

IDENTIFICATION OF A CORE MICROBIOME OF ENDOTRACHEAL TUBES ASSOCIATED WITH *PSEUDOMONAS AERUGINOSA* AND/OR *STAPHYLOCOCCUS EPIDERMIDIS*

A. Hotterbeekx¹, B.B. Xavier¹, P. Moons¹, C. Lammens¹, M. Ieven¹, P. Jorens², E. Vandebroek³, S. Kumar-Singh⁴, H. Goossens¹, S. Malhotra-Kumar¹

¹Medical microbiology, University of Antwerp, Wilrijk, Belgium ; ²Intensive care, University of Antwerp, Wilrijk, Belgium ; ³Intensive care, University Hospital of Antwerp, Wilrijk, Belgium ; ⁴Cell and tissue, University of Antwerp, Wilrijk, Belgium

Objectives: Ventilator associated pneumonia (VAP) is a common complication in mechanically ventilated patients. The microorganisms present on the endotracheal tube (ET) are thought to play a key role in the onset of VAP but the exact mechanism is not yet understood. This study aims to identify the core microbiomes present on ET from ICU patients associated with the pathogens *Pseudomonas aeruginosa* and/or *Staphylococcus epidermidis* as microbial interactions might be involved.

Methods: ET were collected, upon extubation, from mechanically ventilated patients in the ICU of the University Hospital Antwerp. One part was cultured on Blood agar at 37°C for 2 days with 5% CO₂ and obtained colonies were identified by MALDI-TOF. Thirty-nine ET, culture positive for *P. aeruginosa* and/or *S. epidermidis* were selected for further analysis. Prior to DNA extraction, ET adherent material was released and disrupted by sonication and vigorous vortexing. DNA extraction was performed using the Masterpure complete DNA and RNA purification kit (Epicentre). Bacterial DNA was amplified by 16S PCR targeting the V3-V5 region followed by sequencing. Preprocessing and data-analysis was done using the MG-RAST V3.1 service integrating Greengenes, RDP and SILVA databases with a minimum cutoff value of 97% identity. To identify the microbiomes associated with either *P. aeruginosa* or *S. epidermidis*, the data were divided into three groups based on positive culture results for one or both of the target species. Families present on more than 50% of the ET in a group were assigned to the microbiome of that particular group.

Results: On average, patient age was 61 years and intubation length 13 days. To identify the bacterial families specifically associated with the presence of *P. aeruginosa* (group 1), *S. epidermidis* (group 2) or both (group 3), the data were divided in three groups as shown in Fig 1. *Enterobacteriaceae* and *Phyllobacteriaceae* were identified on over 80% of all ET and are considered the core ET microbiome. *Rickettsiaceae* are less prevalent in groups 2 and 3 (10-20% respectively) while high numbers (77%) are found in group 1. The *Corynebacteriaceae* and *Actinomycetaceae* are less frequently found in groups 1 and 3 (average 20-30%) compared to group 2 (81- 52% respectively). *Micrococcaceae* and *Propionibacteriaceae* were found in group 2 as well as group 3 (80-55% respectively) but were present in <10% ET in group 1 while *Coriobacteriaceae* and *Lactobacillaceae* were only found frequently in group 3 (60-80% respectively) but were only present on average 10-30% in group 1 or 2.

Conclusion: These results suggest that particular pathogens are associated with certain bacterial families. The organisms identified in this study will provide a basis for future research to study microbial interactions within the ET microbiome and the potential role in the development of VAP.

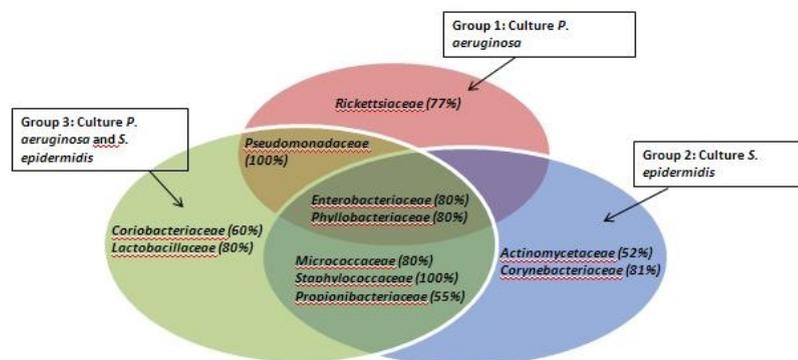


Fig 1: To distinguish the core microbiomes associated with the key pathogens *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, ET tubes with positive culture results for either one (group 1 and 2) or both (group 3) species were selected as shown in Fig 1. Families present in more than 50% of the ET tubes in a certain group were assigned to the microbiome of that group.