A case of postoperative wound infection caused by *Mycobacterium wolinskyi*

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Received March 19, 2024

Abstract

A 69-year-old woman underwent a total hysterectomy for uterine cancer and was undergoing postoperative chemotherapy. One month after surgery, the patient developed a postoperative wound infection caused by methicillin-sensitive *Staphylococcus aureus* (MSSA) in her midline abdominal postoperative wound and was treated with antimicrobial agents. Three months after surgery, a fistula and serous exudate appeared in her midline abdominal postoperative wound and the patient was treated with oral minocycline and topical sodium fusidic acid ointment. Two weeks after starting treatment, exudate culture specimens were collected and a rapidly growing mycobacterium was detected, but its name could not be identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. The rapidly growing mycobacterium detected was identified as *Mycobacterium wolinskyi* by analyses of the *rpoB* and *hsp65* gene sequences. Based on the susceptibility, the patient was treated with minocycline and moxifloxacin for approximately 4 months, and her symptoms improved. In postoperative wound infections which do not respond well to treatment targeting common bacteria, it is important to include nontuberculous mycobacterium as a causative organism and to perform appropriate culture tests.

Key words: *Mycobacterium wolinskyi*, postoperative wound infection, rapidly growing mycobacterium

INTRODUCTION

Mycobacterium wolinskyi (*M. wolinskyi*) is a species of rapid growing mycobacterium (RGM) that is found in water and soil, and was first identified by 16S ribosomal RNA sequencing in 1999[1]. It was previously considered to belong to the *Mycobacterium smegmatis* group, but is now considered to be related to *Mycobacterium mageritense* based on pigmentation and genetic relatedness[2]. Infections caused by *M. wolinskyi* are extremely rare, with only a few cases of

postoperative wound infections reported[3) 4) 5) 6)]. Herein, we report a case of postoperative wound infection caused by *M. wolinskyi*.

CASE REPORT

A 69-year-old woman underwent laparotomy for uterine cancer. Modified radical hysterectomy, bilateral adnexectomy, retroperitoneal filling, and pelvic and para-aortic lymph node dissection were performed. Approximately 4 weeks after the surgery, when the patient was starting postoperative chemotherapy with doxorubicin and cisplatin, erythema and pus were observed in the postoperative wound of the abdomen. The site was drained by incision and treated with cefcapene pivoxil 300 mg/day for 7 days, cephalexin 1000 mg/day for 14 days, and gentamicin ointment. MSSA was cultured from the wound specimen.

Approximately 10 weeks after surgery, clear exudate, a fistula with yellow necrosis, and subcutaneous induration were observed from the same wound (Fig. 1A, 1C), and the patient was treated with sodium fusidic acid ointment and oral minocycline (MINO) 200 mg/day. Two weeks later, however, the patient became aware of pain in the upper part of the wound (Fig. 1B), so we collected a specimen of the exudate for culture. Four weeks after, the fistula in the wound was closing and the subcutaneous induration had disappeared, although erythema was observed around the wound. The pain was relieved but remained.

Laboratory findings (day 13 after chemotherapy) were as follows: total white blood cell (WBC) count of 1,000 cells/mm^3 with 52% neutrophils, 31% lymphocytes, 0% eosinophils, 1% basophils, and 16% monocytes; red blood cell (RBC) count of 244×10^4 cells/mm^3; hemoglobin, 7.3g/dL. The wound culture showed growth of acid fast bacilli, which formed milky-white colonies in 2% Ogawa medium within 2 weeks (Fig. 2). MALDI-TOF Mass Spectrometry analysis using the VITEK® MS Mycobacterium/Nocardia Kit Ver. 3.2 (bioMérieux Japan) identified an acid-fast

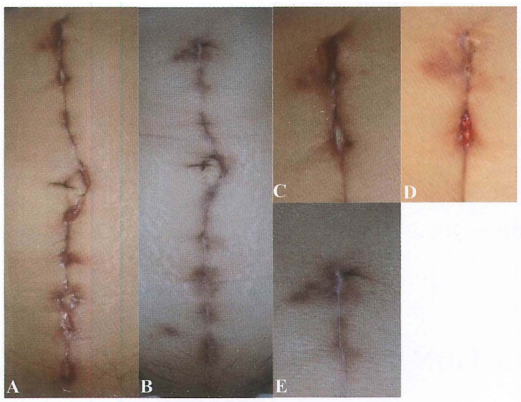

Fig. 1. Appearance of postoperative wound.
A. Entire wound, before MINO administration. B. Entire wound, 1 month after completion of MINO and MFLX administration. C. Enlarged upper wound, before MINO administration. D. Enlarged upper wound, 2 weeks after MINO administration. E. Enlarged upper wound, 1 month after completion of MINO and MFLX administration.

Fig. 2. Morphology of colonies, in which stored strains were regrown. 2% Ogawa medium (Kyokuto®) at 37℃.

Table 1. Antimicrobial susceptibility of the M. wolinskyi isolate

Drug	MIC (μg/mL)	MIC (μg/mL) for category		
		Susceptible	Intermediate	Resistant
Amikacin	≤4	≤16	32	≥64
Tobramycin	≥32	≤2	4	≥8
Sulfamethoxazole/Trimethoprim	9.5/0.5	≤38/2		≥76/4
Doxycycline	1	≤1	2-4	≥8
Imipenem/Cilastatin	16	≤4	8-16	≥32
Meropenem	16	≤4	8-16	≥32
Faropenem	32			
Linezolid	2	≤8	16	≥32
Moxifloxacin	≤0.25	≤1	2	≥4
Sitafloxacin	≤0.25			
Clarithromycin	64	≤2	4	≥8
Azithromycin	≥128			
Clofazimine	0.5			

MIC: Minimum inhibitory concentration

bacillus of the Runyon Classification Group IV, but the species was unknown. To assess the treatment plan, we analyzed the amplified *hsp65* gene and *rpoB* gene sequences of the acid-fast bacillus. The *hsp65* genome sequence detected was 100% (996 bp) identical to *M. wolinskyi* CIP 106348, and the rpoB genome sequence detected was 100% identical to *M. wolinskyi* American Type Culture Collection (ATCC) ® 700010, so we diagnosed postoperative wound infection of uterine cancer caused by *M. wolinskyi*.

Based on the sensitivity results of the micro-liquid dilution method according to the Clinical and Laboratory Standards Institute (CLSI) M24-A2 (Table 1), we prescribed MINO as an alternative to doxycycline, while moxifloxacin (MFLX) 400 mg/day was added. Three months after the start of combination therapy, the pain, exudate, redness, and swelling disappeared. MFLX and MINO were prescribed for a total of 16 weeks. One month after the end of the treatment, there was no worsening of symptoms and there were no subjective symptoms (Fig. 1C, Fig. 2C).

DISCUSSION

Skin-and-soft tissue infections caused by acid-fast bacilli can occur after trauma, surgery, or cosmetic procedures in immunocompetent patients[7]. The Centers for Disease Control and Prevention (CDC) defines a "surgical site infection (SSI)" as an infection that occurs near the wound within 30 days after surgery (90 days after surgery if implants are involved) [8], but SSI due to RGM often develops several months after surgery[7]. Although 60-80% of RGM SSIs are caused by *Mycobacterium fortuitum* [9], the number of reported cases and the types of causative organisms have diversified due to improved detection sensitivity. The diagnosis is made by culture of specific nontuberculous mycobacteria from tissue biopsy[10]. In our case, the patient's symptoms flared up approximately 10 weeks after surgery and after completion of antimicrobial

therapy for a wound infection caused by MSSA, so we suspected the causative bacterium to be an acid-fast bacillus.

M. wolinskyi, a species of RGM, forms non-pigment-producing colonies on solid media. It is characterized by resistance to tobramycin (TOB)[1] and intrinsic resistance to clarithromycin (CAM) due to the *erm* (*40*) gene[11]. In addition, cross-resistance to gentamicin with TOB has been noted[12]. The *M. wolinskyi* detected in our case was also resistant to TOB and CAM. Our case suggests that *M. wolinskyi*, a TOB-resistant pathogen, may have been involved in the infection due to bacterial shift caused by the use of gentamicin ointment. Healthcare-associated infections have been reported, such as an outbreak of six definite cases of *M. wolinskyi* SSI following cardiothoracic surgery at a single medical facility[13] and five wound infections following joint replacement surgery originating at an outdoor hot tub in the home of a nurse[14].

The MALDI-TOF method has difficulty identifying subspecies of mycobacteria which are phylogenetically related, and rare species of mycobacteria[15], so sequencing analysis of the 16S ribosomal RNA gene, *hsp65* gene[16], and *rpoB* gene[17] is essential for the identification of the species of mycobacteria. In particular, the *rpoB* gene, which encodes the bacterial β subunit of the RNA polymerase, contains sufficient information for the identification of many species of RGM. In the present case, species identification by MALDI-TOF was impossible, but the homology analysis of *rpoB* and *hsp65* genes, which were amplified by polymerase chain reaction, identified the species based on the obtained sequences.

Although antimicrobial regimens for RGM often include two or more drugs[18], there is no established regimen, and selection is based on *in vitro* susceptibility patterns. Previous reports of infections caused by *M. wolinskyi* are shown (Table 2). Bloodstream infections were observed in patients after cardiovascular surgery and in patients with long-term intravascular catheter placement during chemotherapy. Skin-and-soft tissue infections and prosthetic joint infections were also observed in immunocompetent hosts. Six other patients had wound infections, all of which were treated with at least two antimicrobial agents, including at least one quinolone and one tetracycline or glycylcycline. Treatment regimens that included these two drugs were

Table 2. Review of case reports about M. wolinskyi infections

	Age/Sex	Type of infection	Antibiotic regimen/Duration	Outcome
Ariza-Heredia EJ et al. 2011[3]	28/F	Surgical Site Infection (post-lung transplantation)	MFLX+DOXY/6 months	Good
Ariza-Heredia EJ et al. 2011[3]	73/M	Surgical Site Infection (post-AICD implantation)	CPFX+MINO/6 months	Good (5 months after)
Hernández-Meneses M et al. 2021[4]	63/F	Surgical Site Infection (post-AICD implantation)	MFLX+DOXY/6 weeks	Good (1 year after)
Santos Lima A et al. 2013[5]	29/F	Surgical Site Infection (post-mammoplasty)	CPFX+DOXY+AMK/6 months (AMK: 10 weeks)	Good
Yoo SJ et al.2013[6]	56/F	Surgical Site Infection (post-cosmetic procedures)	CPFX+DOXY/5 months	Unknown
Bossart S et al. 2016[19]	72/M	Abdominal wall subcutaneous abscess	MFLX+MINO+AMK/6 months (AMK: 1 month)	Good(6 months after)
Fujikura H et al. 2017[20]	66/M	Peritoneal dialysis related peritonitis	LVFX+MINO/39 days	Good(6 months after)
Karakala N et al. 2013[21]	67/F	Peritoneal dialysis related peritonitis	MFLX+DOXY+LZD/1 month	Good(4 months after)
Masuda K et al. 2022[22]	68/F	Peritoneal dialysis related peritonitis	LVFX+MINO/3 months	Good(18 months after)
Ariza-Heredia EJ et al. 2011[3]	78/M	Osteomyelitis	MFLX+TGC+ST/MFLX+ST: 6 months (TGC: 1 month)	Unknown
Ariza-Heredia EJ et al. 2011[3]	84/M	Osteomyelitis	IPM+MFLX+ST/MFLX+ST: 6 months (IPM: 1 month)	Good(1 year after)
Bhatnagar N et al. 2019[23]	62/M	Prosthetic joint infection	AMK+MFLX+LZD/18 weeks (AMK: 6 weeks)	Good(1 year after)
Lee YS et al. 2015[24]	65/F	Prosthetic joint infection	CPFX+DOXY+AMK/4 months	Good(2 year after)
Pulcini C et al. 2006[25]	83/F	Prosthetic joint infection	MFLX+MINO+AMK/6 months (AMK: 1 month)	Good(1 year after)
Tokuda M et al. 2020[26]	70s/F	Prosthetic joint infection	LVFX+CAM/unknown	Unknown
Ariza-Heredia EJ et al. 2011[3]	16/M	Bloodstream infection/Infected aortic root graft	MFLX+DOXY+AMK/lifelong (AMK: unknown)	Unknown
Muranaka E et al. 2022[27]	44/F	Catheter related bloodstream infection	LVFX+MINO+AMK/LVFX: 4 months, MINO: 6 months, AMK: 1 month	Good(1 year after)
Kitajima H et al. 2021[28]	82/M	Prosthetic valve endocarditis	AMK+IPM+CAM→CPFX+MINO/12 months (AMK+IPM+CAM: 6 weeks)	Unknown
Chen YC et al. 2008[29]	22/F	Bloodstream infection	MFLX+MINO+AMK/6 months (AMK: 1 month)	Unknown
Ohno T et al. 2008[30]	55/F	Bloodstream infection	LVFX+MINO+AMK/6 months (AMK: 1 month)	Good(18 months after)
Dupont C et al. 2016[31]	48/M	Bloodstream infection/Infected aortic root graft	MFLX+DOXY+AMK+LZD/6 months	Good(6 months after)

AMK: Amikacin, CAM: clarithromycin, CPFX: ciprofloxacin, DOXY: doxycycline, IPM: imipenem, LVFX: levofloxacin, LZD: linezolid, MINO: minocycline, MFLX: moxifloxacin, ST: sulfamethoxazole-trimethoprim, TGC: tigecycline

applied in 18 of the 21 cases as far as we were aware. 15 cases were still doing well after completion of treatment, and 14 of them were treated with at least a quinolone and a tetracycline or glycylcycline. It should be noted, however, that AMK was also used in most cases where intravenous antimicrobial therapy was the standard of care, such as prosthetic joint infections and bloodstream infections.

The duration of treatment ranges from 6 weeks to 6 months in cases of soft-tissue infection and from 1 month to more than 1 year in all cases. In our case, based on drug sensitivity results and previous case reports, we treated the patient with a combination of MFLX and MINO for approximately 4 months and completed treatment after improvement of subjective symptoms and other findings in the skin-and-soft tissues, and the patient was still doing well at the end of 1 month.

CONCLUSION

When postoperative wound infections do not respond well to treatment targeting common bacteria, or when the time between surgery and onset of symptoms is several months or longer, nontuberculous mycobacteria should be considered as causative organisms and appropriate culture tests should be performed. If nontuberculous mycobacteria cannot be identified by mass spectrometry, genetic analysis should be considered.

CONFLICT OF INTEREST

There is no COI relationship with any company related to the content of the paper that should be disclosed.

IMFORMED CONSENT

Written consent for case reports is obtained from the patient.

REFERENCES

1) Brown, B. A., Springer, B., Steingrube, V. A., Wilson, R. W., Pfyffer, G. E., Garcia, M. J., Menendez, M. C., Rodriguez-Salgado, B., Jost, K. C., Jr, Chiu, S. H., Onyi, G. O., Böttger, E. C., & Wallace, R. J., Jr.: Mycobacterium wolinskyi sp. nov. and Mycobacterium goodii sp. nov., two new rapidly growing species related to Mycobacterium smegmatis and associated with human wound infections: a cooperative study from the International Working Group on Mycobacterial Taxonomy. International journal of systematic bacteriology, 49 Pt 4: 1493–1511, 1999.

2) Brown-Elliott, B. A., & Philley, J. V.: Rapidly Growing Mycobacteria. Microbiology spectrum, 5 (1) , 2017

3) Ariza-Heredia, E. J., Dababneh, A. S., Wilhelm, M. P., Wengenack, N. L., Razonable, R. R., & Wilson, J. W.: Mycobacterium wolinskyi: a case series and review of the literature. Diagnostic microbiology and infectious disease, 71 (4) : 421–427, 2011.

4) Hernández-Meneses, M., González-Martin, J., Agüero, D., Tolosana, J. M., Sandoval, E., Falces, C., San

Antonio, R., Vidal, B., Moreno, A., Ambrosioni, J., Miró, J. M., & Hospital Clínic of Barcelona Infectious Endocarditis Team: Mycobacterium Wolinskyi: A New Non-Tuberculous Mycobacterium Associated with Cardiovascular Infections?. Infectious diseases and therapy, 10 (2) : 1073–1080, 2021.

5) Santos Lima, A., Carneiro Neves, M. M., Machado Gomes, K., Miranda Guarines, K., Luna, C. F., Silva Duarte, R., Lapa Montenegro, L. M., & Charifker Schindler, H.: First case report of infection by Mycobacterium wolinskyi after mammoplasty in Brazil. Infectious disease reports, 5 (2) : e12, 2013.

6) Yoo, S. J., Lee, K. H., Jung, S. N., & Heo, S. T.: Facial skin and soft tissue infection caused by Mycobacterium wolinskyi associated with cosmetic procedures. BMC infectious diseases, 13: 479, 2013.

7) Wentworth, A. B., Drage, L. A., Wengenack, N. L., Wilson, J. W., & Lohse, C. M.: Increased incidence of cutaneous nontuberculous mycobacterial infection, 1980 to 2009: a population-based study. Mayo Clinic proceedings, 88 (1) : 38–45, 2013.

8) Horan, T. C., Andrus, M., & Dudeck, M. A.: CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. American journal of infection control, 36 (5) : 309–332, 2008.

9) Brown-Elliott, B. A., & Wallace, R. J., Jr.: Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. Clinical microbiology reviews, 15 (4) : 716–746, 2002.

10) Bennett, J.E.,Dolin, R. & Blaser, M.J. :Mandell, Douglas, & Bennett's Principles & Practice of Infectious Diseases, 9th ed: 3056, 2019.

11) Nash, K. A., Andini, N., Zhang, Y., Brown-Elliott, B. A., & Wallace, R. J., Jr.: Intrinsic macrolide resistance in rapidly growing mycobacteria. Antimicrobial agents and chemotherapy, 50 (10) : 3476–3478, 2006.

12) Grayson ML, Kucers A. Kucers' The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic, and Antiviral Drugs. 7th ed. Hodder Arnold, London, 2017.

13) Nagpal, A., Wentink, J. E., Berbari, E. F., Aronhalt, K. C., Wright, A. J., Krageschmidt, D. A., Wengenack, N. L., Thompson, R. L., & Tosh, P. K.: A cluster of Mycobacterium wolinskyi surgical site infections at an academic medical center. Infection control and hospital epidemiology, 35 (9) : 1169–1175, 2014.

14) Groenewold, M. R., Flinchum, A., Pillai, A., Konkle, S., Moulton-Meissner, H., Tosh, P. K., & Thoroughman, D. A.: Investigation of a cluster of rapidly growing mycobacteria infections associated with joint replacement surgery in a Kentucky hospital, 2013-2014 with 8-year follow-up. American journal of infection control, 51(4): 454–460, 2023

15) Buchan, B. W., Riebe, K. M., Timke, M., Kostrzewa, M., & Ledeboer, N. A.: Comparison of MALDI-TOF MS with HPLC and nucleic acid sequencing for the identification of Mycobacterium species in cultures using solid medium and broth. American journal of clinical pathology, 141 (1) : 25–34, 2014.

16) McNabb, A., Eisler, D., Adie, K., Amos, M., Rodrigues, M., Stephens, G., Black, W. A., & Isaac-Renton, J.: Assessment of partial sequencing of the 65-kilodalton heat shock protein gene (hsp65) for routine identification of Mycobacterium species isolated from clinical sources. Journal of clinical microbiology, 42(7): 3000–3011, 2004.

17) Adékambi, T., Colson, P., & Drancourt, M. (2003) . rpoB-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. Journal of clinical microbiology, 41 (12) : 5699–5708, 2003

18) De Groote, M. A., & Huitt, G.: Infections due to rapidly growing mycobacteria. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 42 (12) : 1756–1763, 2006.

19) Bossart, S., Schnell, B., Kerl, K., & Urosevic-Maiwald, M.: Ulcers as a Sign of Skin Infection with Mycobacterium wolinskyi: Report of a Case and Review of the Literature. Case reports in dermatology, 8(2):

151–155, 2016.

20) Fujikura, H., Kasahara, K., Ogawa, Y., Hirai, N., Yoshii, S., Yoshihara, S., Ogawa, T., Yonekawa, S., Imakita, N., Nishioka, Y., Yoneda, T., Yoshida, K., Samejima, K. I., Tanabe, K., Saito, Y., Yano, H., & Mikasa, K.: Mycobacterium wolinskyi Peritonitis after Peritoneal Catheter Embedment Surgery. Internal medicine (Tokyo, Japan), 56 (22) : 3097–3101, 2017.

21) Karakala, N., Steed, L. L., & Ullian, M. E.: Peritonitis from Mycobacterium wolinskyi in a chronic peritoneal dialysis patient. International urology and nephrology, 45 (1) : 289–291, 2013.

22) Masuda, K., Yamasaki, Y.: A successfully treated case of PD–related peritonitis caused by Mycobacterium wolinskyi. Journal of Japanese Society for Dialysis Therapy, 55 (4) : 249-253, 2022.

23) Bhatnagar, N., Poojary, A., Maniar, A., Contractor, A., Rohra, S., & Kumar, G.: Mycobacterium wolinskyi: A Rare Strain Isolated in a Persistent Prosthetic Knee Joint Infection: A Case Report. JBJS case connector, 9(3): e0315, 2019.

24) Lee, Y. S., Nam, S. W., Park, Y. S., & Lee, B. K.: Mycobacterium wolinskyi infection after total knee arthroplasty in a healthy woman. Journal of orthopaedic science : official journal of the Japanese Orthopaedic Association, 20 (1) : 229–231, 2015.

25) Pulcini, C., Vandenbussche, E., Podglajen, I., Sougakoff, W., Truffot-Pernot, C., Buu-Hoï, A., Varon, E., & Mainardi, J. L.: Hip prosthesis infection due to Mycobacterium wolinskyi. Journal of clinical microbiology, 44 (9) : 3463–3464, 2006.

26) 德田美香・曽木広信・吉田敦・阿保一茂・松谷暁・水島遼・鎌田啓佑・板倉泰朋・井口成一・鵜澤豊・荒井裕子・菊池賢：Mycolicibacterium wolinskyi による人工膝関節置換術後膝関節炎の 1 例. 日本臨床微生物学会雑誌. 30 巻, 80-84, 2020.

27) Muranaka, E., Hase, R., Utsu, Y., Watari, T., Otsuka, Y., & Hosokawa, N.: Catheter-related bloodstream Mycobacterium wolinskyi infection in an umbilical cord blood transplant recipient: a case report. BMC infectious diseases, 22 (1) : 520, 2022.

28) Kitajima, H., Oba, Y., Ohira, T., Asaoka, T., Atsumi, Y., Nakajima, T., & Okura, T.: First case report of prosthetic valve endocarditis caused by Mycobacterium wolinskyi. Journal of infection and chemotherapy, 27 (5) : 766–769, 2021.

29) Chen, Y. C., Jou, R., Huang, W. L., Huang, S. T., Liu, K. C., Lay, C. J., Chang, S. M., Tseng, C. E., Lai, C. L., & Su, Y. C.: Bacteremia caused by Mycobacterium wolinskyi. Emerging infectious diseases, 14 (11) : 1818–1819, 2008.

30) Ohno, T., Kishimoto, W., Chihara, D., Sakamoto, T., Arimoto-Miyamoto, K., Takeoka, T., Tsuji, M., Kida, K., Ohkusu, K., & Ezaki, T.: First case report of sepsis caused by Mycobacterium wolinskyi in chronic myelogenous leukemia. Diagnostic microbiology and infectious disease, 62 (4) : 433–436, 2008.

31) Dupont, C., Terru, D., Aguilhon, S., Frapier, J. M., Paquis, M. P., Morquin, D., Lamy, B., Godreuil, S., Parer, S., Lotthé, A., Jumas-Bilak, E., & Romano-Bertrand, S.: Source-case investigation of Mycobacterium wolinskyi cardiac surgical site infection. The Journal of hospital infection, 93 (3) : 235–239, 2016.