Re-evaluation of rejection criteria for endotracheal tube (ETT) specimens from adult patients

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The purpose of this study was to determine optimal criteria for microbiology laboratory screening of endotracheal tube (ETT) specimens submitted for bacterial culture from adult patients. ETT specimens from adult patients that were received by two microbiology laboratories were prospectively evaluated and subdivided into one of three study arms with the following criteria: (1) <10 squamous epithelial cells (SECs) per low-power field with bacteria seen on Gram staining (arm 1), (2) >10 SECs per low-power field with bacteria seen on Gram staining (arm 2) and (3) <10 SECs per low-power field with no bacteria seen on Gram staining (arm 3). A fourth study arm (>10 SECs per low-power field with no bacteria seen on Gram staining) was planned but this arm was terminated due to the paucity of specimens meeting these criteria. Isolate evaluation was performed using standard microbiology protocols. A limited chart review was undertaken at one of the institutions, only reviewing patients from which a potential pathogen was recovered on culture.

In total, 141 ETT specimens were evaluated. A potential respiratory pathogen was recovered from 54, 37 and 10% of specimens in study arms 1, 2, and 3, respectively ($P < 0.0001$, comparing between arm 1 and arm 3). For the 23 patients included in the chart review from whom a potential pathogen was recovered on culture, respiratory infection was considered to be present in 50% (6/12) of patients in arm 1, 66.6% (6/9) of patients in arm 2 and 100% (2/2) of patients in arm 3. Therapy was rarely altered based on culture results. In this study, the ETT specimens submitted for bacterial culture were of limited benefit to clinicians. The data presented here support the use of an absence of bacteria on Gram staining as a rejection criterion for ETT specimens. The criterion of >10 SECs per low-power field should be further evaluated in a prospective study of patients with an unequivocal clinical diagnosis of pneumonia.

INTRODUCTION

Semiquantitative culture of endotracheal tube (ETT) secretions is often performed in the diagnostic work-up of patients with suspected ventilator-associated pneumonia (Rotstein et al., 2008; Ruiz et al., 2000; Canadian Critical Care Trials Group, 2006). Culture of poor-quality specimens is not thought to be a good use of laboratory resources (Garcia & Isenberg, 2007). Hence, it is currently recommended that ETT specimens from adult patients that are submitted for culture be rejected by the microbiology laboratory if no bacteria are visualized on Gram staining or if >10 squamous epithelial cells (SECs) are seen per low-power field (Garcia & Isenberg, 2007). The presence of SECs is considered to represent contamination with upper airway secretions (Garcia & Isenberg, 2007). There are currently two published studies that provide evidence to support this practice (Morris et al., 1993; Zaidi & Reller, 1996). Both studies demonstrate that the absence of bacteria on Gram staining is a valid screening criterion (Morris et al., 1993; Zaidi & Reller, 1996). However, only one of these studies (Morris et al., 1993) provides support for screening ETT specimens using the number of SECs per low-power field as a criterion. It has also been observed that the presence of SECs in ETT samples may actually originate from squamous metaplastic changes of the tracheobronchial tree, either because of prolonged intubation or irritation of the respiratory epithelium (Irwin et al., 1980). This would argue against use of SECs in the screening of ETT specimens. As the optimal screening method of ETT secretions is not clear...
based on the available evidence, a study was performed to re-evaluate published ETT screening criteria. The rejection criteria evaluated here were ETT samples that demonstrate >10 SECs per low-power field and ETT samples that do not have any bacteria seen on Gram staining.

**METHODS**

**Study sites.** This study was performed at the Health Sciences Centre and St Boniface Hospital Clinical Microbiology Laboratories. These laboratories are part of Diagnostic Services of Manitoba (DSM) and provide bacteriology services for all seven major hospitals located in Winnipeg, Manitoba, Canada.

**Study protocol.** All ETT specimens from adult patients were eligible for inclusion in the study. Only one specimen per patient was included over the study period. To evaluate the two rejection criteria, specimens were subdivided into one of four study arms: arm 1, ETT secretions for culture with <10 SECs per low-power field with bacteria seen on Gram staining; arm 2, ETT secretions for culture with >10 SECs per low-power field with bacteria seen on Gram staining; arm 3, ETT secretions for culture with <10 SECs per low-power field where bacteria are not seen on Gram staining; arm 4, ETT secretions for culture with >10 SECs per low-power field where bacteria are not seen on the Gram stain.

For each specimen, patient age, patient sex, ward of admission (ICU versus non-ICU), Gram stain result and final culture result were recorded. For quantification of polymorphonuclear (PMN) cells on secretions, for culture with 10 SECs per low-power field where bacteria were observed on Gram staining, the yield of culture was 13% (2/15). For specimens with no bacteria seen on Gram staining, the yield of culture was 48%. For specimens with no bacteria seen on Gram staining, the yield of culture was 59% (29/49).

There were no data available from our institution to estimate the positivity rate of ETT specimens with >10 SECs per low-power field. However, in a study by Zaidi & Reller (1996), 29% of samples with >10 SECs per low-power field grew a potential pathogen.

Based on these data, it was assumed that specimens screened using the two criteria (no organisms on Gram staining or >10 SECs per low-power field) would be positive in, at most, 15–30% of cases. Assuming a culture positivity rate of 60% for Gram-stain-positive specimens, a sample size of 40 patients per study arm would be required to detect a 30% absolute difference in positivity rate between study arms (assumptions: 80% power, higher positivity rate in the Gram-stain-positive arm, acceptable error [alpha] of 0.05). Finding an absolute difference of >30% (and a positivity rate of <5–10% for screened specimens) would support the use of a particular screening criterion. We aimed to include 40–50 specimens in each arm of the study. The study was carried out between September and December, 2010. Specimens were included in the study as they were received by the microbiology laboratories until there were a sufficient number in each study arm. At the Health Sciences Centre, once a given study arm was finished, specimens continued to be classified in that arm (but were not analysed) until study completion in order to estimate the proportion of ETT specimens that would be captured by either screening criterion over a given time period.

**Statistical analysis.** Statistical analysis was performed using JMP software version 9.0 (SAS Institute). Nominal logistic regression was used to determine whether there was a statistically significant difference in potential pathogen recovery between study arms 1 and 2 and study arms 1 and 3. A P-value of ≤0.05 was considered significant.

**RESULTS**

In total, 141 ETT specimens were included in the study. Sixty-seven were obtained from St Boniface Hospital and 74 were obtained from the Health Sciences Centre. The specimens were subdivided as follows: 50 in arm 1, 41 in arm 2 and 50 in arm 3. Arm 4 was terminated due to a lack of specimens meeting the given criteria. Patient demographics and test results are presented in Table 1, broken down by study arm. Approximately two thirds of specimens had a score of 3+ or 4+ for the quantity of PMN cells visible on initial Gram staining, regardless of the study group.

The overall yield for potential pathogen recovery was 54, 37 and 10% for specimens in study arms 1, 2, and 3, respectively (Table 1). The specific pathogens recovered are listed in Table 2. The yield of pathogen recovery in arm 1 was significantly greater than arm 3 (P<0.0001). The difference in pathogen recovery between arm 1 and arm 2 was not statistically significant (P=0.096). Among specimens that yielded a potential pathogen, growth was scored at 3+ or greater for 81% of specimens from arm 1, 67% of specimens from arm 2 and 20% of specimens from arm 3. Overall, only 1/50 specimens (2%) from arm 3 yielded a potential pathogen scored at 3+ or greater for growth.

The hospital charts for 23 out of 25 patients with a potential pathogen isolated on culture at the Health Sciences Centre site were available for review. Clinical data obtained from the chart review are presented in Table S1, available in JMM Online. Fourteen of the 23 patients (60.9%) were considered by the treating service to have a respiratory infection requiring antimicrobial therapy.
respiratory infection was considered to be present in 50% (6/12) of patients with a positive culture in arm 1, 66.6% (6/9) of patients with a positive culture in arm 2 and 100% (2/2) of patients with a positive culture in arm 3. It should be noted that the evidence to support an active respiratory infection (including chest X-ray results) was often equivocal (Table S1). Further to this, in the majority of cases, antibiotic therapy was not altered based on the culture results. For the cases where a respiratory infection was not thought to be present, a positive culture result was considered to represent colonizing flora. In all of these cases, there was no adverse clinical outcome from withholding antimicrobial therapy.

At the Health Sciences Centre, a total of 228 ETT specimens were processed between September 16 and November 15, 2010. These specimens predominantly fell into one of two groups: those with <10 SECs with bacteria seen on Gram staining (arm 1, n=113), and those with <10 SECs with no bacteria seen on Gram staining (arm 3, n=78). Only 30 specimens had >10 SECs with bacteria seen on Gram staining (arm 2). Seven specimens had >10 SECs per low-power field with no bacteria seen on Gram staining (arm 4).

**DISCUSSION**

An optimal screening criterion for ETT secretions would eliminate a large number of specimens that ultimately do not yield a pathogen on culture, while minimizing the rejection of specimens in which a potential pathogen is present. Two screening criteria were investigated in this

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<th>Arm 1 (&lt;10 SECs with bacteria seen on Gram staining)</th>
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<td>S. aureus (MSSA) – 8 isolates</td>
<td>P. aeruginosa – 4 isolates</td>
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<td>S. maltophilia – 7 isolates</td>
<td>M. catarrhalis – 1 isolate</td>
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<td>P. aeruginosa – 7 isolates</td>
<td>S. aureus (MSSA) – 2 isolates</td>
<td>E. cloacae – 1 isolate</td>
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<td>H. influenzae – 3 isolates</td>
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<td>C. striatum – 1 isolate</td>
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report: >10 SECs per low-power field and the absence of bacteria on Gram staining. Absence of bacteria on Gram staining performed well as a screening criterion. In only 1/50 specimens (2%) with no bacteria seen on Gram staining was a potential pathogen recovered at a score of 3+ or greater growth. Additionally, rejecting specimens with no bacteria seen on Gram staining would result in a 34% (78/228) reduction in the number of ETT samples cultured in the laboratory. In contrast, use of >10 SECs per low-power field as a screening criterion performed poorly. In this study, 37% of specimens with >10 SECs per low-power field yielded a potential pathogen on culture (60% of these with a score of 3+ or greater growth). Further to this, based on data from a single site, the rejection of ETT secretions by applying this criterion would only eliminate ~13% (30/228) of specimens.

There are few published reports in the literature evaluating the utility of screening ETT secretions submitted for culture. Morris et al. (1993) assessed the absence of bacteria seen on Gram staining as a screening criterion in adult patients. These investigators reported recovery of a potential pathogen in only 12% of 201 ETT aspirates with no organisms seen on Gram staining. Further to this, when a pathogen was recovered, it was found in low quantity (1+ growth score) and often in association with normal flora (Morris et al., 1993). Zaidi & Reller (1996) evaluated the absence of bacteria on Gram staining as a screening criterion in a paediatric population. Of 225 specimens with no organism seen on Gram staining, only 12% had a growth score of ≤1+ for quantities of Gram-negative bacilli and only 4% of specimens yielded pure or predominant growth of a potential respiratory pathogen (Zaidi & Reller, 1996). The results of these studies are consistent with the data presented here and support the absence of bacteria on Gram staining as a useful criterion for the rejection of ETT specimens submitted for culture.

Morris et al. (1993) also evaluated >10 SECs per low-power field as a screening criterion in adults. Only 15% of the 504 specimens assessed contained >10 SECs per low-power field. Of those, 46% yielded a potential pathogen, although a score of 3+ to 4+ growth of the pathogen was detected in only 8% of specimens. Samples with >10 SECs per low-power field yielded a greater mean number of organisms than samples with <10 SECs per low-power field (Morris et al., 1993). These investigators concluded that rejection of ETT secretions based on a criterion of >10 SECs per low-power field was reasonable. Zaidi & Reller (1996) evaluated >10 SECs per low-power field as a screening criterion in paediatric patients. In contrast to the data presented by Morris, only 3% of ETT aspirates in this study met the screening criterion and of those, 29% yielded pure cultures of Gram-negative rods (Zaidi & Reller, 1996). These investigators concluded that >10 SECs per low-power field was not a useful screening criterion for ETT secretions from paediatric patients. In agreement with both of the studies reviewed above, our data suggest that the number of ETT secretions received by a clinical microbiology laboratory with >10 SECs per low-power field is relatively low (<15%). Similar to the study by Zaidi & Reller (1996), we observed recovery of a potential pathogen in a significant number of these specimens.

A significant limitation of the studies by Morris et al. (1993) and Zaidi & Reller (1996) is that there were minimal efforts to correlate culture results with the clinical condition of the patient. Morris et al. (1993) did not include a chart review as part of their study, while Zaidi & Reller (1996) only reviewed the medical records of select patients that yielded pure growth of an organism on culture with no bacteria seen on Gram staining. In the present study, we attempted to determine the clinical significance of organisms recovered on culture. In ~40% of cases where a potential pathogen was recovered on culture, the result was considered to represent colonization rather than infection and no specific antimicrobial therapy was prescribed. Further to this, for the patients treated as having a possible respiratory infection, the clinical data to support a need for antimicrobial therapy was often equivocal. Antimicrobial therapy was selected/alter based on the culture results in only a minority of cases. These results suggest that in a significant proportion of cases, recovery of a potential pathogen from ETT cultures does not provide clinically useful information, regardless of whether a screening criterion is used. Cultures with >10 SECs per low-power field on Gram staining that grew a potential pathogen appeared to be as useful as those with <10 SECs per low-power field, respecting that ETT cultures in this study, overall, were of questionable benefit.

There are several important limitations to this study that deserve attention. The sample size of each study arm was relatively small, although the results obtained for the laboratory portion of the study were statistically significant. The chart review was also limited to patients at a single institution, and the number of charts reviewed was small. Additionally, approximately 40% of potential pathogens isolated on culture were not considered clinically significant, irrespective of the study arm. This number is high and it is possible that it may not be representative of other institutions. However, given that ETT secretions are often submitted to the microbiology laboratory as part of an empiric work-up for fever in a hospitalized patient, it is suspected that the data here are generally representative of institutions where ETT culture is routinely performed.

In summary, these data suggest that, in general, ETT secretion cultures are of limited benefit to clinicians. The data presented in this study support the use of the absence of bacteria on Gram staining as a screening criterion for ETT secretions submitted to the microbiology laboratory for culture. Use of this criterion would be expected to significantly reduce the number of ETT secretion specimens processed by a microbiology laboratory, while minimizing rejection of samples likely to yield a clinically significant pathogen. Use of >10 SECs per low-power field as a screening criterion for ETT secretions is more controversial. In this study, such a criterion would have resulted in rejection of a significant number of specimens that
harboured a potential pathogen. The clinical significance of the organisms recovered on culture was unclear in many cases but the same was true for specimens with <10 SECs per low-power field. A prospective study of patients with an unequivocal clinical diagnosis of pneumonia would be of benefit to better assess whether the criterion of >10 SECs per low-power field should be used to reject ETT specimens sent for culture. Improved methods for screening ETT secretions for culture are required.

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REFERENCES


