Contamination of Sputum Induction Equipment During Patient Usage

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Sputum induction equipment was evaluated for its capacity to become contaminated by patients harboring Mycobacterium tuberculosis. The mouthpiece, goose-neck, and 10% NaCl solution were found capable of being contaminated by tuberculous patients. Two per cent glutaraldehyde was shown to be an effective means of decontamination. This study indicates that the entire induction apparatus must be cleaned and decontaminated between patients.

A problem in the diagnosis of tuberculosis has been the difficulty in obtaining adequate sputum specimens from certain patients. It has been shown that sputum induction through aerosol inhalation is valuable in outpatient clinics where gastric and tracheal lavage are difficult to perform, where the time allowed for sputum collection is short, and where assurance that the sputum is that of the patient in question. It is also a useful means of collecting sputum from patients who have difficulty in raising sufficient sputum for proper evaluation, from patients on drug therapy, and from those who are unwilling to cooperate in producing voluntary sputum.

Garcia (4) described a method for obtaining bronchial secretions by nebulization and inhalation of sterile distilled water. Payne et al. (7), using the method of Garcia, compared bronchial lavage with nebulized water to morning sputum collection for the detection of tubercle bacilli. They reported that, when the patient inhaled the nebulized water, coughing and expectoration occurred at intervals throughout the lavage. They found that recovery of tubercle bacilli by this method is sufficiently reliable to justify its use when expectoration is absent. They were also of the opinion that such a method of sputum induction had merit for the collection of sputum from outpatients and from those under drug therapy who have little or no expectoration.

Specimens induced by heated aerosols were found to be more satisfactory for cytological examination than the spontaneous sputum employed in routine studies (1). Several researchers have shown that sputum induced by inhalation of a heated, hypertonic aerosol produced a greater number of cultures positive for tubercle bacilli than did gastric specimens from the same group of inpatients under investigation for tuberculosis (2, 3, 5, 8).

In the process of aerosol nebulization for sputum induction, it seemed likely that parts of, or perhaps the entire unit, could easily become contaminated by some patients, since patients do cough directly into the mouthpiece. Therefore, it was decided to determine the level of contamination of various components of the unit both before and after the patients’ use and after decontamination.

MATERIALS AND METHODS

The reservoir in the heating unit (Inhalation Equipment Co., Inc., New York, N.Y.) was filled to the appropriate level with tap water (Fig. 1). The medication cup was three-fourths filled with 10% sterile NaCl solution. The heating unit was turned on, and, when the indicator light turned red, the temperature of the NaCl solution was recorded. A 10-ml amount of the 10% NaCl solution was removed from the medication cup for culturing. Using a sterile cotton swab moistened in 1 ml of sterile phosphate-buffered saline (PBS), an area of 6 square inches was sampled from the interior surface of the metal gooseneck. The mouthpiece was connected to the gooseneck. Three square inches of the interior and 3 square inches of the exterior surfaces of the mouthpiece were sampled. The aerosol pump was connected to the nebulizer and turned on; after a few minutes, the temperature of the vapor coming from the mouthpiece was recorded. The induction process was begun by having

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the patient place the mouthpiece into the mouth, breathing deeply and then exhaling completely. When coughing was induced, it was deliberately directed back into the mouthpiece. Occasionally, saliva ran back into the mouthpiece. The sputum induction process was continued until about 10 ml of sputum was collected in a sputum collection kit (Falcon Plastic, Division of B.D. Laboratories Inc., Los Angeles, Calif.). Sputum induction never exceeded 30 min.

The mouthpiece, gooseneck, and 10% NaCl solution were sampled as before. The sputum induction unit was disassembled completely; after rinsing in tap water, the mouthpiece, cover, medication cup and nebulizer mechanism were placed into 2% glutaraldehyde (Cidex; Arbrook, Somerville, N.J.) and submerged in this disinfectant for 20 min. After decontamination, the equipment was rinsed in running tap water, air-dried, sampled, and wrapped in paper towels. An area of six square inches of the interior surface of the medication cup was sampled as described for the mouthpiece and gooseneck.

Samples of sputum from known tuberculous patients and the 10% NaCl solution samples were digested and decontaminated with N-acetyl-L-cysteine-sodium hydroxide solution according to a previously described method (6). The swab samples in PBS were agitated by using a Vortex mixer, and the fluid was expelled from the swab by firmly pressing it against the interior surface of the tube. The fluid was then processed in the same manner as the sputum and 10% NaCl solution samples. All specimens were sedimented by centrifugation and the supernatant fluid was decanted and discarded; PBS was added to the sediment to bring it up to the original volume. A 0.1-ml amount of this sample and dilutions of 10⁻² and 10⁻³ were plated in duplicate on 7H10 (BBL) agar plates. After incubation, colonies were counted and identified by procedures previously described (6).

RESULTS

In 10 trials, Mycobacterium tuberculosis could not be recovered from the mouthpiece, from the gooseneck (leading from the mouthpiece to the 10% NaCl solution), or from the 10% NaCl solution before the sputum induction process (Table 1). Patient K.G. had 1.6 × 10⁵ colony-forming units (CFU) of M. tuberculosis per ml in the sputum specimen. The mouthpiece, after use, resulted in 1.7 CFU/square inch from the swab specimen. After use, the gooseneck and 10% NaCl solution gave no growth of M. tuberculosis. The temperature of the 10% NaCl solution was 154 F (68 C).

Patient J. M. was evaluated three times for the induction assay. The first trial yielded 1.8 × 10⁵ CFU/ml of M. tuberculosis in the sputum. The mouthpiece was contaminated with 2.0 × 10⁵ CFU/square inch, the gooseneck with 8.0 × 10⁵ CFU/square inch, and the 10% NaCl solution with 5.0 × 10⁴ CFU/ml. The vapor temperature was 110 F (43 C), whereas the temperature of the 10% NaCl solution was 156 F (69 C). The second trial produced 2.5 × 10⁵ CFU/ml of M. tuberculosis. However, M. tuberculosis could not be recovered from the mouthpiece swab, gooseneck swab, and the 10% NaCl solution. The temperature of the 10% NaCl solution during this trial was the highest attained of the 10 experiments, 164 F (73 C). The vapor temperature was 110 F.

Patient E. R. (Table 1) was assayed because M. tuberculosis colonies isolated from this patient were resistant to the usual primary and secondary tuberculosis drugs. The sputum contained 2.0 × 10⁴ CFU/ml of drug-resistant M. tuberculosis. The mouthpiece swab and gooseneck swab were found to contain 2.3 × 10¹ and 1.8 × 10⁴ CFU/square inch, respectively. The 10% NaCl solution showed no mycobacterial contamination. The temperature of the 10% NaCl solution temperature was 162 F (72 C) and that of the vapor was 104 F (40 C).

Patient C. R. was also used for three assays. M. tuberculosis could only be recovered from one sputum specimen and it yielded only 10 CFU/ml. All other specimens were negative for mycobacteria. Two of the three trials showed heavy bacterial and fungal contamination (not identified). The temperatures of the 10% NaCl solution were 156 F, 136 F (58 C), and 158 F (70 C), whereas the vapor temperatures were 108 F, 98 F, (37 C), and 104 F, respectively, in the three assays.

When patient G. M. was first admitted to the hospital, a sputum induction equipment study was performed. The sputum contained 6.9 × 10³
Table 1. Contamination of sputum induction equipment with Mycobacterium tuberculosis during patient usage and decontamination of the equipment with 2% glutaraldehyde

| Patient | Temp (F) | 10% NaCl solution | Phase of sampling | Colony-forming units M. tuberculosis in sample |
|---------|----------|--------------------|------------------|-----------------------------------------------|
|         |          | Vapor              |                  | Mouthpiece<sup>a</sup> (per square inch) | Gooseneck<sup>a</sup> (per square inch) | 10% NaCl solution<sup>a</sup> container (per square inch) | 10% NaCl solution (per ml) | Sputum (per ml) |
| K.G.    | 154      | 112                | Preinduction     | 0<sup>b</sup>                        | 0                        | 0                        | 1.6 × 10<sup>3</sup>      | 0                        |
|         |          |                    | Postinduction    | 1.7                          | 0                        | 0                        |                          |                          |
|         |          |                    | Predécontamination | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postdecontamination | 0                          | 0                        | 0                        |                          |                          |
| J. M.   | 156      | 110                | Preinduction     | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postinduction    | 2.0 × 10<sup>4</sup>       | 8.0 × 10<sup>2</sup> | 5.0 × 10<sup>3</sup> | 1.8 × 10<sup>4</sup>      |                          |
|         |          |                    | Predécontamination | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postdecontamination | 0                          | 0                        | 0                        |                          |                          |
| J. M.   | 160      | 108                | Preinduction     | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postinduction    | 5.3 × 10<sup>2</sup>       | 8.3 × 10<sup>3</sup> | 2.6 × 10<sup>3</sup> | 2.5 × 10<sup>2</sup>      |                          |
|         |          |                    | Predécontamination | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postdecontamination | 0                          | 0                        | 0                        |                          |                          |
| E. R.   | 162      | 104                | Preinduction     | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postinduction    | 2.3 × 10<sup>1</sup>       | 1.8 × 10<sup>1</sup> | 0                        | 2.0 × 10<sup>4</sup>      |                          |
|         |          |                    | Predécontamination | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postdecontamination | 0                          | 0                        | 0                        |                          |                          |
| C. R.   | 156      | 108                | Preinduction     | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postinduction    | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Predécontamination | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postdecontamination | 0                          | 0                        | 0                        |                          |                          |
| C. R.   | 136      | 98                 | Preinduction     | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postinduction    | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Predécontamination | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postdecontamination | 0                          | 0                        | 0                        |                          |                          |
| C. R.   | 158      | 104                | Preinduction     | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postinduction    | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Predécontamination | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postdecontamination | 0                          | 0                        | 0                        |                          |                          |
| G. M.   | 156      | 108                | Preinduction     | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postinduction    | 5.0 × 10<sup>3</sup>       | 5.0 × 10<sup>3</sup> | 5.8 × 10<sup>3</sup> | 6.9 × 10<sup>3</sup>      |                          |
|         |          |                    | Predécontamination | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postdecontamination | 0                          | 0                        | 0                        |                          |                          |
| G. M.   | 156      | 104                | Preinduction     | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postinduction    | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Predécontamination | 0                          | 0                        | 0                        |                          |                          |
|         |          |                    | Postdecontamination | 0                          | 0                        | 0                        |                          |                          |

<sup>a</sup> Six square inches swabbed.
<sup>b</sup> 0 = M. tuberculosis not recovered from 0.1 ml of the original sample.
<sup>c</sup> Heavy contamination.
CFU/ml of M. tuberculosis. The mouthpiece and gooseneck swabs showed 5.0 × 10² CFU/square inch. The 10% NaCl solution contained 5.8 × 10³ CFU/ml. During this study, the temperature of the 10% NaCl solution was 156 F and that of the vapor was 108 F. After 1 month of treatment, this same patient was resampled by the induction process. M. tuberculosis could not be recovered from any of the specimens. However, heavy non-mycobacterial contamination did occur which was similar to that found in patient C. R. The temperatures of the 10% NaCl solution and vapor were 156 and 104 F, respectively.

M. tuberculosis was not recovered from any of the pieces of induction equipment after decontamination.

DISCUSSION

As designed, tuberculous patients could contaminate the sputum induction unit during the induction process. It was believed necessary to prove that the units may become contaminated with M. tuberculosis and that decontamination after each use should be a routine procedure.

Five patients were evaluated by 10 trials for their ability to contaminate the unit during sputum induction. Among the patients utilized were those who were sputum-positive and those who were sputum-negative and thought to be positive because of previous positive cultures. The mouthpiece of the induction apparatus was found to be contaminated with M. tuberculosis 5 of the 10 times tested and with microorganisms other than M. tuberculosis 3 of the 10 times (Table 1). The number of CFU ranged from 1.7 to 530 per square inch of the swab sample. The microbial count from the mouthpiece was usually lower than that in the sputum. The gooseneck was found to be contaminated on four occasions with M. tuberculosis and three times with microorganisms other than M. tuberculosis.

The 10% NaCl solution was contaminated with M. tuberculosis 3 of 10 times. The temperatures of the 10% NaCl solution ranged from 136 F to 164 F. The vapor temperature emitted from the mouthpiece ranged from 98 F to 112 F. The temperatures obviously were not sufficient to destroy all of the mycobacteria present.

Contamination of the sputum induction apparatus can and does occur. Replacing the mouthpiece after patient use is not sufficient to protect the patient who uses the equipment next. It is likely that improper cleaning and decontamination of the equipment could result in the spread of tuberculosis. As the induction process forces NaCl solution into deep areas of the lungs and if that NaCl solution contained M. tuberculosis, this would be a very efficient means of depositing the organisms in a critical area for initiating tuberculosis infection.

The sputum induction equipment was cleaned and decontaminated with 2% glutaraldehyde. The equipment was rinsed, then submerged in the 2% glutaraldehyde for 20 min, rinsed in tap water, and allowed to air-dry. The data indicate that pieces of sputum induction equipment highly contaminated with M. tuberculosis could be decontaminated with glutaraldehyde.

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