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Diagnostic Utility of Tracheal Aspirate Cultures in the Neonatal Intensive Care Unit

Scholarly Pursuit and Thesis Prospectus

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Abstract

Research Question: Are neonatal intensive care unit (NICU) clinicians accurately discriminating between infection and colonization of the upper respiratory tract based on results from endotracheal aspirate cultures (ETAs) of neonates in their evaluation of sepsis? Further, what are the qualitative results of these cultures and how are they related to the antimicrobial treatment method? We hypothesized that the results of the ETAs, regardless of colonization or infection, influence antimicrobial treatment of intubated neonates. The goal of this study is to determine the utility of ETA cultures in routine septic evaluations in the NICU.

Background, Significance, and Rationale for the Question: Ventilatory-associated pneumonia (VAP) is a serious concern that entails high resource utilization in the NICU. Qualitative tracheal aspirate cultures are commonly utilized in VAP's diagnostic work-up. These cultures lack specificity for infection or pathology and have not been shown to differentiate between colonization and infection. The complex microbial community residing in the lungs has been proven to be important for the maintenance of homeostasis. This shift in view of the lungs as a site of colonization calls for new guidelines for interpreting positive ETAs, a routine part of a sepsis work-up in the NICU, and resultant treatment. This study will aid in outlining the microbial characteristics and current practices in treatment of positive ETAs.

Materials and Methods: Using data obtained from tracheal aspirates from neonates, we classified patients based on the following: age, sex, EGA at birth, birth weight, EGA at time of culture, tracheal aspirate source, and simultaneous cultures. Cultures were detailed by organisms grown. Treatment was categorized based on the presence antibiotics used empirically at the time of culture, antibiotics used to treat any positive culture for greater than two days, and documented diagnosis. Surveying attending physicians at Cook Children's Medical Center, we have elucidated patterns of infection identification and antibiotic prescribing practices for intubated neonates. This was done using a questionnaire that automatically given to physicians of neonates with a positive ETA. The survey inquired about the physician's decision to obtain a tracheal culture, whether the results of the culture influenced treatment, their suspected diagnosis at the time of the culture and subsequent diagnosis 48 hours after the culture. The physicians were also asked to describe the factors used to distinguish colonization versus infection. Ultimately, the survey provides insight into determining if physicians' decision to treat positive cultures was in accordance with published guidelines and definitions of VAP and tracheitis.

Results, Conclusions, and Impact: We hypothesized that the tracheal aspirate cultures would be characterized by a variety of colonizing organisms and correlated antimicrobial treatment because of the ETA, regardless of diagnosis. More patients with positive ETAs were prescribed antibiotics due to this result than there were cases of true infection, and even those with suspected tracheal colonization. Physicians also overestimated the incidence of true infection, leading to additional unnecessary antibiotic use. It is imperative that the diagnostic utility of tracheal aspirate cultures be refined as antibiotic resistance continues to become more prevalent and the impact of unnecessary antibiotic use is not neutral for these patients and may have lasting effects.

Introduction, Significance and Rationale

Endotracheal aspirate cultures (ETAs) are routine in the evaluation of suspected infection in mechanically ventilated NICU patients, namely ventilator-associated pneumonia (VAP), ventilator-associated tracheobronchitis (VAT), or sepsis. The use of ETAs is driven by the CDC criteria for diagnosing central line-associated blood stream infections (CLABSI) and lower respiratory tract infection, which includes ETA as a diagnostic step (Horan, Andrus et al. 2008, Control and Prevention 2020). Positive ETAs are defined by the CDC as having greater than 10 bacterial colony-forming units/mL on quantitative culture and/or more than 25 polymorphonuclear neutrophils (PMNs) per low-power field on Gram stain are detected (Willson, Conaway et al. 2014).

There are limited published guidelines on evaluating NICU or pediatric patients for VAT or VAP. (Cernada, Brugada et al. 2014, Ormsby, Conrad et al. 2021). VAT is broadly characterized by respiratory infection (fever, cough, increased sputum) without radiographic evidence of pneumonia (Graf and Stein 2006, Ormsby, Conrad et al. 2021). The treatment for VAP, and by extension VAT, is empiric antibiotics followed by antimicrobial therapy targeted to a positive ETA and sensitivities (Martin-Loeches, Povoa et al. 2015). Studies have shown that VAT diagnosis and treatment are heavily provider-dependent and subjective (Ingolfsland, Gonzalez-Villamizar et al. 2022).

ETAs have low sensitivity and specificity for infection (Claassen and Keenan 2019). The diagnostic uncertainty of ETAs is complicated by the presence of normal respiratory flora. Therefore, bacterial growth from an ETA does not definitively indicate local infection (Kabak, Hudcova et al. 2019). Neonates requiring artificial airways undergo colonization of their upper airway, which can lead to infection through related mucosal damage (Graf and Stein 2006). Bacterial biofilms can form on endotracheal tube (ETT) walls, leading to a positive ETA in the absence of infection (Leroue, Harris et al. 2017). Differentiating colonizing and infecting microbiota is especially difficult because of the frequency of contamination of specimens of the lower respiratory tract with organisms colonizing the upper respiratory tract (Leroue, Harris et al. 2017). Common colonizing organisms of the upper respiratory tract are likely to be implicated in VAT/VAP, so distinguishing between colonization and bacterial infection requires consideration of factors other than growth from an ETA. (Robinson 2004).

There is a paucity of published best practices or protocols for the collection and processing of tracheal aspirate specimens, and opportunities for variability across laboratories are present at many levels

(Prinzi, Parker et al. 2021). A variety of techniques for obtaining ETA samples exist, some of which invite more potential for contamination, such as simple tracheal aspirate suction sampling, while others are more “clean” but also more invasive, such as bronchoalveolar lavage (BAL) (Ormsby, Conrad et al. 2021). Additionally, there is variation among laboratories in the evaluation of ETA samples, including quantification thresholds for reporting microbes and sensitivities (Prinzi, Parker et al. 2021). With such variation in sampling practices and qualities, interpretation of ETAs on an objective, standardized level is further complicated. This in turn affects treatment decisions on the individual level.

Clinicians must differentiate TA results in the context of colonization versus infection often. Misuse of the tracheal aspirate culture as a diagnostic tool without acknowledging its low sensitivity and specificity for VAT leads to a misinterpretation of the results biased in favor of infection and subsequent increased antibiotic utilization. In one study, positive tracheal cultures were not associated with typical screening factors for infection such as clinical, laboratory, or radiological signs, but did correlate with increased risk of prolonged antibiotic exposure (Langston, Pithia et al. 2020). Positive ETAs in isolation cannot be relied upon as an indication for antibiotic therapy, and clear colonization criteria would help in eschewing occurrences of overtreatment in response to these clinical datapoints alone.

VAP and VAT, as nosocomial infections, increase healthcare utilization through increased morbidity and mortality (Ormsby, Conrad et al. 2021). These infections, often treated in the setting of diagnostic uncertainty as a result of positive ETAs, are a common reason for antibiotic use in NICUs. Appropriate use of antibiotics in these infections improves outcomes for neonates, but treatment duration has not been standardized (Grabic, Lake et al. 2019). One pediatric study showed that prolonged antibiotics (> 7 days) did not protect against the evolution to VAP in patients with VAT (Tamma, Turnbull et al. 2011), and adult studies support a 3 day course of antibiotics for VAT (Martin-Loeches, Povoa et al. 2015). Misconstruing colonizing organisms for infectious microbes can lead to unnecessary and prolonged antibiotic use and its attendant complications: adverse effects, such as necrotizing enterocolitis and late-onset sepsis in neonates, the promotion of microbial resistance, and high resource utilization, such prolonged hospital stays and increased days on the ventilator (Prinzi).

Materials and Methods

General Study Details and Resources

The objective of our study is to determine the diagnostic validity and utility of ETAs among NICU patients. We describe the tracheal microbiome of intubated patients in our level IV NICU at Cook Children's Medical Center (CCMC), the microbiological analysis process used at our institution, and the role of ETAs in diagnostic decision-making in our NICU. This study will aid in outlining the response to, sensitivity, and specificity of positive ETA cultures for infection in neonates by surveying NICU physicians, quantifying their current practices for categorizing ETA results as acute infection (VAT, VAP, or sepsis) and the resultant treatment regimen. These aims will aid in the larger goal of creating a standardized and clinically applicable approach to interpreting ETAs and thus the more objective diagnosis and treatment of ventilator-associated respiratory infections in neonates.

All TAs were prospectively monitored from a Level IV NICU over a one-year period beginning February 2022. Positive cultures were used to describe the observed tracheal microbiome of intubated NICU patients. Cultures were assessed by the clinical microbiology lab at CCMC, and species and sensitivities were reported at 24, 48, and 72 hours of growth.

Among positive TAs, we determined a diagnosis of pneumonia, tracheitis, colonization, or contamination via real-time physician surveys (sample survey in supplemental materials) and compared their diagnoses to an independent, retrospective chart review. The survey queried the physician's clinical decision-making process in terms of the indication for the TA, the suspected diagnosis at the time of the culture, and its impact on treatment. Physicians were given the opportunity to add any context or other

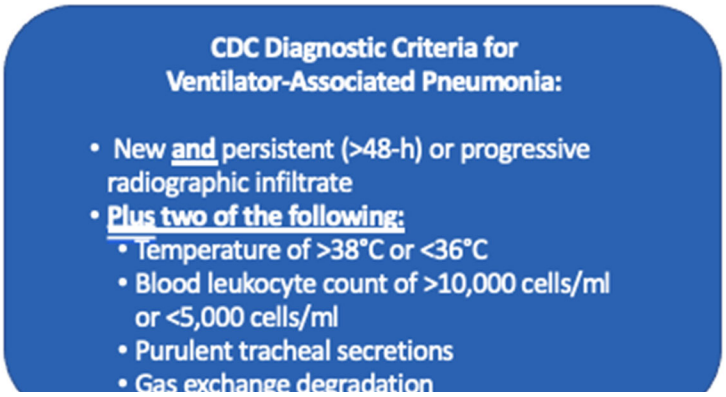

Figure 1: CDC Criteria for diagnosis of VAP (Control and Prevention 2020).

information in a free-text box. Chart review diagnoses were based on current published criteria for the diagnosis of pneumonia or tracheitis in the pediatric population (Figure 1).

The number of clinician and chart review diagnoses of VAP, VAT, and colonization in response to positive TA cultures were compared. Discrepancies in physician survey versus independent chart review were used to assess diagnostic accuracy of VAP and VAT. The sensitivity and positive predictive value of tracheal aspirate cultures for VAP/VAT was calculated both for physician survey results and independent chart review.

Utilizing data from the surveys and chart review, we answer the following research question: In a population of neonatal patients from which tracheal aspirates have been obtained, how many cultures were positive for infection and how was this decision arrived upon? This question will be addressed by the following **Specific Aims (SA):**

SA1: For each positive culture documented, ascertain if it is classified as an infection, colonization, or contaminant based on specific definitions.

A positive tracheal aspirate culture is defined as any culture result with a potentially pathological species. By performing a systematic chart review, the status of infection, colonization, or contaminant was determined using CDC guidelines for infection and established microbiological properties for species identified by the ETA.

SA2: Survey physicians on each case of positive culture from tracheal aspirate to determine whether they classified the culture as infection or colonization and why.

Physicians will be asked to describe the factors, whether clinical, laboratory, or radiological, which allowed them to decide whether a positive tracheal aspirate culture was due to infection, colonization, or contamination.

SA3: Quantify the sensitivity and specificity of tracheal aspirates for determining infection.

Several areas of demographic data will be considered to identify any pertinent patterns in positive tracheal aspirate determination, including age, sex, race/ethnicity, EGA at birth, birth weight, PNA at time of culture, tracheal aspirate source, simultaneous cultures, and comorbidities.

Results

158 TAs were collected in our NICU during the study period. Patient demographics are listed in Table 1.

	Mean	Median
Admission Age (d)	15.9	5.0
Birth Weight (g)	1689.7	1204.0
Birth EGA (weeks)	30.4	30.0
TA Collection CGA (weeks)	39.8	39.9
Length of Stay (d)	97.4	88.0

Figure 2: Demographics of patients who underwent TA culture.

Pathogenic bacterial species grew in 72 cultures (46%), most commonly *Staphylococcus aureus*. Thirteen infants (17%) simultaneously grew a pathogenic organism from blood, urine, or CSF. The distribution of pathogenic organisms in the tracheal microbiome is illustrated in Figure 3.

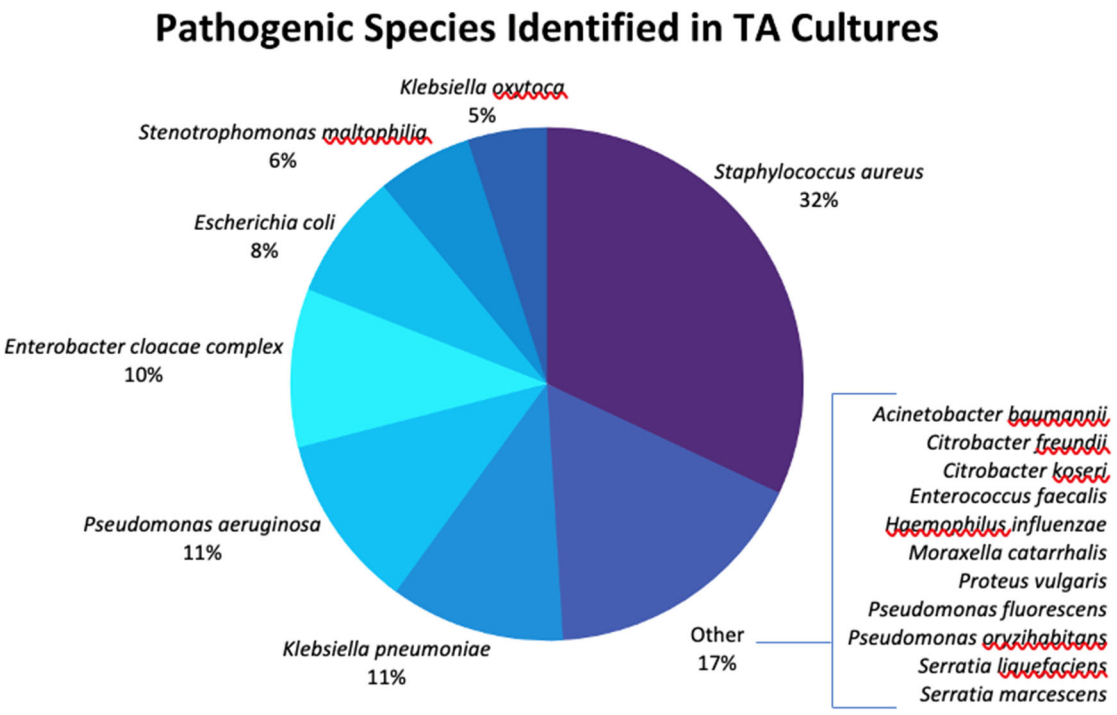

Figure 3: Pathogenic species identified by TA cultures during the study period.

Among infants with pathogenic TA growth, 92% were treated with more than 2 days of antibiotics. Physician diagnoses of treated infants included pneumonia (37%), tracheitis (35%), colonization (26%), and contamination (2%).

Systematic chart review revealed that 28/75 (37%) positive cultures corresponded with cases which did not meet the clinical criteria for infection based on the established CDC guidelines. Physicians accurately identified 28% of those cases.

Based on initial physician diagnosis, the TAs in this population had a specificity of 86% and a positive predictive value of 72%. The independently validated diagnostic utility was reduced to a specificity of 76% and a PPV of 64% (Table 1).

Physician Diagnosis

	Infected	Not Infected
Tracheal Aspirate + Culture	41	16
Tracheal Aspirate - Culture	0	83
Specificity	83.8%	
PPV	71.9%	

Chart Validated Diagnosis

	Infected	Not Infected
Tracheal Aspirate + Culture	47	28
Tracheal Aspirate - Culture	0	83
Specificity	74.8%	
PPV	62.7%	

Table 1: Specificity and positive predictive values of physician and chart validated diagnoses of VAP/VAT.

Most (92%) infants received a prolonged course of antibiotics when a pathogenic organism was identified from TA, even after a physician diagnosis of colonization (26%). Independent chart review reveals physicians also overestimated the incidence of true infection, leading to unnecessary antibiotic use.

Discussion/Innovation

Our study investigated the microbiology and subsequent diagnostic impact of the tracheal aspirate cultures in our level IV NICU. We noted a discrepancy between antibiotic treatment duration and diagnosis of infection, whether tracheitis or pneumonia. Despite a common physician diagnosis of colonization, most infants still received a prolonged course of antibiotics when a pathogenic organism was identified from TA. Independent chart review reveals physicians overestimated the incidence of true infection, leading to additional antibiotic use. Thus, developing and implementing a more rigorous set of clinical guidelines for the interpretation of ETAs is an opportunity to improve antibiotic stewardship in the NICU.

The observed microbiome of intubated NICU patients includes a wide variety of pathogenic organisms. *S. Aureus* was the most commonly identified potentially pathogenic organism from our ETAs, fitting the broader trend of it being the most commonly implicated species in VAP (Tong, Davis et al. 2015). *S. Aureus* also has a role in colonizing the trachea, so in the absence of the clinical criteria meeting the definition for VAT or VAP, antibiotic therapy is not indicated (Kabak, Hudcova et al. 2019). This underscores the necessity of more intentional identification of infection before ruling out colonization and targeting the cultured organism.

Currently, ETAs are a recommended diagnostic tool for the assessment of sepsis cases in neonates. There is not a readily available scaffold for integration of the full array of data offered by this diagnostic step into pediatric care plans. This attenuates the effectiveness and efficiency of the tool clinically. Since appreciating the upper airway as a site of colonization after an endotracheal tube is placed, we now know that diagnosing and treating tracheitis is not as simple as identifying the presence and species of bacteria cultured there (Fernández-Barat, López-Aladid et al. 2020). Now, we must establish that said bacterial species are infecting and causing illness to ensure that the antibiotics are t used only when true infection is present. This is a critical distinction given evolving microbial resistance and a shrinking arsenal of effective antibiotics for clinical use while positive TAs often occur in the absence of true infection.

Antibiotic use at this point in an infant's development can have a permanent, negative impact on their gastrointestinal microbiome, causing tissue damage and death, gastrointestinal issues later in life, and increased susceptibility to infection (Singh and Mittal 2020). In the short term, antibiotics also cause feeding intolerance and poor growth in neonates. Another cause for concern is the mounting evidence

that the more antibiotics are used, the greater risk there is for bacterial mutation and resistance, which continues to rise in hospitals. This problem is especially troubling in intensive care units, and even more so in the vulnerable patient population that is neonates. These patients have little in their immunological arsenal to combat resistant microbes, so this consequence must be avoided as much as possible. A fine balance must be achieved given the high risk for sepsis in this population, further highlighting the prudent use of antibiotics. Making the best use of all data available on when to employ antibiotics in this population is vital, especially the wealth of information provided by ETAs.

By finding discrete, definable patterns in patient care and treatment outcomes as a result of endotracheal aspirate cultures, we are able to better ascertain the relationships between clinical decision making, bacterial infection, and patient outcomes. Since obtaining tracheal aspirate cultures is a mainstay of a clinical diagnoses for sepsis, their routine use should have a routine interpretation that is backed by scientific research and data. This study provides an installment in the creation of an evidence-based framework for interpreting the results of tracheal aspirates in neonates. This is done in the hopes of improving antibiotic stewardship by identifying cases of bacteria colonization in positive ETAs without sacrificing quality of care in the NICU.

Future Directions

Developing and implementing a more rigorous set of clinical guidelines for the interpretation of ETAs is an opportunity to improve antibiotic stewardship in the NICU. Future directions include developing such a scaffold for interpreting positive ETAs and incorporating results into diagnosis and clinical action. The data from this study and this knowledge could aid in initiating a quality improvement project which includes education on standards and guidelines for the definition of VAP and development of an algorithm for diagnostic and treatment decisions in the NICU population.

To increase the strength and impact of the findings of this study, more data could be collected focused on interpreting and examining the effect of treatment of cases of tracheal colonization on length of stay in the NICU, subsequent healthcare visits, and healthcare utilization and costs. Given the long-term effects of antibiotics on neonates and increased risk of infection with longer hospital stays, it is expected that the cost of these interventions will provide sufficient incentive to developing such guidelines and quality improvement initiatives.

Conclusions

Tracheal aspirate cultures are a common diagnostic tool used in the NICU. It is imperfect in its discrimination of cases of infection versus colonization of the upper airway of intubated neonates. Most (92%) infants received a prolonged course of antibiotics when a pathogenic organism was identified from TA, even after a physician diagnosis of colonization (26%) per physician survey. Independent chart review reveals physicians also overestimated the incidence of true infection, leading to unnecessary antibiotic use. It is imperative that this process be refined as antibiotic resistance continues to become more prevalent and the impact of unnecessary antibiotic use is not neutral for these patients and may have lasting effects.

Compliance

This research study has official and up to date Cook Children's Medical Center IRB approval (IRB #2021-055). I have maintained an active CITI Training status throughout the completion of this project.

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