Objective: To obtain in vivo bacterial colonization profiles on endotracheal tubes at different sites in the neonatal airway in an attempt to better characterize one potential element of chondritis.

Design: A case series in which cultures were obtained from calculated segments of 33 endotracheal tubes immediately following extubation. This allowed for sampling at specific levels of the airway corresponding to the trachea, the subglottis, and the oropharynx. Data collected included gender, race, duration of intubation, use of antibiotic therapy, comorbidities, gestational age at birth and extubation, crown-rump length, weight, radiographic distance from tube tip to carina, and culture results.

Setting: Newborn intensive care unit at a tertiary care medical center.

Patients: Twenty-nine neonates intubated for longer than 24 hours (range, 24 hours to 15 days).

Main Outcome Measures: Bacterial and fungal cultures obtained from 3 endotracheal tube segments for each extubation.

Results: A statistically significant difference (P<.05) was found in colonization rates between patients intubated for less than 4 days and those intubated for longer periods. No significant difference was noted in bacterial profile between the 3 sites.

Conclusions: Data demonstrate that bacterial colonization of an indwelling object in the neonatal airway increases with the duration of intubation. Furthermore, 4 days seems to represent a critical period in the formation of such colonization (possibly in the form of a biofilm). These bacteria may contribute to the chondritis known to precede the development of subglottic stenosis. Further studies are indicated to suggest ways to interrupt this process and reduce the incidence of airway injury.


LONG-TERM intubation and ventilatory support for neonates with respiratory distress syndrome was first introduced 3 decades ago1 and has since resulted in the salvage of innumerable premature infants. Prolonged endotracheal intubation, however, can result in the development of clinically significant subglottic stenosis in up to 12% of the patients.2-4 This has resulted in a new group of patients who are tracheotomy dependent in early childhood. Adequate surgical treatment of such patients is difficult and attention has focused on the prevention of subglottic stenosis when long-term intubation is indicated.

Much attention has focused on the endotracheal tube itself. Minimization of trauma from intubation and suctioning has been advocated to limit airway injury. Furthermore, in adults and older children, benefits have been claimed for the use of high-volume low-pressure cuffs, as well as frequent cuff pressure monitoring and soft cuffs at the larynx.5-6 The size of the endotracheal tube relative to the airway lumen has been examined, and recommendations have been made to avoid an excessively tight fit in the subglottis.7,8 In an effort to minimize friction trauma by tube motion, nasotracheal intubation has been supported and tube fixation devices have also been devised.9

Attention has alternatively turned to the underlying cause of subglottic stenosis. The first step in the development of subglottic stenosis is mucosal ischemia induced by cuff or tube pressure. This can occur within hours of intubation and in neonates predominates in the posterior subglottis where endotracheal tube contact with the trachea would be maximal.10 A stepwise process ensues in which mucosal ischemia leads to ulceration and the exposure of underlying cartilage.
PATIENTS AND METHODS

PATIENTS

All newborns orally intubated longer than 24 hours in the neonatal intensive care unit were eligible for inclusion in this study. Neonates undergoing previous airway surgery or in whom the endotracheal tube could not be immediately processed were excluded. This study examined the endotracheal tubes from 29 neonates representing 33 extubations. The duration of intubation ranged from 24 hours to 15 days (mean, 5.4 days; median, 4 days). Information collected about the neonates included gestational age at birth, birth weight, duration of intubation, age at extubation, antibiotic therapy, previous positive culture results, crown-rump and crown-heel length, weight at extubation, and distance of endotracheal tube tip to carina on last chest radiograph. Admission criteria were available for 26 neonates and include 3 with clinical sepsis, 5 for rule-out sepsis, and 6 who underwent corrective cardiac surgery.

CALCULATION OF CORRESPONDING AIRWAY SITES

The goal of this study was to compare bacterial profiles at the distal trachea, subglottis, and pharynx. As such a method was devised to determine the site on the endotracheal tube corresponding to these regions. The distal trachea was taken as the terminal 1 cm on the endotracheal tube if the last chest radiograph demonstrated normal tube position. The position of the subglottis and pharynx required calculation of trachea length and relative positioning of the endotracheal tube.

Exposed cartilage is then susceptible to infection and resultant chondritis with scarring and stenosis on healing. While many of the measures described above focus on reducing mucosal ischemia, few studies have examined the later stages of tissue damage. In particular, the role of potential pathogens in causing or aggravating laryngotracheal chondritis has not been adequately investigated.

No organisms have been identified as unique to the subglottis or as being particularly virulent in exploiting mucosal damage. Several studies to date have attempted to characterize the bacteriologic profile of the subglottis; however, access to the region is difficult. Most studies have examined the bacterial profile at the time of surgery by sampling tissue directly through a tracheotomy site or bronchoscope.\textsuperscript{11,12} That these patients require surgery indicates a more serious condition and may bias results toward patients who have already suffered airway injury. Our study focuses on the endotracheal tube itself and attempts to characterize those organisms that may opportunistically colonize such an indwelling device. This article demonstrates a time-dependent bacterial colonization of endotracheal tubes in intubated neonates and examines whether the subglottic region is unique. Such information may help us to better understand potential pathogens in the formation of neonatal subglottic stenosis.

The length of each neonate’s trachea was calculated based on the normogram devised by Rotschild et al.\textsuperscript{13} The length of the trachea (L) was determined by correlation with the crown-rump length measured in centimeters (CRL):

\begin{equation}
L = [(0.708 + 0.106) \times \text{CRL}]
\end{equation}

The last chest radiograph for each neonate was examined and the distance from the tip of the endotracheal tube to the carina was measured. This distance (C) was not corrected for radiographic magnification as examination demonstrated negligible magnification in this setting. The distance from endotracheal tube tip to the subglottis (S) was then determined by the following formula:

\begin{equation}
S = [(L - C) - 0.3 \text{ cm}]
\end{equation}

The factor of 0.3 cm was added to ensure that the sampled section was not above the level of the vocal folds. The pharynx was then calculated as 3 cm proximal to the subglottis.

COLLECTION OF ENDOTRACHEAL TUBES

A sterile work area was prepared next to each infant at the time of extubation. Included on the field were a ruler, scissors, and culture tubes. Formula 2 was used to calculate the subglottis and pharynx; these points were marked on the sterile ruler. A 1-cm segment of the endotracheal tube corresponding to the oropharynx, subglottis, and distal trachea were cut under sterile conditions immediately on extubation. Specimens were sent to the microbiology laboratory, where gram, Giemsa, and acid-fast bacillus staining was performed. In addition, bacterial, fungal, and acid-fast bacillus cultures were obtained.

There were 33 extubations from 29 neonates. Culture results for each site were obtained in most cases: endotracheal tube tip (n=32), subglottis (n=33), and pharynx (n=31). Positive cultures yielded the identification of an organism while negative cultures yielded no growth after 5 days’ incubation. Approximately 50% of samples demonstrated growth. No statistically significant difference was noted in the culture rate between sites (Table 1). A variety of organisms were cultured but there was a preponderance of \textit{Staphylococcus epidermidis} (Table 2). No significant difference was noted between sites with regard to the organism cultured.

Results from the subglottis were compared with the pharynx and the distal trachea to determine if there was a unique microbial profile in this region (Table 3). In

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of Specimens</th>
<th>No. of Positive Cultures</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETT tip</td>
<td>32</td>
<td>16</td>
<td>50</td>
</tr>
<tr>
<td>Subglottis</td>
<td>33</td>
<td>19</td>
<td>58</td>
</tr>
<tr>
<td>Pharynx</td>
<td>31</td>
<td>14</td>
<td>45</td>
</tr>
</tbody>
</table>

Table 1. Incidence of Cultures With Positive Yields From Endotracheal Tube (ETT) Section by Site


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general, a positive culture result in the subglottis indicated a positive culture result at the other sites. Although not statistically significant, culture results in the subglottis more closely correlated with the distal trachea than with the pharynx. For example, in only one instance was the subglottic culture negative for organisms but in another site was positive; this was in the pharynx. Further, a different organism was found at another site only 3 times; 2 of these cases were in the pharynx.

The incidence of multiple organisms was similarly low. On one occasion each, cultures from the subglottis and pharynx grew 2 organisms. No cultures or stains were positive for Giemsa or acid-fast bacilli. In 2 instances Candida species was identified on culture but not potassium hydroxide preparation and thus was considered a laboratory contaminant. In these 2 cases the clinical course also did not correlate with a fungal infection.

For those patients whose information was complete regarding the use of antibiotic agents (n=22), culture results were analyzed. In the 12 cases in which antibiotic agents were administered, culture results were evenly divided between positive for organisms (n=6) and no growth (n=6). In the 10 cases in which no antibiotic agent was administered, the culture results were 8 positive for organisms and 2 no growth. All cases were treated with ampicillin sodium and/or gentamicin sulfate. Preantibiotic blood cultures that were positive for organisms demonstrated no significant correlation with the incidence of a positive culture of the identity of the organism. A χ² analysis of the use of antibiotic agents and the incidence of negative culture results was not statistically significant.

The incidence of a positive culture after 4 days of intubation was statistically significant by χ² analysis (P<.025, χ² test). Only 39% of the cultures were positive if the neonate was intubated for 4 days or less (7 of 18 cases). Intubations of longer than 4 days resulted in an 80% positive culture rate (12 of 15 cases).

This study did not demonstrate any unique microorganism on endotracheal tubes in the neonatal subglottis. Rather, a variety of organisms were identified inhabiting most regions of the respiratory tract. This agrees with other studies' sampling the airway itself in which polymicrobial flora has been demonstrated. In a study of swab cultures of the subglottis during bronchoscopy, Brown and Manning found 19 different organisms. Similarly, Brown and Montgomery found a variety of organisms in tracheal granulation tissue at T-tube sites. Our study found Staphylococcus species as the most prevalent organisms, agreeing with the findings of Brown and Montgomery. Brown and Manning in contrast demonstrated a preponderance of normal flora followed by Staphylococcus aureus. Both prior investigations also demonstrated a high incidence of Pseudomonas species, which was demonstrated on the endotracheal tube in only one instance.

An examination of the flora in individual cases revealed a sharp contrast between the present study and those previously reported. Our investigation found a low incidence of polymicrobial flora in each case, whereas Brown and Montgomery and Brown and Manning found, on average, more than 2 isolates per patient. This may indicate that endotracheal tube colonization is exclusive for a single, and perhaps more virulent, organism. In contrast, the trachea may enable habitation by many species simultaneously.

Culture results from the endotracheal tubes in this study may, in part, represent luminal bacteria as well. If this were the preponderant source of culture results, one would expect higher rates of positive culture results at the open end (ie, distal tip) where bacteria would enter. As listed in Table 3, no such stratification of culture results was found. Nevertheless, the study method has since been modified to remove the luminal surface from culture preparations.

The use of antibiotic therapy in preventing the sequelae of infected or inflamed cartilage is intuitively rational but not supported by these data. Sasaki et al. in a canine model, found bacterial levels in the larynx to be reduced by the administration of perioperative antibiotic agents and posttracheotomy wound care. From this they inferred that reducing bacterial counts would reduce the duration of an inflammatory response and, thus, promote better healing. Supance tested this paradigm, also in a canine model. He intubated 2 groups of dogs for 14 days with 1 group receiving steroids and antibiotic agents. On postextubation analysis of a cross-sectional area and microscopic examination of tissue, no

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**Table 3. Relationship of Subglottic Culture Results to Other Sites**

<table>
<thead>
<tr>
<th>Culture Result</th>
<th>ETT Tip</th>
<th>Pharynx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subglottis (+); incidence of other site (+)</td>
<td>16/18 (89%)</td>
<td>13/17 (76%)</td>
</tr>
<tr>
<td>Subglottis (+); incidence of other site (+)</td>
<td>1/16 (6%)</td>
<td>2/13 (15%)</td>
</tr>
<tr>
<td>Subglottis (−); incidence of other site (+)</td>
<td>0/14</td>
<td>1/14 (7%)</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage) with the numerator indicating the number of specimens and the denominator indicating the total number of specimens tested.
significant difference was found between the 2 canine groups. He concluded that antibiotic agents lacked efficacy in preventing subglottic stenosis in the canine model.

Our study, although using a different outcome measure, similarly found that antibiotic agents play a small role in influencing potential predisposing factors for stenosis in the subglottis. That is, colonization of the indwelling endotracheal tube was unaffected by systemic antibiotic therapy. Furthermore, Brown and Manning also found no correlation between the results of swab cultures of the subglottis and the use of antibiotic agents. They did, however, recommend empiric penicillin as it has few adverse effects and may help reduce inflammation. Brown and Montgomery likewise recommended antibiotic therapy based on anecdotal evidence of reduction in tracheal granulation tissue with T tubes.

Intubation for longer than 4 days did demonstrate statistical significance for obtaining positive bacterial culture results. This time-dependent colonization was also observed by Brown and Manning. They found 10 days to be significant in the development of pathogenic cultures. In their study, neonates intubated for shorter periods generally demonstrated only normal flora. In an effort to explain these results, one must consider the theory of biofilm formation. Biofilms form on implanted materials and consist of an adherent matrix of bacteria. These matrices are resistant to antibiotic agents and host responses, in contrast to simple colonies of bacteria. In a study by Malaisrie et al., the formation of biofilms on facial plastic implants was studied. They found that incubation of the material with bacteria for 7 days was universally sufficient for the formation of a biofilm.

Scanning electron microscopy would be necessary to confirm that the bacteria cultured in this study were in the form of a biofilm on the endotracheal tube. This arm of our study has recently begun, but the microscopy data are yet incomplete. This investigation found single organisms that, if in a biofilm matrix, would exclude and prevent other organisms from occupying the same region of the indwelling catheter. Further, antibiotic therapy had no affect on the rate of colonization of the endotracheal tube, possibly owing to resistance imparted by a biofilm.

The formation of a biofilm on the endotracheal tube could provide for a resistant colony of bacteria in the neonatal airway. Such bacteria may promote an inflammatory response in the narrow subglottis leading to scarring and the development of subglottic stenosis. Further investigation into biofilm-resistant materials and biofilm-preventing medications or chemicals needs to be pursued. Although frequent endotracheal tube changes may help to prevent the formation of a biofilm, the trauma and risk imposed by such changes are thought to outweigh the benefits. Further, the role of endotracheal tube bacterial colonization in the development of subglottic stenosis remains unproven at present.

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