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MAJOR ARTICLE

Treatment and Outcome of Culture-Confirmed *Mycobacterium marinum* Disease

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Background. *Mycobacterium marinum* is a nontuberculous mycobacterium that causes skin and soft tissue infections. Treatment consists of multiple antibiotics, sometimes combined with surgical debridement. There is little evidence for the choice of antibiotics, the duration of treatment, and the role of susceptibility testing.

Methods. We performed a retrospective cohort study of culture-confirmed *M. marinum* infections in the Netherlands in the 2011–2018 period. Clinical characteristics, in vitro susceptibility, extent of disease, treatment regimens, and outcomes were analyzed. Incidence was assessed from laboratory databases.

Results. Forty cases of *M. marinum* infection could be studied. Antibiotic treatment cured 36/40 patients (90%) after a mean treatment duration of 25 weeks. Failure/relapse occurred in 3 patients, and 1 patient was lost to follow-up. Antibiotic treatment consisted of monotherapy in 35% and 2-drug therapy in 63%. Final treatment contained mostly ethambutol–macrolide combinations (35%). Eleven patients (28%) received additional surgery. We recorded high rates of in vitro resistance to tetracyclines (36% of isolates). Tetracycline resistance seemed correlated with poor response to tetracycline monotherapy. The annual incidence rate was 0.15/100 000/year during the study period.

Conclusions. Prolonged and susceptibility-guided treatment results in a 90% cure rate in *M. marinum* disease. Two-drug regimens of ethambutol and a macrolide are effective for moderately severe infections. Tetracycline monotherapy in limited disease should be used vigilantly, preferably with proven in vitro susceptibility.

Keywords. doxycycline; epidemiology; fish tank finger; *Mycobacterium marinum*; nontuberculous mycobacteria.

Mycobacterium marinum is a nontuberculous mycobacterium capable of causing skin and soft tissue infections. Most infections are associated with skin trauma and contact with contaminated water in fish tanks or pools or direct contact with infected fish [1–3]. The clinical manifestations are variable and range from papules, nodules, or ulcers to verrucous or erythematous plaques. Disease extent varies from mild cutaneous infections to severe infections with involvement of deeper structures, that is, tenosynovitis, osteomyelitis, or septic arthritis [4, 5]. The variable clinical presentation, the rarity of *M. marinum* infections, and the low sensitivity of microbiological diagnostics cause diagnostic delay; in outbreak settings, the sensitivity of culture can be as low as 50% [6].

In absence of clinical trial data, current treatment guidelines recommend treatment with 2 antibiotics until 2 months after resolution of symptoms; ethambutol–macrolide combinations are suggested as they balance efficacy and tolerability [7]. There is little evidence to support the choice of antibiotics and duration of the treatment. The exact role of drug susceptibility testing in the management of *M. marinum* is also not established, in part because most isolates show low minimum inhibitory concentrations (MICs) to the most frequently used antimycobacterial drugs. The notable exception are the tetracyclines doxycycline and minocycline, for which variation in MICs and treatment failure with acquired resistance have been recorded [8].

In this retrospective study, we analyzed treatments and outcomes of culture-confirmed *M. marinum* cases in the

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Netherlands. The aim of this work was to study currently employed treatment regimens, their correlation with in vitro susceptibility, and their outcomes in order to provide a better foundation for future treatments.

METHODS

We performed a retrospective cohort study of patients with a culture-confirmed skin and soft tissue infection caused by *Mycobacterium marinum*. The patients were identified through the database of the Radboudumc mycobacteriology reference laboratory; we included all patients for whom *M. marinum* isolates were cultured or sent in the 2011–2018 period.

All *M. marinum* isolates were identified by the InnoLiPA mycobacteria, version 2, line probe assay, with supplementary *hsp65* gene sequencing [9]. We analyzed the results of drug susceptibility testing if it was performed using broth microdilution, according to Clinical and Laboratory Standards Institute (CLSI) guidelines [10].

Demographic, clinical, and treatment and outcome data were collected from patient records of 8 hospitals in the Netherlands. We applied the following definitions:

Extent of disease was classified as stage I if there was a single lesion <3 cm in diameter, stage II if there was a single lesion >3 cm in diameter, stage III for multiple lesions with lymphatic spread, or stage IV in case of involvement of deeper structures, similar to the staging by Hurst and colleagues, which considers our stage I and II as a single entity [11]. Involvement of deeper structures was defined as an infection with radiological, histological, or cytological signs of tenosynovitis, septic arthritis, or osteomyelitis.

The empirical antibiotic treatment regimen was the first treatment regimen that was prescribed after presentation. Initial treatment was the regimen continued or started at the time of the culture-confirmed diagnosis. The final antibiotic regimen was defined as the regimen used for the majority of the treatment period, after the diagnosis of *M. marinum* was made.

To study treatment outcomes, we defined cure as resolution of lesions at the end of treatment or significant improvement at the end of treatment without a relapse within the next 6 months. We defined failure as no significant improvement of skin lesions or a culture-confirmed relapse within 6 months after the end of treatment.

Ethical approval for this retrospective file study was granted by the medical ethics committee of the Radboudumc (file nr: 2019-5639); this approval was endorsed by the medical ethics committees of participating centers.

To determine the incidence of culture-confirmed *M. marinum* infections, we combined the Radboudumc reference laboratory database data with data from the tuberculosis reference laboratory at the National Institute for Public Health and the Environment (RIVM) to obtain national coverage. Only 1 isolate per patient was used. The annual incidence rates were calculated as the number of incident cases per 100 000 persons using the annual population census of the Netherlands from the Central Bureau of Statistics (Heerlen, the Netherlands; www.cbs.nl).

RESULTS

From January 1, 2011, to December 31, 2018, we identified 71 patients with culture-confirmed *M. marinum* disease. Medical records with treatment details were available for 40 patients. The baseline characteristics of the patients are recorded in Table 1. Sixty-eight percent were men, with a mean age of 58 years. Six patients (15%) used immunosuppressive medication including 1 patient using adalimumab (anti-TNF). Eighty-three percent of patients had evident fish tank exposures, and 58% presented with stage III disease.

Treatment

Eighteen patients received empirical antibiotics at first presentation (Table 1). The initial and final treatment regimens for *M. marinum* disease are listed in Table 2. Upon diagnosis, 21 patients received monotherapy (53%), 17 (43%) started on 2-drug regimens, and 2 patients started on 3-drug regimens.

Fourteen patients (35%) remained on monotherapy throughout. Monotherapy was used for 6/8 patients with stage I, 1/3 with stage II, 7/23 with stage III, and 0/6 with stage IV disease.

Table 1. Baseline Characteristics of 40 Patients With *M. marinum* Disease

Demographics	No. (%)
Age, mean (SD), y	58 (15)
Male	27 (68)
Immunocompromised	6 (15)
Diabetes mellitus	4 (10)
Skin disorder	3 (8)
Exposure	
Aquarium	33 (83)
Diving/swimming	3 (7)
Unknown	4 (10)
Localization	
Upper extremity	36 (90)
Lower extremity	3 (7)
Disseminated	1 (3)
Extent	
Stage 1	8 (20)
Stage 2	3 (8)
Stage 3	23 (58)
Stage 4	6 (15)
Empiric antibiotics	
None	22 (55)
β-lactam antibiotics	10 (25)
Tetracyclines	4 (10)
Macrolides	1 (3)
Co-trimoxazole	1 (3)
Clindamycin	2 (5)

Table 2. Treatment Regimens in 40 Patients Treated for *M. marinum* Disease

Initial Treatment	No. (%)
Doxycycline	10 (25)
Minocycline	4 (10)
Clarithromycin	6 (15)
Cotrimoxazole	1 (2.5)
EMB + CLA	8 (20)
EMB + AZI	3 (7.5)
RIF + EMB	3 (7.5)
RIF + CLA	3 (7.5)
RIF + EMB + CLA	1 (2.5)
RIF + EMB + AZI	1 (2.5)
Final Treatment Regimen	No. (%)
Doxycycline	4 (10)
Minocycline	3 (7.5)
Clarithromycin	5 (12.5)
Cotrimoxazole	2 (5)
EMB + CLA	11 (27.5)
EMB + AZI	3 (7.5)
RIF + EMB	6 (15)
RIF + CLA	3 (7.5)
CLA + DOX	2 (5)
RIF + EMB + AZI	1 (2.5)
Treatment duration	
Mean total treatment duration: 25 ± 14 wk	
Mean duration of treatment after clinical cure: 6 ± 3 wk	
Surgery	11 (28)

Abbreviations: AZI, azithromycin; CLA, clarithromycin; EMB, ethambutol; RIF, rifampicin.

Twenty-four patients (60%) were treated with 2-drug regimens for the majority of the treatment duration. Treatment duration was not significantly different for monotherapy or multidrug therapy (20.6 ± 8.2 vs 27.7 ± 15.7 weeks; $P = .13$). The mean duration of treatment after lesion healing (range) was 6 (2–12) weeks. Eleven patients (28%) received adjunctive surgery.

Thirteen patients (33%) required a total of 17 changes to treatment regimens; 7 patients switched from tetracycline monotherapy to 2-drug regimens because of lack of response (n = 5, 2 with proven doxycycline resistance, 1 doxycycline-susceptible, 2 without susceptibility test) or results of susceptibility testing without record of poor response (n = 2). Three patients switched drug classes because of adverse events; the nature of these adverse events was not recorded.

Outcome

Cure was attained in 36 of 40 cases reviewed (90%); 3 patients experienced failure (n = 1) or a relapse (n = 2) and required retreatment. One patient initially responded but was lost to follow-up. One relapse was attributable to poor treatment compliance.

Susceptibility and its Relation to Outcome

Broth microdilution susceptibility test results were available for 28 patients. The highest rates of resistance in *M. marinum* were observed for ciprofloxacin (18/28; 64%), doxycycline (10/28; 36%), moxifloxacin and rifampicin (both 4/28; 14%), and ethambutol and cotrimoxazole (both 1/28; 4%); no resistance to macrolides was found. The minimum inhibitory concentration (MIC) distribution of doxycycline is presented in Figure 1.

In 22/28 (79%) patients, treatment included only drugs with proven in vitro susceptibility, or treatment was only started when these results were available; 2 of these 22 patients (9%), both with stage III disease, required a change in regimen because of poor response (1 minocycline monotherapy escalated to ethambutol–rifampicin; 1 ethambutol–clarithromycin escalated to rifampicin–ethambutol–clarithromycin).

In 6 patients (6/28; 21%), the initial treatment included a drug to which resistance was observed in vitro. In 5 of these cases, treatment was altered, either because of the results of susceptibility testing (n = 2, doxycycline monotherapy switched to ethambutol–clarithromycin) or lack of clinical improvement (n = 3; 1 switched from rifampicin–ethambutol–clarithromycin to doxycycline–clarithromycin because of rifampicin and ethambutol resistance, 2 switched from doxycycline monotherapy to rifampicin–ethambutol and ethambutol–clarithromycin because of doxycycline resistance). One patient was successfully treated with doxycycline despite in vitro resistance.

The Dutch National Institute for Public Health and the Environment received an additional 134 positive cultures from 2011 to 2018. Incidence rates were stable at 0.11–0.22 (mean, 0.15) per 100 000 persons per year during the study period.

DISCUSSION

In this study, we evaluated treatments and outcomes of 40 cases of culture-confirmed *M. marinum* disease. All patients received antibiotics, mostly ethambutol combined with clarithromycin. Treatment outcomes were good but required prolonged administration of mostly multiple antibiotics. Susceptibility to ethambutol and macrolides was good, but resistance to tetracyclines (34%) was common; 2 out of 3 patients treated with doxycycline monotherapy showed a poor treatment response.

The 2007 American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) statement recommends treatment with 2 active drugs until 1–2 months after symptom resolution, with ethambutol–macrolide combinations representing the best balance between efficacy and tolerability [7]. The statement also mentions the option of monotherapy in minimal disease [7]. Accepting stage I and stage II as minimal disease, 33/40 (83%) patients were treated in accordance with the ATS/IDSA statement in terms of regimen and duration. The use of monotherapy in more advanced disease (stage III) was observed in 7 patients but did not lead to treatment failure. In part, the success of monotherapy may result from the use of susceptibility testing to guide treatment.

Treatment outcomes overall were very good, with a cure rate of 90%. Associations between actual regimens or

treatment durations and the outcome cannot be defined given the low relapse/failure rate and the nature of the investigation. The choice of drugs and treatment duration were in line with previous case series [3–5]. Treatment had to be changed in 33% of patients, equal to the 35% described in 2 previous cohorts [3, 4].

Our cohort stands out for its infrequent use of tetracycline antibiotics, as only 9 patients (22%) received these for the majority of the treatment duration, whereas some large studies have shown rates of 36%–87% [5, 12, 13]. The use of these tetracycline antibiotics was actively discouraged by consultants from the Radboudumc reference clinic because of the frequency of resistance. Yet, the clinical impact of in vitro doxycycline resistance has never been substantiated. The relatively high rate of resistance is largely explained by the fact that the breakpoints lie in the middle of the MIC distribution (Figure 1). In addition, doxycycline or minocycline MICs are determined by 80% growth inhibition, as suggested by CLSI guidelines [10], which is very challenging and allows for inter-reader variability. This makes interpreting susceptibility within cohorts as well as between cohorts difficult. Therefore, a clinical trial with minocycline or doxycycline monotherapy or an in vitro pharmacodynamic model—for example, an animal model or hollow fiber model—is of added value in assessing the impact of MICs on treatment outcome.

Nearly all cases had aquatic exposure, mostly fish tanks. Fish tank maintenance is the best known risk factor, and previously *M. marinum* has been isolated from fish [3, 4, 14, 15]. This exposure also explains the most common localization being the upper extremities, with hands being exposed during fish tank maintenance.

Incidence rates remained stable over the study period. Incidence rates of 0.11–0.22 in the Netherlands are slightly higher than those reported from Denmark by Holden et al. [5], who reported an incidence of 0.05–0.13 per 100 000 persons from 2010 to 2016. Possible explanations could be more fish tank owners, more awareness among physicians, or differences in diagnostic practices, that is, use of biopsies and mycobacterial cultures.

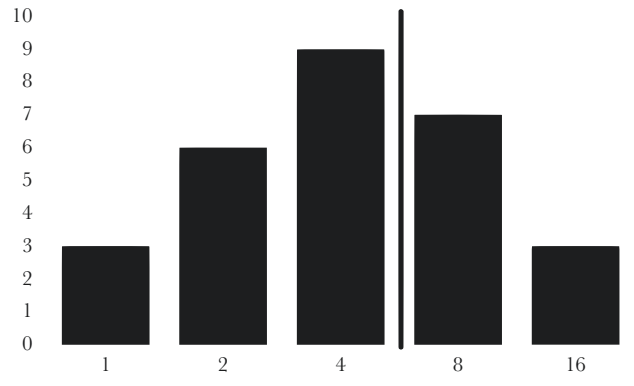

Figure 1. Doxycycline minimum inhibitory concentration distribution in 28 *M. marinum* isolates. The line represents the breakpoint to define resistance according to CLSI document M24-A2, relevant to the study period. Abbreviations: CLSI, Clinical and Laboratory Standards Institute; MIC, minimum inhibitory concentration.

Despite a reasonable cohort size, all limitations inherent to the retrospective medical file reviews apply to our study. Detailed analysis of important aspects including adverse events was not possible for most patients due to incomplete data. Incidence rates are likely underestimated as we only included culture-confirmed infections. Cultures are not always performed, and the sensitivity of cultures might be low [6]. The distribution of extent of disease may be biased because of our inclusion of culture-confirmed cases only; stage I and stage II disease may be more difficult to confirm by culture or may be treated without seeking confirmation of the diagnosis.

In conclusion, this retrospective cohort study underlines that treatment according to the ATS/IDSA statement, that is, with ethambutol–macrolide combinations for stage III disease, leads to a favorable outcome in almost all patients with *M. marinum* disease, provided that treatment is continued for 1–2 months after skin lesions have stabilized or resolved. Given the high rates of in vitro resistance to doxycycline and observed treatment failures, vigilance is warranted when treating (suspected) minimal (stage I or II) *M. marinum* disease with minocycline or doxycycline monotherapy. In vitro susceptibility and treatment outcomes seemed to be correlated in the current cohort, but the true merit of susceptibility testing should be addressed in dedicated studies, preferably a clinical trial.

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Patient consent. Ethical approval for this retrospective file study was granted by the medical ethics committee of the Radboudumc (file nr: 2019-5639); this approval was endorsed by the medical ethics committees of participating centers. Individual patient consent was not required.

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