Nontuberculous Mycobacterial Skin Infections: A 20-Year Retrospective Study

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ABSTRACT:

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Background: Cutaneous nontuberculous mycobacterial infections can present with a wide variety of clinical and histopathological features, and there are currently no standard treatment guidelines.

Objectives: To investigate the clinical characteristics, histopathologic findings, treatment protocols and clinical outcomes of patients with nontuberculous mycobacterial skin infections.

Materials and Methods: The medical records of patients with culture-positive cutaneous nontuberculous mycobacterial infections who attended granuloma clinic at the Department of Dermatology, Siriraj Hospital, between January 1994 and December 2014 were reviewed retrospectively.

Results: Of 98 culture-positive patients, 37 (37.8%) were male, 61 (62.2%) were female, and the mean age was 42.53 (±16.39) years. Legs were the most common affected site. Plaques, nodules, and papules were the most observed presenting morphologies. The major causative organisms were rapidly

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growing mycobacteria, especially *Mycobacterium abscessus* (36.6%). The most frequent histopathologic findings were mixed cell granuloma and suppurative granuloma. *In vitro* antimicrobial resistance rates of rapidly growing mycobacteria were higher than *Mycobacterium marinum*. Empirically, clarithromycin combined with ciprofloxacin was prescribed for rapidly growing mycobacteria (RGM) infections, and doxycycline for *M. marinum* infections. The overall response rate was 95.5%.

Conclusion: Cutaneous lesions with a high index of suspicion of nontuberculous mycobacterial infections are recommended to request for histopathological study, microbiological culture, and antimicrobial susceptibility test. For localized RGM skin infections, a proper initial empirical protocol is a combination therapy with macrolide-based regimens, such as clarithromycin and ciprofloxacin. Doxycycline monotherapy is effective for *M. marinum* infection. The treatments can be modified based on laboratory reports and clinical responses.

Key words: Nontuberculous mycobacteria, Skin infection

บทคัดย่อ:

มนัสมน ชัยรัชนีบูลย์ ธีทัต สุรวรรณ ปรมินทร์ ปัทมาลัย การติดเชื้อมัยโคแบคทีเรียที่ไม่ใช่เชื้อวัณโรคที่ผิวหนัง ศึกษาย้อนหลัง 20 ปี วารสารโรคผิวหนัง 2561; 34: 111-129.

ภาควิชาตจวิทยา คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

การติดเชื้อมัยโคแบคทีเรียที่ไม่ใช่เชื้อวัณโรคที่ผิวหนังมี อาการแสดง และลักษณะทางจุลพยาธิวิทยาที่หลากหลาย อีกทั้งยังไม่มีแนวทางการรักษามาตรฐานในปัจจุบัน

วัตถุประสงค์: เพื่อศึกษาลักษณะทางคลินิก ลักษณะทางจุลพยาธิวิทยา การรักษา และการดำเนินโรคของผู้ป่วยโรคติดเชื้อมัยโคแบคทีเรียที่ไม่ใช่เชื้อวัณโรคที่ผิวหนัง

วิธีการศึกษา: มีการรวบรวมข้อมูลจากเวชระเบียนผู้ป่วยที่ได้รับการวินิจฉัยเป็นโรคติดเชื้อมัยโคแบคทีเรียที่ไม่ใช่เชื้อวัณโรคที่ผิวหนัง เฉพาะผู้ป่วยที่มีผลการเพาะเชื้อเป็นบวก ณ คลินิกโรคแกรนูโลมา หน่วยตรวจโรคผิวหนัง โรงพยาบาลศิริราช โดยรวบรวมย้อนหลังตั้งแต่ปีพุทธศักราช 2537-2557 และนำข้อมูลมาศึกษาวิเคราะห์

ผลการศึกษา: จากข้อมูลเวชระเบียนผู้ป่วยทั้งหมด 98 รายที่เข้าเกณฑ์การศึกษา เป็นเพศชาย 37 คน (ร้อยละ 37.8) เพศหญิง 61 คน (ร้อยละ 62.2) อายุเฉลี่ย คือ 42.53 ปี (±16.39) จากการศึกษานี้พบว่า ตำแหน่งของร่างกายที่พบการติดเชื้อมากที่สุดคือขา ลักษณะทางคลินิกที่พบบ่อยได้แก่ ปื้นนูน ก้อนลึก และตุ่มนูน เชื้อก่อโรคที่พบเป็นส่วนใหญ่ คือ กลุ่มมัยโคแบคทีเรียที่ไม่ใช่เชื้อวัณโรคชนิดที่เติบโตเร็ว โดยเฉพาะ *Mycobacterium abscessus* (ร้อยละ 36.6) ลักษณะทางจุลพยาธิวิทยาที่ตรวจพบมากที่สุด คือ mixed cell granuloma และ suppurative granuloma จากการทดสอบความไวต่อยาต้านจุลชีพในหลอดทดลอง พบว่าเชื้อมัยโคแบคทีเรียที่ไม่ใช่เชื้อวัณโรคชนิดที่เติบโตเร็วมีอัตราการดื้อยาสูงกว่า *Mycobacterium*

marinum ยาอันดับแรกที่เลือกใช้บ่อยที่สุดเมื่อสงสัยการติดเชื้อมัยโคแบคทีเรียที่ไม่ใช่เชื้อวัณโรคชนิดที่เติบโตเร็ว คือ clarithromycin ร่วมกับ ciprofloxacin ส่วน doxycycline ถูกใช้บ่อยที่สุดเมื่อสงสัยการติดเชื้อ *M. marinum* โดยพบว่าการตอบสนองต่อการรักษาในกลุ่มศึกษาทั้งหมดสูงถึงร้อยละ 95.5

สรุปผล: รอยโรคบริเวณผิวหนังที่มีลักษณะน่าสงสัยว่าจะเกิดจากการติดเชื้อมัยโคแบคทีเรียที่ไม่ใช่เชื้อวัณโรค ควรส่งตรวจชิ้นเนื้อทางจุลพยาธิวิทยา ส่งเพาะเชื้อ และส่งตรวจความความไวต่อยาต้านจุลชีพในหลอดทดลอง การติดเชื้อที่ผิวหนังบริเวณแบบเฉพาะที่ ในกลุ่มที่ติดเชื้อมัยโคแบคทีเรียที่ไม่ใช่เชื้อวัณโรคชนิดที่เติบโตเร็ว แนะนำให้เริ่มการรักษาด้วยยาต้านจุลชีพอย่างน้อย 2 ชนิดร่วมกันโดยมีกลุ่ม macrolides เป็นหลัก เช่น clarithromycin ร่วมกับ ciprofloxacin เป็นต้น ส่วนการติดเชื้อ *M. marinum* ให้การรักษาด้วยยาชนิดเดียวได้ เช่น doxycycline เป็นต้น และควรมีการปรับยาตามการตอบสนองทางคลินิกร่วมกับผลการตรวจความไวต่อยาต้านจุลชีพ

คำสำคัญ: มัยโคแบคทีเรียที่ไม่ใช่เชื้อวัณโรค, การติดเชื้อที่ผิวหนัง

Introduction

Nontuberculous mycobacteria (NTM) are ubiquitous, slender, non-motile, acid-fast bacilli identified over 150 species. A number of different environmental sources, including water, soil, wild animals, household, aerosol, or even hospital environments, are identified as their habitats.[1-3] According to growth rate on artificial culture media, NTM are classically divided into rapidly growing mycobacteria (RGM) and slowly growing mycobacteria (SGM). RGM colony formation time is 3-7 days while SGM need more than 7 days to form full-grown colony. Runyon additionally classifies NTM into 4 groups according to their chromogen production properties.[4-7]

Four main clinical manifestations of NTM infections comprise of pulmonary disease, lymphadenitis, cutaneous infection, and disseminated disease. Infection from direct exposure to these organisms is associated with daily lives, occupations, injuries, and hobbies.[1-3] For skin and soft tissue infections (SSTIs), approximately 30 mycobacteria have been reported as human pathogens. Cutaneous presentations and histopathological findings of NTM are diverse and nonspecific.[2,3,8-11] The definite diagnosis of NTM SSTIs requires a positive microbiological culture from a sterile site to avoid colonizers contamination, because NTM are environmental organisms. Moreover, some fastidious organisms are difficult to isolate because they require special media and incubation temperature for growth. The variations in clinical presentation and the long period of time to identify the organisms may contribute to the delays in diagnosis and treatment. Biochemical and molecular diagnostic techniques, such as high-performance liquid chromatography (HPLC), DNA hybridization, gene

amplification-based assays, nucleic acid sequencing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) have been developed to improve the sensitivity and accuracy of NTM identification.[5,7,11,12] Even though we have known that antibiotics alone, or combined with surgical procedure, are the effective treatments, there is no clear consensus in management guidelines for cutaneous NTM infections.[2-3,11] As mentioned above, NTM SSTIs may be underdiagnosed, and the diagnosis and treatments are still challenging.

Previous studies demonstrated an increasing incidence of cutaneous NTM SSTIs over the past decades. No significant difference was found in patient characteristics, yet the majority of causative organisms have changed from SGM to RGM group.[11,13,14] Upsurge of aesthetic procedures and improvement of diagnostic tools may enhance detection of NTM infections. Although many NTM SSTIs have been discovered in Thailand, the epidemiology reports are limited. The predisposing factors for cutaneous NTM infections among Thai include tropical climate and agricultural occupations.[8-10] Additional studies are needed for further understanding about current situation of cutaneous NTM infections in Thailand. Therefore, this study aimed to investigate the clinical manifestations, histopathological and microbiological studies, treatment regimens, and clinical outcomes in a large tertiary hospital data pool.

Materials and Methods

Subjects

A retrospective chart review of patients with cutaneous NTM infections who attended granuloma clinic at the Department of Dermatology, Siriraj Hospital, from January 1994 to December 2014 was conducted. This study was approved by the institutional review boards of the Faculty of Medicine Siriraj Hospital and performed in compliance with the latest version of the Declaration of Helsiki. Since this study involved a retrospective chart review with remained anonymous patients, informed consent was not obtained from the patients included. The diagnosis of cutaneous NTM infections was defined as a positive NTM culture from a biopsy specimen or discharge material of a skin lesion, concomitant with a compatible clinical presentation. Demographic data, underlying diseases, history of previous trauma, cosmetic procedure, or surgery, clinical manifestations, laboratory investigations including histopathological studies, microbiological cultures and antimicrobial susceptibility tests, treatment regimens and outcomes were reviewed. Cured was defined as a complete healing, with or without a negative result of repeated tissue culture of the affected

skin and the antibiotics can be discontinued. Improved was defined as the clinical improvement, i.e., the smaller skin lesions without any new lesions, and the antibiotics, however, were still continued for another period of time. Medical records were retrieved according to fulfilled diagnostic criteria. Incomplete data were excluded. Data were then compared and analyzed between two decades, 1994-2004 and 2004-2014.

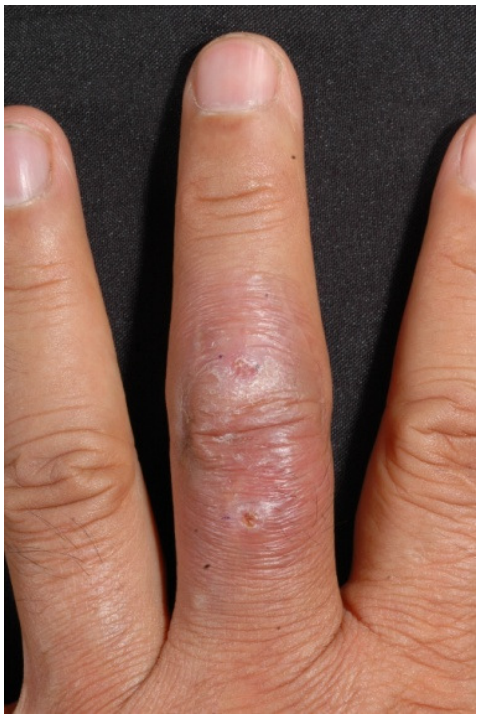

Figure 1 An erythematous plaque with scales and crusts on left middle finger represented a fish-tank granuloma (cutaneous *M. marinum* infection).

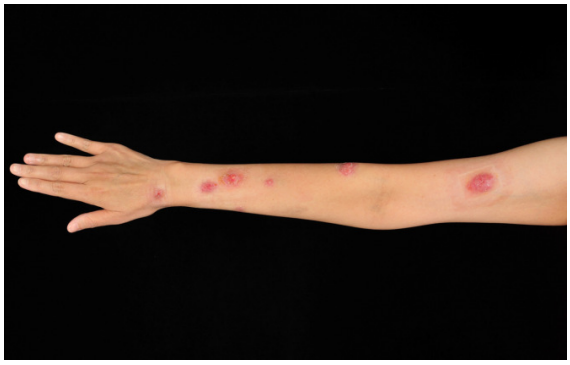

Figure 2 Multiple papulonodular lesions in a sporotrichoid pattern in a patient with cutaneous MAC infection.

Statistical analysis

A chi-squared test was used to compare differences in categorical data while continuous variables were compared by an independent *t*-test. A *p*-value of < 0.05 was considered statistically significant. All analyses were performed using Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA).

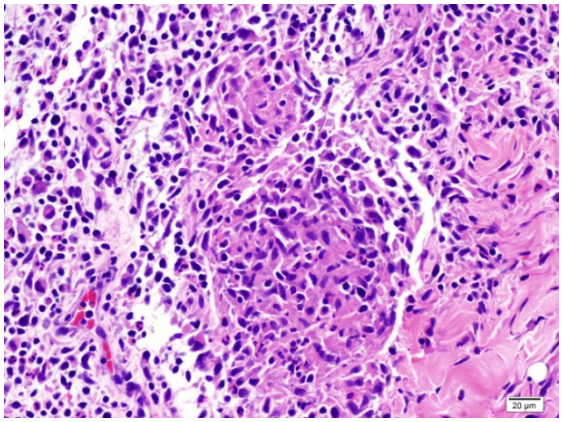

Figure 3 Noncaseating granulomatous inflammation (Hematoxylin-Eosin stain, ×400).

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Results

Clinical characteristics

Medical records of patients diagnosed as NTM SSTIs with culture-positive who attended granuloma clinic at Siriraj Hospital during 1994 to 2014 were reviewed retrospectively. Clinical characteristic data were demonstrated in Table 1. Of the 98 patients who registered in this study, 37 patients (37.8%) were male and 61 patients (62.2%) were female. The mean age (±standard deviation, SD) was 42.53 (±16.39) years. The median duration of symptoms at the first visit was 9 weeks (range, 1-60 weeks). More than half of the patients reported preexisting causes including traumatic injury (26 patients; 46.4%), surgery (13 patients; 23.2%) and cosmetics procedure (9 patients; 16.1%). Of the 26 patients with trauma, 3 of the 4 patients who were presented with aquatic-related injury had culture positive for *M. marinum*. *M. marinum* was also identified in 4 of the 6 patients with history of fish related activity. Most patients in our study, 85 (86.7%) were immunocompetent, and only 13 (13.3%) were immunocompromised. Almost all patients had localized cutaneous infections (92 patients; 93.9%). The number of patients with single lesion was almost equal to multiple lesions. Only 6 patients (6.1%) were presented with disseminated infections involving the skin, but no identifiable immunocompromising factor was found in these cases. The most common infection sites were legs. The most frequently observed morphologies of presenting skin lesions were plaques, nodules, and papules, respectively. The fish-tank granuloma in cutaneous *M. marinum* infection and sporotrichoid distribution in MAC infection were illustrated in Figure 1 and Figure 2. The most common causative species were *M. abscessus* (36.6%), *M. fortuitum* (22.8%), and *M. marinum* (16.8%). MAC infections were found in 2 cases, and both were immunocompromised. Other identified mycobacteria were displayed in the Table 1.

Table 1 Characteristics of nontuberculous mycobacterial skin and soft tissue infections.

Variables	Values
Age at Diagnosis (years), mean (±SD)	42.53 (±16.39)
Sex (n=98)	
Female	61 (62.2%)
Male	37 (37.8%)
Previous History (n=56)*	
Trauma	26 (46.4%)
Aquatic-related injury	4 (15.4%)
Surgery	13 (23.2%)
Cosmetics Procedure	9 (16.1%)
Fish related activity	6 (10.7%)
Immunocompetent Host	85 (86.7%)
Immunocompromised Host	13 (13.3%)
Localized cutaneous Infection	92 (93.9%)
Disseminated Infection involving the skin	6 (6.1%)
Site (n=134)**	
Legs	55 (41.0%)
Head and Neck	19 (14.2%)
Forearms	17 (12.7%)
Arms	15 (11.2%)
Trunk	11 (8.2%)
Hands	7 (5.2%)
Feet	5 (3.7%)
Fingers	3 (2.2%)
Toes	2 (1.5%)
Number of Lesions (n=98)	
Single	50 (51.0%)
Multiple	48 (49.0%)

Table 1 (Cont.) Characteristics of nontuberculous mycobacterial skin and soft tissue infections.

Variables	Values
Morphology (n=130)**	
Plaque	61 (46.9%)
Nodule	33 (25.4%)
Papule	15 (11.5%)
Ulcer	9 (6.9%)
Abscess	5 (3.8%)
Sporotrichoid	4 (3.1%)
Sinus	3 (2.3%)
Causative Organisms (n=101)**	
Rapidly Growing Mycobacteria	
Mycobacterium abscessus	37 (36.6%)
Mycobacterium fortuitum	23 (22.8%)
Mycobacterium chelonae	15 (14.9%)
Slowly Growing Mycobacteria	
Mycobacterium marinum	17 (16.8%)
Mycobacterium avium complex (MAC)	2 (2.0%)
Mycobacterium scrofulaceum	1 (1.0%)
Mycobacterium haemophilum	1 (1.0%)
Mycobacterium intracellulare	1 (1.0%)
Other Mycobacteria, unspecified	2 (2.0%)
Mixed Infections	
M. fortuitum + M. chelonae	1 (1.0%)
M. szulgai + M. terrae	1 (1.0%)

* n < 98 due to no available data in the medical records

** n > 98 due to multiple lesions in some patients

While the mean age at diagnosis of patients who registered in 1994-2003 and 2004-2014 was not significantly different, the number of female patients substantially increased in the second decade (*p*-value = 0.047). Previous trauma was the most frequent external predisposing factor and legs were still reported as common infected sites in both decades. *M. fortuitum* and *M. chelonae* were the predominant causative pathogens in the first decade, whereas *M. abscessus* was the most common in the second decade (Table 2). The patients infected with *M.*

marinum remained unchanged between the 2 periods. Granulomatous inflammation was a major histopathological finding (Figure 3). The most frequent histopathological diagnoses were mixed cell granuloma and suppurative granuloma, respectively (Table 3a). Abscess, psoriasiform dermatitis, and chronic nonspecific inflammation were infrequently observed. AFB stain was positive only in 15.8% of biopsy specimens, and negative in all the discharge materials.

Table 2. Comparison of cutaneous NTM infection features by a decade.

Characteristics	1994-2003 n (%)	2004-2014 n (%)	*p*-value
Age at Diagnosis (years), mean (±SD)	40.13 (±17.64)	44.05 (±15.51)	0.256
Sex			
Female/Male	19/19	42/18	**0.047***
Previous History			
Trauma	11 (55.0%)	15 (44.1%)	0.307
Surgery	6 (30.0%)	7 (20.6%)	0.501
Cosmetics Procedure	1 (5.0%)	8 (23.5%)	0.107
Site			
Legs	19 (32.2%)	36 (48.0%)	0.331
Head and Neck	12 (20.3%)	7 (9.3%)	**0.015***
Forearms	8 (13.6%)	9 (12.0%)	0.441
Arms	5 (8.4%)	10 (13.3%)	0.638
Trunk	7 (11.9%)	4 (5.3%)	0.072
Hands	2 (3.4%)	5 (6.7%)	0.565
Feet	4 (6.8%)	1 (1.3%)	0.052
Fingers	1 (1.7%)	2 (2.6%)	0.844
Toes	1 (1.7%)	1 (1.3%)	0.742
Causative Organisms			
M. abscessus	1 (2.6%)	36 (59.0%)	**< 0.001***
M. fortuitum	13 (33.3%)	10 (16.4%)	**0.046***
M. chelonae	12 (30.8%)	3 (4.9 %)	**< 0.001***
M. marinum	9 (23.1%)	8 (13.1%)	0.187

* *p*-value < 0.05

Table 3a Histopathologic diagnosis of NTM skin and soft tissue infections.

Histopathologic Diagnosis	n (%)	Acid-Fast Bacilli		
		positive	negative	N/A*
Granulomatous inflammation				
Mixed cell granuloma	49 (51.6%)	7 (14.3%)	39 (79.6%)	3 (6.1%)
Suppurative granuloma	23 (24.2%)	6 (26.1%)	15 (65.2 %)	2 (8.7%)
Tuberculoid granuloma	1 (1.1%)	0	1 (100%)	0
Unspecified granuloma	9 (9.5%)	0	7 (77.8%)	2 (22.2%)
Abscess	4 (4.2%)	0	3 (75.0%)	1 (25.0%)
Psoriasiform dermatitis	1 (1.1%)	0	1 (100%)	0
Chronic nonspecific inflammation	8 (8.4%)	2 (25.0%)	2 (25.0%)	4 (50.0%)
Total	95 (100%)	15 (15.8%)	68 (71.6%)	12 (12.6%)

Table 3b Acid-Fast Bacilli staining from discharge smear.

Specimens	n (%)	Acid-Fast Bacilli	
		positive	Negative
Tissue aspiration	2 (66.7%)	0	2 (100%)
Tissue swab	1 (33.3%)	0	1 (100%)
Total	3 (100%)	0	3 (100%)

* N/A = Not Available

Therapeutics and outcomes

The empiric antibiotics were initially prescribed to patients according to the culture isolates, then we modified treatments in some cases according to clinical responses and microbiological results. In our clinic, for patients who had localized, non-severe skin lesions, we generally prescribed clarithromycin combined with ciprofloxacin to most patients with RGM (e.g., *M. fortuitum, M. abscessus, M. chelonae*) infections, and doxycycline monotherapy to *M.marinum* infected patients. The antimicrobial susceptibility of NTM was summarized in table 4. Antimicrobial susceptibility test (AST) from the isolates in this study revealed the broad-spectrum activity of amikacin against *M. abscessus* (42.9%), *M. fortuitum* (53.8%), *M. chelonae* (85.7%), and *M. marinum* (100%).

Clarithromycin also had activity against *M. abscessus* (57.1%), *M. fortuitum* (46.2%), *M. chelonae* (50%), and *M. marinum* (100%). Ciprofloxacin sensitivity rates were 83.3% to *M. marinum*, and 50% to *M. fortuitum*, but no activity against *M. abscessus* and *M. chelonae.* RGM tended to resist to doxycycline, while *M. marinum* was sensitive approximately 60% to this drug. The treatments and clinical results was display in table 5. A combination therapy with ciprofloxacin and clarithromycin was the most often prescribed regimen, especially for RGM infected patients. Azithromycin plus rifampicin and ethambutol were given to a SLE patient with cutaneous MAC infection for 17 months and the disease was cured. Surgical treatment alone was performed in 4 patients who presented with single small lesion. Surgical procedures in combination with medical treatment were given to 15 patients in this study. Patients with disseminated disease were hospitalized for intravenous antibiotics administration. Median duration of antimicrobial treatments was 24.0 weeks (4-84 weeks in range). Ten patients were lost to follow-up after 1 or 2 visits. Of the 88 patients who completed the study, the clinical response rate was 95.5%.

Table 4 Antimicrobial susceptibility test report of nontuberculous mycobacteria.

AST		Rapidly Growing Mycobacteria			Slowly Growing Mycobacteria		
		M. abscessus (n=28)	*M. fortuitum* (n=13)	*M. chelonae* (n=7)	*M. marinum* (n=6)	*M. intracellulare* (n=1)	*M. terrae* (n=1)
AMK	S	42.9% (12)	53.8% (7)	85.7% (6)	100% (6)	100% (1)	N/A
AMC	S	0% (0)	0% (0)	N/A	N/A	N/A	N/A
FEP	S	0% (0)	0% (0)	N/A	N/A	N/A	N/A
FOX	S	3.6% (1)	0% (0)	0% (0)	0% (0)	0% (0)	N/A
CRO	S	0% (0)	0% (0)	N/A	N/A	N/A	N/A
CIP	S	0% (0)	50.0% (6)	0% (0)	83.3% (5)	0% (0)	0% (0)
CLR	S	57.1% (16)	46.2% (6)	50.0% (3)	100% (6)	100% (1)	0% (0)
DOX	S	0% (0)	11.1% (1)	0% (0)	60.0% (3)	0% (0)	N/A
EMB	S	0% (0)	0% (0)	0% (0)	100% (5)	0% (0)	0% (0)
ETO	S	N/A	N/A	N/A	0% (0)	N/A	N/A
IPM	S	0% (0)	0% (0)	0% (0)	0% (0)	N/A	N/A
INH	S	N/A	N/A	N/A	0% (0)	N/A	N/A
LZD	S	20.0% (1)	0% (0)	N/A	100% (1)	N/A	N/A
MIN	S	0% (0)	100% (1)	N/A	N/A	N/A	N/A

Table 4 (Cont.) Antimicrobial susceptibility test report of nontuberculous mycobacteria.

AST		Rapidly Growing Mycobacteria			Slowly Growing Mycobacteria		
		M. abscessus (n=28)	*M. fortuitum* (n=13)	*M. chelonae* (n=7)	*M. marinum* (n=6)	*M. intracellulare* (n=1)	*M. terrae* (n=1)
MXF	S	0% (0)	100% (1)	N/A	100% (1)	N/A	N/A
RFB	S	N/A	N/A	N/A	100% (1)	N/A	N/A
RIF	S	0% (0)	0% (0)	0% (0)	80.0% (4)	0% (0)	0% (0)
STR	S	N/A	N/A	N/A	0% (0)	N/A	N/A
TGC	S	0% (0)	0% (0)	N/A	N/A	N/A	N/A
TOB	S	0% (0)	N/A	N/A	N/A	N/A	N/A
SXT	S	0% (0)	27.3% (3)	0% (0)	100% (5)	0% (0)	N/A

AST = Antimicrobial Susceptibility Test; S = Susceptible; N/A = Not available; AMK = Amikacin; AMC = Amoxicillin-clavulanic acid; FEP = Cefepime; FOX = Cefoxitin; CRO = Ceftriaxone; CIP = Ciprofloxacin; CLR = Clarithromycin; DOX = Doxycycline; EMB = Ethambutol; ETO = Ethionamide; IPM = Imipenem; INH = Isoniazid; LZD = Linezolid; MIN = Minocycline; MXF = Moxifloxacin; RFB = Rifabutin; Rifampicin = RIF; STR = Streptomycin; TGC = Tigecycline; TOB = Tobramycin; SXT = Trimethoprim-sulfamethoxazole.

Table 5 Common therapeutic modalities for NTM infections and outcomes.

Medication (n=94)	
Monotherapy	**19 (20.2%)**
Doxycycline	14 (14.9%)
Minocycline	2 (2.1%)
Others	3 (3.2%)
Combination therapy	**75 (79.8%)**
Ciprofloxacin + Clarithromycin	54 (57.4%)
Ciprofloxacin + Clarithromycin + Doxycycline	4 (4.3%)
Ciprofloxacin + Doxycycline	2 (2.1%)
Ciprofloxacin + Clarithromycin + Amikacin	2 (2.1%)
Ciprofloxacin + Clarithromycin + Co-trimoxazole	2 (2.1%)
Azithromycin + Rifampicin + Ethambutol	1 (1.1%)
Others	10 (10.6%)

Table 5 (Cont.) Common therapeutic modalities for NTM infections and outcomes. (Continue)

Surgery (n=19)	
Combined with medication	15 (78.9%)
Monotherapy	3 (15.8%)
Combination therapy	12 (63.2%)
Surgery alone	4 (21.1%)
Duration	
Duration of symptoms at first visit (n=74)	1-60 (median = 9.0) weeks
Duration of antimicrobial treatments (n=60)	4-84 (median = 24.0) weeks
Result (n=98)	
Cured	54 (61.4%)
Improved	30 (34.1%)
Not improved	4 (4.5%)
Loss to follow-up	10 (10.2%)

Discussion

NTM SSTIs incidence has been increasing in many different geographic areas due to the emerging immunocompromised host, the increasing in aesthetic procedures, and the improvements in mycobacteria detection methods.[11, 15] Previously, most studies reported *M. marinum* as the most common pathogenic organisms, with clinical diagnoses of fish-tank granuloma or swimming pool granuloma,[16-19] but some authors identified RGM as the predominant causative species.[8,20] Geographic area of fieldworks and subject enrollment criteria may determine the incidences. However, the most recent studies suggested the upward trend of RGM infections worldwide.[11, 15, 21] The organism generally enters the body through direct cutaneous inoculation, but only a small number of patients can recall their external cutaneous injuries.[18, 21] The skin eruptions often appear as chronic, indolent, minimal tender, papules, plaques, nodules, abscesses, ulcers, or sporotrichoid lesions, and they are often recalcitrant to routine antibacterial agents.[11, 15, 18, 19] The diagnosis of NTM SSTIs is challenging, because the clinical manifestations and histopathologic findings are non-specific in most cases. These infections may be indistinguishable clinically from those caused by other bacteria. The differential diagnosis in a granulomatous dermatosis group is frequently documented, since granulomas are commonly observed histopathologic findings.[2, 3, 6]

This study was conducted as a single center retrospective review of patients who had positive NTM cultures concomitant with compatible cutaneous lesions, reported in a dermatology clinic over 20 years. The cutaneous NTM infected patients increased 1.6 folds in the second decade (2004-2014) compared to the first decade (1994-2003). Demographic data of our patients were not different from other surveys. The mean age was 42.53 (±16.39) years and female patients were more common.[8, 11, 15, 18, 19, 21] Previous trauma was the most frequent history related to infections (26 patients; 46.4%). Lower extremities were the most frequent infected sites, in concordance with Lee WJ, *el al* retrospective study.[13] However, many studies demonstrated that the most frequent affected sites were upper extremities.[11, 15, 18, 19, 21] The majority of NTM causing cutaneous infections over the entire period of our study, regardless of host's immune status, was RGM group. *M. abscessus* was the most common pathogenic species, and statistically increased in the last decade. Our result concurs with most updated literatures that reported RGM as the major cause of NTM SSTIs.[11, 15, 21] In contrast to studies from Dodiuk-Gad R, *et al*, and others, in which *M. marinum* was reported as the most common pathogenic organism.[16-19] In our study, only 4 of the 17 patients (23.5%) with positive culture for *M. marinum* had fish-related activity, while higher proportion (44.7%) was displayed in the study from Ang P, *et al*.[21] The cosmetic procedures, such as filler injection, mesotherapy, or traditional treatments including acupuncture, may contribute to an increase incidence of NTM infections, which has been confirmed by many authors among recent years.[22-24] The patients with history of previous cosmetic procedures in our study increased from 1 (5.0%) in the first decade to 8 (23.5%) in the second decade. Nonetheless, we found no statistically significant difference between the 2 decades in cosmetic or surgical procedures associated with NTM SSTIs. The relationship between the increased incidence of NTM infections and the growth of immunocompromised population has been postulated by some researchers.[12, 19, 25] However, only 13.3% of immunocompromised patients were observed in our study, corresponding to a review of Wentworth AB, *et al*, which demonstrated an increased incidence of NTM infections even in immunocompetent host, and approximately 20% of their subjects were immunosuppressed.[11] Even though granulomatous dermatitis was the main histopathologic finding, only 15% of the biopsy specimens showed positive AFB stain in the present study. Due to the low sensitivity of AFB stain, AFB negativity in the tissues cannot exclude cutaneous NTM infections.[26-29] Moreover,

positive AFB stain is unable to distinguish NTM from *M. tuberculosis*.

Antimicrobial susceptibility test (AST) demonstrated high rate of sensitivity to amikacin and clarithromycin in RGM and SGM. Cowman S, *et al* reported clarithromycin susceptible rates of RGM is 94-100% and amikacin is 68-100%.[30] *M. marinum* showed high rate of sensitivity to most antibiotics, including amikacin, ciprofloxacin, clarithromycin, doxycycline, ethambutol, linezolid, moxifloxacin, mifabutin, rifampicin, and co-trimoxazole. Most experts agree that *M. marinum* has a well-known sensitivity pattern, then currently, we generally not do the AST for *M. marinum*. Given the possibility of mutational resistance to the drugs in RGM, many experts recommend the combination of macrolide and fluoroquinolone for patients with RGM skin infections.[3, 31] In our clinic, a combination of clarithromycin and ciprofloxacin was the most frequent regimen prescribed to patients with RGM infections, and the treatment was successful clinically. In some cases, we modified the treatments later according to AST results and clinical responsiveness. The discordance of *in vitro* and *in vivo* response to antimicrobial agents was frequently observed in the literatures, and we noticed it in our study as well. We agree with many experts that monotherapy, such as clarithromycin, minocycline, doxycycline, rifampicin, ethambutol, and co-trimoxazole, is proper in limited *M. marinum* cutaneous infections, still the combination is suggested in severe cases.[2-3, 31] We have normally used doxycycline as an empiric treatment for *M. marinum* cutaneous infections and the clinical result was favourable with minimal adverse effects. Of the 17 patients diagnosed as *M. marinum* cutaneous infections, 11 patients were treated with doxycycline or minocycline monotherapy. Interestingly, overall response rate was 100%. It differs from previous study of Edelstein H, which reported 71% effectiveness of minocycline treatment and the case report from Cummins DL, *et al*, which presented the treatment failure of doxycycline pharmacotherapy in treatment of *M. marinum* cutaneous infections.[32, 33] Because there are currently no treatment guidelines for NTM SSTIs, the treatment is mostly based on personal experience and case reports or case series. There is still no clear recommendation for treatment duration. Most experts recommend continuing antimicrobial drugs until 4-8 weeks after the lesions have healed. The median treatment duration in the present study was 24 weeks. Our result was concurrent with most experts who recommended a minimum of 3-6 months of therapy depending on the extent, severity of infections, and response to treatment.[3] For immunosuppressed hosts, disseminated diseases, or multiple medical

conditions, hospitalisation with multidisciplinary care team and intravenous empirical antimicrobial drugs are preferred. Surgery should be integrated in operable lesions. Drainage of abscesses and surgical debridement are also essential components of treatment. In the company of these strategies, the satisfactory outcomes in atypical mycobacterial SSTIs occurs. Follow-up is suggested until assurance with no recurrence.

There are limitations in this study due to the retrospective review. Some clinical and laboratory data were incomplete. This report included only patients who attended outpatient dermatology clinic. In addition, several cases of highly suspicion of NTM SSTIs with granulomatous inflammation, but culture negative, were excluded. As a result, the incidence of cutaneous NTM infections in the present study may be underreported.

Conclusions

The incidence of cutaneous NTM, particularly RGM infections, is increasing worldwide. Clinical and laboratory integration is the key for the diagnosis and treatment strategies. Histopathological examination and mycobacterial culture are recommended in all suspected cases. Ciprofloxacin-clarithromycin combination for RGM and doxycycline monotherapy for *M. marinum* infections, are effective regimens. Further studies are required to develop management guidelines for NTM SSTIs in the future.

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