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LETTER

Microbiota in ICU, not only a gut problem

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Dear Editor,

The editorial by Ruppé et al. [1] related to the article by Freedberg et al. [2] about gut microbiota in ICU patients describing the association between *Enterococcus* dominance in the gut at ICU admission and death was very interesting. This paper provides a useful insight for future research on gut microbiota in critically ill patients. Dysbiosis can be a trigger to promote many nosocomial infections [3] because of lack of equilibrium among bacteria species.

Given this finding, we deem that, in ICU setting, studies should not be limited to gut microbiota, but it could be crucial to investigate even lung microbiota considering the potential application in mechanically ventilated patients.

The role of lung microbiota in ICU mechanically ventilated patients is still underexplored. In fact, its potential to predict and to prevent infections in a clinical condition at high risk of complications related to mechanical ventilation (MV) such as ventilator-associated pneumonia (VAP) is unknown. Few data are available on lung microbiota during MV and on pathogens which eventually colonize the endotracheal tube (ETT) and cause VAP.

ETT is an interface between patient and ventilator, which can modify the oral microbiota composition, and can be colonized by commensal oral or respiratory bacteria. These bacteria can translocate to the lower airways and cause a high increase in the likelihood of VAP insurgence.

As described on Table 1 (part A), some studies have analyzed the colonization of dental plaque performed by standard culture techniques showing a change in microbial composition of the oral cavity and an incorporation of at least one respiratory pathogen potentially responsible for VAP (*Staphylococcus aureus* and *P. aeruginosa*). Other studies have analyzed the microbiota of dental plaque or the ETT directly, confirming a microbial shift in colonization with a predominance of non-oral bacteria during a different stage of intubation. In addition, the detection of some bacteria (i.e. *Pseudomonas* or *S. aureus*) in ETT correlate with worse outcome (see table for references).

Few preliminary studies of lung microbiota during MV are available (Table 1, part B). The studies showed a reduction in number and abundance of species during MV. The correlation between modification in microbiota composition and VAP development is not conclusive. Lu et al. [4] described a difference in constituent ratio of some bacteria (*Klebsiella* sp., *Acinetobacter* sp., *Streptococcus* sp.) among patients with VAP compared with no VAP patients. Moreover, Zakharkina et al. [5] observed that patients with VAP have more profound dysbiosis

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Table 1 Data on oral and lung microbiota among mechanically ventilated patients

References	Objective	*n*	Timing sample collection	Culture/molecular analysis	Results/conclusions
Part A: analysis of oral microbiota					
Sands et al. (2016)	To analyse microbiome of dental plaque in patients undergoing mechanical ventilation (MV)	13 patients 38 dental plaques	Intubation start Midpoint intubation End intubation or admission to ward	16S rRNA gene PCR bacterial primers of dental plaque	1. 40 genera were identified, 6 species are not members of dental plaque community including *Enterococcus*, and *Staphylococcus* spp. 2. Microbial shift in the composition of dental plaque is evident between stages of MV with an increment for all non oral bacteria (*E. coli/Shigella flexneri*, *S. aureus*, *S. pseudo-pneumoniae*) 3. Dental plaque composition further change following extubation, even if some microbial species can still be identified
Sands et al. (2017)	To assess colonization of *P. aeruginosa* and *S. aureus* within dental plaque To detect isolation in LRT during and after MV	107 patients, 848 dental plaque sample (592 during MV)	Plaques samples: in 24 h from MV 3 times in the first week and then weekly Subglottic aspiration, (BAL, NBL, TA) based con clinical judgement	Standard culture and PCR for DNA analysis for detection for *S. aureus and P. aeruginosa*	1. 43 patients were colonized by *S. aureus* 23 patients were colonized by *P. aeruginosa* 10 patients had a co-isolation *S. aureus* and *P. aeruginosa* 35 patients (33%) had a change in microbial composition with incorporation with at least one targeted pathogen 2. VAP occurred in 41/107 patients 18 patients had *S. aureus* and *P. aeruginosa* within dental plaque during MV, 24 patients had the same respiratory pathogen in dental plaque and in the lower airways. 3. Colonization of S. aureus returned after extubation
Hotterbeekx et al. (2016)	To study organisms and species diversity in ETT To determine the core microbiomes on the ETTs which were culture positive for the key organism *P. aeruginosa* and *S. epidermidis* To link this findings to patients parameters	203 ETT collected each for patients.	During MV (not specified)	16SrRNA analysis on ETT noted that it was colonized with *P. aeruginosa* and *S. epidermidis* on standard culture methods	44 out of 203 patients developed VAP 1. Presence of visible mass in the ETT lumen do not correlate with development of VAP for patient survival 2. Relative abundance of *Pseudomonaceae* and *Staphylococcaceae on ETT* correlated to change of patient survival 3. *Actynomyces* and *Corynebacterium* were more identified in ETT of surviving patients. *Bifidobacterium adolescentis* was completed absent among non survivors 4. *Pseudomonas* spp. correlated to worse patient outcome.
Souza et al. (2017)	To explore possible associations between respiratory pathogens from tracheal aspirate and oral biofilm To identify the most common respiratory pathogens present in the oral biofilm in VAP patients	32 patients	Oral sample at time of the admission and after 48 h, tracheal sample (timing unknown)	Culture analysis of oral biofilm and tracheal aspirate	1. Bacterial presented in tracheal aspirates were also detected in oral sample especially in patients with VAP diagnosis 2. Change in oral flora between admission and 48 h after admission determined a reduction of species number

Table 1 (continued)

References	Objective	*n*	Timing sample collection	Culture/molecular analysis	Results/conclusions
Part B analysis of lung microbiota					
Lu et al. (2014) [4]	To characterize the microflora during VAP	25 infants	Specimen collected and analyzed at the 1, 3, 5 days of ventilation	16S rRNA analysis on sputum specimen	1. Flora composition between VAP and no VAP group No differences within 1 day of intubation From 1 to 3 days: constituent ratio of *Klebsiella* sp., *Acinetobacter* sp., *Streptococcus* sp. in the VAP group was higher and the ratio of *Serratia* and *Achromobacter* sp. were lower From 3 to 5 days *Streptococcus* sp. was higher in the VAP 2. Analysis of diversity between VAP and no VAP group Richness of the Non VAP group was higher than that of VAP group between 1 and 3 days after intubation no differences were observed between 3 and 5 days
Toma et al. (2014)	To characterize the repertoire of pulmonary bacteria in intubated patients with possible VAP	61 patients which performed tracheal aspirate based on clinical indication; 44 samples have a DNA available, and 27 samples produced sufficient PCR amplimers for NGS	One timing each patients	16S rRNA analysis in tracheal aspirate of MV patients	1. All the 27 samples identified bacterial species; culture results were negative in 20 samples. 2. Microbiological and NGS identification of bacteria coincided in 17 (85%) samples
Kelly et al. Microbiome (2016)	To define the dynamic of the respiratory microbiome during MV To identify features of bacterial community structure associated with LTRI To assess the correlation between LTRI by culture and NGS sequencing To detect dominant bacterial species not recognized by cultures	15 patients with respiratory failure intubated vs healthy subjects	At intubation and each 48-72 h during the duration of MV	16S rRNA analysis of endotracheal aspirate sample and oropharyngeal secretion	1. Endotracheal and oral samples had a lower diversity of flora composition than healthy controls at baseline and during mechanical ventilation time 2. Patients with lower respiratory tract infection had a low community diversity and dominance of a single taxon 3. In 13 patients bacterial cultures resulted as positive with concordance of the OTU dominance 4. In 2 cases NSG detected 2 pathogens responsible of infections normally not found in LRTI
Smith et al. (2016)	To evaluate performance of massive parallel sequencing (MPS) in examining culture negative BAL	15 patients mechanically ventilated for surgical treatment	Clinical requirement	16S rRNA analysis of bronchoalveolar lavage	1. High diversity of bacteria and low rate of abundance were shown 2. Role of antibiotics in dynamics of microbiota evolution were not conclusive

Table 1 (continued)

References	Objective	*n*	Timing sample collection	Culture/molecular analysis	Results/conclusions
Zakharkina et al. (2017) [5]	To analyze lung microbiota in MV patients	35 patients, 18 non infected, 17 with pneumonia	At intubation and each 24 h	16S rRNA analysis of bronchoalveolar lavage	1. During mechanical ventilation there was a decrease of diversity of lung microbiota (Shannon index decrease on 83% of patients) Duration of mechanical ventilation was associated with a decrease in diversity measured by Shannon index) Antibiotic exposure was not associated with a decrease in diversity 2. Dysbiosis (measured as weightened UniFrac distance) in patients with VAP was more profound .than in patients who did not develop pneumonia
Qi et al. (2018)	To analyse LRT microbiota in *P.aeruginosa* VAP To evaluate relationship between LRT microbial characteristics and patients prognosis	36 patients with *P. aeruginosa* VAP 18 controls patients intubated	At diagnosis of *P. aeruginosa* VAP then at day 7 and day 14	16S rRNA analysis of TA	Microbiota composition of LRT in patients with *P. aeruginosa* infections was significantly different from that the control group (unweighted frac distance R^2 0.18431, $p = 0.001$): Diversity (Shannon index) was lower in *P. aeruginosa* VAP At phylum level Proteobacteria was significantly increased in *P. aeruginosa* VAP Among patients with *P. aeruginosa* VAP two cluster were identified: Pro cluster (high abudance of *Proteobacteria)* and Fir-Bac cluster (high abudance of Firmicutes and Bacteroidetes) Pro-cluster prophyle was associated with baseline gastrointestinal disease; Fir-Bac cluster is associated with baseline lung disease Antibiotic exposure did not influence the type of cluster Some genera were associated with severity of VAP, as Burkholderia, Alcaligenes, Pseudomonas, Massilia, Flavobacterium and Enterobacter Microbiota composition was not associated with clinical outcome

Table 1 (continued)

Relevant findings of lung microbiota analysis:
1. Mechanical ventilation is associated with decrease in diversity and abundance of lung microbiota composition
2. VAP is associated with additional depression of diversity and abundance species
3. Lung microbiota composition is associated with disease severity
4. Role of antibiotics in microbiota changes and its relationship in pathogenesis of VAP need to be investigated
5. No data on association of lung microbiota and risk of VAP development are present
MV mechanical ventilation, *VAP* ventilator associated pneumonia, *BAL* bronchoalveolar lavage, *NBL* non directed bronchoalveolar lavage, *TA* Tracheal aspirate, *ETT* endotracheal tube, *LTRI* lower respiratory tract infections, *NGS* next generation sequences, *OTU* operational taxonomic unit, *MPS* massive parallel sequencing, *LRT* lower respiratory tract
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that controls patients. Exposure of antibiotics is not associated with changes in microbiota composition, but these data need to be confirmed,

This conclusion opens a large field of investigation addressed to understand how lung microbiota characteristic can predispose and correlate to VAP insurgence.

Further studies aimed at analyzing the change of lung microbiota during MV and to evaluate characteristics associated with VAP development are needed.

We think that the identification of basal risk factors associated with a higher likelihood ratio to develop VAP could be useful to implement measures of prevention and diagnosis. Studies on microbiota in ICU will be a challenge to improve patient management.

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Compliance with ethical standards

Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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