Recurrent Catheter-Related Infection Caused by a Single Clone of Mycobacterium chelonae with Two Colonial Morphotypes

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We describe herein a recurrent catheter-related (Port-A-Cath; Smiths Industries Medical Systems [SIMS] Deltec, Inc., St. Paul, Minn.) infection caused by multidrug-resistant Mycobacterium chelonae with two colonial morphotypes in a 53-year-old woman with gastric adenocarcinoma. Four isolates recovered from this patient within a 3-month period were found to belong to a single clone on the basis of the isolates’ identical antibiotypes as determined by the E test and their identical random amplified polymorphic DNA patterns.

*M. chelonae, a rapidly growing mycobacterium, is an opportunistic pathogen which causes a wide variety of clinical syndromes (2, 3, 5, 8, 9, 12, 15, 18). Nosocomial infections associated with the use of various indwelling devices and diagnostic instruments have been reported (7, 16, 17). The propensity of the organism to adhere to the cardiac valve or vessel walls resulting in endocarditis or aortitis has also been described (3, 5, 9). However, none of the previous reports have described the use of molecular typing methods to document the long-term persistence of M. chelonae in the catheter and/or the walls of vessels, which contributed the recurrent nature of intravenous catheter-related infection caused by this organism (18).

Case report. A 53-year-old woman with gastric adenocarcinoma underwent subtotal gastrectomy in 1993. She began to receive weekly intravenous infusions of fluorouracil (2,500 mg) in November 1996. The first catheter (Port-A-Cath; Smiths Industries Medical Systems [SIMS] Deltec, Inc., St. Paul, Minn.) was implanted via the right subclavian vein on 25 November 1996. She first developed fever and shaking chills several minutes after each manipulation of the Port-A-Cath device in June 1997. Two sets of cultures of blood aspirated via the catheter and cultured in a BACTEC 6A aerobic bottle (Becton Dickinson, Sparks, Md.) on 7 July 1997 both yielded M. chelonae with a smooth colonial morphotype (isolate A). The chest roentgenography was negative. She received intravenous amikacin (750 mg every day) therapy on 15 October. Acid-fast bacilli. Culture of the biopsied skin tissue yielded M. chelonae with a smooth-colony morphotype (isolate B). However, a culture of the catheter tip and two sets of blood cultures collected immediately after the removal of the catheter all yielded M. chelonae with two colonial morphologies, i.e., small and smooth (isolate C) and large and rough (isolate D). Cultures of sputum, throat and nasal swabs, stool, urine, and skin were all negative for M. chelonae.

The patient was admitted again on 13 October 1997. A wedge-shaped consolidation over the lower lobe of the right lung and a nodular patch over the right midlung field were found by chest roentgenography and sonography. A skin biopsy of the soft tissue from the second insertion site revealed granulomatous inflammation and numerous acid-fast bacilli. The third catheter was removed on 15 October. A smear of the blood from the catheter showed many clusters of acid-fast bacilli. Culture of the biopsied skin tissue yielded M. chelonae with a smooth-colony morphotype (isolate B). However, a culture of the catheter tip and two sets of blood cultures collected immediately after the removal of the catheter all yielded M. chelonae with two colonial morphologies, i.e., small and smooth (isolate C) and large and rough (isolate D). Cultures of sputum, throat and nasal swabs, stool, urine, and skin were all negative for M. chelonae.

Microbiology. All isolates (isolates A to D) grew well on the Trypticase soy agar supplemented with 5% sheep blood agar (BBL Microbiology Systems, Cockeysville, Md.) and Middlebrook 7H11 agar (BBL Microbiology Systems) within 3 days of incubation. Two different colonial morphotypes were observed on both the blood agar plate (Fig. 1) and the Trypticase soy agar. Growth on MacConkey agar (BBL Microbiology Systems) of these isolates was evident on the fourth day of incubation. The isolates were differentiated from Mycobacterium fortuitum and Mycobacterium abscessus and identified as M. chelonae because they had all of the following characteristics: failure to grow in 5% NaCl; positive citrate and arylsulfatase reactions at 3 days; negative nitrate reduction and mannitol
utilization reactions; negative for alkaline phosphatase, trypsin, and $\beta$-glucosidase (API ZYM system; bioMérieux Vitek, Inc., Hazelwood, Mo.); and resistance to polymyxin (300-U) and cephalothin (30-µg) disks (BBL Microbiology Systems) (8, 11, 16).

**Cellular fatty acid analysis.** The procedure for the extraction and derivation of methyl esters of mycobacterial lipids was performed as described previously (4, 11). All isolates had major cellular fatty acid peaks ($\geq$3% of total fatty acid) of 14:0 (tetradecanoic acid), 16:0 (hexadecanoic acid), 18:1 (octadecanoic acid), 18:0 (octadecenoic acid), and TBSA (tuberculostearic acid) and minor peaks of 16:1 (hexadecanoic acid), 17:0 (heptadecanoic acid), and 2-0H-20:0 (2-hydroxyeicosanoic acid). The cellular fatty acid profile was characteristic for the identification of the *M. chelonae* group (7).

**Antimicrobial susceptibilities.** In vitro susceptibilities of these isolates, determined by the E test (PDM Epsilometer; AB Biodisk, Solna, Sweden), were measured on Mueller-Hinton agar supplemented with 5% sheep blood (BBL Microbiology Systems), and the results were read after 72 h of incubation (6). All isolates had identical antibiotic MICs of each agent for the four isolates were ≤2 gradient discrepancies: MICs of $>\geq 256$ µg/ml for cefoxitin, cefmetazole, tobramycin, minocycline, and erythromycin; MICs of $>\geq 32$ µg/ml for ampicillin-sulbactam, imipenem, ofloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole, and rifampin; MICs of 16 to 32 µg/ml for amikacin; and MICs of 1.5 to 2 µg/ml for clarithromycin.

**RAPD patterns.** Random amplified polymorphic DNA (RAPD) patterns of these isolates were determined by means of arbitrarily primed PCR as described in our previous report (4). A total of six oligonucleotide primers were used: M13 (5'-TTATGTAAACGAGCGCAGT-3'), H3 (5'-AGACG TCCAC-3'), H4 (5'-GGAGTCCG-3'), H9 (5'-TGTAGCT GGG-3'), ERIC1 (5'-GTAATCCAGAGCGTAAAT-3'), and ERIC2 (5'-AGATAGTGAAGGGTGAGC-3') (Operon Technologies, Inc., Alameda, Calif.). For comparison, one clinical isolate of *M. chelonae* (isolate E) was also included in this study as a control strain. The four isolates (isolates A to D) had identical RAPD patterns (i.e., they shared every band), and these patterns were different from that of the control strain. Figure 2 shows the RAPD patterns for three of the primers (H3, H4, and M13).

The finding in this report obtained by a molecular typing method confirms that *M. chelonae* caused recurrent central venous catheter-related sepsis, which further resulted in a pulmonary embolism and soft-tissue infection of the insertion site. The recurrent nature of the infection was probably caused by the presence of this organism in the thrombus along the vessel wall for a period of at least 3 months, although the thrombus was not removed for microbiological study.

Though two kinds of colonial morphology of *M. chelonae* on Middlebrook 7H11 agar are well known, i.e., one is round and smooth and the other is rough and wrinkled (8), infection caused by a single clone of this organism which simultaneously possessed these two obviously different colonial morphotypes has not been previously reported. The identity of RAPD patterns with six primers was clearly demonstrated for these two morphotypes, suggesting that they belonged to a single clone. However, we cannot explain why the rough morphotype was not seen in the first two positive cultures.

*M. chelonae* is resistant to numerous antimicrobial agents, showing variable susceptibilities to amikacin, tobramycin, doxycycline, erythromycin, cephalosporins, imipenem, and quinolones (1, 10, 13, 14). Clarithromycin is the most active drug against this organism, with 100% of isolates being susceptible at an MIC of $\leq 1$ µg/ml (1). In contrast, our isolates were not susceptible to any of the antibiotics tested, including clarithromycin. However, after the removal of the infected catheter, our patient responded satisfactorily to treatment with clarithromycin, ciprofloxacin, and amikacin for 1 month, followed by 2 months of treatment with clarithromycin and ciprofloxacin. Combination therapy for infections due to this multidrug-resistant organism seems advisable, although some of the agents described had poor in vitro activity.

The findings in this case illustrate that *M. chelonae* should be included in the differential diagnosis of central venous catheter-related sepsis, particularly for immunocompromised hosts. And this organism has the propensity to adhere to the vascular wall. When recurrent infections occur at different sites of Port-A-Cath implantation, clinicians should make an effort to find any vascular lesions along the route of the catheter.

**REFERENCES**


