Nontuberculous Mycobacterial Skin Infections : Clinical and Bacteriological Studies

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Abstract

Objective : Nontuberculous mycobacterial (NTM) skin infections were analysed in terms of clinical manifestation in different species to provide clues for the clinical diagnosis and sensitivity patterns of these species were studied for planning appropriate therapy.

Design : A retrospective study was performed in 123 suspected cases of NTM infections from January 1994 to December 2000. NTM infection was documented by culture result of the infected tissue obtained by skin biopsy. Drug susceptibility test was done as requested.

Result : Rapid growers (*M. fortuitum-chelonae*) were found in 26 cases (65%) and *M. marinum* was responsible for 12 cases (30%) and caused only localized skin lesions on arms or legs as indurated plaque, Disseminated skin infections manifested as multiple abscesses were found in 2 cases caused by *M. avium* in an HIV-infected male patient and mixed infection of *M. szulgai* and *M. terrae* in an immunocompetent female patient after a dental procedure. Both sexes were affected equally in overall number but male predominated in *M. marinum* infection and females predominated in rapid growers. All ages can be affected but most cases were middle aged. Scrofuloderma - like cervical lymphadenitis and cutaneous abscesses were the common manifestation of rapid grower infections. Hyperkeratotic verrucous plaques (tuberculosis verrucosa cutis - like) and sporotrichoid lesions were the common manifestations of *M. marinum* infection. *M. marinum* is sensitive to minocyclin, clarithromycin, amikacin, rifampicin and ethambutol and a good clinical response was obtained with doxycyclin 100 mg orally twice a day for 3 months. Clarithromycin and amikacin showed *in vitro* activity against the same strain of *M. fortuitum* but most strains of rapid growers resisted antituberculous drugs and also various antibiotics.

Conclusion : Clinical manifestations can be used as clues for diagnosis. Medical therapy is recommended for *M. marinum* infection and surgical treatment is recommended for rapid growers.

Key word : Nontuberculous Mycobacterial Skin Infections, Clinical Study, Bacteriologic Study, Sensitivity Patterm

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Nontuberculous mycobacteria (NTM) have been widely recognized as human pathogens since the 1950's. Their prevalence has increased dramatically nowadays for unknown reasons but better clinical and cultural recognition may play important roles (1). NTM often manifests as a primary pulmonary infection but cutaneous lesions may be the first or only sign of infection(2). Most NTM organisms have been isolated from water and soil so that geographical factors may be responsible for the causative species of the disease(1,2). Problems in the diagnosis of cutaneous NTM infection are frequently found because the clinical appearance of the lesions is not always characteristic and a positive culture is not always obtained. No controlled trials of treatment regimens have been studied for NTM skin infection and optimum duration of treatment is also unknown(3).

To answer these difficult questions, the authors tried to collect clinical data of all cases with suspicious cutaneous lesions of NTM infections as well as histopathologic and cultural results of biopsy tissue from affected skin lesions. In this article, the authors report the analysis of 40 cases from which NTM was recovered by culture among 123 cases during an 8-year period (1994-2001).

MATERIAL AND METHOD

The records of 123 patients who attended the granuloma clinic, Department of Dermatology, Faculty of Medicine, Siriraj Hospital, Mahodol University, Bangkok, Thailand from January 1994 to December 2001 were analyzed. The patients who attended the clinic had various forms of chronic skin lesions as well as cervical lymphadenitis which had ruptured and formed chronic sinus tract through the skin at the side of the neck. Age, sex, number of lesions and clinical details such as morphology of the skin lesions and underlying diseases were recorded. Skin biopsy was performed in the affected site after obtaining informed consent from the patients for histopathological and microbiological studies. Tissues about 0.5 x 0.5 x 0.5 cm^3 and lymph nodes were aseptically excised and bisected into 2 pieces. One piece of each specimen was kept in a dry sterile bottle and sent to the Division of Mycology and Mycobacteriology, Department of microbiology for microbiologic study and another piece was fixed in formalin and sent to the Department of Pathology for histopathological study. NTM infections were documented by culture results and supported by granulomatous inflammation with or without acid fast bacilli in the tissue section.

In the Division of Mycology and Mycobacteriology, the tissue was processed in a safety cabinet class II (Gelman BH 2000, Gelman Sciences, Australia), by cutting into small pieces with a new sterile blade in a Petridish. The pieces of infected tissue were inoculated on two Lowenstein Jensen

(LJ) slants and incubated simultaneously at 25°C and 37°C. The tubes were examined weekly for mycobacterial growth for 3 months.

The mycobacterial colonies were confirmed by Kinyoun acid fast staining and identified to species by standard biochemical tests[4]. Pure culture of each species in the exponential phase was prepared for 1 McFarland cell suspension measured by Colorimeter (bioMerieux Inc., Missouri, USA) or compared to the standard solution of 1 McFarland and used for antimycobacterial susceptibility tests. Only 17 bacterial isolates were processed for the susceptibility test. Minimal inhibitory concentration (MIC) of amikacin (AK), clarithomycin (CH), ciprofloxacin (CI) or ofloxacin (OF) and minocyclin (MC) were determined by E-test[5]. Each cell suspension of mycobacteria (1 McFarland turbidity) was thoroughly spread on Middlebrook 7H10 supplemented with 10 per cent OADC (oleic acid, albumin, dextrose, catalase) agar plate (⌀ 90 mm) with a sterile cotton swab and left to dry in a safety cabinet. The E-test strip of each drug was placed according to the manufacturer's instructions. The plates were sealed with plastic wrap and incubated upside down at 25°C for *M. marinum* or 37°C for *M. fortuitum* complex and *M. avium* complex. The MICs were determined as the drug concentration of clear eclipse around the strip read with naked eyes after 1 to 2 weeks of incubation. The MIC - breakpoints for sensitivity to ciprofloxacin, clarithromycin and minocyclin were ≤ 16, 1, 2 and 6 μg/ml[6].

The patients were treated with antibiotics according to the species of the causative organisms recovered from culture or by empirical means according to the clinical ground. Surgical treatment was performed in the cases who had a single small and easily respectable lesion after pathological result and/or microbiological results were obtained or as a combined therapy with medical treatment. For patients who did not respond well to treatment, sensitivity tests were required if the organism could be recovered from the lesion and parenteral antibiotics such as amikacin were tried. The patients were followed-up monthly at the clinic as out patients until complete resolution and at least 6 months after stopping the treatment.

RESULT

Clinical findings

NTM infections were found in 40 cases, 22 females and 18 males, age range from 2-72 years (average 42.1 years). The causative species, sex of the patients and sites of the skin lesions are shown in Table 1 and cutaneous manifestations of each species are shown in Table 2. The main clinical features were chronic localized skin infections and cervical lymphadenitis with contiguous infection through overlying skin at the side of the neck (scrofuloderma-like lesions). Rapid growers (*M. fortuitum* and *M. chelonae*) were the main causative species of cutaneous and lymph node infection whereas *M. marinum* can cause only skin infection. NTM infections in the present series affected both sexes almost equally but were different in specific species. Males predominated in *M. marinum* infections and females predominated in rapid grower species. Acral part of the body (hands, arms and legs) was the site of predilection for cutaneous infections. All cases of localized skin infections and scrofuloderma-like lesions were non-HIV patients. Disseminated infection was found in only 2 cases caused by *M. avium* in an HIV-infected male patient and mixed infection of rare species, *M. szulgai* and *M. terrae*, in a previously immunocompetent female patient.

In localized infections, most cases presented as a single skin lesion of indolent and asymptomatic

Table 1. Causative species by sex and site.

	Total cases	F	M	Site (number of cases)
M. chelonae	11	10	1	Cervical nodes (2) Arms (7) Legs (2)
M. fortuitum	15	10	5	Cervical nodes (6) Arm (3) Legs (6)
M. marinum	12	1	11	Arms (6) Legs (6)
M. avium	1	0	1	Disseminated (HIV+)
Mixed infections (*M. szulgai* + *M. terrae*)	1	1	0	Disseminated (HIV-)
Total	40	22	18	

F = female, M = male

Table 2. Causative species and clinical manifestations.

	TVC*	Scrof**	Sporo***	Nodule	Abscess	Plaque	Ulcer	Total
M. chelonae	0	2	0	4	2	3	0	11
M. fortuitum	2	6	0	2	0	4	1	15
M. marinum	3	0	3	0	0	6	0	12
M. avium	0	0	0	0	1	0	0	1
Mixed infections	0	0	0	0	1	0	0	1
Total	5	8	3	6	4	13	1	40

* TVC = tuberculosis verrucosa cutis-like lesion
** Scrof = scrofuloderma-like lesion
*** Sporo = sporotricoid lesion

Fig. 1. Tuberculosis verrucosa cutis-like lesion in *M. marinum* infection.

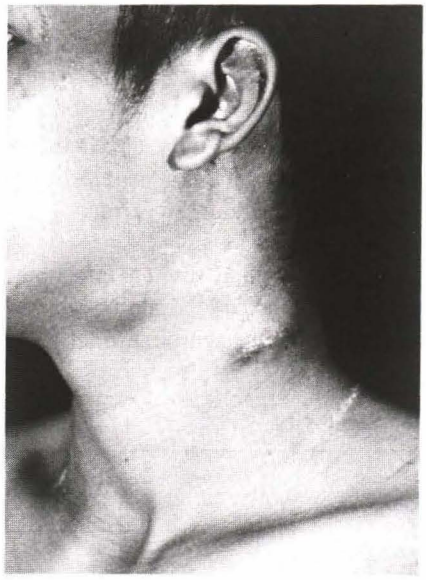

Fig. 2. Cervical lymphadenitis (scrofuloderma-like lesion) in *M. chelonae* infection.

nature. The patients still remained in good health without fever and weight loss during the course of the disease. In cases who had scrofuloderma-like lesions, low grade fever may be found but no significant weight loss and no chest symptoms such as chronic cough or dyspnoea. Because of a quite asymptomatic nature, the diseases were left untreated for a few weeks to months. Some cases were treated with antibiotics, especially the penicillin group, without good response, before the causative organisms were identified.

Most common cutaneous manifestations are indurated erythematous plaques with an irregular surface caused by *M. marinum* and rapid growers. Hyperkeratotic verrucous plaques like tuberculosis verrucosa cutis were caused by *M. marinum* (Fig. 1) and *M. fortuitum*. Sporotricoid lesions were caused only by *M. marinum*, whereas, nodule and abscess were caused by *M. chelonae* and ulcer was seen in *M. fortuitum* infection. The affected lymph nodes in the scrofuloderma-like condition (Fig. 2) were cervical groups which were affected bilaterally in all cases. The affected nodes were rubbery firm in consistency and focally matted together. There was spontaneously ruptured draining discharge through the overlying skin. Acid fast bacilli could be seen by direct smear of discharge from these sinus tract, abscesses and from

Fig. 3. Mixed *M. terrae* and *M. szulgai* infection.

the base of the ulcer but could not be found, even in the tissue section, from other kinds of lesions such as indurated plaques, tuberculosis verrucosa cutis-like lesions, nodules and papules.

The case with *M. avium* infection in the present series had multiple draining abscesses at both lower legs without gastrointestinal symptoms. Numerous acid fast bacilli were found from purulent discharge and treated with antituberculous drugs for a few months before the causative organisms were identified. The patient did not respond well with antibiotics (clarithromycin and ciprofloxacin) and lost to follow-up.

Mixed infection of *M. terrae* and *M. szulgai* occurred in one female patient after a dental procedure 2 years before the causative organism was identified. She had localized chronic infection at the left mandible after tooth extraction and was treated as actinomycosis with an unsatisfactory result. The infection progressed to nearby soft tissue and resulted in perforation of her cheek. (Fig. 3) Disseminated infection as multiple abscesses throughout her body occurred after that. She did not respond to antimicrobial therapy and died with overwhelming infection.

Sensitivity pattern (Table 3)

M. marinum is sensitive to many antibiotics such as minocycline, clarithromycin and amikacin and also antituberculous drugs such as rifampicin and ethambutol. Resistance to isoniazid, streptomycin and ciprofloxacin were found by some strains of *M. marinum.*

Table 3. Sensitivity.

Culture	Drug									
	CH	MC	AK	CI	INH	RIF	ETB	STR	OFR	Doxy
1. *M. marinum*	-	-	-	-	R	S	S	S	S	-
2. *M. marinum*	S	S	S	S	-	-	-	-	-	-
3. *M. marinum*	S	S	S	S	-	-	-	-	-	-
4. *M. marinum*	S	S	S	R	-	-	-	-	-	-
5. *M. marinum*	S	S	S	R	-	-	-	-	-	-
6. *M. marinum*	S	S	S	R	-	-	-	-	-	-
7. *M. marinum*	S	-	-	S	R	S	S	R	-	-
8. *M. chelonae*	-	-	-	-	R	R	R	R	R	-
9. *M. chelonae*	R	R	R	R	-		-	-	-	-
10. *M. chelonae*	R	R	-	R	-	-	-	-	-	-
11. *M. chelonae*	R	R	-	R	-	-	-	-	-	-
12. *M. fortuitum*	R	R	R	R	-	-	-	-	-	-
13. *M. fortuitum*	S	S	S	-	-	-	-	-	-	-
14. *M. fortuitum*	-	-	R	-	R	R	R	R	R	-
15. *M. fortuitum*	-	R	R	R	R	R	R	R	R	R
16. *M. fortuitum*	R	-	S	R	R	R	R	R	-	R
17. *M. terrae*	R	-	-	R	R	R	R	R	R	-

R = resist, S = sense, - = not done
CH = Clarithromycin, MC = Minocyclin, AK = Amikacin, CI = Ciprofloxacin, INH = Isoniazid,
RIF = Rifampicin, ETB = Ethambutol, STR = Streptomycin, OFR = Ofloxacin, Doxy = Doxycyclin
(For INH, RIF, ETB, STR and OFR using Proportion method with LJ or M7H10 containing drug as recommended for *M. tuberculosis*)
(For CH, MC, AK, CI, and Doxy using The E-test method on M7H10 as recommended by the manufacturer)
LJ = Lowenstein - Jenesn medium
M7H10 = Middlebrook 7H10agar

Conventional antituberculous drugs as well as various antibiotics had no activity against rapid growers. Clarithromycin and amikacin showed *in vitro* activity against some strains of *M. fortuitum*. *M. terrae* which was isolated from our case with overwhelming mixed infection with *M. szulgai* showed resistance against various antibiotics as well as antituberculous drugs.

Treatment and outcome

Because *M. marinum* is sensitive to various kinds of antibiotics as well as antituberculous drugs, minocycline or doxycycline 100 mg orally twice daily were tried in every case of *M. marinum* infection. Clinical response was obtained after one month of treatment and complete healing with scar within 3 months. No recurrence was found. Surgical excision was tried in cases of rapid growers localized skin infection with satisfactory response. Clarithromycin 500 mg/day combined with ciprofloxacin 500 mg/day were tried in scrofuloderma-like cases for at least 3 months but most cases were treated for 6-12 months followed by surgical excision of the remaining lesions. For inoperable cases, the treatment was continued until resolution was observed. Nobody died of the disease except the case with disseminated mixed infection of *M. terrea* and *M. szulgai*.

DISCUSSION

NTM infections in the present series were commonly found in middle age and showed sex predomination in specific species, males predominated in *M. marinum* infection and females predominated in rapid growers infection. Although predominate age and sex were observed, occupations of patients did not correlate well with acquired infections except a few cases of *M. marinum* infections in this series who had handled sea food and aquariums as previously reported from the Institute of Dermatology, Thailand [7]. Affected sites can be influenced by route of infection and nature of the causative agents. *M. marinum* which can be isolated from water, grows optimally at 30°C and grows poorly at 37°C[8] usually caused localized infection at the acral part such as arms and legs as found in the present series but disseminated infection was occasionally reported in immunocompromised patients such as renal transplant recipients [9], systemic lupus erythematosus[10], or long-term steroid therapy[11,12]. Rapid growers which were the most common species found in this series can affect various sites of the body such as skin[13], lung [13], bone[13], central nervous system[14], cornea (after laser surgery)[15,16], otitis media[17], prosthetic heart valve[8] and catheter related infection[14] but cervical lymphadenitis as in the present series was seldom reported. Cervical lymphadenitis caused by rapid growers has been reported, 5 cases from the north-eastern part of Thailand[18], 2 cases from Ramathibodi Hospital, a Medical School Hospital in Bangkok[19], and 1 case from Taiwan[20]. *M. terrae* and *M. szulgai* are rare human pathogens but have been reported from various parts of the world[21-27] as the cause of lung[21-24], bone and joint[25-27] infections. Mixed infection of *M. terrae* and *M. szulgai* occurred in a female patient after a dental procedure 2 years before the causative organism was identified. *M. avium* is an important opportunistic pathogen in AIDS patients. The infection was acquired mainly through the gastrointestinal tract[28] and disseminated through hematogenous spreading. Primary skin infections caused by *M. avium* were also reported in healthy children[29,30] as multiple subcutaneous nodules which spontaneously ruptured forming sinus tract to skin surface. Our case was an AIDS patient who had multiple draining abscesses without gastrointestinal symptoms.

Cutaneous morphology of the skin infection though was not specific to any species but from the present study the authors noticed that cervical lymphadenitis with draining sinus tract (scrofuloderma-like) and abscess formation were caused by rapid growers and this finding was not found in *M. marinum* infection. Hyperkeratotic verrucous plaque and sporotricoid lesion was more frequently found in *M. marinum* infections. Disseminated infection manifested as multiple abscesses which discharges from these lesion is a good specimen to identify the causative agent by direct smear and staining with Ziehl-Neelsen technique and culture. Skin biopsy should be performed for histopathological study and acid fast bacilli can also be detected by special stain directly in the specimen and by tissue culture.

The sensitivity pattern provides a great deal of information for clinicians to plan the appropriate treatment. *M. marinum* was sensitive to many antibiotics as well as antituberculous drugs except isoniazid. Minocycline[31,32], doxycycline[33], ciprofloxacin[9], cotrimoxazole[10,34], ethambutol[9] and rifampicin[9,34] were reported as a single drug or

combined therapy with satisfactory results. Surgical treatment should be considered in rapid growers infections if possible because they resist many antimicrobial agents.

SUMMARY

Rapid growers (*M. fortuitum-chelonae*) and *M. marinum* were the most common species found in the present series. Cervical lymphadenitis is the most common manifestation caused by rapid growers and localized skin infections at acral areas are usually caused by *M. marinum*. Single oral drug therapy such as minocycline/doxycycline or cotrimoxazole is recommended for *M. marinum* infection and surgical treatment should be considered in rapid growers infections.

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การติดเชื้อกลุ่มมัยโคแบคทีเรียที่ผิวหนัง : อาการทางคลินิกและการศึกษาทางจุลชีววิทยาของเชื้อ

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การติดเชื้อกลุ่มมัยโคแบคทีเรีย (ชนิดที่ไม่ใช่วัณโรค) ที่ผิวหนังและต่อมน้ำเหลืองซึ่งมีการแตกทะลุออกมายังผิวหนัง นับเป็นปัญหาในการวินิจฉัยและรักษา เนื่องจากไม่มีอาการทางคลินิกที่จำเพาะและอาจมีอาการคล้ายการติดเชื้อวัณโรคได้ จำเป็นต้องมีการตรวจเพาะเชื้อและทำการทดสอบความไวต่อยาต้านจุลชีพ คณะผู้วิจัยได้ทำการศึกษาผู้ป่วยจำนวน 40 ราย ที่เพาะเชื้อขึ้น จากจำนวนทั้งสิ้น 123 ราย ที่รวบรวมในช่วงเวลา 8 ปี (พ.ศ. 2537-2543) ที่ภาควิชาตจวิทยา คณะแพทยศาสตร์ศิริราชพยาบาล เชื้อที่พบบ่อยเป็น rapid growers (*M. fortuitum – chelonae*) 26 ราย (คิดเป็นร้อยละ 65) และ *M. marinum* 12 ราย (คิดเป็นร้อยละ 30) ที่เหลือเป็น *M. avium* 1 ราย และติดเชื้อร่วมกัน 2 ชนิด คือ *M. szulgai* และ *M. terrae* 1 ราย ผู้ป่วยทุกรายยกเว้นรายที่ติดเชื้อ *M. avium* ไม่ได้ติดเชื้อ HIV ผู้ป่วยส่วนใหญ่อยู่ในวัยทำงาน พบผู้ป่วยเพศชาย ได้บ่อยกว่าในการติดเชื้อ *M. marinum* และเพศหญิงบ่อยกว่าในการติดเชื้อ rapid growers แต่อาชีพไม่มีส่วนสัมพันธ์กับการติดเชื้ออย่างชัดเจนนัก การติดเชื้อ rapid growers มักจะทำให้เกิดการอักเสบของต่อมน้ำเหลืองบริเวณคอและแตกออกคล้ายฝีประคำร้อย (scrofuroderma – like lesions) หรือเป็นฝีตามแขน ขา การติดเชื้อ *M. marinum* จะก่อให้เกิดรอยโรคที่ผิวหนังแบบ hyperkeratotic verrucous plaque คล้าย tuberculosis verrucosa cutis และ sporotrichoid lesions เชื้อ *M. marinum* มีความไวต่อยาต้านวัณโรคและยาต้านจุลชีพหลายชนิด ตอบสนองต่อการรักษาด้วย การให้รับประทาน Doxycycline ในขนาด 100 มก วันละ 2 ครั้งนาน 3 เดือน เชื้อ rapid growers มีความดื้อต่อยาต้านจุลชีพ หากรอยโรคมีขนาดเล็กแนะนำให้รักษาด้วยการผ่าตัดออก

คำสำคัญ : การติดเชื้อกลุ่มมัยโคแบคทีเรียที่ผิวหนัง, อาการทางคลินิก, จุลชีววิทยาของเชื้อ, ความไวต่อยา

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