ravindran R, Luciw PA, Jacobs WR, Fennelly G, Larsen MH, et al. 2012. A recombinant attenuated *Mycobacterium tuberculosis* vaccine strain is safe in immunosuppressed simian immunodeficiency virus-infected infant macaques. Clin Vaccine Immunol. 19(8):1170–1181.

Jernigan JA, Farr BM. 2000. Incubation period and sources of exposure for cutaneous *Mycobacterium marinum* infection: Case report and review of the literature. Clin Infect Dis. 31(2):439–443.

Kennedy GM, Hooley GC, Champion MM, Mba Medie F, Champion PAD. 2014. A novel ESX-1 locus reveals that surface-associated ESX-1 substrates mediate virulence in *Mycobacterium marinum*. J Bacteriol. 196(10):1877–1888. doi:10.1128/JB.01502-14

Kurokawa S, Kabayama J, Fukuyasu T, Hwang SD, Park CI, Park SB, del Castillo CS, Hikima J, Jung TS, Kondo H, et al. 2013. Bacterial classification of fish-pathogenic *Mycobacterium species* by multigene phylogenetic analyses and MALDI Biotyper identification system. Mar Biotechnol 15(3):340–348.

Lawler A. 1994. Human *Myobacterium marinum* Aronson infections. J Aquaculture Aquatic Sci. 6(4):93–95.

Lewis FM, Marsh BJ, von Reyn CF. 2003. Fish tank exposure and cutaneous infections due to *Mycobacterium marinum*: tuberculin skin testing, treatment, and prevention. Clin Infect Dis. 37(3):390–397. doi:10.1086/376628

Li BR, Zou Y, Wei Q. 2009. Sturgeon aquaculture in China: Status of current difficulties as well as future strategies based on 2002–2006/2007 surveys in eleven provinces. J Appl Ichthyol. 25(6):632–639. doi:10.1111/j.1439-0426.2009.01366.x

Linell F, Norden A. 1954. *Mycobacterium balnei*. a new acid-fast Bacillus occurring in swimming pools and capable of producing skin lesions in humans. Acta Tuberculosea Scandinavica. 33:1–84.

Mainous ME, Smith SA. 2005. Efficacy of common disinfectants against *Mycobacterium marinum*. J Aquatic Ani Health. 17(3):284–288.

Marrakchi H, Lanéelle M-A, Daffé M. 2014. Mycolic acids: Structures, biosynthesis, and beyond. Chem Biol. 21(1):67–85.

Najiah M, Lee KL, Noorasikin H, Nadirah M, Lee SW. 2011. Phenotypic and genotypic characteristics of Mycobacterium isolates from fighting fish Betta spp. in Malaysia. Res Veterinary Sci. 91(3):342–345. doi:10.1016/j.rvsc.2010.09.010

Nenoff P, Uhlemann R. 2006. Mycobacteriosis in mangrove killifish *(Rivulus magdalenae)* caused by living fish food *(Tubifex tubifex)* infected with *Mycobacterium marinum*. Dtsch Tierarztl Wochenschr 113(6):230–232.

Noga EJ. 2010. Mycobacteriosis. In: Fish disease: diagnosis and treatment. 2nd ed. Ames, IO: Blackwell Publishing , John Wiley & Sons; p. 204–208.

Noga EJ. 2011. Fish disease: diagnosis and treatment. Ames, IO: John Wiley & Sons.

Noga EJ, Wright JF, Pasarell L. 1990. Some unusual features of mycobacteriosis in the cichlid fish *Oreochromis mossambicus*. J Comp Pathol. 102(3):335–344.

Nordén Å, Linell F. 1951. A new type of pathogenic mycobacterium. Nature. 168:826.

Novotny L, Dvorska L, Lorencova A, Beran V, Pavlik I. 2004. Fish: a potential source of bacterial pathogens for human beings. Veterinary Med. 49:343–358.

Onwueme KC, Vos CJ, Zurita J, Ferreras JA, Quadri LE. 2005. The dimycocerosate ester polyketide virulence factors of mycobacteria. Prog Lipid Res. 44(5):259–302.

Ortega J, Noguera A, Garcia-Quiros A, Viana D, Selva L, de Juan L, Romero B, Garcia-Parraga D, Crespo JL, Corpa JM. 2014. Lesional patterns associated with mycobacteriosis in an Atlantic horse mackerel, *Trachurus trachurus (L.)*, aquarium population. J Fish Dis. 37(6):591–595. doi:10.1111/jfd.12145

Pandey AK, Sassetti CM. 2008. Mycobacterial persistence requires the utilization of host cholesterol. Proc Natl Acad Sci USA. 105(11):4376–4380. doi:10.1073/pnas.0711159105

Pasnik DJ, Smith SA. 2005. Immunogenic and protective effects of a DNA vaccine for *Mycobacterium marinum* in fish. Vet Immunol Immunopathol. 103(3):195–206.

Pattyn, R. (1984). *Mycobacterium marinum*. In: Kubica GP, Wayne LG, editors. The mycobacteria: A sourcebook. New York (NY): Markel Dekker; p. 1137.

Pedley S, Bartram JRG, Dufour A, Cotruvo J. 2004. Pathogenic mycobacteria in water: a guide to public health consequences, monitoring, and management. London (UK): IWA Publishing.

Petrini B. 2006. *Mycobacterium marinum*: Ubiquitous agent of waterborne granulomatous skin infections. Eur J Clin Microbiol Infect Dis. 25(10):609–613.

Poort M, Whipps C, Watral V, Font W, Kent M. 2006. Molecular characterization of a Mycobacterium species in non−native poeciliids in Hawaii using DNA sequences. J Fish Dis 29(3):181–185.

Post, G. 1987. Textbook of fish health. 2nd ed. Ascot: TFH Publications; p. 65–68.

Prouty MG, Correa NE, Barker LP, Jagadeeswaran P, Klose KE. 2003. Zebrafish-*Mycobacterium marinum* model for mycobacterial pathogenesis. FEMS Microbiol Lett. 225(2):177–182.

Rallis E, Koumantaki-Mathioudaki E. 2007. Treatment of *Mycobacterium marinum* cutaneous infections. Expert Opin Pharmacother 8(17): 2965–2978. doi:10.1517/14656566.8.17.2965

Ranzani-Paiva MJT, Ishikawa CM, Acd E, Silveira VRD. 2004. Effects of an experimental challenge with *Mycobacterium marinum* on the blood parameters of *Nile tilapia, Oreochromis niloticus* (Linnaeus, 1757). Brazilian Arch Biol Technol 47(6):945–953.

Reed MB, Domenech P, Manca C, Su H, Barczak AK, Kreiswirth BN, Kaplan J, Barry CE 3rd. 2004. A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. Nature. 431(7004):84–87.

Rhodes MW, Kator H, Kaattari I, Gauthier D, Vogelbein W, Ottinger CA. 2004. Isolation and characterization of mycobacteria from striped bass Morone saxatilis from the Chesapeake Bay. Dis Aquatic Organ. 61(1-2):41–51. doi:10.3354/dao061041

Roberts RJ. 2001. Fish pathology. London: WB SAUNDERS.

Ruley KM, Ansede JH, Pritchett CL, Talaat AM, Reimschuessel R, Trucksis M. 2004. Identification of Mycobacterium marinum virulence genes using signature-tagged mutagenesis and the goldfish model of mycobacterial pathogenesis. FEMS Microbiol Lett. 232(1):75–81.

Salati F, Meloni M, Fenza A, Angelucci G, Colorni A, Orru G. 2010. A sensitive FRET probe assay for the selective detection of Mycobacterium marinum in fish. J Fish Dis. 33(1):47–56.

Salogni C, Zanoni M, Covi M, Pacciarini ML, Alborali GL. 2007. Outbreak of Mycobacterium marinum in farmed rainbow trout (Oncorhynchus mykyss) and brown trout (Salmo trutta). Ittiopatologia. 4:227–237.

Sassetti CM, Rubin EJ. 2003. Genetic requirements for mycobacterial survival during infection. Proc Natl Acad Sci USA. 100(22):12989–12994. doi:10.1073/pnas.2134250100

Sechi LA, Colorni A, Duprè I, Molicotti P, Fadda G, Zanetti S. 2002. Strain variation in Mediterranean and Red Sca Mycobacterium marinum isolates. Microbiol Bologna. 25(3):351–356.

Senaratne RH, Sidders B, Sequeira P, Saunders G, Dunphy K, Marjanovic O, Reader JR, Lima P, Chan S, Kendall S, et al. 2008. Mycobacterium tuberculosis strains disrupted in mce3 and mce4 operons are attenuated in mice. J Med Microbiol. 57(Pt 2):164–170. doi:10.1099/jmm.0.47454-0

Slany M, Jezek P, Bodnarova M. 2013. Fish tank granuloma caused by Mycobacterium marinum in two aquarists: Two case reports. BioMed Res Int. Volume 2013 (2013), Article ID 161329.

Slany M, Makovcova J, Jezek P, Bodnarova M, Pavlik I. 2014. Relative prevalence of Mycobacterium marinum in fish collected from aquaria and natural freshwaters in central Europe. J Fish Dis. 37(6):527–533. doi:10.1111/jfd.12135.

Smith, I. 2003. Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. Clin Microbiol Rev. 16(3):463–496.

Sodjit T, Somrisi T, Chinabut S. 1993. A study of mycobacteriosis transmission in Siamese fighting fish (Betta splendens Regan). The AAHRI Newsletter 2, December 1993.

Stamm LM, Morisaki JH, Gao LY, Jeng RL, McDonald KL, Roth R, Takeshita S, Heuser J, Welch MD, Brown EJ. 2003. Mycobacterium marinum escapes from phagosomes and is propelled by actin-based motility. J Exp Med. 198(9):1361–1368. doi:10.1084/jem.20031072

Stoop EJM, Schipper T, Rosendahl Huber SK, Nezhinsky AE, Verbeek FJ, Gurcha SS, Besra GS,Vandenbroucke-Grauls CM, Bitter W, van der Sar AM. 2011. Zebrafish embryo screen for mycobacterial genes involved in the initiation of granuloma formation reveals a newly identified ESX-1 component. Dis Models Mechan 4(4):526–536. doi:10.1242/dmm.006676

Strike TB, Feltrer Y, Flach E, Macgregor SK, Guillaume S. 2016. Investigation and management of an outbreak of multispecies mycobacteriosis in Australian lungfish (Neoceratodus fosteri) including the use of triple antibiotic treatment. J Fish Dis. 40(4):557–570. doi:10.1111/jfd.12535

Sullivan JT, Young EF, McCann JR, Braunstein, M. 2012. The Mycobacterium tuberculosis SecA2 system subverts phagosome maturation to promote growth in macrophages. Infect Immun. 80(3):996–1006. doi:10.1128/iai.05987-11

Swaim LE, Connolly LE, Volkman HE, Humbert, O, Born DE, Ramakrishnan L. 2006. Mycobacterium marinum infection of adult Zebrafish causes caseating granulomatous tuberculosis and is moderated by adaptive immunity. Infect Immun. 74(11):6108–6117.

Swift S, Cohen H. 1962. Granulomas of the skin due to Mycobacterium balnei after abrasions from a fish tank. N Engl J Med. 267(24):1244–1246.

Talaat AM, Reimschuessel R, Trucksis M. 1997. Identification of mycobacteria infecting fish to the species level using polymerase chain reaction and restriction enzyme analysis. Vet Microbiol. 58(2-4):229–237.

Tobin DM, Ramakrishnan L. 2008. Comparative pathogenesis of Mycobacterium marinum and Mycobacterium tuberculosis. Cell Microbiol. 10(5):1027–1039.

Tønjum T, Welty D, Jantzen E, Small P. 1998. Differentiation of Mycobacterium ulcerans, M. marinum, and M. haemophilum: mapping of their relationships to M. tuberculosis by fatty acid profile analysis, DNA-DNA hybridization, and 16S rRNA gene sequence analysis. J Clin Microbiol. 36 (4):918–925.

Trigo G, Martins TG, Fraga AG, Longatto-Filho A, Castro AG, Azeredo J, Pedrosa J. 2013. Phage therapy is effective against infection by Mycobacterium ulcerans in a murine footpad model. PLoS Negl Trop Dis. 7(4):e2183.

Tsai HC, Kunin CM, Lee SSJ, Chen YS, Wann SR, Liu YW, Liu YC . 2007. Fish gambler's tenosynovitis caused by Mycobacterium marinum: environmental investigation of a fishing pond in Southern Taiwan. Diagn Microbiol Infect Dis. 59 (2):227–230.

Turenne CY, Tschetter L, Wolfe J, Kabani A. 2001. Necessity of quality-controlled 16S rRNA gene sequence databases: identifying nontuberculous Mycobacterium species. J Clin Microbiol. 39(10):3637–3648. doi:10.1128/jcm.39.10.3638-3648.2001

Ucko M, Colorni A. 2005. Mycobacterium marinum infections in fish and humans in Israel. J Clin Microbiol. 43(2):892–895. doi:10.1128/jcm.43.2.892-895.2005

Ucko M, Colorni A, Kvitt H, Diamant A, Zlotkin, A, Knibb WR. 2002. Strain variation in Mycobacterium marinum fish isolates. Appl Environ Microbiol 68(11):5281–5287.

van der Sar AM, Abdallah AM, Sparrius M, Reinders E, Vandenbroucke-Grauls CM, Bitter W. 2004. Mycobacterium marinum strains can be divided into two distinct types based on genetic diversity and virulence. Infect Immun 72 (11):6306–6312. doi:10.1128/iai.72.11.6306-6312.2004

van der Woude AD, Stoop EJ, Stiess M, Wang S, Ummels R, van Stempvoort G, Piersma SR, Cascioferro A, Jimenez CR, Houben EN, et al. 2014. Analysis of SecA2-dependent substrates in Mycobacterium marinum identifies protein kinase G (PknG) as a virulence effector. Cell Microbiol. 16 (2):280–295. doi:10.1111/cmi.12221

Vincent V, Brown-Elliott B, Jost K Jr, Wallace R Jr. 2003. Mycobacterium: phenotypic and genotypic identification. Manual of clinical microbiology. 8th ed. Washington (DC): ASM Press.

Von Betegh L. 1910. More reviews on experimental tuberclosis of marine fish. Zentrablatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Abteilung 153:54.

Watkins BY, Joshi SA, Ball DA, Leggett H, Park S, Kim J. et al. 2012. Mycobacterium marinum SecA2 promotes stable granulomas and induces tumor necrosis factor alpha in vivo. Infect Immun. 80(10):3512–3520.

Watral V, Kent ML. 2007. Pathogenesis of *Mycobacterium spp.* in Zebrafish (*Danio rerio*) from research facilities. Comp Biochem Physiol Toxicol Pharmacol: CBP. 145(1):55–60. doi:10.1016/j.cbpc.2006.06.004

Weerdenburg EM, Abdallah AM, Mitra S, de Punder K, van der Wel NN, Bird S, Appelmelk BJ, Bitter W, van der Sar AM. 2012. ESX-5-deficient *Mycobacterium marinum* is hypervirulent in adult Zebrafish. Cell Microbiol. 14(5):728–739. doi:10.1111/j.1462-5822.2012.01755.x

Weerdenburg EM, Abdallah AM, Rangkuti, F, El Ghany MA, Otto TD, Adroub SA, Molenaar D, Ummels R, Ter Veen K, van Stempvoort G, et al. 2015. Genome-wide transposon mutagenesis indicates that *Mycobacterium marinum* customizes its virulence mechanisms for survival and replication in different hosts. Immunity. 83(5):1778–1788.

Wheeler A, Graham B. 1989. Atypical mycobacterial infections. South Med J. 82(10):1250.

Wongworawat M, Holtom P, Learch T, Fedenko A, Stevanovic M. 2003. A prolonged case of *Mycobacterium marinum* flexor tenosynovitis: radiographic and histological correlation, and review of the literature. Skeletal Radiol. 32 (9):542–545.

Wu TS, Chiu CH, Su LH, Chia JH, Lee MH, Chiang PC, Kuo AJ, Wu TL, Leu HS.2002. *Mycobacterium marinum* infection in Taiwan. J Microbiol Immunol Infect. 35(1):42–46.

Yanong RP, Pouder DB, Falkinham JO III. 2010. Association of mycobacteria in recirculating aquaculture systems and mycobacterial disease in fish. J Aquatic Ani Health. 22 (4):219–223.

Zanoni RG, Florio D, Fioravanti ML, Rossi M, Prearo M. (2008). Occurrence of Mycobacterium spp. in ornamental fish in Italy. Journal Fish Disease 31(6):433–441. doi:10.1111/j.1365-2761.2008.00924.x

Zeeli T, Samra Z, Pitlik, S. 2003. Ill from eel? Lancet Infect Dis. 3(3):168.

Zhang DF, Ji C, Zhang XJ, Li TT, Li AH, Gong XN. 2015. Mixed mycobacterial infections in farmed sturgeons. Aquaculture Res. 46(8):1914–1923. doi:10.1111/are.12346