Respiratory Infection Complicating Long-term Tracheostomy*

The Implication of Persistent Gram-negative Tracheobronchial Colonization

Michael S. Niederman, M.D.;† Redento D. Ferranti, M.D.; Annemarie Zeigler, R.M.T.; William W. Merrill, M.D.; and Herbert Y. Reynolds, M.D., F.C.C.P.‡

Colonization of the lower respiratory tract by enteric Gram-negative bacilli (EGNB) has been a frequent finding in patients with long-term tracheostomies; however, the association of hospitalization and certain features of serious illness with this phenomenon has not been clearly established. Because such factors can render the oropharynx more susceptible to EGNB colonization, we sought to discover whether they can also have this effect on the tracheobronchial tree and its microflora. Thus, we collected serial paired culture samples from these two mucosal sites in 15 subjects with long-term tracheostomies and examined patterns and rates of colonization and related these findings to clinical parameters. In 49 sets of cultures, we found that EGNB (especially Pseudomonas species) were present in significantly fewer upper-airway cultures (36.7 percent) than lower-airway cultures (75.5 percent) (p = 0.009). At the tracheobronchial site, seven subjects had persistent EGNB colonization, all with Pseudomonas species, while only one subject had this finding at the oropharyngeal site (p = 0.015). Patients with persistent tracheobronchial colonization were more ill than those without this finding. They were treated with higher doses of prednisone (p = 0.06), received antibiotics more often, and developed purulent tracheobronchitis more often (100 percent vs 25 percent) than patients without persistent colonization. In addition, in the month following the culture survey, four subjects developed pneumonia, and three of these had previous persistent tracheobronchial colonization. The findings suggest that in this population of patients, there was an enhanced susceptibility for more frequent and more persistent EGNB colonization of the lower respiratory tract than the upper respiratory tract and that these two mucosal sites became colonized independently of one another. Furthermore, persistent colonization was associated with greater clinical illness and may have predisposed patients to symptomatic infection.

Infections of the lower respiratory tract pose a serious threat to patients with long-term tracheostomies, with nosocomial pneumonia developing in up to 66 percent of these individuals. The etiology of this problem may be related to the observed 60 to 100 percent incidence of enteric Gram-negative bacillary colonization of the tracheobronchial tree, a site usually sterile in healthy subjects.6,6

Previous studies of hospitalized individuals have shown that the frequency of isolation of enteric Gram-negative bacilli (EGNB) from the oropharynx parallels the degree of illness, but the role of specific host factors in leading to the patterns seen in the lower respiratory tract of patients with tracheostomies has not been established.6,7 Additionally, the relationship between oropharyngeal and tracheobronchial colonization and the relative susceptibilities of these sites to EGNB attachment are not fully known. In a previous study designed to examine simultaneously collected culture samples from these two mucosal sites in a population of patients with tracheostomies, Bartlett et al.1 found a disparity between the microflora of the upper and lower respiratory tract. This observation raised the possibility that these two sites had inherently different propensities to bacterial colonization and that the trachea acquired its EGNB from a source other than the upper airway; however, these data have not been confirmed or related to specific host factors. Also, because multiple serial cultures were not collected from most patients, it could not be determined whether any particular organism predominated at either site.

We were interested in extending these observations because we expected to find that EGNB would preferentially localize in the tracheobronchial tree, rather than the oropharynx of patients with tracheostomies. This expectation arose from our recent observation that the degree of in vitro bacterial adherence of Pseudomonas aeruginosa to isolated tracheal cells (of healthy subjects) exceeds the degree of binding of the same organism to buccal cells.8 Because bacterial adherence to buccal epithelial cells is thought to be a mechanism

*From the Pulmonary Section, Department of Medicine, Yale University School of Medicine, New Haven, Conn., and the Department of Medicine, Gaylord Hospital, Wallingford, Conn.
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‡Supported by National Institutes of Health grant HL-22302.
Reprint requests: Dr. Niederman, Pulmonary Section, Nassau Hospital, Mineola, New York 11501

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facilitating oropharyngeal colonization, we speculated that when EGNB had equal access to both the upper and lower respiratory tract (as is the case with long-term tracheostomy), the inherently greater tracheal binding (compared to buccal binding) of bacteria might lead to an enhanced susceptibility, or "tropism," for more frequent and more persistent isolation of EGNB from the tracheobronchial tree.

To investigate whether this hypothesis could aid our understanding of the patterns of respiratory tract flora seen in patients with long-term tracheostomies and to relate these findings to specific host factors, we collected sequential paired culture specimens from both the buccal surface and the tracheal lumen of 15 such subjects. The data that we collected, including the identity of specific EGNB and their persistence at both respiratory mucosal sites, suggested a possible pathogenetic mechanism for colonization of the lower respiratory tract in this population that differed from the mechanisms described for patients with an intact trachea.

**Materials and Methods**

All individuals with long-term tracheostomies hospitalized at a local rehabilitative facility were potentially eligible for this study; however, we eliminated anyone with acute pneumonia, as defined by a thorough history, physical examination, and chest roentgenogram. Subjects meeting these criteria were selected for study if they were to remain in the hospital for at least four weeks and were free of pneumonia during this time. For one month after completion of the study, patients remaining in the hospital were observed weekly for the development of pneumonia. Follow-up data were available on any patients discharged before this period of observation was complete.

Fifteen subjects were included, and all gave informed consent after approval of the study's protocol by the Caylord Hospital Human Investigation Committee. The duration of the tracheostomy ranged from one month to ten years (mean of 25 months), and the tracheostomy had been performed for continuing care of either chronic pulmonary disease (13 subjects) or chronic neuromuscular disease (two subjects). There were seven men and eight women, with ages ranging from 23 to 77 years (mean of 61 years). Mechanical ventilation was being used in six subjects, and 13 subjects were receiving supplemental oxygen (ranging from 24 to 50 percent; mean of 31 percent). Ten subjects were treated daily with prednisone, with dosage ranging from 10 to 30 mg (mean of 10.5 mg); and 12 subjects received antibiotics during the time of the study.

Each subject had serial simultaneous biweekly cultures obtained from both the buccal and tracheal mucosa during a minimum period of observation of four weeks (at least three sets of cultures). Oropharyngeal cultures were obtained in the early morning, before the subjects ate breakfast, by rubbing the buccal mucosa bilaterally with a sterile cotton swab. The swab was then placed into modified Stuart's bacterial transport medium (Marion Scientific). Tracheobronchial cultures were obtained by sterile suctioning of sputum directly from the tracheal lumen through the stoma of the tracheostomy. All cultures were inoculated within 30 minutes of collection and were placed onto blood agar, MacConkey's medium, and into thioglycollate broth. They were incubated at 35°C with ambient oxygen concentrations, and all Gram-negative bacteria were identified from culture plates, in a qualitative fashion. If no bacterial growth was seen after 24 hours on the plates, the thioglycolate broth was subcultured onto plates, and any resulting Gram-negative organisms were identified. Broth cultures were included in this study because they have been reported to be more sensitive for detecting EGNB during surveillance culture studies than plates alone.

Culture data were then related to clinical parameters. Patients with EGNB isolated from a mucosal culture, in the absence of roentgenographic evidence of pneumonia, were said to have colonization. Any subject with EGNB in all cultures at any site was said to have persistent colonization at that site. If the respiratory secretions of a patient without pneumonia became more copious and purulent-appearing, tracheobronchitis was diagnosed. By these definitions, tracheobronchitis, a clinical diagnosis, could exist with or without colonization, a microbiologic diagnosis.

Respiratory assistance devices were changed and cleaned according to standard hospital policy, but water reservoirs from each device were not cultured. The tubing of the mechanical ventilator was changed daily, the ventilator's humidifier cascade equipment and water supply were changed every other day, and the ventilator's bacterial filter in the inspiratory line was cleaned weekly. For patients receiving supplemental oxygen without mechanical ventilation, the tubing, water, and the humidification system were changed every other day. All subjects had suctioning of their tracheal secretions as frequently as needed, but new sterile equipment and irrigating saline solution were used for each treatment. In addition, all subjects received nebulized medications during the study, and nebulizer equipment was cleaned after each treatment.

Data were stored and analyzed using a software package provided by the CLINFO Project, National Institutes of Health. Matched data were analyzed by paired t-test and χ² analysis and group means by Student's t-test.

**Results**

**Frequency of EGNB**

The frequency of EGNB isolation was determined in multiple cultures from two respiratory mucosal sites (Table 1). During the study, 49 paired sets of serial buccal and tracheal mucosa cultures were collected from 15 subjects. The EGNB were isolated in 37 (76 percent) of 49 tracheal cultures, 28 of which contained *Pseudomonas* species. These included *P aeruginosa*, *P maltophilia*, and other *Pseudomonas* species isolates. At the buccal site, 18 (37 percent) of 49 cultures contained EGNB, of which nine contained *Pseudomonas* species. The frequency of cultures free of EGNB at the tracheal site (24 percent; 12/49) was significantly less than the frequency of specimens free of EGNB colonization at the buccal site (63 percent; 31/49), with p = 0.009. The frequency of *Pseudomonas* species was significantly greater in the tracheal mucosa cultures than in the buccal mucosa cultures.

**Table 1—Colonization Incidence in Multiple Cultures from Two Respiratory Mucosal Sites**

<table>
<thead>
<tr>
<th>Data</th>
<th>Buccal Mucosa</th>
<th>Tracheal Mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of cultures</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>Cultures without EGNB</td>
<td>31*</td>
<td>12*</td>
</tr>
<tr>
<td>Cultures with <em>Pseudomonas</em> species</td>
<td>9*</td>
<td>28*</td>
</tr>
<tr>
<td>Cultures without <em>Pseudomonas</em> species but with other EGNB</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Total No. of EGNB isolates (some cultures with multiple organisms)</td>
<td>28</td>
<td>53</td>
</tr>
</tbody>
</table>

*p = 0.009 by two-tailed paired t-test for buccal vs tracheal mucosa.

Respiratory Infection Complicating Long-term Tracheostomy (Niederman et al)
species in the tracheobronchial tree (57 percent or 25 of all 49 cultures) significantly exceeded the frequency of these organisms at the buccal site (18 percent or nine of all 49 cultures), with p = 0.009.

Broth cultures had been employed to enhance the detection of EGNB; however, of 48 mucosal specimens that had been free of EGNB on standard plates, only five subsequently contained these organisms in broth cultures, leaving 43 samples free of EGNB (Table 1). Enteric Gram-negative bacteria that were absent from plates but present in broth subcultures included two isolates of Escherichia coli and one isolate each of P aeruginosa, Serratia marcescens, and Klebsiella pneumoniae.

Identity of EGNB Isolated

The organisms isolated and their relative frequency are shown in Table 2. Because some cultures contained multiple bacteria, 18 buccal cultures contained 25 EGNB isolates, while 37 tracheal cultures contained 53 EGNB isolates. The most frequent organisms observed at the buccal site were Pseudomonas species, Enterobacter species, and K pneumoniae. At the tracheal site, in addition to Pseudomonas species, the most common isolates were Enterobacter species and S marcescens. As shown in Table 2, there were 20 EGNB isolates present simultaneously at both sites in paired cultures.

Persistence of Colonization

With a serial multiple-culture survey conducted over at least a four-week period, five subjects (33 percent) never had EGNB isolated from buccal mucosa, and three subjects (20 percent) never had these organisms isolated from tracheal mucosa cultures (Table 3). Transient colonization with Pseudomonas species or other EGNB was observed at the buccal site in nine subjects and at the tracheal site in five subjects. Among the remaining colonized individuals, one had persistent EGNB colonization, with Pseudomonas species, at both sites; and six had persistent EGNB colonization, also with Pseudomonas species, at only the tracheal site. In three of these six subjects, Pseudomonas species were never found in the oropharynx. By χ² analysis for untied pairs, the frequent

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of Isolates (Percent of Total)</th>
<th>Present Simultaneously at Both Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas species</td>
<td>9 (32) 23 (83)</td>
<td>8</td>
</tr>
<tr>
<td>S marcescens</td>
<td>1 (4) 7 (13)</td>
<td>1</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>11 (3) 8 (4)</td>
<td>3</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>4 (12) 5 (9)</td>
<td>3</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>14 (4) 4 (5)</td>
<td>2</td>
</tr>
<tr>
<td>K pneumoniae</td>
<td>18 (5) 3 (6)</td>
<td>3</td>
</tr>
<tr>
<td>Acinetobacter calcoaceticus</td>
<td>2 (1) 0 (2)</td>
<td>2</td>
</tr>
<tr>
<td>K oxytoca</td>
<td>2 (1) 0 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>1 (4) 0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>1 (4) 0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>28 (9) 53 (17)</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 4—Influence of Specific Parameters on Colonization Patterns Observed in the Tracheobronchial Tree

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>Pseudomonas Species Not Persistently</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pseudomonas Species Persistently</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>6</td>
</tr>
<tr>
<td>No mechanical ventilation</td>
<td>9</td>
</tr>
<tr>
<td>Antibiotic therapy</td>
<td>12</td>
</tr>
<tr>
<td>No antibiotic therapy</td>
<td>3</td>
</tr>
<tr>
<td>Tracheobronchitis</td>
<td>9</td>
</tr>
<tr>
<td>No tracheobronchitis</td>
<td>6</td>
</tr>
<tr>
<td>Mean prednisone dose, mg</td>
<td>15.3*</td>
</tr>
<tr>
<td>Mean oxygen, percent</td>
<td>33.7†</td>
</tr>
<tr>
<td>Mean duration of tracheostomy, mo</td>
<td>23.2†</td>
</tr>
</tbody>
</table>

* p = 0.06 when patients with and without persistent colonization are compared.
† p > 0.25 when patients with and without persistent colonization are compared.
finding of persistent tracheal colonization without associated persistently positive buccal cultures was significant (p = 0.015).18

Influence of Host Factors

Antibiotic therapy was used in 12 subjects, including all seven of the individuals who developed persistent tracheal colonization by EGNB (Table 4). Antibiotic therapy preceded persistent colonization with Pseudomonas species in five of these subjects. The five remaining antibiotic-treated subjects did not develop persistent EGNB tracheal colonization.

The role of mechanical ventilation in the development of colonization is also shown in Table 4. While persistent EGNB tracheal colonization developed in four subjects receiving mechanical ventilation, two others treated in this manner did not develop this complication. In addition, three subjects developed persistent EGNB tracheal colonization while not being treated with mechanical ventilation.

All of the subjects with persistent tracheal colonization with Pseudomonas species had chronic pulmonary disease. Of the two individuals in the study with neuromuscular disease, one had transient tracheobronchial EGNB, and one never had EGNB in the lower respiratory tract.

The mean daily dosage of prednisone in the subjects developing persistent EGNB tracheal colonization (15.3 mg) exceeded the dosage of the subjects without persistent tracheal colonization (6.25 mg), and this difference approached significance (p = 0.06). There was no significant difference (p>0.25) in the concentration of supplemental oxygen or in the duration of the tracheostomy when individuals with and without persistent EGNB tracheal colonization were compared.

In the month following this study, four subjects developed pneumonia, and three of these individuals had preceding persistent tracheal colonization with Pseudomonas species.

Tracheobronchitis and Colonization

Nine subjects developed tracheobronchitis during the period of observation, and they had Pseudomonas species isolated more frequently (p = 0.016) and cultures free of EGNB less frequently (p = 0.017) than the six patients who never developed tracheobronchitis. Seven of these individuals had persistent tracheobronchial colonization with Pseudomonas species during the study (Table 4).

When antibiotics were prescribed during an episode of tracheobronchitis, they were specifically directed against Pseudomonas species in five of the subjects, with agents to which these organisms were sensitive. The therapeutic regimens included an appropriate cephalosporin in one individual and courses of an aminoglycoside in the others (three parenteral and one via airway nebulization). These five persistently colonized subjects received a ten-day to 14-day course of antibiotics which reduced the purulence of the sputum and, thus, successfully treated their episode of tracheobronchitis; however, in these subjects, specific antibiotic therapy did not eliminate lower-airway colonization with Pseudomonas species, and these organisms remained whether individuals had tracheobronchitis or just simple colonization.

Discussion

Classic studies of hospitalized individuals have established that EGNB are present in the respiratory tract with a frequency that parallels certain aspects of serious illness. Thus, Johanson et al,4 using a multiple-culture survey technique, observed that these organisms were present in the oropharynx of only 6 percent of normal subjects, while they could be found in as many as 35 percent of moderately ill and 73 percent of moribund patients. More recently, Irwin et al13 confirmed the high rate of oropharyngeal isolation of EGNB in ill individuals but showed it to be a transient phenomenon; most patients in a skilled nursing facility did not harbor these organisms persistently when observed serially over a 31-week period.

Studies of the tracheobronchial tree and its microflora have also demonstrated that the frequency of finding EGNB can be related to the severity of the patient's illness. It has been shown that while healthy subjects were free of EGNB in the lower respiratory tract, 10 to 20 percent of those with chronic bronchitis had these organisms when swab cultures were collected through a rigid bronchoscope.14,15 In more ill hospitalized patients, tracheobronchial EGNB have been found in 28 percent of all patients in intensive care units and in 50.5 percent of all expectorated or suctioned samples of sputum.7 When examined, specific aspects of serious illness did not appear to affect the susceptibility to EGNB attachment any differently in the tracheobronchial tree than in the oropharynx, but long-term paired cultures of both sites were not collected.

Patients with long-term tracheostomies have an observed frequency of tracheobronchial EGNB growth even higher than that reported in other hospitalized subjects. Studying 101 individuals in a surgical intensive care unit after a recent tracheostomy, Bryant et al3 found that 94 had lower-airway colonization with potential pathogens, most often with P aeruginosa or other EGNB. Brook5 conducted a one-year survey of less acutely ill tracheostomized pediatric patients with severe neurologic disease, using biweekly tracheal cultures, and all of 27 patients had a microflora, again most often consisting of P aeruginosa and other EGNB. Of note, when Pseudomonas aeruginosa was
present, it remained in the tracheal aspirates longer than any other organism and could not be eradicated by aminoglycoside therapy. While both of these studies documented the high frequency of EGNB at the tracheobronchial site, neither examined whether these organisms were also present simultaneously in the upper respiratory tract. Bartlett et al.\(^4\) addressed this question in a population of stable patients with long-term tracheostomies and found that the oropharynx and trachea had different microflora patterns. Although the results of serial oropharyngeal cultures were not reported, each of six subjects had a second tracheal culture 20 to 60 days after the first, and the numerically dominant colonizing species showed a tendency to persist. This observation was in contrast with the transient pattern of oropharyngeal EGNB reported by Irwin et al.\(^5\)

The data in the current study, like those of other investigators, have shown that patients with long-term tracheostomies, especially those with serious illness, frequently harbor EGNB in the respiratory tract. However, by collecting sequential paired cultures from the upper and lower respiratory tract biweekly (over at least a four-week period) and by relating these results to a patient's clinical parameters, we were able to examine a new question: whether, in the face of serious illness, these two frequently colonized mucosal sites had different susceptibilities to EGNB attachment. We found that EGNB could be cultured more frequently (76 percent vs 37 percent of all cultures; \(p = 0.009\)) from the tracheobronchial tree than from the oropharynx (Tables 1 and 3). These data are in agreement with the finding by Bartlett et al.\(^4\) of different microflora patterns at the same two sites; however, our analysis of serial culture data led to the new observation that EGNB were present more persistently (46 percent vs 7 percent of all subjects; \(p = 0.015\)) in the lower than in the upper airway when both sites were faced with the same systemic alterations in host defense. The tenacity of certain EGNB for the lower airway was also shown by the observation that in none of five subjects from whom EGNB were persistently cultured could *Pseudomonas* species colonization be eliminated even when antibiotic therapy directed at these organisms had been given to treat tracheobronchitis.

It appeared that the repeated isolation of EGNB from the tracheobronchial tree, especially *Pseudomonas* species, occurred in the more seriously ill patients with tracheostomies. Persistently colonized subjects received higher daily doses of prednisone (\(p = 0.06\)) than those without this finding, although there was no difference in the duration of the tracheostomy or the percentage of inhaled oxygen used. These individuals also were treated more often with antibiotics (100 percent vs 63 percent) and mechanical ventilation (57 percent vs 25 percent) and developed tracheobronchitis (100 percent vs 25 percent) with a greater frequency than those who did not have persistent EGNB in the lower airway (Table 4). Also, all of these patients had underlying chronic pulmonary disease. From these data, it appeared that continued tracheobronchial growth of EGNB could have been the result of serious illness. Alternatively, it may have been the cause of further morbidity because in the month following the culture-survey period, three of seven of the persistently colonized subjects, compared to only one of eight without this finding, developed nosocomial pneumonia.

Although the presence of a tracheostomy allowed bacteria to bypass nasopharyngeal defense mechanisms and have direct access to the lower respiratory tract, the more frequent predominance of EGNB in the tracheobronchial (and not oropharyngeal) flora was surprising. This observation suggested that in patients with long-term tracheostomies, the usual mechanism of airway colonization, aspiration of pathogenic EGNB from a colonized oropharynx,\(^6\) may not have been operative. Rather, EGNB may have directly entered and become attached to the trachea. Also in support of such a mechanism was the observation that in three of the seven individuals from whom *Pseudomonas* species could always be cultured from the lower airway, these organisms were never isolated from the oropharynx.

It is most likely that EGNB entered both mucosal sites from the general environment and not from respiratory assistance equipment. Although this equipment was not cultured, it was cleaned and washed according to accepted practices, and humidification systems were changed in a manner that has been shown to minimize airway contamination.\(^7\) While all subjects received treatments with a nebulizer through their tracheostomies and this could have introduced bacteria into the tracheobronchial tree,\(^8\) the absence of this therapy in the oropharynx did not prevent EGNB from colonizing this site, although often transiently, in 67 percent of subjects.

To collect culture data, we supplemented standard bacteriologic plate methods with broth cultures because the latter technique has been reported to add sensitivity for the detection of EGNB.\(^9\) In fact, the broth did demonstrate these organisms in five specimens that had been free of EGNB growth on standard plates; however, 43 samples did not contain Gram-negative bacteria by either method, and the plates were extremely sensitive for detecting the only organisms to show persistence of colonization, yielding 36 of the 37 isolates of *Pseudomonas* species. Thus, it appeared that the presence and absence of specific EGNB isolates from the respiratory tract were reliably detected by standard plate methods alone and that supplemental broth cultures added very little. For this reason, standard plate culture methods seem adequate.
for routine clinical use in patients with long-term tracheostomies.

This study has extended and confirmed previous observations about airway colonization among patients with long-term tracheostomies. In contrast to the oropharynx, the tracheobronchial tree of these patients harbored EGNB more often and more persistently, especially among more seriously ill individuals. Although the mechanism for this phenomenon is unknown, our earlier observation\(^a\) of a greater degree of adherence by \textit{P. aeruginosa} to tracheal cells than to buccal cells may have relevance. It is possible that the presence of a tracheostomy let these in \textit{vivo} differences in adherence express themselves as the enhanced in \textit{vivo} susceptibility of the lower-respiratory-tract mucosa to EGNB that was observed in this study. In addition, serious illness may have preferentially altered the tracheobronchial, but not oropharyngeal, mucosa, rendering it even more receptive to bacterial attachment than it was in the absence of this influence. Also, the violation of tracheal integrity may have directly caused secondary lower-airway mucosal changes that resulted in more frequent and more persistent EGNB colonization and an increased risk of Gram-negative pneumonia. Thus, it is possible that the presence of a tracheostomy itself, in conjunction with the degree of serious illness seen in patients requiring this therapy, may have led to infectious complications. This knowledge should reinforce the need to carefully consider the indications for applying this important type of treatment.

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