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CLINICAL RESEARCH ARTICLE

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Tobacco smoke exposure, the lower airways microbiome and outcomes of ventilated children

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BACKGROUND: Tobacco smoke exposure increases the risk and severity of lower respiratory tract infections in children, yet the mechanisms remain unclear. We hypothesized that tobacco smoke exposure would modify the lower airway microbiome.
METHODS: Secondary analysis of a multicenter cohort of 362 children between ages 31 days and 18 years mechanically ventilated for >72 h. Tracheal aspirates from 298 patients, collected within 24 h of intubation, were evaluated via 16 S ribosomal RNA sequencing. Smoke exposure was determined by creatinine corrected urine cotinine levels ≥30 µg/g.
RESULTS: Patients had a median age of 16 (IQR 568) months. The most common admission diagnosis was lower respiratory tract infection (53%). Seventy-four (20%) patients were smoke exposed and exhibited decreased richness and Shannon diversity. Smoke exposed children had higher relative abundances of *Serratia* spp., *Moraxella* spp., *Haemophilus* spp., and *Staphylococcus aureus*. Differences were most notable in patients with bacterial and viral respiratory infections. There were no differences in development of acute respiratory distress syndrome, days of mechanical ventilation, ventilator free days at 28 days, length of stay, or mortality.
CONCLUSION: Among critically ill children requiring prolonged mechanical ventilation, tobacco smoke exposure is associated with decreased richness and Shannon diversity and change in microbial communities.

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IMPACT:

- Tobacco smoke exposure is associated with changes in the lower airways microbiome but is not associated with clinical outcomes among critically ill pediatric patients requiring prolonged mechanical ventilation.
- This study is among the first to evaluate the impact of tobacco smoke exposure on the lower airway microbiome in children.
- This research helps elucidate the relationship between tobacco smoke exposure and the lower airway microbiome and may provide a possible mechanism by which tobacco smoke exposure increases the risk for poor outcomes in children.

INTRODUCTION

Tobacco smoke exposure (TSE) contributes to an estimated 168,000 pediatric deaths per year worldwide, primarily attributed to increasing the incidence and severity of lower respiratory tract infections (LRTI).[1,2] In the United States, 38.1% of children are exposed to tobacco smoke.[3] TSE is associated with increased healthcare utilization and the risk of LRTI, otitis media, sinusitis, asthma, and obesity.[4–7] Tobacco smoke exposed children with influenza are more likely to require intubation and admission to the pediatric intensive unit (PICU) with increased lengths of stay compared to those without TSE.[8] TSE is associated with increased hospitalization and severity of illness in children with respiratory syncytial virus (RSV).[9]

The association between TSE and increased risk of critical illness has been recognized in adults. Among adult patients with non-pulmonary sepsis, current smokers were twice as likely to develop acute respiratory distress syndrome (ARDS). Despite being younger with fewer comorbidities and lower severity of disease than patients without TSE, patients with TSE had similar severity of lung injury, suggesting that TSE may increase the risk of ARDS in younger, healthier patients.[10,11]

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The molecular basis of TSE-associated health risks includes broad effects on lung inflammation, immune response, infection response, and tissue damage.[12] Recent studies suggest that these effects may in part be mediated by or result in changes in the respiratory tract microbiome.[13–15] Among critically ill adults with severe blunt trauma, those with TSE had increased relative abundance of *Haemophilus spp*., *Streptococcus spp*., and *Fusobacterium spp*. in the lower airways, which persisted after 48 h of invasive mechanical ventilation. Furthermore, microbial changes associated with TSE, specifically enrichment of *Enterobacteriaceae*, were associated with development of ARDS.[16]

Children most often experience secondary exposure from inhalation of smoke from someone else using cigarettes or tertiary smoke exposure from clothing and other surfaces. Therefore, investigations of the relationship between TSE and critical illness are warranted in this population.

In a secondary analysis of a prospective multicenter cohort of critically ill children requiring prolonged ventilation, we evaluated the association between TSE, the lower respiratory tract microbiome, and clinical outcomes. We hypothesized that children with TSE would have a microbiome characterized by higher bacterial burden, decreased bacterial diversity, and increased relative abundance of pathogenic bacteria, and worse clinical outcomes.

METHODS

Data and specimens for this study were collected as part of a prospective cohort study of mechanically ventilated children admitted to eight PICUs in the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development's Collaborative Pediatric Critical Care Research Network from 2015 to 2017.[17] Children 31 days to 18 years requiring endotracheal intubation and mechanical ventilation for >72 h were eligible. For this study, we analyzed data from patients who had a urine sample collected and 16 S rRNA sequencing data from a tracheal aspirate obtained the day of intubation.

The study was approved by the University of Utah central Institutional Review Board. Full details of the consent process, study methodology, and data collection have been previously published.[17]

Tracheal aspirates were collected within 24 h of intubation according to a standardized protocol and frozen at −80 °C until analyzed.[17] Urine was collected within 96 h of intubation from an indwelling foley catheter, bed pans, other urine receptacles, or cotton balls. After collection, samples were frozen at −80 °C.

Laboratory analysis

Tracheal aspirates underwent 16 S rRNA gene sequencing to evaluate the lower airway microbiome; full methods have been previously described.[17–20] The relative abundance of each taxon was calculated (number of sequences for specific taxon/total number of sequences*100). Shannon diversity and evenness indices were used to characterize the bacterial community.

To determine TSE, urine was analyzed for total urinary cotinine content through the Human Health Exposure Analysis Resource[21] via liquid chromatography-tandem mass spectrometry assay as previously described.[22] The lower limit of detection was 0.1 ng/mL. To account for urinary dilution and changes in glomerular filtration across age and critical illness severity, raw cotinine levels were corrected for creatinine concentration using the following formula: cotinine (ng/mL)/creatinine (mg/dL).[23] To account for a right skew, creatinine-corrected cotinine was log2 transformed and is reported as μg cotinine/g creatinine.

Statistical analysis

The main exposure, creatinine corrected cotinine level, was defined two ways: (1) binary variable using a threshold of ≥30 μg/g[24–29] and (2) continuous variable adjusted for time of collection from hospital admission. Log linear regression assessed the relationship between TSE and total bacterial load, richness, and Shannon diversity. For subgroup analyses, patients were classified based on clinical data and laboratory results as bacterial LRTI, viral LRTI (either viral only or combined viral and bacterial), clinical LRTI (pathogen not identified), or non-infectious etiology of respiratory failure (additional details in online supplement). Relative abundance from 16 S rRNA gene sequencing for known pathogens[30] between TSE and no TSE groups was assessed using Wilcoxon rank sums. Species-level data were available for a subset of organisms; otherwise, taxa are reported at the genus level, the most specific taxonomic rank available.

Log linear regression and logistic regression models evaluated the association of clinical outcomes for continuous and binary outcome TSE variables, respectively. Clinical outcomes of interest were diagnosis of ARDS based on Berlin criteria,[31] duration of invasive mechanical ventilation, ventilator free days to day 28, PICU length of stay (LOS), hospital LOS, and mortality. Covariates chosen a priori for the final model were Pediatric Risk of Mortality (PRISM) Score III,[32] comorbidities, and baseline dependence on non-invasive ventilation.

A sensitivity analysis was performed excluding the 88 patients that had parental or self-reported TSE but urine creatinine corrected cotinine levels <30 μg/g (additional details in online supplement).

RESULTS

Of the 454 analyzable patients in the parent study, 362 (80%) had urine samples obtained. All 362 patients had tracheal aspirate samples from the day of intubation that were evaluated for total bacterial load. However, only 298 (66%) patients had tracheal aspirate samples with sufficient bacterial load to generate 16 S rRNA gene sequencing data for analysis of richness and Shannon diversity.[17,33] The median age was 16 months (IQR 5–68) and 59% were male (Table 1). The most common primary diagnosis was LRTI (53%) and the median PRISM III score was 5 (IQR 1–10). Antibiotics were administered to 36% of patients in the seven days prior to admission, and 99.7% of patients received antibiotics on the day of intubation. There was no significant difference among patients with TSE and no TSE comparing clinical microbiology results available within the first 48 h of admission (Supplementary Table 1). Urine creatinine corrected cotinine levels ranged from 0.1 to 663 μg/g (median 5, IQR: 1, 22 μg/g) and median time to collection was 1.78 days from admission (IQR 1.04, 2.74 days). Seventy-four (20%) patients had urinary cotinine levels ≥30 μg/g, a threshold established to represent TSE.[24–27,34] Based on parental reporting, 138 (38%) of the cohort had passive TSE (97.1%) or the patient actively smoked cigarettes (2.9%). Twenty-four (11%) patients who had no parental report of TSE had evidence of TSE based on creatinine corrected urine cotinine levels. In contrast, 88 (64%) of the patients who had parental reporting of TSE, had no evidence of TSE based on cotinine measurement. The median length of intubation was 7 days (IQR 5–10 days), median PICU LOS was 10 days (IQR 7–15 days), and median hospital LOS was 17 days (IQR 12–29 days). Ninety-seven (27%) developed ARDS and 7 (6%) patients died.

Cotinine and lower respiratory tract microbiome

Among the entire cohort, binary analysis, defining TSE using a creatinine corrected cotinine cutoff of levels ≥30 μg/g, demonstrated that those with TSE had a greater, but not statistically significant, bacterial load ($p = 0.06$). However, urine cotinine levels were not associated with total bacterial load on continuous cotinine designation (Fig. 1A, D). TSE, based on the binary definition, was associated with significantly lower ($p < 0.01$) richness but showed no association with richness in the analysis based on the continuous cotinine measurements (Fig. 1B, E). Patients with TSE defined as a binary variable had on average 31.1% less richness than those who were not smoke exposed (Table 2). Shannon diversity was significantly lower among children with TSE in the binary ($p < 0.01$) and the continuous measure ($p = 0.02$) analyses (Fig. 1C, F). Patients with TSE had on average 52.7% decrease in Shannon diversity than those without TSE (Table 2).

On average, patients with TSE had higher relative abundance of *Serratia* spp, *Moraxella* spp., *Haemophilus* spp, and *Staphylococcus aureus while* patients without TSE were found to have higher relative abundance of *Mycoplasma* spp., *Fusobacterium* spp.,

Table 1. Patient characteristics.

		Tobacco Smoke Exposure Based on Creatinine Adjusted Cotinine Levels		
	All ($n = 362$)	**No TSE ($n = 288$)**	**TSE ($n = 74$)**	***P* Value**
Age (months), median (IQR)	16 (5, 68)	18 (6, 92)	6 (3, 19)	<0.01
Male Sex	214 (59%)	172 (60%)	42 (57%)	0.74
Ethnicity Not Hispanic or Latino	284 (78%)	217 (75%)	67 (91%)	0.01
Race				0.51
Asian	19 (5%)	17 (6%)	2 (3%)	
Black or African American	71 (20%)	51 (18%)	20 (27%)	
White	216 (60%)	172 (60%)	44 (59%)	
Admit category				0.20
Medical	317 (88%)	254 (88%)	63 (85%)	
Surgical	26 (7%)	22 (8%)	4 (5%)	
Trauma	19 (5%)	12 (4%)	7 (9%)	
Admit primary diagnosis				0.24
Lower respiratory tract infection	193 (53%)	157 (55%)	36 (49%)	
Sepsis/SIRS/Septic shock	39 (11%)	34 (12%)	5 (7%)	
Trauma	17 (5%)	10 (3%)	7 (9%)	
Other	86 (24%)	65 (23%)	21 (28%)	
PRISM III Score, median (IQR)	5 (1, 10)	5 (1, 10)	5 (1, 9)	0.73
Parental or Self-Reported Smoke Exposure	138 (38%)	88 (31%)	50 (68%)	<0.01
Antibiotics Given on the Day of Intubation	361 (99.7%)	287 (99.5%)	74 (100%)	0.30
Received Antibiotics within 7 days prior to Admission	125 (36%)	109 (39%)	16 (22%)	0.01
Any Comorbidities	177 (49%)	147 (51%)	30 (41%)	0.99
Immunocompromised	29 (8%)	25 (9%)	4 (5%)	0.50

Escherichia spp., *Streptococcus pyogenes, Streptococcus mitis* group, *Streptococcus anginous*, and *Pseudomonas aeruginosa* (Fig. 2).

Among patients diagnosed with a bacterial LRTI ($n = 43$), there was no difference in total bacterial load between the patients with and without TSE. However, patients with bacterial LRTI and TSE had significantly lower richness ($p = 0.03$) and Shannon diversity ($p = 0.02$) (Fig. 3, $n = 33$). Patients with bacterial LRTI and TSE demonstrated higher relative abundance of *Moraxella* spp. Those with a diagnosis of bacterial LRTI without TSE had an increase in *Streptococcus* spp. For taxa identified at the species level, those with TSE had a higher relative abundance of *S. aureus*. Non TSE patients had higher relative abundance of *S. pyogenes*, *S. mitis group*, *S. anginous*, and *P. aeruginosa* (Fig. 4).

Patients with viral LRTI ($n = 117$) demonstrated no differences in total bacterial load based on TSE. However, patients with viral LRTI and TSE had significantly lower richness ($p = 0.03$) and Shannon diversity ($p = 0.02$) (Fig. 3). Those with viral LRTI without TSE demonstrated a higher relative abundance of *Moraxella* spp. compared to those with TSE. In contrast to the entire cohort and non-smoke exposed patients with bacterial LRTI, non-smoke exposed patients with viral LRTI had a higher relative abundance of *S. aureus*, although the difference was small (Fig. 4).

Patients with clinically defined LRTI (no pathogen identified on bacterial or viral testing; $n = 52$) had no differences in total bacterial load, richness, or Shannon diversity based on TSE status (Fig. 3). However, patients with no TSE and clinically defined LRTI did demonstrate a higher relative abundance of Mycoplasma *spp*. At the species level, those without TSE had a higher relative abundance of *S. mitis group* and *S. aureus*. (Fig. 4).

There were no differences in total bacterial load, richness, or Shannon diversity between those with and without TSE among patients with a non-infectious reason for respiratory failure ($n = 99$) (Fig. 3). However, those with TSE had higher relative abundance of *Moraxella* spp. *Serratia spp., S. aureus*, and *S. mitis group* whereas those without TSE had higher relative abundance of *Haemophilus* spp. and *Fusobacterium* spp (Fig. 4).

Cotinine and clinical outcomes

TSE was not associated with diagnosis of ARDS within 48 h, duration of mechanical ventilation, ventilator free days to day 28, PICU length of stay (LOS), hospital LOS, or mortality (Table 3). There were no differences in clinical outcomes when comparing patients with and without TSE based on LRTI subgroup classification.

DISCUSSION

This prospective, multicenter study evaluated the association between creatinine corrected urine cotinine levels, the lower airway microbiome, and clinical outcomes of critically ill children requiring prolonged mechanical ventilation. TSE was associated with lower richness, and lower Shannon diversity. In patients with TSE, the lower airway bacterial microbiome was represented by higher relative abundance of *Moraxella* spp., *Haemophilus* spp., and *S. aureus*. These changes were largely driven by differences observed among patients with bacterial and viral LRTI. TSE was not associated with clinical outcomes.

Overall, in critically ill children requiring prolonged mechanical ventilation, our findings demonstrate that TSE is associated with changes in the lower airway microbiome, yet the clinical implications of these differences were not readily discernable. Given the emerging role of the lung microbiome in modifying inflammatory signaling and acute lung injury,[35] our findings highlight the need for ongoing investigations evaluating the mechanistic effects of TSE on the respiratory tract response to acute respiratory failure, especially in the setting of LRTI.

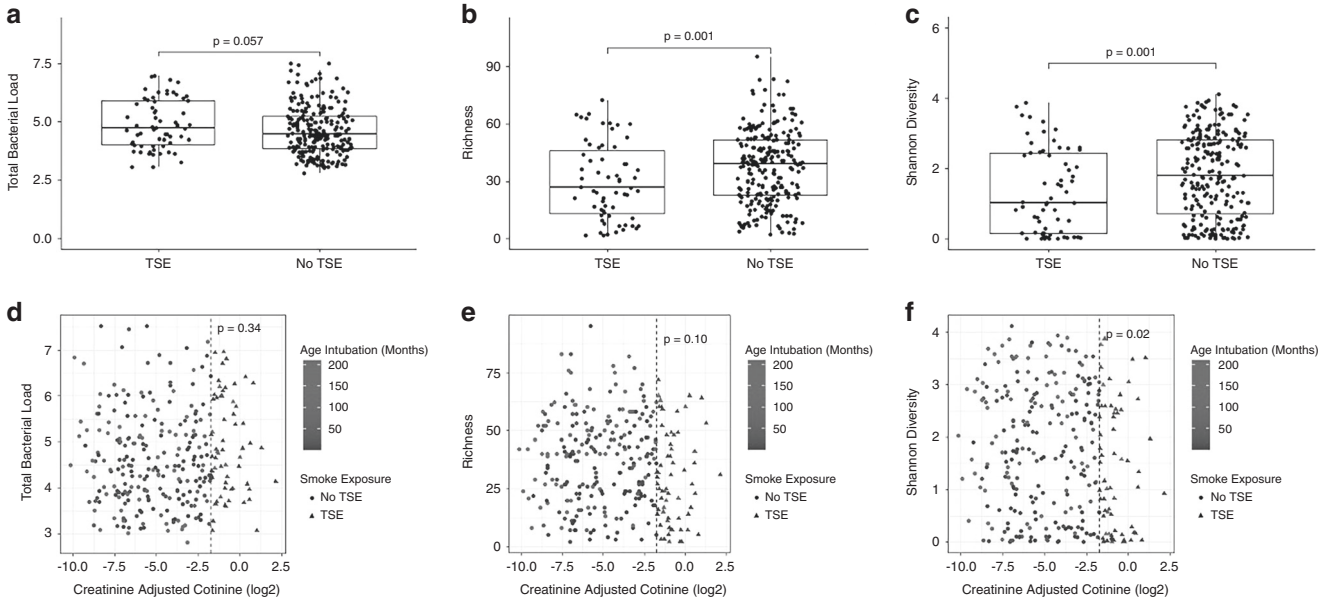

Fig. 1 Comparison of total bacterial load, richness and Shannon diversity between patients with and without TSE. Box plot **a**–**c** compare total bacterial load, richness and Shannon diversity between patients with TSE defined as creatinine adjusted urine cotinine level ≥30 µg/g creatinine to patients without TSE. Scatter plot **d**–**f** evaluate the respective metrics using creatinine adjusted urine cotinine as a continuous measure accounting for time of sample collection. Age of individual patients in months is denoted by color of symbol. TSE tobacco smoke exposure.

Table 2. Effect of tobacco smoke exposure on microbiome by analysis.

Metric	Cotinine Analysis	Effect Estimate (95% CI)
Total Bacterial Load	Continuous	1.01 (0.99 to 1.02)
	Binary	1.08 (1.01 to 1.15)
Richness	Continuous	0.97 (0.94 to 1.01)
	Binary	0.69 (0.56 to 0.85)
Shannon diversity	Continuous	0.93 (0.87 to 0.99)
	Binary	0.47 (0.31 to 0.72)

The TSE-related changes identified in richness and Shannon diversity within this cohort were largely driven by differences observed in patients with bacterial and viral LRTI. Changes in bacterial microbiome characteristics were not observed based on TSE status in the subgroups of patients with clinically diagnosed LRTI or non-infectious respiratory failure when sampled at the time of intubation. One potential explanation for this finding is that TSE may not be, in itself, responsible for changes in the lower airway microbiome, but may alter the host and immunologic response to infection leading to downstream changes in the microbiome in those with infectious LRTI. Alternatively, TSE may alter the baseline microbiome in such a way that makes children more susceptible to infection and pathogen expansion. TSE induced changes in interferon and interleukin expression, alveolar macrophage function, and T-cell response may contribute to changes in the microbiome in the setting of infection.[12] The use of host transcriptomics and metagenomic next generation sequencing to evaluate both the microbiome, viral copy number, and host response in the setting of TSE may offer more insight.

Patients with a creatinine corrected urine cotinine level of ≥30 µg/g had a greater predominance of *S. aureus*, *Haemophilus* spp., and *Moraxella* spp. compared to non-TSE patients. *S. aureus* is a well described pulmonary pathogen in both community-acquired and hospital-acquired pneumonia.[36,37] *Haemophilus* spp. have also been implicated in ventilator-associated pneumonia in children.[37] While we did find statistically significant differences in the taxa, the clinical relevance is unclear given the small size of the difference and the sample size. Like the alterations seen with richness and Shannon diversity, these changes were largely found in patients with infectious LRTI. Patients with bacterial LRTI, viral LRTI, clinically diagnosed LRTI, and non-infectious respiratory failure all demonstrated variable patterns of relative abundance at the genera and species level based on TSE status.

These findings suggest that disease context may determine the influence of TSE on lower airway microbial communities, and that other factors may contribute to changes in the relative abundance of bacterial species. For example, Panzer et al. evaluated the microbiome at the time of intubation in adult trauma patients and compared active and passive smokers to non-smoke exposed patients. They observed increased relative abundance of *Haemophilus* spp., *Streptococcus* spp., *and Fusobacterium* spp. in patients who smoked or were smoke exposed.[16] In our pediatric cohort, we similarly found an increase in *Fusobacterium* spp. and *S. mitis* group among TSE patients who were intubated for non-infectious causes. Future research focusing on larger, specific subgroups of patients with ARDS or bacterial LRTI may better delineate the potential impact of TSE on the lower airway microbiome.

Urine cotinine levels were not associated with the measured clinical outcomes in this cohort of severely critically ill children as participation required at least 72 h of mechanical ventilations support. These results are contrary to previously published pediatric and adult studies that evaluated the impact of TSE on clinical outcomes.[4,10,11,16] In a study by Wilson et al., hospitalized pediatric patients with influenza and TSE were more likely to subsequently require intensive care, had longer hospital LOS, and were more likely to require intubation compared with non-TSE patients with influenza.[8] Outcomes in this severely ill cohort are likely driven by patient and clinical course characteristics whereas patients of lower illness severity may have outcomes that are more affected by environmental exposures such as tobacco smoke. The effect of TSE on a population of hospitalized children that spans all severity levels will be essential to more accurately determine the impact of TSE on outcomes across a broader range of clinical presentations.

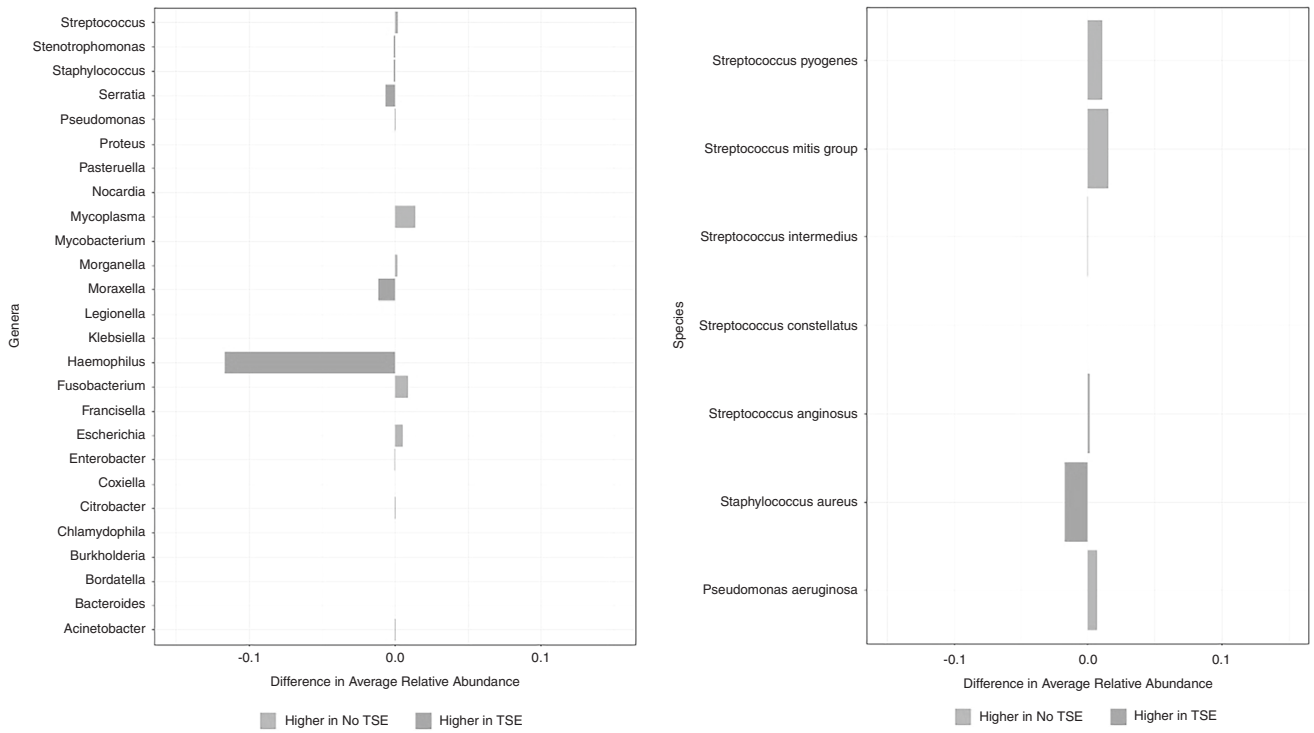

Fig. 2 TSE leads to alteration of relative abundance of lower airways bacteria. Comparison of average differences in relative abundance of bacterial genera (left) and species (right) between patients with TSE and without TSE. TSE tobacco smoke exposure.

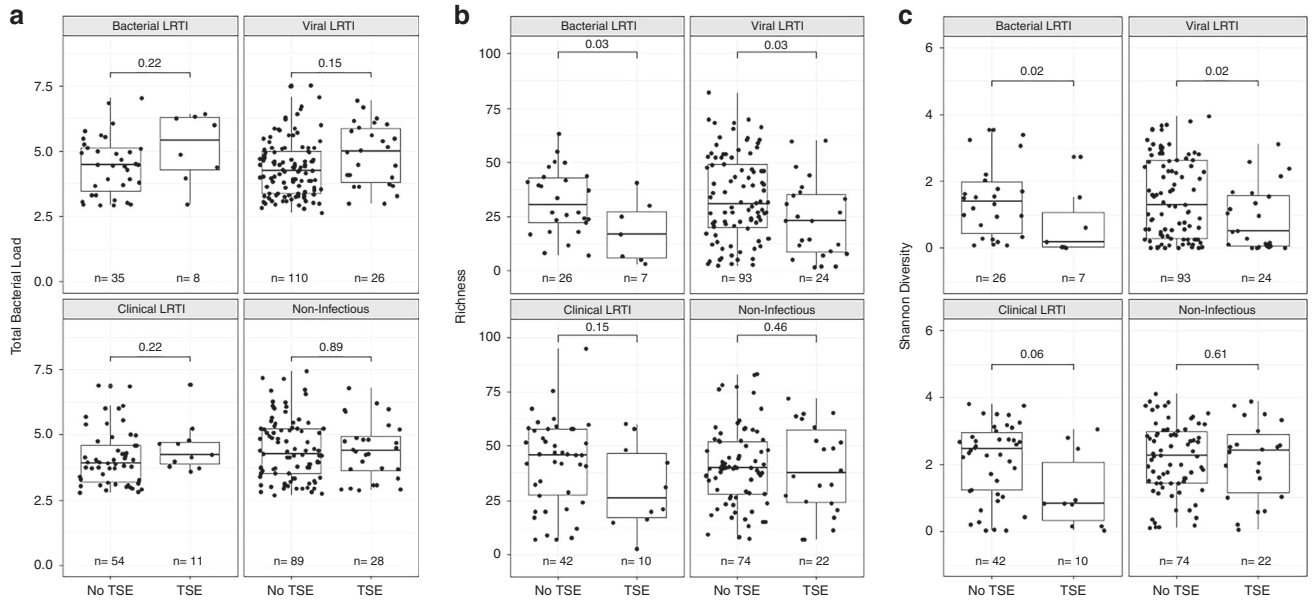

Fig. 3 The impact of TSE on the lower airways microbiome is dependent on etiology of respiratory failure. Box plots comparing total bacterial load (**a**), Richness (**b**), and Shannon diversity (**c**) between patients with bacterial LRTI, viral LRTI, clinically diagnosed LRTI, and non-infectious diagnosis based on TSE status in each group using binary comparison of urine cotinine level ≥ 30 µg/g creatinine. Numbers of patients included in each comparison are listed below each box plot. LRTI lower respiratory tract infection, TSE tobacco smoke exposure.

The most accurate way to determine TSE in children remains unclear. In our cohort, 38% of parents reported that their child had some degree of TSE. However, using a creatinine corrected urine cotinine cutoff of ≥30 µg/g, only 20% of our cohort met the criteria for TSE. This discrepancy may be related to the interval time difference between the patient's last TSE and the urine collection for cotinine analysis. The average time to urine collection was 42.72 h after admission to the hospital and the half-life of urine cotinine is 16–24 h.[26] It is, therefore, likely that the levels of cotinine were underestimating the exposure level in some smoke-exposed patients and may have contributed to misclassification. However, 24 children whose parents reported no history of TSE, did in fact, have creatinine corrected cotinine levels ≥30 µg/g. The discrepancy between parental reporting and urine cotinine measurements are in concordance with studies that have demonstrated that parental report is not sufficient in determining TSE.[38,39]

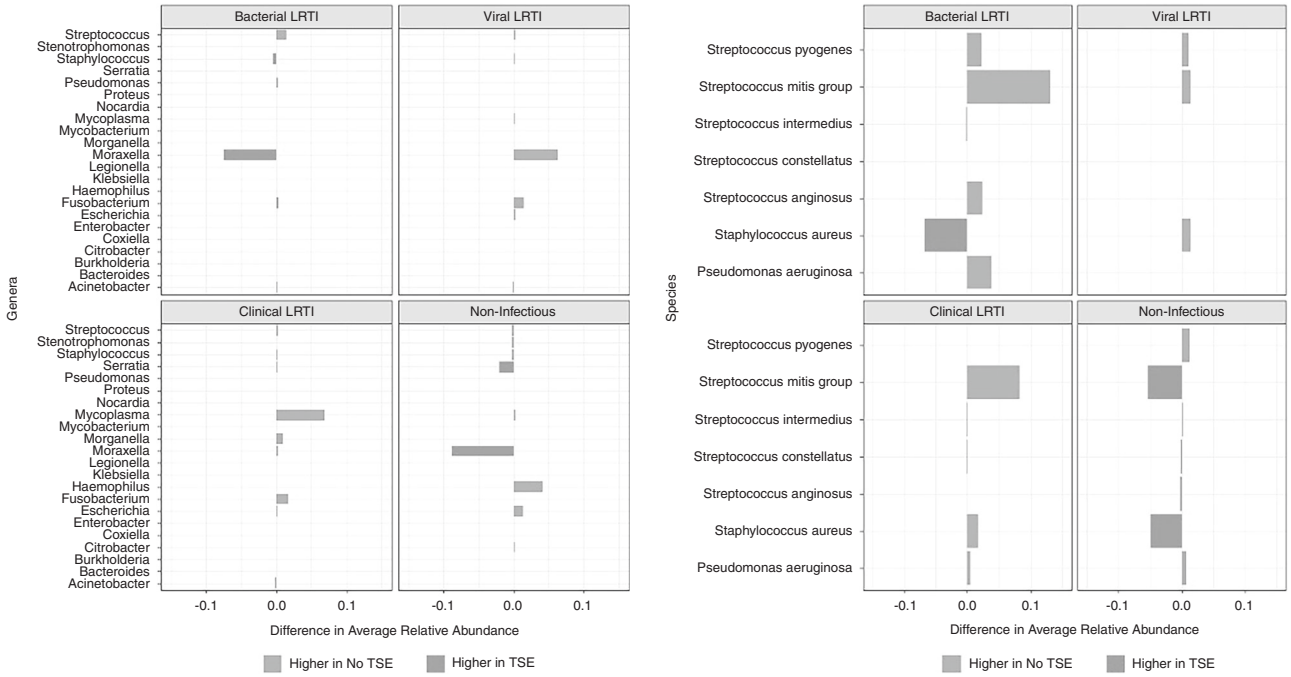

Fig. 4 TSE has different effects on relative abundance of lower airways bacteria based on etiology of respiratory failure. Comparison of average differences in relative abundance of bacterial genera (left) and species (right) between patients with tobacco smoke exposure and those without based on etiology of respiratory failure.

Table 3. Clinical outcomes.

Clinical outcome	Cotinine analysis	Effect estimate (95% CI)	*P*-value
Diagnosis of Acute Respiratory Distress Syndrome	Binary	0.71 (0.38, 1.30)	0.65
	Continuous	0.98 (0.90, 1.07)	0.77
Duration of Invasive Mechanical Ventilation	Binary	0.95 (0.81, 1.12)	0.77
	Continuous	0.98 (0.96, 1.00)	0.20
Ventilator Free Days to Day 28	Binary	2.67 (0.30, 23.50)	0.53
	Continuous	1.27 (0.91, 1.75)	0.41
PICU Length of Stay	Binary	0.92 (0.78, 1.09)	0.55
	Continuous	0.97 (0.95, 1.01)	0.06
Hospital Length of Stay	Binary	0.95 (0.79, 1.13)	0.58
	Continuous	0.97 (0.94, 1.01)	0.05
Mortality	Binary	2.83 (0.65, 12.36)	0.26
	Continuous	1.51 (0.98, 1.36)	0.16

Continuous variables have been log transformed. *PICU* Pediatric Intensive Care Unit.

The results of this study are strengthened by the prospective, multi-center design. However, there are also several limitations of our study. First, patients in our study were intubated greater than 72 h, making the results less generalizable to less severely ill populations. Second, while we attempted to correct for time of collection (from time of hospital admission) in our continuous urine cotinine analysis, it may not have adequately corrected for the period from the last exposure, and we may have underestimated the number of patients who were smoke exposed. Thirdly, the TSE group was significantly younger and had significant differences in ethnicity. These findings are consistent with what has been found on the population level in the United States.[3] It is difficult, however, to know whether or not these differences contributed to the findings of this study. Fourth, there were significant differences between the two groups among those who received antibiotics prior to hospitalization. However, nearly all of the patients enrolled in this study received antibiotics at the time of intubation and a single dose of antibiotics has been shown to alter the microbiome of other body compartments and may impact our ability to evaluate the lower airways microbiome via tracheal aspirates.[40,41] Fifth, tracheal aspirates specimens may not adequately reflect lower lung bacteria. However, other methods such bronchiolar alveolar lavage are not frequently performed in children. Finally, this was an ancillary study that may have not been adequately powered to effectively evaluate differences between groups.

CONCLUSION

In conclusion, among critically ill children admitted to the PICU requiring prolonged mechanical ventilation, creatinine corrected urine cotinine levels were associated with increased total bacterial

load, decreased richness and Shannon diversity of the lower airway microbiome, especially among patients with bacterial and viral LRTI. Specifically, patients with TSE had higher relative abundance of *Haemophilus* spp. *Moraxella* spp., and *S. aureus*. There were no identified associations between TSE and clinical outcomes. Additional prospective studies will be required to evaluate the implications of TSE on the severity and outcomes of critically ill children and whether the alteration of the airway microbiome could be a possible mechanism by which TSE conveys increased risk of poor outcomes. Future studies should focus on evaluating TSE earlier in the course of illness and obtaining airway microbiome specimens prior to antibiotic administration and in conjunction with measures of the host response to better understand the impact of TSE on this patient population.

DATA AVAILABILITY

Deidentified data is publicly available at: https://hheardatacenter.mssm.edu/Search/Study. Microbiome data are available via: PRJNA533819 (data generated from methods development PRJNA436139 were also used for this study).

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AUTHOR CONTRIBUTIONS

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CONSENT TO PUBLISH/PARTICIPATE

The study was approved by the University of Utah central Institutional Review Board and consent was obtained from all patients.

ADDITIONAL INFORMATION

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