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Review

Reconsidering ventilator-associated pneumonia from a new dimension of the lung microbiome

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ABSTRACT

Complex microbial communities that reside in the lungs, skin and gut are now appreciated for their role in maintaining organ, tissue and immune homoeostasis. As lungs are currently seen as an ecosystem, the shift in paradigm calls for the consideration of new algorithms related to lung ecology in pulmonology. Evidence of lung microbiota does not solely challenge the traditional physiopathology of ventilator-associated pneumonia (VAP); indeed, it also reinforces the need to include molecular techniques in VAP diagnosis and accelerate the use of immunomodulatory drugs, including corticosteroids, and other supplements such as probiotics for VAP prevention and/or treatment.

With that stated, both microbiome and virome, including phageome, can lead to new opportunities in further understanding the relationship between health and dysbiosis in VAP. Previous knowledge may be, however, reconsidered at a microbiome scale.

1. The microbiome generation

Microbiome analysis determines the composition and function of a microorganism community in a particular location. Many human biological processes are associated with microorganisms and their functional products.

The microbiome consists of an ecological community of commensal, symbiotic and pathogenic microorganisms inhabiting the human body. Known as "dysbiosis", changes occurring in the microbiome are deeply involved in a patient's health condition.

The human body is estimated to comprise eukaryotic cells and colonizing microorganisms in a 1:1 ratio such that host cells and microbiota are of nearly the same number in an individual [1]. The highest concentration of microbes is present in the gut, skin and oral cavity.

Additionally, the microbiome in humans does not remain constant throughout life; rather, it changes as a person ages. Culture and geographic location will impact the microbiome, as well as an individual's health status [2].

These complex microbial communities inhabiting environments such as the lungs, skin or gut are now appreciated for their role in maintaining organ, tissue and immune homoeostasis. A striking example is that of germ-free mice: early observations have found that these study subjects have absent/impaired secondary lymphoid architecture with a resulting loss of lymphoid cells [3].

Lastly, commensal microbiota can have both systemic and site-specific, autonomous immune effects. For example, *Staphylococcus epidermidis* colonization of the skin promotes IFN-γ production from CD4 T cells, protecting against infections caused by the parasite *Leishmania major*. In contrast, *S. epidermidis* colonization of the gut had no effect [4]. In any other situation, it has been well established that alterations of the gut microbiome can influence immune responses at distal sites.

2. Towards a new conceptual framework of VAP

Evidence of lung microbiota challenges the traditional physiopathology of ventilator-associated pneumonia (VAP). Indeed, it strengthens the need to include molecular techniques in VAP diagnosis and follow-up, and accelerates the search for alternative VAP therapies like immunomodulatory drugs or probiotics [5-7]. Until now, lung microbiome studies during VAP have reinforced previous findings of negative consequences occurring in VAP prognosis as a result of gut microorganisms being present in lung respiratory

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specimens. All of this thus leads to the important question of how to define the value of lung microbiota knowledge, especially as it relates to VAP prevention, diagnosis, treatment and prognosis, so as to reshape traditional concepts and improve clinical management of patients accordingly.

Disruption of lung microbiota homoeostasis occurs during VAP; its association to the disease remains an expanding field of investigation [8]. From a microbiome perspective, the respiratory system is an ecosystem and new algorithms regarding lung ecology are thus needed for consideration in pulmonology.

2.1. Definition of dysbiosis

Considered sterile for a considerable time, the lungs are inhabited by the respiratory microbiome shown with culture-independent techniques (Fig. 1A). When any change arises in the composition of resident commensal microbial communities relative to the community found in healthy individuals, it is known as dysbiosis. Given the emerging importance of the microbiota in host development, these observed changes in microbial composition have been recently shown to be contributing factors to the onset and/or persistence of many diseases.

2.2. Airways' microbiota dysbiosis in VAP

The alpha-diversity of lung microbiota has been recently suggested to infer critically ill patients' health status. In a study by Zakarkhina et al., when comparing diversity at intubation with that at extubation or VAP onset using 16S rRNA gene sequencing, authors identified a significant decrease in ETA microbial alpha diversity associated to duration of mechanical ventilation [9]. There is substantial evidence supporting that alpha diversity is different between positive and negative culture samples. Additionally, two samples with similarly low alpha diversity can differ highly in beta diversity [10, 11].

Several findings support that beta diversity also plays a role in VAP [9, 12-15]. For instance, Woo S et al. found different composition of predominant respiratory microbiota between pneumonia and non-pneumonia groups (*Pseudomonas, Corynebacterium* and *Rothia* versus *Streptococcus* and *Prevotella,* respectively) [12]. Similarly, Emonet S. et al. found that *Gammaproteobacteria* (Gram-negative bacteria) at Day 3 of VAP were significantly more abundant in ETA from patients with VAP, whereas the absolute abundance of *Streptococcus, Enterococcus, Lactobacillus* and *Staphylococcus* was significantly higher in ETA retrieved from controls at ICU admission [15]. Lastly, the respiratory microbiota of patients with SARS-CoV-2 has been described as pathogen-enriched. This is similar to that observed in patients with community-acquired pneumonia, when compared to a control group of healthy patients who presented with commensal-enriched microbiota [16].

Some of these investigations have identified new potential markers of poor prognosis in ICU patients [9]. For example, a shift towards an *Acinetobacter*-dominant microbiome in patients with COPD was associated with weaning failure, suggesting that the bacterial community structure can impact weaning outcomes [17]. Additionally, Dickson et al. found that gut-associated bacteria (*Pasteurellaceae* spp. and *Enterobacteriaceae* spp.) were associated with acute respiratory distress syndrome (ARDS) and bacterial lung DNA burden predicted fewer ventilator-free days) [18, 19]. More recently, Sommerstein et al. dispelled the idea that the presence of *Enterobacteriaceae* in the oropharynx during the first 5 days of mechanical ventilation was later associated to *Enterobacteriaceae* VAP [13]. Metataxonomic analysis indicated that a low abundance of *Streptococcus* at the time of intubation may be associated with 28−day mortality with 86% sensitivity and 63% specificity [12]. Others found that the quantity of human DNA in ETA the day of VAP diagnosis predicted VAP with high sensitivity (94%) and specificity (83%) [15].

The overall findings support that bacterial diversity decreases within the context of mechanical ventilation. However, pre-existing dysbiosis at the beginning of mechanical ventilation or ICU stay can influence the loss of diversity as well [12, 13, 19]. A positive feedback loop of inflammation and dysbiosis then exists in critically ill patients. In such scenario, both normalizing respiratory microbiota diversity and restoring mucosal immunity could improve clinical management of VAP considerably [20, 21].

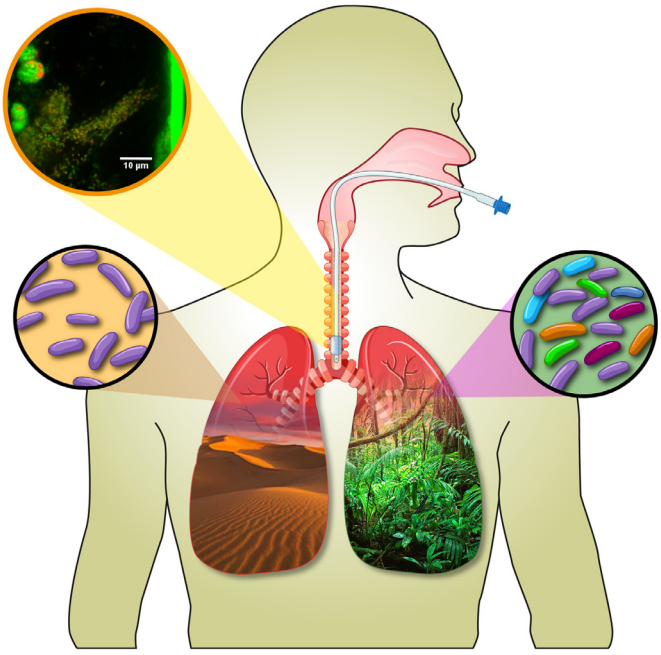

Fig. 1. a. Traditional versus The New Paradigm of Ventilator-associated Pneumonia (VAP).

The figure illustrates a traditional versus new conceptual framework for VAP.

A close-up look of the inner surface of the endotracheal tube shows an image taken of *Pseudomonas aeruginosa* in vivo biofilm with a confocal laser scanning microscopy. In the image, after staining with the LIVE/DEAD™ BacLight™ bacterial viability Kit (ThermoFisher scientific), *Pseudomonas* fluoresces in green when bacilli were alive and in red, when dead. The ETT biofilm, traditionally considered as a potential source of pathogens within the context of VAP, becomes a new ecosystem, influencing lung ecology during mechanical ventilation at a microbiome scale.

On the left, the lung with a desert alludes to the former concept of lung sterility, in which pathogens (in purple) were seen as the unique bacterial species present in the lungs of mechanically-ventilated (MV) patients. In this view, appropriate antimicrobial therapy had no other microbiological effects in lung than local pathogen clearance and potential emergence of antimicrobial resistance.

In contrast, the lung comprising a jungle alludes to the new concept of the lung microbiome, in which pathogens (in purple) are not the only bacterial species. Rather, an autochthonous community of microorganisms (in other colors, not purple) is present in the lungs of MV patients. The pathogen thus competes with endogenous microbiota and the immune system to colonize the lung niche. In this view, narrow-spectrum antimicrobial therapy could achieve pathogen clearance but possibly at the cost of weakening endogenous flora and directly or indirectly contributing to the emergence of antimicrobial resistance with potential mid to long term consequences.

Fig. 1b. Diagram shows infection progression comparing the traditional versus new model.

In the left lung (traditional model), infection progression is shown by a gradual increase in pathogen (in purple) counts. This model is essentialy focused on pathogen progression within the lung. In the right lung (new model), infection progression is linked to the concept of dysbiosis in the lung and in other body compartments, such as the oropharynx and gut. In this new scenario, antibiotic therapy and other ICU factors can contribute to infection progression by enhancing dysbiosis. Also, as mentioned throughout the review, such dysbiosis unifies the microbiological signature of different compartments and results in similarities in the composition of dominant taxa.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

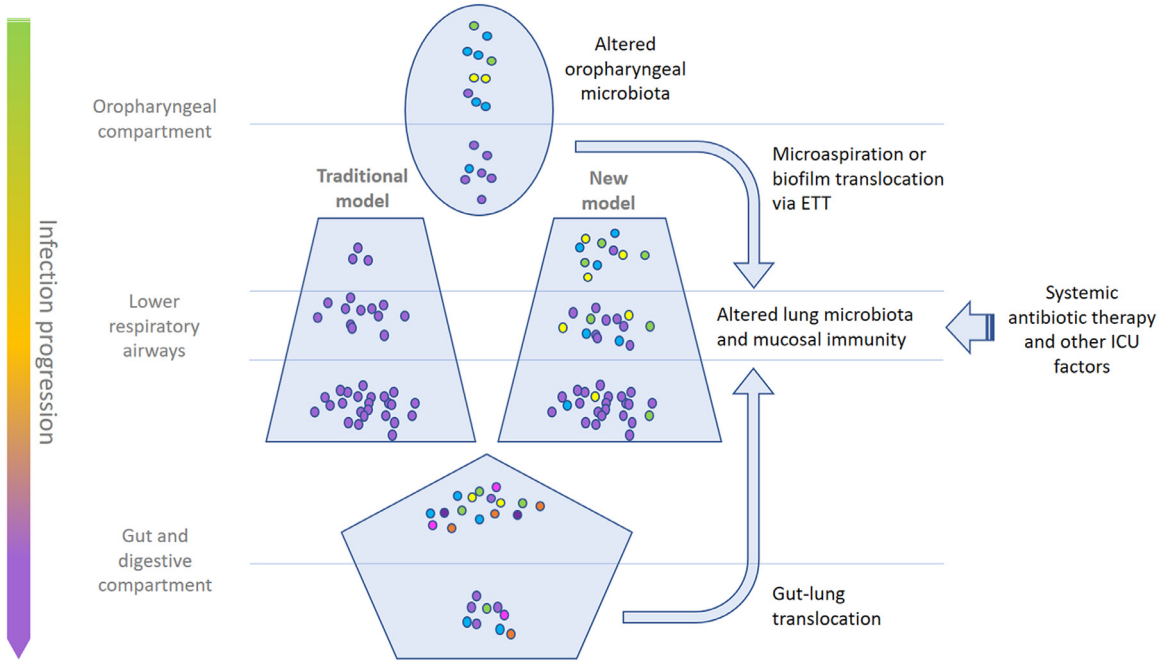

Fig. 1 Continued.

Lastly, shifting focus from bacterial contamination in the airways to the new framework of microbiota dysbiosis (Fig. 1B) will allow critically ill patients to be stratified and targeted with different, more personalized therapeutic strategies. The optimization of such treatments, as well as the deterrent to the emergence of antimicrobial resistance could result in improvements in patient outcomes.

2.3. Orotracheal intubation: a microbiome dysbiosis promoter

Orotracheal intubation impairs natural lung defense mechanisms, e.g. coughing and mucus clearance, and connects the oropharynx with the lung ecosystem, which is usually separated by the glottis and larynx [22]. In addition, by allowing microorganisms to form biofilms known to be tolerant to antibiotics and the immune system, ETTs may alter the abundance and composition of lung microbiota [23, 24](Fig. 1A).

Although microflora in ETT biofilms is far more complex when compared to previous cultures, findings have demonstrated that nosocomial pathogens compose microbial dominance in ETT biofilms [25]. By using bacterial 16S rRNA and fungal ITS-II sequencing on 203 ETT biomass, Hotterbeekx et al. found that 89% of ETT phyla belonged to the family *Proteobateriaceae*; 86%, *Phylobacteriaceae* and 77%, *Enterobacteriaceae* [26]. It is noteworthy to highlight that when the relative abundance of *Pseudomonadaceae* and *Staphylococcaceae* was <4.6% and <70.8%, respectively, ventilated intensive care patients had the highest chance of survival. In contrast, discriminative analysis showed that *Actinomyces, Corynebacterium* or *Bifidobacterium adolescentis* were more frequent or exclusively found, respectively, in the ETTs of survivors [26]. However, as ETT-biofilm will be analyzed at patient's extubation, causal associations between ETT-biofilm and VAP can only be done at VAP diagnosis by obtaining samples from within the ETT.

3. Involvement of microbiome in VAP diagnosis

VAP continues to be diagnosed, prevented and treated following the traditional physiopathologic concept of bacterial contamination of sterile airways. However, given the limitations in culture-dependent methods for VAP diagnosis, multiplex PCR arrays and metagenomics may prove as promising diagnostic tools.

For instance, metagenomics of 16S can bypass consequences caused by cultures that falsely test negative due to empirical antibiotic therapy. Such treatment is often administered to intubated patients before cultures are obtained. Several studies have revealed a dominance of unexpected bacteria not typically identified by culture or considered respiratory pathogens with 16S rRNA gene sequencing.

Semi-quantitative cultures may be more imprecise than DNA copies achieved by culture-independent methods, even though the latter must standardize differentiation between carriage and infection. Sequencing analyses of 16S affords a representative overview of both pathogens and other concomitant microbiota. As such, synergistic associations amongst microorganisms can be studied. Additionally, as clinicians can have the whole microbiological picture of antimicrobial therapy consequences, antimicrobial therapy may be optimized.

A significant challenge in next-generation sequencing is that result interpretation requires time and a highly trained skillset. Similarly, next-generation sequencing has been associated with an increase in gross costs. Availability of friendly high-throughput data analysis tools for rapid diagnosis will therefore be crucial for clinical practice [27]. With high sensitivity and specificity, nanopore sequencing (Oxford technologies, UK) that combine rapid library preparation and real-time data acquisition have shown a turnaround of approximately 6–8 h from sample collection to sample result [28].

We need further studies regarding the following points: what is the best microbiota sample in the lung? What relevance do microbiota diversity and similarities between BAL and TA in critically ill patients hold? What is the cut-off point of the bacterial load to obtain a representative sample from the lung? Similarly, besides the sampling issue, DNA extraction methods can also impact results. DNA isolation and human DNA depletion methods are other factors that affect the representative microbiota population. Saponin-based host DNA depletion has demonstrated good performance when removing a large amount of human DNA present in respiratory samples (ratio of human: microbial DNA > 99:1) [28, 29]. Another option is eukaryotic lysis buffers like QIAamp DNA Microbiome Kit (Catalogue 51704, Qiagen, Hilden, Germany) during bacterial DNA isolation [30].

3.1. reconsidering optimal samples in VAP

Metagenomics allows differences between bronchoalveolar lavage (BAL) and tracheal aspirates (TA) to be inspected at a much more precise resolution than standard cultures. Long considered suboptimal due to a higher risk of oral contamination, TA could now gain interest in terms of diagnosis and microbiome studies if no disadvantages are presented at a metagenomic scale against BAL. This can be considered relevant, given that TA requires a less invasive intervention than BAL and is a widely used respiratory specimen [31-33].

Depending on the implications that arise in VAP diagnosis, differences in microbiota richness, diversity and composition among BAL samples and TA specimens could prove relevant. Kalantar et al. made matched comparisons between mini-bronchoalveolar lavage (mBAL) and TA and found no differences in microbiota composition and diversity, even in patients with confirmed vs suspected pneumonia [34]. In addition, oral microbiota and abundance of dominant pathogens were similar between mBAL and TA, indicating that pathogen dominance of the lung microbiome during infection may drive compositional similarity between mBAL and TA. Recent interest has been raised concerning exhaled breath condensate fluid samples as a potential non-invasive diagnostic tool for VAP [35].

Other important aspects of metagenomics are the quality of information obtained and the way by which it is measured. A separate study concerning microbial biomass in the lung suggested that representative microbiota samples need densities comprising between 8E+04 and 8E+06 16S copies/ml BAL. They thereafter recommended pre-screening sample bacterial densities (16S copies/ml of sample) to predict accuracy and precision of expected sequencing for any given sample set [36, 37].

3.2. Oral cavity microbiota in VAP patients

Oral and oropharyngeal bacteria are believed to have a role in VAP. In mechanically ventilated patients, oral pathogens can easily translocate into lower airways; similarly, a shift in oral flora has been linked to a shift in TA and BAL samples [38]. VAP-causing bacteria are typically species found in nosocomial environments (*Pseudomonas, Staphylococcus* or *Enterobacteriaceae* among others) and are not usually predominant in oropharyngeal flora and healthy subjects.

Kalanuria et al. found that oropharyngeal microbial dysbiosis occurred in a high proportion of mechanically-ventilated patients and isolated at least one pathogen originating from dental plaque in the lower respiratory tract of all 13 patients [39]. A shift in oral flora towards dominant nosocomial pathogens has been linked to VAP in mechanically-ventilated patients. Indeed, coincidence of pathogens between dental plaque and lower airways was found in a high proportion (58%) of patients with VAP who were mechanically ventilated for long periods [40].

3.3. The gut-lung axis

The dynamics of the aerodigestive tract become inverted during critical illness and microbiota translocation from gut to lungs is enhanced. Whereas in healthy subjects, the oropharynx is the primary source of microbiota for the lungs and stomach, the overgrown microbial reservoir of the stomach and small intestine become the primary microbiota source for the oropharynx and lungs in critically ill patients [13, 18, 41]. Vulnerable conditions and medication (depressed consciousness and sedation, endotracheal intubation, Proton-pump inhibitors) of critically ill patients favour a shift in oropharynx flora and possibly facilitate colonisation of respiratory airways by both nosocomial pathogens and the enrichment of *Proteobacteria* displacing normal respiratory communities [42].

The gut microbiome is the main enhancer of innate host immunity against infections, such as the production of antimicrobial peptides (bacteriocins) [43] and an adaptive response role via regulation and differentiation of Th17 cells [44]. By means of mesenteric lymph nodes, gut lymph nodes send mediators as certain gut bacteria from the intestinal lumen to the lung where, once there, tissue damage can then occur [45].

Lung communities are dominated by gut-associated bacteria following sepsis. Ecological analysis revealed the gut as the likely source of bacterial dysbiosis in the lung that leads to VAP and ARDS as aforementioned [13, 18].

3.4. Virome and mycobiome

The human virome is composed of eukaryotic viruses which infect human cells, smaller eukaryotes like protozoans or fungi, and bacteriophages and viruses in food. The International Committee on Taxonomy of Viruses in 2014 listed 104 families, 505 genera, and 3186 species of all known viruses [46]. Such types like dsDNA, ssDNA, dsRNA, ssRNA− ssRNA+ viruses, and dsDNA and ssRNA retroviruses can affect any tissue within the body. Lists of viral pathogens and prophages are maintained by the Virus Pathogen Database and Analysis Resource (ViPR) and PHAST, respectively [47].

Viral infection occurs when surface proteins bind to host cell receptors, and replication and lysis takes place. It is well known that human enteric viruses can benefit from bacterial members of the gut microbiome enhancing its infectivity thanks to bacterial surface polysaccharides [48]. Therefore, understanding interactions between microbiota and virome, as well as their clinical implications is a great challenge in VAP and other diseases.

The bacteriophage community in the human gut contains a set of core bacteriophages shared among people but also other types of bacteriophages either rarely shared or unique to a person. Such shared bacteriophages comprise the healthy gut phageome found in a smaller percentage in individuals with gastrointestinal/irritable bowel disease [49]. Phages' functionality may be, however, associated with their lytic/lysogenic cycles. For instance, it has been shown that in response to temperature and UV radiation, certain phages are able to decrease bacterial counts. In addition, intestinal permeability, also regulated by phages community, allows for phage movement in the human gut associated with different disease phenotypes. Finally, the impact of viruses of human protists (non-fungal microbial eukaryotes) on VAP pathogenesis is barely described [46]. A similar balance is expected to occur in lung phageome, even though there is a lack of studies to support this hypothesis.

Although respiratory virome and mycobiome have not been well described yet, a newly, unstudied viral family, *Redondoviridae*, has been associated with critical illnesses, such as respiratory failure and periodontitis [50]. *Candida albicans* and *Candida glabrata* were the first (40%) and third most common (18%) microorganisms isolated in ETTs of mechanically-ventilated patients and were significantly associated with the *Prevotella* genus [26]. Importantly, fungi such as *Aspergillus* may be underestimated in VAP; further metagenomic studies are needed to elucidate its involvement in clinical outcomes, both short and long term, and determine which technical approach is the most suitable to describe mycobiome in VAP respiratory specimens [51].

4. Microbiome involvement in VAP prevention & treatment

4.1. The role of antibiotics & antiseptics

Narrow-spectrum antibiotics recommended during VAP treatment are effective against the pathogen isolated by culture; however, they will decrease the already low microbiome diversity in patients with VAP and strengthen the resistance of usually neglected concomitant flora. One large study including 115 ICU patients revealed a low abundance of *Firmicutes* and *Bacteroidetes* and an increased

abundance of *Proteobacteria* when compared to healthy individuals [52]. Further, antibiotic treatments invite an increase in fungal colonization and can exaggerate the allergic response. For example, changes in gut microbiota in critically ill patients include depletion of butyrate-producing microbes and a subsequent overgrowth of virulent strains of *Escherichia/Shigella, Salmonella, Enterococcus, Clostridium difficile* or *Staphylococcus* [53].

Several studies support that oral washes with chlorhexidine, a universal VAP prevention measure for the ICU, are effective in reducing VAP, especially in cardiac surgery patients. Recent data shows that this beneficial effect could be explained by the drug's ability to prevent pathogenic oral flora from progressing. Although not exempt from controversy, oral washes with chlorhexidine plus digestive decontamination did decrease the carriage of third-generation cephalosporin-resistant *Enterobacterales* [54-56].

4.2. The role of enteral or parenteral nutrition

Although usually restricted in nutrients, environmental conditions in the lungs then become extremely nutrient-enriched (oedema, oxygen, temperature, cytokines, effect of antimicrobials on endogenous microbiota), promoting the growth of potential pathogens. Nutrition and other supplements such as probiotics appear to play a significant role in modulating the microbiome of critically ill patients. Enteral nutrition is recommended to avoid derangements of the intestinal epithelium and microbiome associated with starvation [57]. However, microbiota can be impacted by dietary emulsifiers and various glycerol derivatives added to such formulations to extend shelf life and texture. Fat-rich diets are associated with a microbiome dominated by *Bacteroidetes* and *Actinobacteria,* whereas fibre-rich diets potentiate *Firmicutes* and *Proteobacteria.*

Lately, recent evidence has supported findings of enteral nutrition being associated with high prevalence of *Clostridium difficile* Infection [58].Thus, enteral feeding is associated not solely with a loss in the diversity of the gut microbiome. It is also associated with a loss in unique microbial signatures of different body sites, indicating widespread colonization [59].

4.3. The use of probiotics and other ICU conditions

More recently, modulation of gut microbiota via the use of probiotics has shown to heighten the frequency of B cells expressing IgA in the colon and lymph nodes, and secondarily, increase both lymph node T follicular helper (Tfh) cells and IL-23-expressing dendritic cells [60]. All of them changes that are likely to boost host defenses at mucosal sites as it occurs in response to vaccination.

As such, the use of probiotics in critical illnesses has been primarily investigated for the prevention of VAP or sepsis. It remains controversial given that multiple studies, including randomized controlled trials, are available on both sides of the debate [61]. In addition, substantial differences in study design, type, duration and dose of any probiotic treatment warrant enough reason to limit the strength of any conclusion drawn regarding the effects of such microbial formulations.

To illustrate the former point, in a metanalysis comprising 5 RCT, investigators concluded that probiotics did not significantly decrease the incidence of VAP; however, the administration of probiotics did reduce the risk of VAP caused by *Pseudomonas aeruginosa* [62]. This information was later contradicted in a more recent metanalysis by Hong Weng et al. which included 13 RCT and concluded that incidence of VAP was reduced by the use of probiotics without any impact on other outcomes such as diarrhoea, length of ICU stay, length of hospital stay and mortality [63]. The effects of probiotics on duration of mechanical ventilation should merit further research. In particular, a probiotics capsule containing live Bacillus subtilis and Enterococcus faecalis (Medilac-S) of 0.5 g administered three times daily through a nasogastric feeding tube was found to be beneficial in reducing the incidence of VAP and delaying VAP occurrence [64]. Other formulations such as those including *Lactobacillus acidophilus* LA-5 1.75×10^9 CFU, *Lactobacillus Plantarum* 0.5×10^9 CFU, *Bifidobacterium lactis* BB-12 1.75×10^9 CFU *και Saccharomyces boulardii* 1.5×10^9 CFU per capsule (LactoLevure, UniPharma, Athens, Greece) should be prospectively investigated for both prevention and long-term stability.

Finally, altered chemical gradients (pH and oxygen) in critical illnesses can also reshape the structure and function of microbial communities. These gradients influence community metabolism and virulence factor production, treatment and resistance. For example, low pH and oxygen promoted fermenting anaerobes and *P. aeruginosa* strains whilst depleting its virulence [65].

Overall, evidence of a lung ecosystem will not only impact antimicrobial optimization, nutrition, dietetic supplements, medicalization and other ICU settings; it will also pave the way for new therapeutic strategies that boost the host microbiome and target key cellular processes during infections.

4.4. New therapeutic approaches in VAP

4.4.1. The role of corticosteroids

In vitro studies have demonstrated a U-shaped response of bacterial growth to pro-inflammatory cytokines. Exposure to a lower concentration of cytokines restricts extracellular and intracellular bacterial growth and preserves efficient bacterial clearance by human monocytic cells. Conversely, higher concentrations of pro-inflammatory cytokines enhance intracellular and extracellular bacterial growth in a dose-dependent manner [66]. Within this context, glucocorticoids can be beneficial in preventing immune reprogramming associated to a high production of inflammatory cytokines, as well as in pathogen clearance, normalization of respiratory microbiota diversity and restoration of mucosal immunity [67]. However, further research is needed on this topic since chronic inhaled corticosteroids in COPD and asthma patients have been associated with altered respiratory microbiotas [68].

4.4.2. Immunomodulatory drugs

New therapeutic approaches aimed at normalizing the microbiome diversity and restoring mucosal immunity is a promising strategy. For example, Roquilly A et al. have suggested restoring the IL-12/ IFN-γ axis commonly observed to fail in critically ill patients and associated with hospital-acquired pneumonia (HAP). Their approach touches upon the fact that in the healthy lung, commensal-derived metabolites promote tolerance through mucosal immunity. In this scenario of symbiosis, bacteria identification by dendritic cells produces IL-12 to stimulate natural killer cells IFN-γ secretion in such a way that balance is maintained without damage to the epithelium. In contrast, during HAP, an imbalance in the IL-12/ IFN-γ axis induces dysbiosis between microbiota and the immune system and causes epithelial injuries [21].

5. Future directions

With the aforementioned stated, both microbiome and virome will provide new opportunities to better understand the relationship between health and dysbiosis in VAP [69]. New studies are therefore needed to understand the mechanisms of potential disease progression from a translational perspective. In particular, such investigative pursuits would endeavor to describe further associations between the lung and gut virome, as well as such impact on microbiota and host immunity. As it stands, the role of viral lytic/lysogenic cycles in restoring gut microbiome balance is unknown. Interestingly, phages or other microorganisms could be

used as new therapeutic approaches in VAP [70, 71], specially to deal with multi-resistant bacteria.

In such a scenario, microbiome databases and big data, such as that of the Human Pan-Microbe Community, would be mandatory in order to facilitate better interpretation of microbiome patterns involved in respiratory pathologies and build robust, predictive algorithms for specific diseases like VAP [72-74].

Lastly, priorities would need to be established as they concern microbiome applications to clinical practice. It is indeed difficult to believe that today, the microbiome could serve as a potential diagnostic tool given the contradiction that exists with expansive data management and the need for rapid diagnoses in the clinic. Yet, artificial intelligence paves the way to design biological and clinical algorithms as predictive disease tools and cluster patients by phenotypes to potentially enhance clinical management of patients. As high-throughput technology allows us to dive into a new dimension of understanding diseases, there remains only the arduous task of reconsidering prior knowledge in a different light.

6. Contributors

All authors searched participated in the search strategy and selected literature of reference for this topic. LFB and RLA wrote the manuscript. LFB, RLA and AT designed the contents and reviewed the final contents of the review.

Outstanding questions

Important questions for future research include: 1) To describe further associations between the lung and gut virome and mycobiome, as well as its impact on microbiota and host immunity; 4) To study synergistic or antagonistic relationships between commensal microbiota and pathobiome 2) To define microbiome patterns involved in respiratory pathologies and build robust, predictive algorithms for VAP; 3) To optimize VAP treatment by fostering personalized medical strategies based on restoring mucosal immunity and striking a balanced respiratory microbiota.

Search strategy and selection criteria

Data for this review were identified by searches performed in MEDLINE, Current Contents, PubMed, and references from relevant articles using terms such as “ventilator-associated pneumonia”; “next-generation sequencing”; “microbiota”; “endotracheal tubes”; “mycobiome”; “virome”. Abstracts and reports from meetings were included only when they were related directly to previously published work. Only articles published in English between 1992 and 2020 were included.

Declaration of Competing interest

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