Endemic Contamination of Clinical Specimens by *Mycobacterium gordonae*

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Contamination of clinical specimens by *Mycobacterium gordonae* is a significant endemic problem in many laboratories. To investigate this problem, 84 cases at 1 hospital were retrospectively identified during 20 months. The overall rate of specimen contamination was 2.4%, and 72 of the contaminated specimens were respiratory. A case-control comparison showed that the risk of respiratory specimen contamination was significantly increased if the specimen was expectorated (odds ratio [OR], 3.62; 95% confidence interval [CI], 1.36–9.50) or if the patient consumed fluids within 2 days before specimen collection (OR, 8.92; 95% CI, 1.40–71.20). Cultures of tap water, ice, and iced drinking water all yielded *M. gordonae* at 10^2–10^6 cfu/mL. A culture survey of consenting patients showed contamination of 8 (24%) of 34 sputum specimens collected immediately after a tap water mouth rinse. These findings demonstrate that endemic specimen contamination arises from mycobacteria in hospital tap water and provide a foundation for control efforts.

*Mycobacterium gordonae*, like other nontuberculous mycobacteria, is found primarily in natural waters and tap water systems [1, 2]. It rarely causes human infection or colonization [3–6] and generally is viewed as a contaminant when isolated from clinical specimens. However, even as a contaminant, *M. gordonae* may have troublesome clinical consequences. Because pigment that distinguishes colonies of *M. gordonae* from *Mycobacterium tuberculosis* sometimes is not evident initially, presumptive therapy for tuberculosis may be initiated pending species identification [7–9].

The source of *M. gordonae* in clinical specimens has been investigated when clusters of positive cultures, or pseudoepidemics, have occurred. These investigations have implicated contaminated tap water or ice [8–11], a topical anesthetic [12], and a commercially prepared antibiotic solution [7]. Endemic contamination of specimens by *M. gordonae* appears to be an even greater problem, on the basis of observations that *M. gordonae* is one of the most common mycobacteria isolated in hospital laboratories [6, 13] and that ~1% of clinical specimens are culture-positive for *M. gordonae* [7]. Accordingly, we initiated an investigation to learn the rate and source of endemic *M. gordonae* specimen contamination in our hospital (University of Chicago Hospital, Chicago) and to guide subsequent control efforts.

**Materials and Methods**

Case definition and case detection. We considered a specimen to be contaminated by *M. gordonae* if the specimen was the only one from a particular patient that yielded *M. gordonae* on culture and if there was no histopathologic evidence of mycobacterial infection. Patients from whom multiple specimens yielded *M. gordonae* were considered to be colonized unless there was compelling (e.g., histopathologic) evidence of infection. We retrospectively reviewed records from the Clinical Microbiology Laboratory of the University of Chicago Hospital, to identify all culture-positive specimens during 1 January 1997 through 31 August 1998, and we examined patients’ medical records to determine whether contamination, colonization, or infection had occurred.

Case-control study. We conducted a case-control study to evaluate possible risk factors for *M. gordonae* contamination of respiratory specimens. Contaminated respiratory specimens from University of Chicago Hospital inpatients were designated as case-specimens, and 64 culture-negative specimens were chosen as controls. The controls were selected randomly from all culture-negative respiratory specimens from inpatients whose specimens all were culture-negative. If >1 specimen was selected from a patient, only the first specimen was included. The following information was collected concerning case- and control-specimens: laboratory technologist who processed the specimen, results of other specimens processed on the same day, day of hospitalization that the specimen was obtained, patient age and sex, presence of chronic lung disease, patient access to drinking water during the 2 days before specimen collection, and systemic antibiotic treatment during the week before specimen collection.

Continuous variables were compared by use of the *t* test or Mann-Whitney *U* test, and proportions were compared by χ² test or Fisher’s 2-tailed test.

Evaluation of specimen collection and processing. Procedures for specimen collection in patient care areas and specimen processing in the Clinical Microbiology Laboratory were observed to detect opportunities for *M. gordonae* contamination. In brief, specimens from nonsterile sites, such as sputum, were collected in sterile
screw-cap containers and transported to the Clinical Microbiology Laboratory, where they were stored at 4°C until being processed at the start of the next weekday. Up to 10 mL of sputum was transferred inside a biological safety hood into a sterile 50-mL centrifuge tube, and an equal volume of freshly mixed 4% sodium hydroxide, sterile 2.9% sodium citrate, and N-acetyl-l-cysteine powder was added for digestion and decontamination. Specimens were agitated on a vortex mixer, then allowed to stand for 15 min before 30 mL of sterile phosphate buffer was added. The liquefied specimen was centrifuged, most of the supernatant was decanted, and the sediment was inoculated onto separate slants of Middlebrook 7H11 and Lowenstein-Jensen agar and into an enriched broth with 14C-labeled substrate for radiometric detection of growth (BACTEC 12B medium; Becton Dickinson Microbiology Systems, Baltimore) supplemented with polyoxyethylene stearate, polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin. A portion of the decontaminated sputum sediment also was inoculated onto slides for auramine O and Kinyoun staining, followed by microscopic examination. Probable mycobacteria that grew during incubation of inoculated media at 35°C for 6 weeks were identified by auramine O or Kinyoun staining, pigment production, and DNA probes for \textit{M. tuberculosis} and other mycobacteria [6].

To evaluate sputum processing in the Clinical Microbiology Laboratory, 2 sputum specimens that had been sterilized in an autoclave at 121°C for 20 min were submitted each weekday for routine mycobacterial culture until 100 sterilized specimens had been processed. Laboratory technologists were not informed that test specimens were being submitted, and the unusual milky appearance of these specimens was explained as being due to a new method of sputum induction.

\textit{Environmental cultures.} To determine whether \textit{M. gordonae} was present in the hospital environment, 500-mL samples of hot and cold tap water were collected from a patient room in each of 3 nursing units and from the mycobacteriology and media preparation rooms in the Clinical Microbiology Laboratory. Also, 500-mL samples of drinking water or melted ice were collected from the ice machines on 8 nursing units and from 10 bedside pitchers of iced drinking water. Each sample was concentrated by being passed through a 0.2-µm filter that was then placed in 5 mL of sterile water and agitated on a vortex mixer for 90 s. The filter was discarded, and the concentrate was digested and decontaminated in the same manner as sputum, except that a pipette was used to remove the supernatant after centrifugation, leaving a final sediment volume of 2.5 mL. Next, 1.0- and 0.1-mL portions of the sediment were inoculated onto separate plates of Middlebrook 7H11 agar, and 0.4 mL was inoculated into BACTEC 12B medium, as described above. The plates were incubated at 35°C for up to 6 weeks, and colonies resembling \textit{M. gordonae} were identified by pigment production and by hybridization with a DNA probe (Accuprobe; Gen-Probe, San Diego).

\textit{Culture survey of patients.} To determine whether \textit{M. gordonae} was present in the oropharynx or respiratory tract of inpatients, a mouth rinse specimen and a sputum specimen were requested from 35 patients from whom a sputum specimen for mycobacterial culture had been ordered by their physician. Patients were asked first to rinse their mouths and gargle with ~30 mL of water from their bedside container of iced drinking water. This mouth rinse specimen was collected in a sterile container, then a sputum specimen was expectorated into another sterile container. These specimens were processed in the Infection Control Program Laboratory using the procedures for sputum followed in the Clinical Microbiology Laboratory.

\section*{Results}

\textit{Description of cases.} During 20 months, 109 specimens from 95 patients yielded \textit{M. gordonae} on culture. Eighty-four patients with a single positive specimen were classified as having a case of \textit{M. gordonae} contamination. The other 11 patients with multiple positive specimens were considered to have probable \textit{M. gordonae} colonization. For these 11 patients, the specimens that repeatedly yielded \textit{M. gordonae} were sputum.

At least 1 case occurred each month, and no seasonal variation was evident (figure 1). Seventy-eight of the cases occurred on a day when there were no other cases, and the remaining 6 cases were distributed as 2 cases per day on 3 days. Fifty-three cases were in inpatients at our hospital, 25 were in outpatients, and 6 were in patients at 2 affiliated institutions that submitted specimens to the Clinical Microbiology Laboratory. At our hospital, contaminated specimens were submitted from 16 inpatient units and 7 outpatient clinics. The greatest number of contaminated specimens submitted from any unit was 19. Contaminated specimens were sputum in 61 cases, bronchoalveolar lavage fluid in 11, urine in 4, and feces, gastric aspirate, skin biopsy, or drainage fluid in 2 cases each.

The overall specimen contamination rate during the study period was 2.4% (84 of 3450 specimens). For comparison, the rate of positive cultures for \textit{M. tuberculosis} during the same period was 1.4%. As shown in table 1, the rate of contamination varied by specimen type, and only specimens from nonsterile body sites yielded \textit{M. gordonae}.

The medium from which \textit{M. gordonae} was isolated was broth only in 37 cases, Middlebrook 7H11 agar only in 10 cases,

\begin{table}[h]
\centering
\caption{Rate of \textit{Mycobacterium gordonae} contamination of various types of specimens during 1 January 1997–31 August 1998.}
\begin{tabular}{lll}
\hline
Type of specimen & No. of specimens & No. (%) of specimens contaminat\textit{ed} \\
\hline
Expectorated sputum\textsuperscript{a,b} & 1056 & 60 (5.7) \\
Bronchoalveolar lavage fluid\textsuperscript{c} & 314 & 11 (3.5) \\
Endotracheal aspirate\textsuperscript{d} & 65 & 1 (1.5) \\
Urine\textsuperscript{e} & 65 & 4 (6.2) \\
Feces\textsuperscript{f} & 33 & 2 (6.0) \\
Gastric aspirate\textsuperscript{g} & 107 & 2 (1.9) \\
Drainage fluid\textsuperscript{h} & 70 & 2 (2.9) \\
Skin\textsuperscript{i} & 31 & 2 (6.5) \\
Tissue biopsy & 582 & 0 \\
Blood & 127 & 0 \\
Cerebrospinal fluid & 562 & 0 \\
Other fluids from sterile sites & 438 & 0 \\
\hline
\end{tabular}
\textsuperscript{a} Specimens from these nonsterile sites routinely were digested with \textit{N}-acetyl-l-cysteine and decontaminated with 4% sodium hydroxide.
\textsuperscript{b} Specimens obtained by spontaneous expectoration or induced by inhaled nebulized hypertonic saline could not be distinguished; therefore, the data are combined in the category “expectorated sputum.”
\end{table}
Lowenstein-Jensen agar only in 3 cases, and ≥2 media in 34 cases. In none of the cases were mycobacteria detected by microscopic examination of the specimen stained with auramine O or Kinyoun reagents.

Four of the 84 patients with *M. gordonae* contamination of a sputum specimen received presumptive antituberculous therapy on the basis of the positive mycobacterial culture. The duration of treatment was 2–18 days (mean, 11 days), and 1 patient was readmitted to the hospital for 2 days.

**Case-control study.** Forty-four inpatients at our hospital had a contaminated respiratory specimen, and these specimens were classified as case-specimens for the case-control study. Compared with 64 randomly selected uncontaminated respiratory specimens, case-specimens were significantly more likely to have been produced by expectoration (table 2). Also, patients from whom contaminated specimens were obtained were significantly more likely to have taken oral fluids during the 2 days before specimen collection. None of the other patient or specimen characteristics examined was significantly associated with contamination. The proportion of case-specimens and control-specimens processed by each of 11 laboratory technologists also was tabulated. None of the technologists was significantly associated with contaminated specimens (*P* > .05 for each), and no technologist processed >12 case-specimens.

**Environmental cultures.** In patient care areas, *M. gordonae* was recovered from hot and cold tap water in patient bathrooms, ice to be added to drinking water, and bedside containers of iced drinking water (table 3). The concentration of *M. gordonae* in these specimens usually was 0.01–1.0 cfu/mL. *M. gordonae* also was recovered from tap water at work area sinks in the mycobacteriology and media preparation rooms of the Clinical Microbiology Laboratory and from a countertop in the media preparation room.

**Evaluation of specimen processing.** No errors in aseptic practices or deviations from written procedures were detected during frequent observation of specimen processing in the Clinical Microbiology Laboratory. All 100 sterilized sputum specimens submitted to evaluate specimen processing were culture-negative for mycobacteria.

**Culture survey of patients.** Mouth rinse and sputum samples for mycobacterial culture were obtained from 34 inpatients. *M. gordonae* was isolated from 23 (68%) of the tap water mouth rinse specimens and 8 (24%) of the sputum samples. All culture-positive sputum samples were from patients whose mouth rinse specimen also was culture-positive. The 8 patients whose sputum specimen was positive had 15 other sputum specimens submitted for routine mycobacterial culture within 1 week. Only 1 of these yielded *M. gordonae*: none of the 4 specimens collected on the same day as the culture survey specimen was positive. *M. tuberculosis* was recovered from 3 culture survey specimens, and all 3 patients also had *M. tuberculosis* in respiratory specimens submitted for routine mycobacterial culture.

**Discussion**

Our investigation demonstrates that endemic *M. gordonae* contamination of respiratory specimens is caused by *M. gordonae* in hospital tap water. We hypothesize that, as the initial step, *M. gordonae* in iced tap water is deposited in the mouth or oropharynx when iced water is ingested. Next, *M. gordonae* present in the mouth or oropharynx contaminates respiratory specimens during expectoration, tracheal suctioning, or bronchoscopy. This formulation is supported by our culture studies showing *M. gordonae* in most iced drinking water and mouth rinse specimens and contamination of sputum only when the patient's mouth rinse also was culture-positive. Further, our epidemiologic studies showed an association of sputum contamination with access to drinking water and an increased rate.
of contamination of respiratory specimens that had direct contact with the mouth and oropharynx, that is, expectorated sputum. Laboratory contamination was deemed unlikely, because of negative results for cultures of mock specimens, absence of clustering of cases, and the lack of association of cases with any technologist.

Introduction of small numbers of nontuberculous mycobacteria into the mouth appears to be an effective means of contaminating respiratory specimens. We detected only 0.01–1.0 cfu of contaminating respiratory specimens. We detected only 0.01±1.0 cfu of 

\[ M. gordonae \]

onized with hospital tap water, but the residual volume in the mouth immediately after gargling the water was sufficient to contaminate 24% of sputum specimens. An even higher rate of sputum contamination (72%) was reported by Murphey et al. [14], who had volunteers first gargle hospital tap water containing \[ M. gordonae \], \[ M. avium±intracellulare \], \[ M. xenopi \], or possibly shower water [17]. Tap water used to rinse bronchoscopes also has been the source of respiratory specimen contamination by \[ M. gordonae \] [8, 12], \[ M. xenopi \] [15], \[ Mycobacterium abscessus \] [18], and \[ Mycobacterium chelonae \] [19]. Contamination of nonrespiratory specimens, such as urine and stool, has been ascribed to the use of tap water to rinse bedpans or irrigate bowel lumen during colonoscopy [15]. The only other important source of contaminant mycobacteria has been deionized tap water used to prepare antibiotics [7] or reagents [20, 21] for laboratory detection of mycobacteria.

As illustrated by this investigation, endemic contamination problems often are difficult to solve. Cases were not clustered geographically or temporally, and multiple types of specimens and culture media were involved. The strongest observation—that \[ M. gordonae \] was recovered only from specimens that underwent digestion and decontamination—could have been compatible with a ward tap water source [15], contaminated laboratory fluids [7, 21], or laboratory technician error [22]. Consequently, we examined each of the potential modes of contamination by means of a case-control study, environmental cultures, and cultures of mock specimens to reach a definitive conclusion.

Endemic contamination of respiratory specimens by \[ M. gordonae \] appears to be a substantial problem, with an incidence as high as 14% for induced sputum [10]. Even though DNA

### Table 2. Characteristics of respiratory specimens contaminated by \[ Mycobacterium gordonae \], compared with uncontaminated control specimens.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case specimens (( n = 44 ))</th>
<th>Control specimens (( n = 64 ))</th>
<th>OR (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age, years</td>
<td>50.5 ± 15.6</td>
<td>51.9 ± 19.6</td>
<td>—</td>
<td>.71</td>
</tr>
<tr>
<td>Male sex</td>
<td>25 (57)</td>
<td>36 (56)</td>
<td>1.02 (0.47–2.20)</td>
<td>.95</td>
</tr>
<tr>
<td>Type of specimen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expectorated sputum</td>
<td>38 (86)</td>
<td>42 (66)</td>
<td>3.62 (1.36–9.50)</td>
<td>.02</td>
</tr>
<tr>
<td>Endotracheal aspirate</td>
<td>1 (2)</td>
<td>2 (3)</td>
<td>0.72 (0.00–5.72)</td>
<td>1.00</td>
</tr>
<tr>
<td>Bronchoalveolar lavage</td>
<td>5 (11)</td>
<td>20 (31)</td>
<td>0.28 (0.10–0.80)</td>
<td>.02</td>
</tr>
<tr>
<td>Patient location</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical unit</td>
<td>35 (80)</td>
<td>44 (69)</td>
<td>1.77 (0.73–4.29)</td>
<td>.21</td>
</tr>
<tr>
<td>Intensive care unit</td>
<td>6 (14)</td>
<td>16 (25)</td>
<td>0.47 (0.17–1.30)</td>
<td>.15</td>
</tr>
<tr>
<td>Other</td>
<td>3 (7)</td>
<td>4 (6)</td>
<td>1.10 (0.26–4.65)</td>
<td>1.00</td>
</tr>
<tr>
<td>Duration of hospitalization before specimen submitted, days</td>
<td>3.0 ± 2.9</td>
<td>4.1 ± 7.1</td>
<td>—</td>
<td>.95</td>
</tr>
<tr>
<td>Chronic pulmonary disease</td>
<td>21 (48)</td>
<td>25 (39)</td>
<td>1.42 (0.66–3.08)</td>
<td>.37</td>
</tr>
<tr>
<td>Oral intake of fluids during 2 days before specimen collection</td>
<td>43 (98)</td>
<td>53 (83)</td>
<td>8.92 (1.40–71.20)</td>
<td>.03</td>
</tr>
<tr>
<td>Systemic antibiotic therapy during 7 days before hospitalization</td>
<td>32 (73)</td>
<td>50 (78)</td>
<td>0.75 (0.31–1.79)</td>
<td>.52</td>
</tr>
</tbody>
</table>

\( a \) Data are no. (%) or mean ± SD.

### Table 3. Detection of \[ Mycobacterium gordonae \] in the hospital environment.

<table>
<thead>
<tr>
<th>Location, type of specimen</th>
<th>No positive/ no tested</th>
<th>Log cfu/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient bathroom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold tap water</td>
<td>2/3</td>
<td>( 10^{-2} )</td>
</tr>
<tr>
<td>Hot tap water</td>
<td>1/3</td>
<td>( 10^{-1} )</td>
</tr>
<tr>
<td>Nutrition station</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice</td>
<td>2/3</td>
<td>( 10^{-2}–10^{0} )</td>
</tr>
<tr>
<td>Drinking water</td>
<td>0/3</td>
<td>—</td>
</tr>
<tr>
<td>Patient room</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iced drinking water from bedside pitcher</td>
<td>9/10</td>
<td>( 10^{-2}–10^{0} )</td>
</tr>
<tr>
<td>Microbiology laboratory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold tap water</td>
<td>2/2</td>
<td>( 10^{-1} )</td>
</tr>
<tr>
<td>Hot tap water</td>
<td>2/2</td>
<td>( 10^{-1}–10^{0} )</td>
</tr>
</tbody>
</table>
probes for rapid species identification can minimize the clinical consequences, preventive measures are warranted. In accordance with the recommendation of Metchock et al. [6], we advise that patients do not rinse their mouths or gargle with tap water before sputum collection. Also, it may be worthwhile to evaluate other measures, such as a mouth rinse with sterile water immediately before sputum expectoration or withholding tap water and iced beverages for several hours before expectoration.

References