Unmasking the Contribution of Oral Flora to the Biofilm Accretion-Endotrach Microbiota during Mechanical Ventilation using 16S Microarray Detection

Christopher Waters, Hamed M. Mollah, Alison Wilson, Bruce Paster, John G. Thomas

1 West Virginia University, Health Sciences Center, School of Medicine, Department of Pathology, Morgantown, WV; 2 West Virginia University Hospitals, Morgantown, WV; 3 Forsythe Institute, Cambridge, MA.

INTRODUCTION:
As part of our continuing studies addressing the oral-systemic link (OSL), we previously identified a rich dental microbiota, particularly dental (Red Complex) pathogens Streptococcus and P. gingivalis (using PCR) in the humoral endotracheal tube (ETT) biofilm accretion materials of mechanically ventilated (MV) ICU patients.

HYPOTHESIS:
Here, as part of a 40 patient clinical pilot study to evaluate an optically directed ETT luminal clearing device, we incorporated the use of a commercial 16S microarray (Fig. 1) assay to focus on oral-dental flora; our aim was to 1) enumerate and 2) quantify the microbiota, comparing Pre and Post-clearing of 20 extubated ETTs.

METHOD:
1) Extubated ETTs from WVUH ICU were received in two hours from 20 adult patients, ages 18-92 (Mean 51.7), averaging 3.2 days (Range 1-14) of MV with multiple comorbidities included in the IRB approved study. 2) The optically directed clearing device, (Fig. 2a) composed of four linked units, was used to remove, in approximately 10 seconds, lumen biofilm (BF) accretion in an ETT positioned in an anatomically accurate head design, head position at 30°, lung to oral end. 3) Pre-Clearing (Fig. 2b) Biofilm accretions were characterized including volume, viscosity, color, Gram Stain, and H/E. Pre-clearing 4 mL of fluid was added, the ETT vortexed, and material collected. Both Pre and Post accretion samples were stabilized using the SalivaGene Collection Module Stabilization Set. DNA extraction was sent to Forsythe Institute for 16S rRNA Gene Sequencing using Microarray.

RESULTS:
For the 20 patients, 40 samples (Pre and Post) revealed a total of 101 difference bacterial species, predominantly oral Streptococci with 87 in the Pre in the Post 48 in the Asco. 4 subject (8 samples) were totally negative. 11 subject (22 samples) had detectable species in both samples, while 5 subjects (10 samples) had detectable species in either/or Pre-Post, enormous diversity and unpredictable.

In comparing Pre vs. Post species signature of top 10 detected, the major majority the same where the same different order. The 10 species related to the same Streptococcus cluster Cl (96%), Lactobacillus cluster 1 (45%), and Streptococcus anginosus and intermedius (45%). Proportion of isolates ranged from 0 to 5%. No traditional VAP associated isolates were detected.

CONCLUSION:
Oral flora predominated, primarily Streptococcus in ETT accretion, and highlights 1) the unrecognized pathophysiology of endogenous microbes as primary ETT colonizers and 2) the importance of oral care during MV as a preventative strategy. Further, oral flora is incredibly adherent, remaining as a residual coating on the ETT lumen, although >90-99% was removed via our IVIS study. (Poster ASM 752/053)

DISCUSSION:
Previous Results indicated oral colonization occurs in a bi-phasic cascade, Gram + to Gram -, endogenous (oral) to exogenous (environment). These results support those preliminary findings and suggests at -5 days, a major break point. No traditional VAP pathogens were recovered attributable to short LOS.

CLINICAL APPLICATIONS:
VAP is today called Air Way Disease, linking oral flora, to ETT colonization to lung infection. The use of a non-antibiotic strategy to reduce the ETT bio-burden with routine frequent use of a unique clearing device warrants further immediate clinical evaluation. The Post frequency emphasises the need for clearing at twice daily.

REFERENCES:
3. David Wilkins et al., The Role of the Human Oral Microflora in VAP (2011 annual Clinical 7 of Dental Health 1:17

SUPPORT
West Virginia University, Department of Surgery, EndOClear, LLC, CA IRB Approved Grant 1001497.5