Non-tuberculous Mycobacterial Pseudo-outbreak of an Intestinal Culture Specimen Caused by a Water Tap in an Endoscopy Unit

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Abstract:
Objective Gastrointestinal lesions of non-tuberculous mycobacteria (NTM) are regarded as opportunistic infections. A large number of positive specimens of NTM were identified in an intestinal fluid culture in the endoscopy unit and it was considered to be a pseudo-outbreak.

Methods We reviewed the hospital, laboratory, and colonoscopy records of 263 consecutive patients whose intestinal fluids were analyzed for a mycobacterial culture by colonoscopy at St. Marianna University Hospital, between January 2009 and December 2018. The endoscopy reprocessing procedures were reviewed and samples of water used in the endoscopy unit were cultured.

Results An intestinal fluid culture of 154 (58.6%) patients tested positive for NTM (M. intracellulare; 125 cases, M. gordonae; 14 cases, M. avium; 4 cases, M. abscessus; 3 cases, and 8 other cases). In 182 cases (69.2%), an intestinal mucosal culture was performed simultaneously with a fluid culture and tested positive for NTM in 2 cases. Next, we examined the endoscopy unit for any possible environmental contamination. NTM were detected in the tap water used to prepare the antifoaming solution in the endoscopy unit. The water faucets in the endoscopy unit were considered to be the source of the contamination of NTMs.

Conclusion We observed that a large number of cases tested positive due to contaminated water that had been used in an endoscopy unit, thus leading to a pseudo-outbreak of NTM.

Key words: non-tuberculous mycobacteria, intestinal fluid culture, pseudo-outbreak, colonoscopy, water tap

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Introduction

Non-tuberculous mycobacteria (NTM) consist of more than 150 species and are globally ubiquitous in both, natural and man-made, environments (1, 2). The respiratory and gastrointestinal (GI) tracts are a common gateway for NTM infection. The M. avium complex (MAC), which comprises two closely related species, M. avium and M. intracellulare, is the most frequent causative organism of pulmonary NTM disease, although they have little virulence in natural hosts. GI lesions of NTM are regarded as opportunistic infections that occur mainly in immunodeficient states, such as in patients with an HIV infection. The dysfunctional immune system in AIDS patients, usually reflected by the CD4+ cell count, is a major risk factor for the disseminated MAC (3). In the reported cases of MAC infection of the GI tract in patients with an advanced HIV infection, the duodenum was the most common site, followed by the rectum, ileum, colon, esophagus, jejunum, and stomach (4).

In September 2018, the infection control team of our hospital pointed out that an unusual number of positive cases of NTMs were being detected in an intestinal fluid culture obtained by colonoscopy from patients without any apparent

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AIDS symptoms over a 10-year period, thus suggesting environmental contamination. The medical records were retrospectively reviewed to determine the patient characteristics and calculate the procedure-specific NTM isolation rates. Subsequently, we investigated the source of the pseudo-outbreak in the endoscopy unit.

**Materials and Methods**

This was a retrospective analysis of 263 consecutive patients whose intestinal fluid mycobacterial culture was obtained by colonoscopy and analyzed between January 2009 and December 2018 at St. Marianna University Hospital, Kawasaki, Japan, an academic medical center performing approximately 16,000 endoscopies annually. The institutional review board of the hospital approved the study. The patients’ medical records provided the information related to their medical history and microbiology tests.

The colonoscopy procedures were performed by gastroenterologists in the endoscopy unit. The procedures were performed with colonic lavage [2 L polyethylene glycol (PEG)-based lavage solution] over three to four hours in 256 patients (97%). Patients who underwent colonoscopy had the PEG dissolved in their tap water at home. During colonoscopy observations, the colonic lumen was cleansed with dimethylpolysiloxane antifoaming solution (Kaigen Pharma, Osaka Japan). Purified water was used from a supply of water bottles. The endoscopes were routinely cleaned with Endoclens® (Johnson and Johnson, Tokyo, Japan), an automated endoscope preprocessor that flushed with a detergent solution, disinfected with 0.55% orthophthalaldehyde and rinsed with filtered tap water, according to Japan Gastrointestinal Endoscopy Society guidelines (5, 6). A endoscopic biopsy was performed at the inflamed lesion using disposable forceps for the pathological specimen analysis. In case there was no apparent inflamed lesion, then a biopsy was performed according to the physician’s preference.

For the mycobacterial culture, the specimens (Fig. 1) were incubated in Mycobacteria Growth Indicator Tube® (Becton, Dickinson and Company, Tokyo, Japan) at 37 °C up to 6 weeks and Ziehl-Neelsen staining of colonies was performed to identify acid-fast organisms. Polymerase chain reaction (PCR) was used to identify mycobacteria to the genera level, and a restriction analysis was used to identify it to the species level.

The infection control team reviewed the patients’ microbiology charts, obtained cultures of source water, faucets, washers and endoscopes, and observed endoscope cleaning and disinfecting procedures.

**Statistical analyses**

A statistical analysis was performed using the χ² test and Student’s t-test was used to compare the data. Results with p <0.05 were considered to be statistically significant. The SPSS Statistics software program 13.0 (SPSS, Chicago, USA) was used for all statistical analyses.

**Results**

**Patient baseline characteristics**

The demographic and clinical characteristics of the 263 patients, for whom intestinal fluid mycobacterial culture obtained by colonoscopy was performed, are shown in Table 1. The patients included 143 males and 120 females, with an average age of 52±20.3 years (range: 2-91 years). The indications for colonoscopies were as follows: positive fecal immunochemical test 32, diarrhea 160, hematochezia 100, abdominal pain 101, health surveillance 67, and inflammatory bowel diseases 33. None of the patients had any AIDS symptoms. 88.2% of the patients for whom intestinal fluid culture was performed showed inflammation, which was observed by colonoscopy. Of these, endoscopic findings suggesting inflammation such as redness, erosion, or a reduced vascular pattern were observed in 232 patients (Table 1).

**Intestinal fluid and mucosal culture of NTM obtained by colonoscopy**

An intestinal fluid culture of 154 (58.6%) patients was positive for NTM (<i>M. intracellulare</i>; 125 cases, <i>M. gor-
loss of fluid culture was positive in 102 cases (58.2%). Of these performed intestinal mucosal cultures, an intestinal mucosal culture was performed simultaneously for NTM. Of these, or the rate of NTM infection (Table 3). Of these, there was no significant correlation among the patient characteristics, the indications for colonoscopy, the endoscopic findings, or the rate of NTM infection (Table 3). Of these, an intestinal mucosal culture was performed simultaneously for NTM. Of these, or the rate of NTM infection (Table 3). Of these, there was no significant correlation among the patient characteristics, the indications for colonoscopy, the endoscopic findings, or the rate of NTM infection (Table 3).

| Characteristic                  | n=263 |     |
|--------------------------------|-------|-----|
| Age (year)                     | 55.2±20.3 |     |
| Sex, male/female               | 143/120 |     |
| Indication for colonoscopy     |       |     |
| Diarrhea                       | 160 (60.8) |     |
| Hematochezia                   | 100 (38.0) |     |
| Abdominal pain                 | 101 (38.4) |     |
| (suspect of) IBDS              | 33 (12.5) |     |
| Health surveillance            | 67 (25.5) |     |
| Positive fecal immunochemical  | 32 (12.1) |     |
| Endoscopic findings suggesting | 232 (88.2) |     |

Values are presented as mean±SD or number (%). IBD: inflammatory bowel disease

**Table 1. Baseline Characteristics of Patients Whose Intestinal Fluid Mycobacterial Culture was Analyzed by Colonoscopy.**

**Figure 2. Annual number of positive and negative intestinal fluid cultures for NTM from 2009 to 2018.**

| Characteristics                                         |
|--------------------------------------------------------|
| Intestinal fluid culture for NTM; positive / total     |
| 154/263                                                |
| M. intracellulare                                      |
| 125 (81.2)                                             |
| M. gordonae                                            |
| 14 (9.1)                                               |
| M. avium                                              |
| 4 (2.6)                                                |
| M. abscessus                                           |
| 3 (1.9)                                                |
| others                                                 |
| 8 (5.2)                                                |
| Intestinal mucosal culture for NTM; positive / total   |
| 2/182                                                  |
| M. intracellulare                                      |
| 1 (50.0)                                               |
| M. peregrinum                                          |
| 1 (50.0)                                               |

Values are presented as number (%). NTM: non-tuberculous mycobacteria

**Table 2. Species of Culture for Non-tuberculous Mycobacteria (NTM) Obtained by Colonoscopy.**

donae; 14 cases, M. avium; 4 cases, M. abscessus; 3 cases, and 8 other cases; Table 2). The annual number of NTM intestinal fluid cultures and the isolation rate from 2009 to 2018 were examined. The endoscope was reprocessed according to the guidelines, and remained unchanged during this period. Our results showed that positive rates did not change significantly over time. However, the number of NTM positive culture specimens had increased, which attracted the attention of infection control personnel (Fig. 2). There was no significant correlation among the patient characteristics, the indications for colonoscopy, the endoscopic findings, or the rate of NTM infection (Table 3). Of these, an intestinal mucosal culture was performed simultaneously in 182 cases (69.2%). Among them, 2 cases were positive for NTM (M. intracellulare and M. peregrinum respectively). Of these performed intestinal mucosal cultures, an intestinal fluid culture was positive in 102 cases (58.2%). M. tuberculosis was positive in 3 cases of intestinal culture, 2 of which also presented positive intestinal fluid cultures. All three cases were treated for tuberculosis. The abnormally high prevalence of NTM in the intestinal fluid culture of patients without any obvious evidence of an immunodeficient condition suggested environmental contamination of the specimen.

**Investigation of the source of pseudo-outbreak in the endoscopy unit**

Therefore, we investigated the source of possible NTM contamination during endoscope reprocessing and colonoscopic examination procedure. NTM was not detected from the endoscopes that had been cleansed and disinfected with the standard procedure in our endoscopy unit (5, 6). Next, we examined possible environmental contamination in the endoscopy unit. NTM (M. intracellulare) was detected from the two faucets in the endoscopy unit, which were used to prepare the dimethylpolysiloxane antifoaming solution during endoscopic examinations. When purified water was passed through the forceps channel of properly reprocessed endoscope and sampled, NTM were not detected. In contrast, when the tap water that was previously collected from the faucet was passed through the forceps channel and sampled, M. intracellulare was detected. Thus, the water taps were considered to be the source of the NTM infection. Once the faucets were replaced, NTM was no longer detected in tap water.

**Discussion**

The risk of cross-contamination was almost non-existent when flexible endoscopes are reprocessed following the accepted guidelines. Until infection control personnel of our hospital noted an unusual number of NTM positive specimens from the endoscopy unit, we overlooked water tap as a source of infection. Chongwe et al. reported that NTM was isolated from 3% of colonic lavage sample before endoscopy (7). According to the annual isolation rate of intestinal fluid cultures for NTM in the present study (Fig. 2), colonization of NTM in the water taps may have occurred before 2010. The number of intestinal fluid culture cases had in-
creased since 2017. It is probable that the colonoscopist noticed this increase in the NTM in the intestinal fluid culture, likely encouraging them to perform intestinal fluid cultures more often. Since NTM was detected in the tap water collected from the faucets, the reported detection rate of NTM in intestinal fluid culture might not reflect the true picture and it can be speculated that the rate of NTM may have remained unchanged during this period.

In contrast to pulmonary infection, the clinical significance of recovery of NTM from intestinal specimens from patients without an immunocompromised state seems unclear. Most of the reported cases of intestinal infection of NTM were associated with disseminated AIDS (4, 8). There have been reports on the evidence relating inflammatory bowel disease (IBD) pathogenesis to NTM, however, the present study indicated that there was no significant association between IBD and a positive intestinal fluid culture (9). On the other hand, pulmonary infection by NTM has been recognized with clinical significance (2). As a result, bronchoscopy has been reported to be a source of both pseudo-infections and infectious outbreaks of NTM due to inadequate disinfection of flexible bronchoscopes or insufficient sterilization of endoscopic devices (10-12). There are several reports on contamination of NTM from water supply of endoscopy unit. M. xenopi in the tap water contaminated the glutaraldehyde-disinfected bronchoscopes during reprocessing (13).

The niches used by MAC and other NTM organisms are soil, water, and dust, and many reports indicate that MAC species are also present in common households (2). Several studies have shown that showerheads, water taps, and the end-points of drinking water distribution systems, can be MAC reservoirs in households (14-16). Specimen contamination with MAC from hospital water has also been reported (17). The reported rates of NTM contamination for household tap water and for hospital water samples were substantially high. The most frequently isolated strains were M. intracellulare, M. genavense, and M. haemophilum (18). The densities of Mycobacterium spp. were generally higher at distal sites relative to the entry point of the water distribution system. The NTM might travel in the reservoir water all the way through the water distribution system up to the tap. Long-term colonization of showerheads and tap water indicates that MAC and other NTM species attach to surfaces, withstand water flow, and grow inside of showerheads and plumbing pipes. Feazel et al. (19) used scanning electron microscopy to directly observe a biofilm that formed inside a showerhead. In addition, NTM and other opportunistic human pathogens are enriched by >100-fold in many showerhead biofilms. NTM seems to form biofilms by overcoming competition with other fast-growing microbes.

There are several reports on NTM colonization prevention in water distribution systems. Copper pipelines might be effective for preventing NTM colonization. Inkinen et al. surveyed water samples supplied through a copper pipeline and a polyethylene pipeline (20). Mycobacterium spp. sequences were absent from the copper pipe-line samples and they were detected only in the cold polyethylene pipeline water and biofilm samples. Chlorination is usually performed to disinfect drinking water treatment plants. However, the ability of NTM to tolerate chlorine could allow NTM to inhabit drinking water. While NTM were not found in water samples in which the concentration of residual chlorine was greater than 0.5 mg/L, the Japanese water supply law requires a minimum of 0.1 mg/L at the water tap (2). In addition, higher temperatures in plumbing might effectively reduce NTM colonization (21). The decimal reduction time (D value)- the time needed to inactivate 90% of the bacterial population- of M. avium at 70°C was 2.3 seconds, and at 60°C, it was 240 seconds.

The present study is the first report on NTM contamination of intestinal culture specimens via water tap in the endoscopy unit. Tap water is used to prepare intestinal lavage solution at endoscopy units in many hospitals. Mycobacterial intestinal fluid culture may increase the positive rate and may lead to erroneous diagnostic decisions. In fact, in the

Table 3. Demographic Information and Indication for Colonoscopy.

| Variable                         | Intestinal fluid culture positive or negative for NTM | p value |
|----------------------------------|------------------------------------------------------|---------|
|                                  | Positive (n=154) | Negative (n=109) |
| Age (year)                       | 54.5±20.7       | 56.2±19.9        | 0.512   |
| Sex, male/female                 | 77/77          | 66/43            | 0.117   |
| Indication for colonoscopy       |                    |                   |         |
| Diarrhea                         | 88             | 72              | 0.183   |
| Hematochezia                     | 61             | 40              | 0.726   |
| Abdominal pain                   | 58             | 42              | 0.989   |
| (suspect of) IBD                 | 16             | 17              | 0.286   |
| Health surveillance              | 36             | 31              | 0.433   |
| Positive fecal immunochemical test | 22         | 10             | 0.290   |
| Endoscopic findings suggesting inflammation | 134 | 98 | 0.601 |

Values are presented as mean±SD or number. NTM: non-tuberculous mycobacteria, IBD: inflammatory bowel disease.
present study, two cases with a positive intestinal fluid culture for NTM were treated for NTM. Even if NTM is detected in the intestinal mucosal culture, NTM should not be treated in the absence of immunocompromised state until possible contamination is ruled out. On the other hand, *M. tuberculosis* cultures of intestinal mucosa seems to reflect the clinical findings. Accordingly, when performing mycobacterial culture of the intestinal tract during colonoscopy, intestinal fluid cultures should be avoided. Instead, intestinal mucosal cultures should be performed.

Taking these results into account, we have developed action plans to prevent further NTM contamination in the endoscopy unit. The water faucets of the endoscopy unit were enabled with a touchless sensor. Since water droplets could remain on the faucet, possibly forming NTM biofilm, we replaced them with a manual opening faucet. We added a step to the hygiene protocol in the endoscopy unit of discharging hot water from the faucet for a certain time before starting the daily practice to reduce any possible NTM contamination.

In summary, we experienced a pseudo-outbreak of NTM caused by a contaminated water tap in the endoscopy center. Contamination of intestinal fluid specimens by environmental NTM in hospital water supplies may lead to an erroneous diagnosis. Thus, based on our results, we recommend that when performing mycobacterial culture of the intestinal tract by colonoscopy, mucosal cultures should be performed instead of intestinal fluid cultures.

**The authors state that they have no Conflict of Interest (COI).**

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