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(54) **Title:** RESPIRATORY MICROBIOME SIGNATURES

(57) **Abstract:** The present invention relates to a method for determining a risk of having or developing a respiratory condition in a subject, based on the detection of the presence or abundance of bacteria of specific genus in a respiratory sample of said subject.

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RESPIRATORY MICROBIOME SIGNATURES

FIELD OF INVENTION

[0001] The present invention relates to the field of the diagnosis, prognosis, prevention and/or treatment of respiratory complications in critically ill patients. In particular, the present invention relates to respiratory microbiome signatures that are associated with a risk of development of respiratory complications, including, without limitation, acute respiratory distress syndrome (ARDS), hospital-acquired pneumonia (HAP) or prolonged mechanical ventilation (MV).

BACKGROUND OF INVENTION

[0002] Acute respiratory distress syndrome (ARDS) and hospital-acquired pneumonia (HAP) are the most frequent respiratory complications in critically ill patients, particularly in critically ill patients receiving mechanical ventilation (MV). Despite improvements in their prevention and treatment, these conditions remain frequently associated with prolonged mechanical ventilation need and death (Roquilly, A. *et al.,* (2020), Clin Infect Dis 73, e1601–e1610, Implementation of French Recommendations for the Prevention and the Treatment of Hospital-acquired Pneumonia: A Cluster-randomized Trial.; Bellani, G. *et al.,* (2016), Jama 315, 788–800, Epidemiology, Patterns of Care, and Mortality for Patients With Acute Respiratory Distress Syndrome in Intensive Care Units in 50 Countries.; Magill, S. S. *et al.,* (2018), New Engl J Med 379, 1732–1744, Changes in Prevalence of Health Care–Associated Infections in U.S. Hospitals).

[0003] There is thus still a need for means to individually predict patient outcomes and develop innovative therapeutical approaches.

[0004] High throughput sequencing of respiratory fluids showed that lungs, initially considered sterile, are colonized by a highly diverse microbiota, collectively known as the lung microbiome (Morris, A. & Flores, S. C., (2017), American Journal of Respiratory and Critical Care Medicine 195, 15-16, Study of the Lung Microbiome. Have We Reached the End of the Beginning?; Charlson, E. S. *et al.*, (2011), Am J Resp Crit Care 184, 957–963, Topographical Continuity of Bacterial Populations in the Healthy Human Respiratory Tract). A group of bacteria found in most healthy individuals, composed of five genera *Prevotella*, *Streptococcus*, *Veillonella*, *Fusobacterium*, and *Haemophilus*, was identified and proposed as a pulmonary core microbiota essential for lung homeostasis (Man, W. H., Piters, W. A. A. de S. & Bogaert, D., (2017), Nat Rev Microbiol 15, 259–270, The microbiota of the respiratory tract: gatekeeper to respiratory health).

[0005] Lung microbiome investigations in patients hospitalized in intensive care units (ICU) have provided supportive evidence for respiratory microbial dysbiosis during critical illness (Dickson, R. P. *et al.,* (2016), Nat Microbiol 1, nmicrobiol2016113, Enrichment of the lung microbiome with gut bacteria in sepsis and the acute respiratory distress syndrome; Dickson, R. P. *et al.*, (2020), Am J Resp Crit Care 201, 555–563, Lung Microbiota Predict Clinical Outcomes in Critically Ill Patients; Fromentin, M., Ricard, J.-D. & Roux, D., (2021), Intens Care Med 1–15 doi:10.1007/s00134-020-06338-2, Respiratory microbiome in mechanically ventilated patients: a narrative review). However, the definitions of respiratory microbiome signatures associated with HAP and ARDS have not been achieved.

[0006] In the present invention, Applicants define robust respiratory microbiome signatures associated with ARDS, HAP and prolonged MV, that may be of importance for development or selection of personalized treatments and innovative microbiome-based therapies.

[0007] Applicants also demonstrate that microbiome-targeted approaches based on these respiratory microbiome signatures (such as, for example, restoration of Streptococcus spp lung colonization) can prevent and treat pneumonia.

SUMMARY

[0008] The present relates to a method for determining a risk of developing a respiratory complication in a subject or for identifying a subject at risk of developing a respiratory complication, said method comprising a step of detecting the presence or abundance of a least one bacterium of a genus selected from the group comprising *Staphylococcus*, *Ralstonia*, *Enterococcus*, *Flavobacterium*, *Veillonella*, *Prosthecobacter*, *Streptococcus*, *Atopobium*, *Oribacterium*, a genus of the *Lactobacillales* order, and *Rothia* in a respiratory sample of the patient.

[0009] In one embodiment, the subject is critically ill patient.

[0010] In one embodiment, the respiratory sample is selected from the group comprising an endotracheal aspirate (ETA), a bronchoalveolar lavage (BAL), and an oropharyngeal swab (OS).

[0011] In one embodiment, the presence or abundance of the at least one bacterium is assessed through the detection of sequences specific of said at least one bacterium, preferably by PCR or RT-PCR.

[0012] In one embodiment, the respiratory complication is selected from the group comprising hospital acquired pneumonia (HAP), acute respiratory distress syndrome (ARDS), ARDS related death and prolonged mechanical ventilation (MV).

[0013] In one embodiment, the respiratory complication is HAP, and the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus selected from *Streptococcus*, *Atopobium*, *Oribacterium* and *Veillonella* in a respiratory sample of the patient, preferably an endotracheal aspirate of the patient.

[0014] In one embodiment, the respiratory complication is ARDS or ARDS related death, and the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus selected from Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella and Prosthecobacter in a respiratory sample of the patient.

[0015] In one embodiment, the respiratory complication is ARDS or ARDS related death, and the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus selected from *Staphylococcus*, *Ralstonia* and *Enterococcus* in an endotracheal aspirate of the patient.

[0016] In one embodiment the respiratory complication is ARDS or ARDS related death, and the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus selected from *Flavobacterium*, *Veillonella* and *Prosthecobacter* in a bronchoalveolar lavage of the patient.

[0017] In one embodiment, the respiratory complication is prolonged mechanical ventilation, and the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus selected from a genus of the *Lactobacillales* order, *Rothia* and *Streptococcus* in a respiratory sample of the patient, preferably in an endotracheal aspirate or in a bronchoalveolar lavage of the patient, more preferably in an endotracheal aspirate of the patient.

[0018] The present invention further relates to a method of providing an adapted care to a subject at risk of having or developing a respiratory complication, the method comprising the steps of:

- identifying a subject with a risk of having or developing a respiratory complication in a subject by the method as described herein, and
- providing an adapted care to a subject identified with a risk of having or developing a respiratory complication.

[0019] The present invention further relates to a composition comprising at least one bacterium of the *Flavobacterium* genus, or of the *Veillonella* genus, or of the *Prosthecobacter* genus, or of the *Streptococcus* genus, or of the *Atopobium* genus, or of the *Oribacterium* genus, or of one of the genera of the *Lactobacillales* order, or of the *Rothia* genus or metabolites thereof or mixtures thereof, for use in the prevention or treatment of a respiratory complications in a subject.

DEFINITIONS

[0020] "**Acute respiratory distress syndrome (ARDS)**" refers to any acute syndrome of acute hypoxemic respiratory failure.

[0021] **"Hospital-acquired pneumonia (HAP)"** refers to any lung inflammation caused by bacteria, viruses and/or parasites developing in patients hospitalized for more than 48 hours and not present at admission.

[0022] "**Subject**" refers to an animal, preferably a mammal, more preferably a human. In one embodiment, the subject is a patient, i.e., a recipient of health care services. In one embodiment, the subject is a critically ill patient. In one embodiment, the subject is a critically ill patient admitted in an intensive care unit. In one embodiment, the subject is a patient receiving mechanical ventilation.

[0023] "**Signature**" or "**microbiome signature**" are used interchangeably and refer to a group of bacteria species, bacteria orders, bacteria phyla or bacteria families whose presence or abundance or metabolomic functions is(are) indicative of a biological condition, and/or of a risk of developing a respiratory complication and/or a risk of unfavorable response to treatment in a subject. In one embodiment, a signature refers to one (unique) bacteria species, bacteria order, bacteria phylum or bacteria family. In another embodiment, a signature refers to a group of at least 2 bacteria species, bacteria orders or bacteria families. In one embodiment, a signature refers to a group of at least 3, 4, 5, 6 or 7 bacteria species, bacteria orders or bacteria families. In one embodiment, a signature refers to a group of at least 8, 9, 10 or more bacteria species, bacteria orders or bacteria families.

[0024] "**Critically ill patient(s)**" refers to patient(s) who either suffer from life-threatening conditions or are at risk of developing them. A "critically ill patient" may in particular refer to a patient suffering from sepsis or septic shock, and/or severe trauma, and/or acute brain injury and/or to a patient suffering from pneumonia (including community-acquired pneumonia). "**Critical care**" relates to the process of looking after

patients who either suffer from life-threatening conditions or are at risk of developing them.

[0025] "**Intensive care units**" also named "**critical care units**" or "**intensive therapy units**" refers to specialist hospital wards that provide monitoring and treatment for people who are in need thereof, notably who are seriously and/or critically ill. "**Patient(s) hospitalized in intensive care units**" may be for example patient(s) in need of mechanical ventilation (MV), or patient(s) under mechanical ventilation, notably under prolonged mechanical ventilation. Patient(s) hospitalized in intensive care units may be for example, patient(s) with severe head injury or patient(s) with serious short-term condition such as heart attack or stroke. Patient(s) hospitalized in intensive care units may be patient(s) with severe burns.

[0026] "**Treatment**" refers to both therapeutic treatment and prophylactic or preventative measures the aim of this treatment or these measures is to prevent or slow down (lessen) a targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

[0027] "**Sample**" generally refers to any biological material obtained via suitable methods known to the person skilled in the art from a subject. The sample may be collected in a clinically acceptable manner, *e.g.*, in a way that biological material such as cells, microorganisms, nucleic acids (such as DNA and RNA) and/or proteins are preserved. Specifically, a "**sample**" in the context of the invention may include bodily fluids such as a respiratory sample. Examples of respiratory samples include, but are not limited to, bronchoalveolar lavage (BAL) fluids, endotracheal aspirate (ETA) fluids, oropharyngeal swabs (OS) or the like.

[0028] "**Population**", "**cohort**", "**cohort study**", as used herein, are interchangeable and refer to a group of subjects with shared factors, influences or condition.

[0029] "**Risk classification**" as used herein, means a grouping of subjects by the level of risk (or likelihood) that the subject will experience a particular clinical outcome. A subject may be classified into a risk group or classified at a level of risk based on the

methods of the present disclosure. A "**risk group**" is a group of subjects or individuals with a similar level of risk for a particular clinical outcome.

[0030] "**About**" as used herein, means approximately, roughly, around, or in the region of. When the term "**about**" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. When the term "**about**" is used in conjunction with and preceding a figure it modifies said figure by extending said figure by plus or less 10% of the value of said figure.

[0031] "**Therapeutically effective amount**" as used herein, means the level or amount of composition that is aimed at, without causing significant negative or adverse side effects to the target, (1) delaying or preventing the onset of respiratory complication; (2) slowing down or stopping the progression, aggravation, or deterioration of one or more symptoms of respiratory complication; (3) bringing about ameliorations of the symptoms of respiratory complication; (4) reducing the severity or incidence of respiratory complication; or (5) curing respiratory complication. A therapeutically effective amount may be administered prior to the onset of respiratory complication, for a prophylactic or preventive action. Alternatively, or additionally, the therapeutically effective amount may be administered after initiation of respiratory complication, for a therapeutic action or maintenance of a therapeutic action.

[0032] "**Pharmaceutically acceptable excipient**" refers to an excipient or carrier that does not produce an adverse, allergic or other untoward reaction when administered to a mammal, preferably a human. It includes any and all solvents, such as, for example, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents. A pharmaceutically acceptable excipient or carrier refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. For human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by the regulatory offices such as the FDA (US Food and Drug Administration) or EMA (European Medicines Agency).

[0033] "**Relative abundance**" of at least one bacterium of one predetermined genus in a respiratory sample of a patient refers to the abundance of said at least one bacterium of

said predetermined genus in the respiratory sample of said patient by reference to the abundance of all bacteria of the genus identified in said respiratory sample.

DETAILED DESCRIPTION

[0034] The present invention relates to a method for determining a risk of developing a respiratory complication in a subject or for identifying a subject at risk of developing a respiratory complication, said method comprising a step of detecting the presence or abundance, in a respiratory sample of the patient, of a least one bacterium of a genus selected from the group comprising *Abiotrophia, Achromobacter, Acidovorax, Acinetobacter, Actinobacillus, Actinomyces, Afipia, Aggregatibacter Alloiococcus, Anaerococcus, Anaeroplasma, Aquabacterium, Arcobacter, Atopobium, Bacillus, Bacteroides, unc_Bacteroidales, Bdellovibrio, Bifidobacterium, Bulleidia, Burkholderia, Butyrivibrio, Campylobacter, Capnocytophaga, Caulobacter, Chryseobacterium, Collinsella, Corynebacterium, Devosia, Dialister, Enterococcus, unc_Enterococcaceae, Faecalibacterium, Filifactor, Fimbriimonas, Finegoldia, Flavobacterium, Fluviicola, Fusobacterium, Gardnerella, Gemmata, unc_Gemellaceae, unc_Gemellales, Granulicatella, Haemophilus, Lachnoanaerobaculum,* a genus of the *Lactobacillales* order, *unc_Lactobacillales, Leptotrichia, Megasphaera, Methylobacterium, Microbacterium, Mogibacterium, Moryella, Mycoplasma, Neisseria, Novosphingobium, Olsenella, Opitutus, Oribacterium, Oscillospira, Paracoccus, paraprevotella, Parvimonas, Pediococcus, Pelomonas, Pedobacter, Peptococcus, Peptoniphilus, Peptostreptococcus, Phascolarctobacterium, Planctomyces, Porphyromonas, Prevotella, unc_Prevotellaceae, Propionibacterium, Prosthecobacter, Pseudomonas, Ralstonia, Reyranella, unc_Rhizobiaceae, Rhodococcus, Rothia, Ruminococcus, Scardovia, Schwartzia, Sediminibacterium, Selenomonas, Serratia, Shuttleworthia, Slackia, Sphaerochaeta, Staphylococcus, Streptococcus, unc_Streptococcaceae, Stenotrophomonas, Tannerella, TG5, Treponema* and *Veillonella.*

[0035] The present invention relates to a method for determining a risk of developing a respiratory complication in a subject or for identifying a subject at risk of developing a

respiratory complication, said method comprising a step of detecting the presence or abundance of a least one bacterium of a genus selected from the group comprising *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella, Prosthecobacter, Streptococcus, Atopobium, Oribacterium,* a genus of the *Lactobacillales* order, and *Rothia* in a respiratory sample of the patient.

[0036] In some embodiments, the method of the invention further comprises a step of mathematically combining the abundance (preferably the relative abundance) detected for each of the bacteria listed hereinabove, thereby obtaining a score. As used herein, a score refers to a digit value. In some embodiments, said mathematical combination is an equation comprising sums and/or subtractions.

[0037] In some embodiments, the method of the invention is intended to determine a risk of developing a respiratory complication in a subject, notably in a subject hospitalized in an intensive care unit. In some embodiments, the method of the invention is intended to identify a subject at risk of developing a respiratory complication.

[0038] In some embodiments, the respiratory complication is a life-threatening condition where lungs cannot provide the subject's vital organs with enough oxygen. In some embodiments, the respiratory complication is an acute respiratory distress syndrome (ARDS). In some embodiments, the acute respiratory distress syndrome (ARDS) is a respiratory complication resulting from a serious preexisting health condition.

[0039] In some embodiments, the respiratory complication is a pneumonia that occurs at least about 48 hours after hospitalization of the subject and that does not appear to be incubating at the time of hospitalization. In some embodiments, the respiratory complication is a pneumonia that occurs 48 hours or more after hospitalization of the subject and that does not appear to be incubating at the time of hospitalization. In some embodiments, the respiratory complication is a hospital-acquired pneumonia (HAP), also named nosocomial pneumonia. In some embodiments, the respiratory complication is a type, named ventilator-associated pneumonia (VAP), of hospital-acquired pneumonia (HAP) that develops more than 48 hours after endotracheal intubation and/or mechanical ventilation.

[0040] In some embodiments, the method comprises a step of detecting the presence of a least one bacterium of a genus selected from the group comprising *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella, Prosthecobacter, Streptococcus, Atopobium, Oribacterium,* a genus of the *Lactobacillales* order, and *Rothia* in a respiratory sample of the patient. Detecting the presence of at least one bacterium of a selected genus can be performed by means known of one ordinary skilled in the art. For example, the presence of at least one bacterium of a selected genus can be assessed by any well-known microbiologic method. In one embodiment, the presence of at least one bacterium of a selected genus can also be assessed through the detection of nucleotide sequences specific of said at least one bacterium. In some preferred embodiments, the presence of at least one bacterium of a selected genus can be assessed through the detection of at least one nucleotide sequence specific of said at least one bacterium, such as, for example, by PCR or RT-PCR. In one embodiment, the at least one nucleotide sequence specific of said at least one bacterium is a 16S rRNA encoding gene sequence or a 16S rRNA sequence.

[0041] In some embodiments, the method comprises a step of determining the abundance of a least one bacterium of a genus selected from the group comprising *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella, Prosthecobacter, Streptococcus, Atopobium, Oribacterium,* a genus of the *Lactobacillales* order, and *Rothia* in a respiratory sample of the patient. Evaluation of the abundance of at least one bacterium of a selected genus can be performed by means known of one ordinary skilled in the art. For example, the abundance of at least one bacterium of a selected genus can be assessed by any well-known microbiologic method. In some preferred embodiments, the abundance of at least one bacterium of a selected genus can be assessed through quantitative or semi-quantitative analysis of nucleotide sequences specific of said at least one bacterium. In some preferred embodiments, the abundance of at least one bacterium of a selected genus can be assessed through quantitative or semi-quantitative analysis of at least one nucleotide sequence specific of said at least one bacterium, such as, for example, by PCR or RT-PCR. In one embodiment, the at least one nucleotide sequence specific of said at least one bacterium is a 16S rRNA encoding gene sequence or a 16S rRNA sequence.

[0042] In some embodiments, said method comprises a step of detecting the presence or abundance of one bacterium of a genus selected from the group comprising *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella, Prosthecobacter, Streptococcus, Atopobium, Oribacterium,* a genus of the *Lactobacillales* order, and *Rothia* in a respiratory sample of the patient.

[0043] In some embodiments, said method comprises a step of detecting the presence or abundance of one bacterium of the genus *Staphylococcus* in a respiratory sample of the patient.

[0044] In some embodiments, said method comprises a step of detecting the presence or abundance of one bacterium of the genus *Ralstonia* in a respiratory sample of the patient.

[0045] In some embodiments, said method comprises a step of detecting the presence or abundance of one bacterium of the genus *Enterococcus* in a respiratory sample of the patient.

[0046] In some embodiments, said method comprises a step of detecting the presence or abundance of one bacterium of the genus *Flavobacterium* in a respiratory sample of the patient.

[0047] In some embodiments, said method comprises a step of detecting the presence or abundance of one bacterium of the genus *Veillonella* in a respiratory sample of the patient.

[0048] In some embodiments, said method comprises a step of detecting the presence or abundance of one bacterium of the genus *Prosthecobacter* in a respiratory sample of the patient.

[0049] In some embodiments, said method comprises a step of detecting the presence or abundance of one bacterium of the genus *Streptococcus* in a respiratory sample of the patient.

[0050] In some embodiments, said method comprises a step of detecting the presence or abundance of one bacterium of the genus *Atopobium* in a respiratory sample of the patient.

[0051] In some embodiments, said method comprises a step of detecting the presence or abundance of one bacterium of the genus *Oribacterium* in a respiratory sample of the patient.

[0052] In some embodiments, said method comprises a step of detecting the presence or abundance of one bacterium of one genus of the *Lactobacillales* order in a respiratory sample of the patient.

[0053] In some embodiments, said method comprises a step of detecting the presence or abundance of one bacterium of the genus *Rothia* in a respiratory sample of the patient.

[0054] In some embodiments, said method comprises a step of detecting the presence or abundance of more than one (such as, for example, 2, 3, 4, 5, 6, 7, 8, 9 or 10) bacteria of a genus selected from the group comprising *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella, Prosthecobacter, Streptococcus, Atopobium, Oribacterium,* a genus of the *Lactobacillales* order, and *Rothia* in a respiratory sample of the patient.

[0055] In some embodiments, the at least one bacterium of the genus *Staphylococcus* is a bacterium of a species or sub-species of the genus *Staphylococcus*.

[0056] In some embodiments, the at least one bacterium of the genus *Ralstonia* is a bacterium of a species or sub-species of the genus *Ralstonia*.

[0057] In some embodiments, the at least one bacterium of the genus *Enterococcus* is a bacterium of a species or sub-species of the genus *Enterococcus*.

[0058] In some embodiments, the at least one bacterium of the genus *Flavobacterium* is a bacterium of a species or sub-species of the genus *Flavobacterium*.

[0059] In some embodiments, the at least one bacterium of the genus *Veillonella* is a bacterium of a species or sub-species of the genus *Veillonella*.

[0060] In some embodiments, the at least one bacterium of the genus *Prosthecobacter* is a bacterium of a species or sub-species of the genus *Prosthecobacter*.

[0061] In some embodiments, the at least one bacterium of the genus *Streptococcus* is a bacterium of a species or sub-species of the genus *Streptococcus*.

[0062] In some embodiments, the at least one bacterium of the genus *Atopobium* is a bacterium of a species or sub-species of the genus *Atopobium*.

[0063] In some embodiments, the at least one bacterium of the genus *Oribacterium* is a bacterium of a species or sub-species of the genus *Oribacterium*.

[0064] In some embodiments, the at least one bacterium of one genus of the genera of the *Lactobacillales* order is a bacterium of a family of the order of *Lactobacillales*.

[0065] In some embodiments, the at least one bacterium of the genus *Rothia* is a bacterium of a species or sub-species of the genus *Rothia*.

[0066] In some embodiments, the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus *Staphylococcus* in the respiratory sample, **and** of detecting the presence or abundance of at least one bacterium of a genus *Ralstonia* or *Enteroccus* in the respiratory sample.

[0067] In some embodiments, the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus *Staphylococcus* in the respiratory sample, **and** of detecting the presence or abundance of at least one bacterium of a genus *Ralstonia* in the respiratory sample, **and** of detecting the presence or abundance of at least one bacterium of a genus *Enteroccus* in the respiratory sample.

[0068] In some embodiments, the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus *Streptococcus* in the respiratory sample, **and** of detecting the presence or abundance of at least one bacterium of a genus *Atopobium*, *Oribacterium* and *Veillonella* in the respiratory sample.

[0069] In some embodiments, the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus *Streptococcus* in the respiratory sample, **and** of detecting the presence or abundance of at least one bacterium of a genus *Veillonella* in the respiratory sample.

[0070] In some embodiments, the subject is a critically ill patient. In some embodiments the subject is a critically ill patient admitted in an intensive care unit. In some embodiments the subject is a critically ill patient in an intensive care unit and receiving mechanical ventilation (MV) or prolonged mechanical ventilation.

[0071] In one embodiment, the subject is a critically ill patient admitted in an intensive care unit for less than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 days, preferably less than 14 days and more preferably less than 7 days.

[0072] In one embodiment, the subject is a mammal, such as, for example, a human, a non-human primate, a horse or a cow.

[0073] In some embodiments, the subject is a human.

[0074] In some embodiments, the subject is a pet, such as, for example, a dog, a cat, a guinea pig, a hamster, a rat, a mouse, a ferret, a rabbit, a bird or an amphibian.

[0075] In some embodiments, the respiratory sample is selected from the group of respiratory samples comprising an endotracheal aspirate (ETA), a bronchoalveolar lavage (BAL), and an oropharyngeal swab (OS).

[0076] In some embodiments, the presence or abundance of a least one bacterium of a genus selected from the group comprising *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella, Prosthecobacter, Streptococcus, Atopobium, Oribacterium,* a genus of the *Lactobacillales* order, and *Rothia* in a respiratory sample of the patient, is assessed through the detection of at least one sequence specific of said at least one bacterium, preferably by PCR or RT-PCR.

[0077] In some embodiments, the presence and/or abundance of a least one bacterium of a genus selected from the group comprising *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella, Prosthecobacter, Streptococcus, Atopobium, Oribacterium,* a genus of the *Lactobacillales* order, and *Rothia* in a respiratory sample of the patient, is assessed by multiplex PCR. In these embodiments, assessment of the presence and/or abundance of a least one bacterium listed above, are to be delivered in few hours after

sampling, thus enabling the determination of a risk of developing a respiratory complication in a subject or for identifying a subject at risk of developing a respiratory complication at the bedside.

[0078] In some embodiments, said at least one sequence specific of said at least one bacterium is a sequence representative of a metabolic function supported by said at least one bacterium. In these embodiments, the presence or abundance of said sequence representative of a metabolic function is determined by deep sequencing of the respiratory microbiome.

[0079] In some embodiments, said at least one sequence specific of said at least one bacterium is a 16S rRNA encoding gene sequence. In some embodiments, said at least one sequence specific of said at least one bacterium is a 16S rRNA sequence, such as, for example, the sequence of the V4 region of the 16S rRNA encoding gene sequence.

[0080] In some embodiments, the specific sequence of the 16S rRNA encoding gene of said at least one bacterium of the genus *Staphylococcus* is a sequence comprising or consisting of **SEQ ID NO: 1** or a variant thereof comprising at least 90% of identity with **SEQ ID NO: 1**, preferably comprising at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of identity with **SEQ ID NO: 1.**

[0081] **SEQ ID NO: 1** (Staphylococcus epidermidis strain ATCC 14990)

TTTATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAAT
ACATGCAAGTCGAGCGAACAGACGAGGAGCTTGCTCCTCTGACGTTAGCGG
CGGACGGGTGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTT
CGGGAAACCGGAGCTAATACCGGATAATATATTGAACCGCATGGTTCAATA
GTGAAAGACGGTTTTGCTGTCACTTATAGATGGATCCGCGCCGCATTAGCTA
GTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCGTAGCCGACCTGAGAG
GGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGC
GTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACA
AATGTGTAAGTAACTATGCACGTCTTGACGGTACCTAATCAGAAAGCCACG

GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCC
GGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGA
AAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTG
CAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATAT
GGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGAT
GTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCC
GTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGC
TAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCA
AAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA
AGCAACGCGAAGAACCTTACCAAATCTTGACATCCTCTGACCCCTCTAGAG
ATAGAGTTTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGT
CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTT
AAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACA
AACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTG
GGCTACACACGTGCTACAATGGACAATACAAAGGGCAGCGAAACCGCGAG
GTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTC
GACTATATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGGTG
AATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTA
ACACCCGAAGCCGGTGGAGTAACCATTTGGAGCTAGCCGTCGAAGGTGGGA
CAAATGATTGGGGTGAAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCGGC
TGGATCACCTCCTTT

[0082] In some embodiments, the specific sequence of the 16S rRNA encoding gene of said at least one bacterium of the genus *Ralstonia* is a sequence comprising or consisting of **SEQ ID NO: 2** or a variant thereof comprising at least 90% of identity with **SEQ ID NO: 2**, preferably comprising at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of identity with **SEQ ID NO: 2.**

[0083] **SEQ ID NO: 2** (Ralstonia pickettii strain ATCC 27511)

AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTACACATGCA
AGTCGAACGGCAGCATGATCTAGCTTGCTAGATTGATGGCGAGTGGCGAAC
GGGTGAGTAATACATCGGAACGTGCCCTGTAGTGGGGGATAACTAGTCGAA

AGATTAGCTAATACCGCATACGACCTGAGGGTGAAAGTGGGGGACCGCAAG
GCCTCATGCTATAGGAGCGGCCGATGTCTGATTAGCTAGTTGGTGAGGTAA
AGGCTCACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGACGATCAGCCAC
ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAA
TTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAA
GGCCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAATGGCTCTGGTTAAT
ACCTGGGGTCGATGACGGTACCGGAAGAATAAGGACCGGCTAACTACGTGC
CAGCAGCCGCGGTAATACGTAGGGTCCAAGCGTTAATCGGAATTACTGGGC
GTAAAGCGTGCGCAGGCGGTTGTGCAAGACCGATGTGAAATCCCCGAGCTT
AACTTGGGAATTGCATTGGTGACTGCACGGCTAGAGTGTGTCAGAGGGGGG
TAGAATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGGAATACCGA
TGGCGAAGGCAGCCCCCTGGGATAACACTGACGCTCATGCACGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCA
ACTAGTTGTTGGGGATTCATTTCCTTAGTAACGTAGCTAACGCGTGAAGTTG
ACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGG
ACCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACGCGAAAAAC
CTTACCTACCCTTGACATGCCACTAACGAAGCAGAGATGCATTAGGTGCTCG
AAAGAGAAAGTGGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG
AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCTCTAGTTGCTAC
GAAAGGGCACTCTAGAGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGG
ATGACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTTCACACGTCATACAA
TGGTGCATACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCAGAAAA
TGCATCGTAGTCCGGATCGTAGTCTGCAACTCGACTACGTGAAGCTGGAATC
GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGTCTTGTA
CACACCGCCCGTCACACCATGGGAGTGGGCTTTACCAGAAGTAGTTAGCCT
AACCGCAAGGAGGGCGATTACCACGGTAGGGTTCATGACTGGGGTGAAGTC
GTAACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCT

[0084] In some embodiments, the specific sequence of the 16S rRNA encoding gene of said at least one bacterium of the genus *Enterococcus* is a sequence comprising or consisting of **SEQ ID NO: 3** or a variant thereof comprising at least 90% of identity with

SEQ ID NO: 3, preferably comprising at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of identity with **SEQ ID NO: 3.**

[0085] **SEQ ID NO: 3** (Enterococcus faecalis EnGen0336 strain T5)

TATTATGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAAT
ACATGCAAGTCGAACGCTTCTTTCCTCCCGAGTGCTTGCACTCAATTGGAAA
GAGGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTACCCATCAGAGG
GGGATAACACTTGGAAACAGGTGCTAATACCGCATAACAGTTTATGCCGCA
TGGCATAAGAGTGAAAGGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCG
GTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCCACGATGCATAG
CCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACT
CCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACC
GAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGT
TAGAGAAGAACAAGGACGTTAGTAACTGAACGTCCCCTGACGGTATCTAAC
CAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGG
CAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTAA
GTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGG
AGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATG
CGTAGATATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAA
CTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGG
TAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTC
AGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAG
GTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGAC
CACTCTAGAGATAGAGCTTTCCCTTCGGGGACAAAGTGACAGGTGGTGCAT
GGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTTATTGTTAGTTGCCATCATTTAGTTGGGCACTCTAGCGAGACTGC
CGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTT
ATGACCTGGGCTACACACGTGCTACAATGGGAAGTACAACGAGTCGCTAGA
CCGCGAGGTCATGCAAATCTCTTAAAGCTTCTCTCAGTTCGGATTGCAGGCT
GCAACTCGCCTGCATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCACGC

CGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAG
AGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTTGGAGCCAGCCGCCTA
AGGTGGGATAGATGATTGGGGTGAAGTCGTAACAAGGTAGCCGTATCGGAA
GGTGCGGCTGGATCACCTCCTTT

[0086] In some embodiments, the specific sequence of the 16S rRNA encoding gene of said at least one bacterium of the genus *Flavobacterium* is a sequence comprising or consisting of **SEQ ID NO: 4** or a variant thereof comprising at least 90% of identity with **SEQ ID NO: 4**, preferably comprising at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of identity with **SEQ ID NO: 4.**

SEQ ID NO: 4 (Flavobacterium glycines strain NBRC 105008)
GATGAACGCTAGCGGCAGGCTTAACACATGCAAGTCGAGGGGTATAGGTTT
TCGGACCTAGAGACCGGCGCACGGGTGCGTAACGCGTATGCAATCTACCTT
TCACAGAGGGATAGCCCAGAGAAATTTGGATTAATACCTCATAGTATTATG
AAGTGGCATCATTTTATAATTAAAGTTCCAACGGTGAAAGATGAGCATGCG
TCCCATTAGCTAGATGGTAAGGTAACGGCTTACCATGGCTACGATGGGTAG
GGGTCCTGAGAGGGAGATCCCCCACACTGGTACTGAGACACGGACCAGACT
CCTACGGGAGGCAGCAGTGAGGAATATTGGTCAATGGACGCAAGTCTGAAC
CAGCCATGCCGCGTGCAGGATGACGGTCCTATGGATTGTAAACTGCTTTTGT
ACGAGAAGAAACACTCCTTCGTGAAGGAGCTTGACGGTATCGTAAGAATAA
GGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATCCAAGCG
TTATCCGGAATCATTGGGTTTAAAGGGTCCGTAGGCGGTTTAGTAAGTCAGT
GGTGAAAGCCCATCGCTCAACGGTGGAACGGCCATTGATACTGCTAGACTT
GAATTATTAGGAAGTAACTAGAATATGTAGTGTAGCGGTGAAATGCTTAGA
GATTACATGGAATACCAATTGCGAAGGCAGGTTACTACTAATGTATTGACG
CTGATGGACGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
ACGCCGTAAACGATGGATACTAGCTGTTGGGAGCAATTTCAGTGGCTAAGC
GAAAGTGATAAGTATCCCACCTGGGGAGTACGGGCGCAAGCCTGAAACTCA
AAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA
TGATACGCGAGGAACCTTACCAAGGCTTAAATGTAGATTGACCGGTTTGGA
AACAGACCTTTCGCAAGACAATTTACAAGGTGCTGCATGGTTGTCGTCAGCT

CGTGCCGTGAGGTGTCAGGTTAAGTCCTATAACGAGCGCAACCCCTGTTGTT
AGTTGCCAGCGAGTGATGTCGGGAACTCTAGCAAGACTGCCGGTGCAAACC
GTGAGGAAGGTGGGGATGACGTCAAATCATCACGGCCCTTACGCCTTGGGC
TACACACGTGCTACAATGGCCGGTACAGAGAGCAGCCACTTCGTGAGGAGG
AGCGAATCTATAAAGCCGGTCACAGTTCGGATCGGAGTCTGCAACTCGACT
CCGTGAAGCTGGAATCGCTAGTAATCGGATATCAGCCATGATCCGGTGAAT
ACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCTGGGGGT
GCCTGAAGTCGGTGACCGCAAGGAGCTGCCTAGGGTAAAACTAGTAACTAG
GGCTAAG

[0087] In some embodiments, the specific sequence of the 16S rRNA encoding gene of said at least one bacterium of the genus *Veillonella* is a sequence comprising or consisting of **SEQ ID NO: 5** or a variant thereof comprising at least 90% of identity with **SEQ ID NO: 5**, preferably comprising at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of identity with **SEQ ID NO: 5.**

SEQ ID NO: 5 (Veillonella parvula DSM 2008)
AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGC
AAGTCGAACGAAGAGCGATGGAAGCTTGCTTCTATCAATCTTAGTGGCGAA
CGGGTGAGTAACGCGTAATCAACCTGCCCTTCAGAGGGGGACAACAGTTGG
AAACGACTGCTAATACCGCATACGATCTAACCTCGGCATCGAGGAAAGATG
AAAGGTGGCCTCTATTTATAAGCTATCACTGAAGGAGGGGATTGCGTCTGA
TTAGCTAGTTGGAGGGGTAACGGCCCACCAAGGCGATGATCAGTAGCCGGT
CTGAGAGGATGAACGGCCACATTGGGACTGAGACACGGCCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGC
AACGCCGCGTGAGTGATGACGGCCTTCGGGTTGTAAAGCTCTGTTAATCGG
GACGAAAGGCCTTCTTGCGAACAGTTAGAAGGATTGACGGTACCGGAATAG
AAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAA
GCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGATCAGTCAGT
CTGTCTTAAAAGTTCGGGGCTTAACCCCGTGATGGGATGGAAACTGCTGATC
TAGAGTATCGGAGAGGAAAGTGGAATTCCTAGTGTAGCGGTGAAATGCGTA
GATATTAGGAAGAACACCAGTGGCGAAGGCGACTTTCTGGACGAAAACTGA

CGCTGAGGCGCGAAAGCCAGGGGAGCGAACGGGATTAGATACCCCGGTAG
TCCTGGCCGTAAACGATGGGTACTAGGTGTAGGAGGTATCGACCCCTTCTGT
GCCGGAGTTAACGCAATAAGTACCCCGCCTGGGGAGTACGACCGCAAGGTT
GAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTT
TAATTCGACGCAACGCGAAGAACCTTACCAGGTCTTGACATTGATGGACAG
AACCAGAGATGGTTCCTCTTCTTCGGAAGCCAGAAAACAGGTGGTGCACGG
TTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA
ACCCCTATCTTATGTTGCCAGCACTTTGGGTGGGAACTCATGAGAGACTGCC
GCAGACAATGCGGAGGAAGGCGGGGATGACGTCAAATCATCATGCCCCTTA
TGACCTGGGCTACACACGTACTACAATGGGAGTTAATAGACGGAAGCGAGA
TCGCGAGATGGAGCAAACCCGAGAAACACTCTCTCAGTTCGGATCGTAGGC
TGCAACTCGCCTACGTGAAGTCGGAATCGCTAGTAATCGCAGGTCAGCATA
CTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGA
AAGTCGGAAGTGCCCAAAGCCGGTGGGGTAACCTTCGGGAGCCAGCCGTCT
AAGGTAAAGTCGATGATTGGGGTGAAGTCGTAACAAGGTAGCCGTATCGGA
AGGTGCGGCTGGATCACCT

[0088] In some embodiments, the specific sequence of the 16S rRNA encoding gene of said at least one bacterium of the genus *Prosthecobacter* is a sequence comprising or consisting of **SEQ ID NO: 6** or a variant thereof comprising at least 90% of identity with **SEQ ID NO: 6**, preferably comprising at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of identity with **SEQ ID NO: 6.**

[0089] **SEQ ID NO: 6** (Prosthecobacter fluviatilis)

AACGAACGCTGGCGGCGTGGATAAGACATGCAAGTCGAACGGGATCATCG
GAGTAGCAATACAACGGTGGTCTAGTGGCGAACGGGTGCGTAACACGTGGA
TACATTCCGGAAAGCGGGGGATAGCCCAGGGAAACTTGGATTAATACCGCA
TGTGGTTGAAAAACTAAAGGCAGGGACCGCAAGGCCTGCCACTTTCCGATT
GGTCCGCGGCCTATCAGCTAGTTGGCGGGGTAAAAGCCCACCAAGGCTACG
ACGGGTAGCTGGTCTTAGAGGACGACCAGCCACACTGGAACTGAGACACGG
TCCAGACACCTACGGGTGGCAGCAGTCGAGAATCTTTCACAATGGGGGCAA
CCCTGATGGAGCGACGCCGCGTGGAGGATAAGGTCTTCGGATTGTAAACTC

CTGTCATGTGTGAACAAGGTTGCGAGGTTAATAGTCTTGCAAATTGATGGTA
ACACAAGAGGAAGAGACGGCTAACTCTGTGCCAGCAGCCGCGGTAATACA
GAGGTCTCAAGCGTTGTTCGGAATCACTGGGCGTAAAGGGTGCGTAGGTGG
CGTGGTAAGTCAGATGTGAAAGCCCGGGGCTCAACCTCGGAACAGCATCCG
ATACTACCATGCTAGAGTACTGAAGAGGTGACTAGAATTCTCGGTGTAGCA
GTGAAATGCGTAGATATCGAGAGGAACACCAACGGCGAAGGCAGGTCACT
GGGCAGTTACTGACACTGAGGCACGAAGGCCAGGGGAGCAAACGGGATTA
GATACCCCGGTAGTCCTGGCAGTAAACGGTGCACGTTTGGTGTGGGCGCAA
TCGACCGCGTCCGCGCCGGAGCTAACGCGTTAAACGTGCCGCCTGGGAAGT
ACGGTCGCAAGATTAAAACTCAAAGAAATTGACGGGGACCCGCACAAGCG
GTGGAGTATGTGGCTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTG
ACATGCATTGTGTCTACGGTGAAAGCCGTATAGGGTAGCAATACCCGCTTTG
CACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAG
TCCCGCAACGAGCGCAACCCCTGTGATTTGTTGCCACCGGCGTATGCCGAGC
ACTCGAATCAGACTGCCTCGATCAACGAGGAGGAAGGTGGGGATGACGTCA
AGTCCGTATGGCCCTTACGACCAGGGCTGCACACGTACTACAATGCCCAGT
ACAATGTGAACCGAGACCGCGAGGTGGAGGAAATCAATAAAACTGGGCTC
AGTTCAGATTGGAGTCTGCAACTCGACTTCATGAAGCTGGAATCGCTAGTA
ATGGTACATCAGCTACGGTACCGTGAATACGTTCCCGGGTCTTGTACACACC
GCCCGTCACATCATGGAAGCTGGCTTCGCCCGAAGTGCGTGCGCCAACCGC
AAGGAGGCAGCGCCCTAAGGCAAGACTGGTGACTGGGATGAAGT

[0090] In some embodiments, the specific sequence of the 16S rRNA encoding gene of said at least one bacterium of the genus *Streptococcus* is a sequence comprising or consisting of **SEQ ID NO: 7** or a variant thereof comprising at least 90% of identity with **SEQ ID NO: 7**, preferably comprising at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of identity with **SEQ ID NO: 7.**

[0091] **SEQ ID NO: 7** (Streptococcus constellatus subsp. pharyngis SK1060 = CCUG 46377)

ACAGGATGCACCGTAGTTTACTACACCGTATTCTGTGAGTTGCGAACGGGTG
AGTAACGCGTAGGTAACCTGCCTGGTAGCGGGGGATAACTATTGGAAACGA

TAGCTAATACCGTATAAGAACATTTACTGCATGGTAGATGTTTAAAAGGTGC
AAGAGCATCACTACCAGATGGACCTGCGTTGTATTAGCTAGTTGGTGAGGT
AACGGCTCACCAAGGCAACGATACATAGCCGACCTGAGAGGGTGATCGGCC
ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGG
AATCTTCGGCAATGGGGGGAACCCTGACCGAGCAACGCCGCGTGAGTGAAG
AAGGTTTTCGGATCGTAAAGCTCTGTTGTTAAGGAAGAACGTGTGTGAGAG
TGGAAAGTTCACACAGTGACGGTACTTAACCAGAAAGGGACGGCTAACTAC
GTGCCAGCAGCCGCGGTAATACGTAGGTCCCGAGCGTTGTCCGGATTTATTG
GGCGTAAAGCGAGCGCAGGCGGTTAGATAAGTCTGAAGTGAAAGGCAGTG
GCTCAACCATTGTAGGCTTTGGAAACTGTTTAACTTGAGTGCAGAAGGGGA
GAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACAC
CGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGC
GTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG
AGTGCTAGGTGTTAGGTCCTTTCCGGGACTTAGTGCCGCAGCTAACGCATTA
AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGA
AGAACCTTACCAGGTCTTGACATCCCTCTGACCACTCTAGAGATAGAGTTTC
CCTTCGGGGCAGAGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
GAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTGTTAGTTGCCA
TCATTCAGTTGGGCACTCTAGCGAGACTGCCGGTAATAAACCGGAGGAAGG
TGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGC
TACAATGGCTGGTACAACGAGTCGCAAGCCGGTGACGGCAAGCTAATCTCT
GAAAGCCAGTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTC
GGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGG
CCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCG
GTGAGGTAACCGTAAGGAGCC

[0092] In some embodiments, the specific sequence of the 16S rRNA encoding gene of said at least one bacterium of the genus *Atopobium* is a sequence comprising or consisting of **SEQ ID NO: 8** or a variant thereof comprising at least 90% of identity with **SEQ ID NO: 8**, preferably comprising at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of identity with **SEQ ID NO: 8.**

[0093] **SEQ ID NO: 8** (Atopobium massiliense strain Marseille-P4126)

GACGGAGAGTTCGATCCTGGCTCAGGATGAACGCTGGCGGCGCGCCTAACA
CATGCAAGTCGAACGATTAAAGCACCTTCGGGTGTGTATAAAGTGGCGAAC
GGCTGAGTAACACGTGGGCAACCTGCCCTTTGCACTGGGATAGCCTCGGGA
AACCGAGGTTAATACCGGATACTCCATATATATCGCATGATGTATATGGGA
AAGCTCCGACGGCAAAGGATGGGCCCGCGGCCTGTTAGCTAGTTGGTGGGG
TAACGGCCTACCAAGGCAATGATGGGTAGCCGGGTTGAGAGACCGACCGGC
CAGATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGG
GAATCTTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGCGGGAT
GAAGGCCTTCGGGTTGTAAACCGCTTTCAGCAGGGACGAGGCCGCAAGGTG
ACGGTACCTGCAGAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTA
ATACGTAGGGGGCAAGCGTTATCCGGATTCATTGGGCGTAAAGCGCGCGTA
GGCGGTTTGTTAGGTCAGGAGTTAAATCTGGGGGCTCAACCCCTATCCGCTC
CTGATACCGGCAGGCTTGAGTCTGGTAGGGGAAGGCGGAATTCCAAGTGTA
GCGGTGAAATGCGCAGATATTTGGAAGAACACCGGTGGCGAAGGCGGCCTT
CTGGGCCACGACTGACGCTGAGGCGCGAAAGCTAGGGGAGCGAACAGGAT
TAGATACCCTGGTAGTCCTAGCCGTAAACGATGGACACTAGGTGTGGGGAG
ATTATACTTTCCGTGCCGCAGCCAACGCATTAAGTGTCCCGCCTGGGGAGTA
CGGTCGCAAGACTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCAG
CGGAGCATGTGGCTTAATTCGAAGCAACGCGAAGAACCTTACCAGGGCTTG
ACATTTAGGTGAAGCAGTGGAAACACTGTGGCCGAAAGGAGCCTAAACAG
GTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG
CAACGAGCGCAACCCTTGTCGCATGTTGCCAGCGGTTAAAGCCGGGCACCC
ATGCGAGACCGCCGGCGTTAAGCCGGAGGAAGGTGGGGACGACGTCAAGT
CATCATGCCCCTTATGTCCTGGGCTGCACACGTGCTACAATGGCCGGCACAG
AGGGCTGCAACTGCGCGAGCAGAAGCGAATCCCTAAAGCCGGTCCCAGTTC
GGATTGGAGGCTGCAACCCGCCTCCATGAAGTCGGAGTTGCTAGTAATCGC
GGATCAGCACGCCGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCG
TCACACCACCCGAGTCGTCTGCACCCGAAGTCGTCGGCCTAACCTGCAAAG
GAGGGAGACGCCGAAGGTGTGGAGGGTAAGGGGGGTGAAGTCGTAACAAG
GTAGCCGTACCGGAAGGTGCGGCTGGATCACCTCCTTT

[0094] In some embodiments, the specific sequence of the 16S rRNA encoding gene of said at least one bacterium of the genus *Oribacterium* is a sequence comprising or consisting of **SEQ ID NO: 9** or a variant thereof comprising at least 90% of identity with **SEQ ID NO: 9**, preferably comprising at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of identity with **SEQ ID NO: 9.**

[0095] **SEQ ID NO: 9** (Oribacterium sinus 16S ribosomal RNA gene, partial sequence)

CGAACGAGATGCGTATCGGAAAGCTTCGGCCGGAAGATACGTCCTCTAAAG
GCGGACGGGTGAGTAACACGTGGGTAACCTGCCTTATGGAGGGGGATAACA
GAGAGAAATCACTGCTAATACCGCATAAGCACACAGTACCGCATGGTACAG
GGTGAAAAGATTTATCGCCATAAGATGGACCCGCGTCTGATTAGCCAGTTG
GCAGGGTAAAAGCCTACCAAAGCAACGATCAGTAGCCGATCTGAGAGGAT
GACCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGC
AGTGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCGACGCCGCGTG
AGTGAAGAAGTATTTCGGTATGTAAAGCTCTATCAGCAGGGAAGATAATGA
CAGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAA
TACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAGGGAGCGTAG
ACGGAATGGCAAGTCTGAAGTGAAATACCCGGGCTCAACCTGGGAACTGCT
TTGGAAACTGTTGTTCTAGAGTGTTGGAGAGGTAAGTGGAATTCCTGGTGTA
GCGGTGAAATGCGTAGATATCAGGAAGAACACCGGAGGCGAAGGCGGCTT
ACTGGACAATAACTGACGTTGAGGCTCGAAAGCGTGGGGATCAAACAGGAT
TAGATACCCTGGTAGTCCACGCTGTAAACGATGAATACTAGGTGTCGGGGT
GCAAAGTATCTCGGTGCCGTCGCTAACGCAATAAGTATTCCACCTGGGGAG
TACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCG
GTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAGTCTT
GAGATCCCATTGACAGAGTATGTAATGTACTTTCCCTTCGGGGCAATGGTGA
CAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTC
CCGCAACGAGCGCAACCCCTATAGTTAGTAGCCAGCAGTAAGATGGGCACT
CTAACTAGACTGCCAGGGATAACCTGGAGGAAGGCGGGGATGACGTCAAAT
CATCATGCCCCTTATGACTTGGGCTACACACGTGCTACAATGGCGTAAACAA
AGGGAAGCAAGAGGGTGACCTTAAGCAAATCTCAAAAATAACGTCTCAGTT

CGGACTGTAGTCTGCAACCCGACTACACGAAGCTGGAATCGCTAGTAATCG
CAGATCAGCATGCTGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCG
TCACACCATGGGAGTTGGTAATGCCCGAATACAGTGTCCCAAGAA

[0096] In some embodiments, the specific sequence of the 16S rRNA encoding gene of said at least one bacterium of one genus of the *Lactobacillales* order is a sequence comprising or consisting of **SEQ ID NO: 10** or a variant thereof comprising at least 90% of identity with **SEQ ID NO: 10**, preferably comprising at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of identity with **SEQ ID NO: 10.**

[0097] **SEQ ID NO: 10** (Lactobacillus hominis DSM 23910 = CRBIP 24.179)

AGAGTTTGATCATGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGC
AAGTCGAGCGAGCTTGCCTAGATGATTTCGGTGCTTGCACCGAATGAAACT
AGATACAAGCGAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCA
AGAGACTGGGATAACACCTGGAAACAGATGCTAATACCGGATAACAACATC
TAGCGCATGCTAGAAGTTTGAAAGATGGGTTTCTATCACTCTTGGATGGACC
TGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAATGATGC
ATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCA
AACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCT
GATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTG
TTGGTGGTGAAGAAAGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAAT
CACCTAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGTGCAGGCGGTTC
AATAAGTCTGATGTGAAAGCCTTCGGCTCAACCGGAGAATTGCATCAGAAA
CTGTTGAACTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG
GAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTC
TGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGC
CTCTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACC
GCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAG
CATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCC
AGTGCAAACCTAAGAGATTAGGTGTTCCCTTCGGGGACGCTGAGACAGGTG

GTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTTGTCATTAGTTGCCATCATTAAGTTGGGCACTCTAATGA
GACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATG
CCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGAA
GCGAACCTGCGAAGGCAAGCGGATCTCTGAAAGCCGTTCTCAGTTCGGACT
GTAGGCTGCAACTCGCCTACACGAAGCTGGAATCGCTAGTAATCGCGGATC
AGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACA
CCATGAGAGTCTGTAACACCCAAAGCCGGTGAGATAACCATTAAAGGAGTC
AGCCGTCTAAGGTAGGACAGATGATTAGGGTGAAGTCGTAACAAGGTAGCC
GTAGGAGAACCTGCGGTTGGATCACCTCCTTA

[0098] In some embodiments, the specific sequence of the 16S rRNA encoding gene of said at least one bacterium of the genus *Rothia* is a sequence comprising or consisting of **SEQ ID NO: 11** or a variant thereof comprising at least 90% of identity with **SEQ ID NO: 11**, preferably comprising at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of identity with **SEQ ID NO: 11.**

[0099] **SEQ ID NO: 11** (Rothia dentocariosa ATCC 17931)

TTGTAACGGAGAGTTTGATTCTGGCTCAGGACGAACGCTGGCGGCGTGCTT
AACACATGCAAGTCGAACGATGAAGCCTAGCTTGCTAGGTGGATTAGTGGC
GAACGGGTGAGTAATACGTGAGTGACCTACCTTTGACTCTGGGATAAGCCT
GGGAAACTGGGTCTAATACCGGATACGACCAATCTCCGCATGGGGTGTTGN
TGGAAAGCGTTATGGAGTGGTTTTAGATGGGCTCACGGCCTATCAGCTTGTT
GGTGAGGTAATGGCTTACCAAGGCGACGANGGGTAGCCGGCCTGAGAGGG
TGACCGGCYACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG
CAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGT
GAGGGATGACGGCCTTCGGGTTGTAAACCTCTGTTAGCATCGAAGAAGCGA
AAGTGACGGTAGGTGCAGAGAAAGCGCCGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAG
CTTGTAGGCGGTTGGTCGCGTCTGCTGTGAAAGGCTGGGGCTTAACCCTGGT
TTTGCAGTGGGTACGGGCTAACTAGAGTGCAGTAGGGGAGACTGGAATTCC
TGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAG

GCAGGTCTCTGGGCTGTAACTGACGCTGAGAAGCGAAAGCATGGGGAGCGA
ACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTG
TGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGC
CTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCCN
GCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTA
CCAAGGCTTGACATATACTGGACTGCGTCAGAGATGGCGTTTCCCTTCGGGG
CTGGTATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGTGATG
GTGGGGACTCATAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGAT
GACGTCAAATCATCATGCCCCTTATGTCTTGGGCTTCACGCATGCTACAATG
GCCGGTACAGAGGGNTGCGATACTGTGAGGTGGAGCTAATCCCTAAAAGCC
GGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCG
CTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGT
ACACACCGCCCGTCAAGTCACGAAAGTTGGTAACACCCGAAGCCGGTGGCC
TAACCCTGGTGGGGGGAGCCGTCGAAGGTGGGACTGGNGATNGGG.

[0100] In one embodiment, the presence or abundance of at least one bacterium is assessed by assessing the binding of a specific probe to a nucleic acid present in the respiratory sample. In one embodiment, said probe is specific for a specific 16S rRNA encoding gene, such as for example, the V4 region of specific 16S rRNA encoding gene. In one embodiment, before assessing the binding of a specific probe to a nucleic acid present in the respiratory sample, a PCR step is performed in order to amplify 16S rRNA encoding gene sequences present in the respiratory sample, such as for example to amplify the v4 region of the 16S rRNA encoding gene sequences present in the respiratory sample, wherein the respiratory sample is a Bronchoalveolar Lavage (BAL) sample, an endotracheal aspirate (ETA) sample, and/or an oropharyngeal sample (OS). Examples of primers that may be used to amplify the V4 region include, but are not limited to SEQ ID NO: 25 and SEQ ID NO: 26.

[0101] In some embodiments, the presence or abundance of at least one bacterium of the genus *Staphylococcus* is assessed using a probe having the sequence **SEQ ID NO: 12** (5'-TCG CAC ATC AGC GTC AGT T -3').

[0102] In some embodiments, the presence or abundance of at least one bacterium of the genus *Ralstonia* is assessed using a probe having the sequence **SEQ ID NO: 13** (5'-TCC TAT AGC ATG AGG CCT TG -3').

[0103] In some embodiments, the presence or abundance of at least one bacterium of the genus *Enterococcus* is assessed using a probe having the sequence **SEQ ID NO: 14** (5'-AAC CTA CCC ATC AGA GGG -3').

[0104] In some embodiments, the presence or abundance of at least one bacterium of the genus *Flavobacterium* is assessed using a probe having the sequence **SEQ ID NO: 15** (5'- GGT CTG TTT CCA AAC CGG -3').

[0105] In some embodiments, the presence or abundance of at least one bacterium of the genus *Veillonella* is assessed using a probe having the sequence **SEQ ID NO: 16** (5'-AGA CGC AAT CCC CTC CTT -3').

[0106] In some embodiments, the presence or abundance of at least one bacterium of the genus *Prosthecobacter* is assessed using a probe having the sequence **SEQ ID NO: 17** (5'- CCG GAA TGT ATC CAC GTG -3').

[0107] In some embodiments, the presence or abundance of at least one bacterium of the genus *Streptococcus* is assessed using a probe having the sequence **SEQ ID NO: 18** (5'-CCA CTC TCC CCT TCT GCA C -3').

[0108] In some embodiments, the presence or abundance of at least one bacterium of the genus *Atopobium* is assessed using a probe having the sequence **SEQ ID NO: 19** (5'-GGT CGG TCT CTC AAC CC -3').

[0109] In some embodiments, the presence or abundance of at least one bacterium of the genus *Oribacterium* is assessed using a probe having the sequence **SEQ ID NO: 20** (5'-TTA CTG CTG GCT ACT AAC TAT AGG G -3').

[0110] In some embodiments, the presence or abundance of at least one bacterium of the genus *Lactobacillales* is assessed using a probe having the sequence **SEQ ID NO: 21** (5'-YCA CCG CTA CAC ATG RAG TTC CAC T -3').

[0111] In some embodiments, the presence or abundance of at least one bacterium of the genus *Rothia* is assessed using a probe having the sequence **SEQ ID NO: 22** (5'- AAC CAC TCC ATA ACG CTT TC -3').

[0112] In some embodiments, the respiratory complication is selected from the group comprising hospital acquired pneumonia (HAP), acute respiratory distress syndrome (ARDS), ARDS related death and need of prolonged mechanical ventilation (MV).

[0113] In some embodiments, the respiratory complication being HAP, the method comprises a step of determining the presence or the abundance, in a respiratory sample of the patient, preferably in an endotracheal aspirate (ETA) of the patient, of at least one bacterium of a genus selected from *Fusobacterium, Microbacterium, Tannerella, Haemophilus, Aquabacterium, Phascolarctobacterium, Faecalibacterium, Mogibacterium, Enterococcus, Lachnoanaerobaculum, Pelomonas, Acinetobacter, Porphyromonas, Abiotrophia, Ruminococcus, Oscillospira, Rhodococcus, Neisseria, Collinsella, Prevotella, TG5, Bacteroides, Filifactor, Butyrivibrio, Sphaerochaeta, Schwartzia, Granulicatella, Peptococcus, Corynebacterium, Peptostreptococcus, Olsenella, Dialister, Mycoplasma, Peptoniphilus, Slackia, Alloiococcus, Capnocytophaga, paraprevotella, Caulobacter, Reyranella, Achromobacter, Flavobacterium, Atopobium, Treponema, Veillonella, Rothia, Stenotrophomonas, Leptotrichia, Campylobacter, Fimbriimonas, Bulleidia, Sediminibacterium, Burkholderia, Actinomyces, Moryella, Streptococcus, Oribacterium, Chryseobacterium, Selenomonas, Ralstonia, Actinobacillus, Megasphaera, Pseudomonas, Bifidobacterium, Aggregatibacter* and *Shuttleworthia*.

[0114] In one embodiment, the method of the present invention further comprises a step of mathematically combining the abundance (preferably the relative abundance) detected for the bacteria listed hereinabove, thereby obtaining a score.

[0115] In one embodiment, the method of the invention comprises summing the abundance (preferably the relative abundance) of at least one bacterium of a genus selected from *Fusobacterium, Microbacterium, Tannerella, Haemophilus, Aquabacterium, Mogibacterium, Lachnoanaerobaculum, Porphyromonas, Abiotrophia,*

Neisseria, Collinsella, Prevotella, TG5, Filifactor, Sphaerochaeta, Schwartzia, Granulicatella, Peptococcus, Corynebacterium, Peptostreptococcus, Olsenella, Dialister, Mycoplasma, Peptoniphilus, Slackia, Alloiococcus, Capnocytophaga, Paraprevotella, Caulobacter, Reyranella, Atopobium, Treponema, Veillonella, Rothia, Leptotrichia, Campylobacter, Fimbriimonas, Bulleidia, Sediminibacterium, Actinomyces, Moryella, Streptococcus, Oribacterium, Selenomonas, Actinobacillus, Megasphaera, Pseudomonas, Bifidobacterium, Aggregatibacter, Shuttleworthia and/or subtracting the abundance of at least one bacterium of a genus selected from *Ralstonia, Chryseobacterium, Burkholderia, Stenotrophomonas, Achromobacter, Flavobacterium, Butyrivibrio, Bacteroides, Ruminococcus, Oscillospira, Rhodococcus, Pelomonas, Acinetobacter, Enterococcus, Phascolarctobacterium, Faecalibacterium.*

[0116] In one embodiment, the method of the invention comprises summing the abundance (preferably the relative abundance) of bacteria of a genus selected from *Fusobacterium, Microbacterium, Tannerella, Haemophilus, Aquabacterium, Mogibacterium, Lachnoanaerobaculum, Porphyromonas, Abiotrophia, Neisseria, Collinsella, Prevotella, TG5, Filifactor, Sphaerochaeta, Schwartzia, Granulicatella, Peptococcus, Corynebacterium, Peptostreptococcus, Olsenella, Dialister, Mycoplasma, Peptoniphilus, Slackia, Alloiococcus, Capnocytophaga, Paraprevotella, Caulobacter, Reyranella, Atopobium, Treponema, Veillonella, Rothia, Leptotrichia, Campylobacter, Fimbriimonas, Bulleidia, Sediminibacterium, Actinomyces, Moryella, Streptococcus, Oribacterium, Selenomonas, Actinobacillus, Megasphaera, Pseudomonas, Bifidobacterium, Aggregatibacter, Shuttleworthia* and subtracting the abundance of at least one bacterium of a genus selected from *Ralstonia, Chryseobacterium, Burkholderia, Stenotrophomonas, Achromobacter, Flavobacterium, Butyrivibrio, Bacteroides, Ruminococcus, Oscillospira, Rhodococcus, Pelomonas, Acinetobacter, Enterococcus, Phascolarctobacterium, Faecalibacterium.*

[0117] In one embodiment, the method of the invention comprises a step of determining the presence or the abundance, in a respiratory sample of the patient, preferably in an endotracheal aspirate (ETA) of the patient, of at least one bacterium of a genus selected from *Stenotrophomonas, Veillonella, Enterococcus, Reyranella, Rothia, Ralstonia,*

Mycoplasma, Microbacterium, Aggregatibacter, Neisseria, Acinetobacter, Tannerella, Haemophilus, Bacteroides, Pseudomonas, Corynebacterium.

[0118] In some embodiments, the respiratory complication being HAP, the method comprises a step of determining the presence or the abundance of at least one bacterium of a genus selected from *Streptococcus, Atopobium, Oribacterium* and *Veillonella* in a respiratory sample of the patient, preferably in an endotracheal aspirate (ETA) of the patient. In some embodiments, the presence or abundance of at least one bacterium of the following genus is determined: *Streptococcus, Atopobium, Oribacterium* and *Veillonella.*

[0119] In some embodiments, the respiratory complication being HAP, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of a genus selected from *Streptococcus, Atopobium, Oribacterium* and *Veillonella* in a respiratory sample of the patient, preferably in an endotracheal aspirate (ETA) of the patient. In some embodiments, the presence or abundance of at least one bacterium of the following genus is determined: *Streptococcus, Atopobium, Oribacterium* and *Veillonella.*

[0120] In some embodiments, the respiratory complication being HAP, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of a genus selected from *Streptococcus, Atopobium, Oribacterium* and *Veillonella* in a respiratory sample of the patient, the respiratory sample of the patient being an endotracheal aspirate (ETA) of the patient. In some embodiments, the presence or abundance of at least one bacterium of the four following genus is determined: *Streptococcus, Atopobium, Oribacterium* and *Veillonella.*

[0121] In one embodiment, the method of the invention comprises summing the abundance (preferably the relative abundance) of at least one (preferably of the 4) bacterium of a genus selected from *Streptococcus, Atopobium, Oribacterium* and *Veillonella.*

[0122] In one embodiment, a high score is indicative of a low risk of developing HAP. In one embodiment, a score inferior or equal to 0.9 is indicative of a high risk of HAP.

[0123] In one embodiment, the respiratory complication is HAP and the method of the present invention comprises a first step of determining the presence or abundance, notably a first step of detecting the presence or determining the abundance, of at least one bacterium of the *Streptococcus* genus in a respiratory sample of the patient, a second optional step of determining the presence or abundance of at least one bacterium of the *Atopobium* genus in the respiratory sample of the patient, a third optional step of determining the presence or abundance of at least one bacterium of the *Oribacterium* genus in the respiratory sample of the patient and a fourth optional step of determining the presence or abundance of at least one bacterium of the *Veillonella* genus in the respiratory sample of the patient. In one preferred embodiment, the respiratory sample is an endotracheal aspirate (ETA) of the patient.

[0124] In one embodiment, the respiratory complication is HAP and the method of the present invention comprises a first step of determining a relative abundance of at least one bacterium of the *Streptococcus* genus in a respiratory sample of the patient and comparing the relative abundance of said at least one bacterium of the *Streptococcus* genus to a first predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus, a second optional step of determining a relative abundance of at least one bacterium of the *Atopobium* genus in the respiratory sample of the patient and comparing the relative abundance of said at least one bacterium of the *Atopobium* genus to a second predetermined threshold value of relative abundance of said at least one bacterium of the *Atopobium* genus, a third optional step of determining a relative abundance of at least one bacterium of the *Oribacterium* genus in the respiratory sample of the patient and comparing the relative abundance of said at least one bacterium of the *Oribacterium* genus to a third predetermined threshold value of relative abundance of said at least one bacterium of the *Oribacterium* genus, and a fourth optional step of determining a relative abundance of at least one bacterium of the *Veillonella* genus in the respiratory sample of the patient and comparing the relative abundance of said at least one bacterium of the *Veillonella* genus to a third predetermined threshold value of relative abundance of said at least one bacterium of the *Veillonella* genus.

[0125] In one embodiment, the second step is performed if the relative abundance of said at least one bacterium of the *Streptococcus* genus is greater than or equal to the first predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient.

[0126] In one embodiment, the third step is performed if the relative abundance of said at least one bacterium of the *Atopobium* genus is greater than or equal to the second predetermined threshold value of relative abundance of said at least one bacterium of the *Atopobium* genus in the respiratory sample of the patient.

[0127] In one embodiment, the fourth step is performed if the relative abundance of said at least one bacterium of the *Oribacterium* genus is greater than or equal to the third predetermined threshold value of relative abundance of said at least one bacterium of the *Oribacterium* genus in the respiratory sample of the patient.

[0128] In some embodiments, wherein the respiratory complication is HAP, the first predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus is determinable and relates to the relative abundance of said at least one bacterium of the *Streptococcus* genus in a respiratory sample of subjects of a reference population of subjects. The reference population of subjects refers to a group of subjects with shared factors, influences, or condition.

[0129] In some embodiments, wherein the respiratory complication is HAP, the first predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus is less than 0.2. In some embodiments, wherein the respiratory complication is HAP, the first predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus is less than 0.05, such as, for example, is lower than about 0,01. In some embodiments, wherein the respiratory complication is HAP, the first predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus is less than 0.05, such as, for example, is lower than about 0,125. In some embodiments, wherein the respiratory complication is HAP, the first predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus is 0.01, 0.05, 0.1 or 0.125. In some

embodiments, wherein the respiratory complication is HAP, the first predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus is about 0.01.

[0130] In some embodiments, wherein the respiratory complication is HAP, the second predetermined threshold value of relative abundance of said at least one bacterium of the *Atopobium* genus is determinable and relates to the relative abundance of said at least one bacterium of the *Atopobium* genus in a respiratory sample of subjects of a reference population of subjects. The reference population of subjects refers to a group of subjects with shared factors, influences, or condition.

[0131] In some embodiments, wherein the respiratory complication is HAP, the second predetermined threshold value of relative abundance of said at least one bacterium of the *Atopobium* genus is less than 0.03. In some embodiments, wherein the respiratory complication is HAP, the second predetermined threshold value of relative abundance of said at least one bacterium of the *Atopobium* genus is 0.0001, 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.011, 0.012, 0.013, 0.014, 0.015, 0.016, 0.017, 0.018, 0.019, 0.020, 0.021, 0.022, 0.023, 0.024, 0.025, 0.026, 0.027, 0.028, 0.029, 0.030. In some embodiments, wherein the respiratory complication is HAP, the second predetermined threshold value of relative abundance of said at least one bacterium of the *Atopobium* genus is about 0.005.

[0132] In some embodiments, wherein the respiratory complication is HAP, the third predetermined threshold value of relative abundance of said at least one bacterium of the *Oribacterium* genus is determinable and relates to the relative abundance of said at least one bacterium of the *Oribacterium* genus in a respiratory sample of subjects of a reference population of subjects. The reference population of subjects refers to a group of subjects with shared factors, influences, or condition.

[0133] In some embodiments, wherein the respiratory complication is HAP, the third predetermined threshold value of relative abundance of said at least one bacterium of the *Oribacterium* genus is less than 0.002. In some embodiments, wherein the respiratory complication is HAP, the third predetermined threshold value of relative abundance of

said at least one bacterium of the *Oribacterium* genus is 0.00001, 0.00005, 0.0001, 0.0002, 0.0003, 0.0004, 0.0005, 0.0006, 0.0007, 0.0008, 0.0009, 0.0010, 0.0011, 0.0012, 0.0013, 0.0014, 0.0015, 0.0016, 0.0017, 0.0018, 0.0019 or 0.002. In some embodiments, wherein the respiratory complication is HAP, the third predetermined threshold value of relative abundance of said at least one bacterium of the *Oribacterium* genus is about 0.0003.

[0134] In some embodiments, wherein the respiratory complication is HAP, the fourth predetermined threshold value of relative abundance of said at least one bacterium of the *Veillonella* genus is determinable and relates to the relative abundance of said at least one bacterium of the *Veillonella* genus in a respiratory sample of subjects of a reference population of subjects. The reference population of subjects refers to a group of subjects with shared factors, influences, or condition.

[0135] In some embodiments, wherein the respiratory complication is HAP, the fourth predetermined threshold value of relative abundance of said at least one bacterium of the *Veillonella* genus is less than 0.04. In some embodiments, wherein the respiratory complication is HAP, the fourth predetermined threshold value of relative abundance of said at least one bacterium of the *Veillonella* genus is about 0.001, 0,004, 0.005, 0.01, 0.02, 0.03, or 0.04. In some embodiments, wherein the respiratory complication is HAP, the fourth predetermined threshold value of relative abundance of said at least one bacterium of the *Veillonella* genus is about 0.004. In some embodiments, wherein the respiratory complication is HAP, the fourth predetermined threshold value of relative abundance of said at least one bacterium of the *Veillonella* genus is about 0.04.

[0136] In some embodiments, wherein the respiratory complication is HAP, the respiratory sample is an endotracheal aspirate (ETA) of the patient.

[0137] In some embodiments, wherein the respiratory complication is HAP, the method comprises a step of determining the relative abundance of at least one bacterium of the *Streptococcus* genus in a respiratory sample of the patient, preferably in an endotracheal aspirate (ETA) of the patient,

wherein when the relative abundance of said at least one bacterium of the *Streptococcus* genus is determined to be lower than the first predetermined threshold value, such as, for example lower than about 0.01 or 0,125, in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient, then a high risk for HAP outcome is concluded for the patient.

[0138] In some embodiments, wherein the respiratory complication is HAP, the method comprises a step of determining the relative abundance of at least one bacterium of the *Atopobium* genus in a respiratory sample of the patient, preferably in an endotracheal aspirate (ETA) of the patient,

wherein when the relative abundance of said at least one bacterium of the *Atopobium* genus is determined to be lower than the second predetermined threshold value, such as, for example lower than 0.005, in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient, then a high risk for HAP outcome is concluded for the patient.

[0139] In some embodiments, wherein the respiratory complication is HAP, the method comprises a step of determining the relative abundance of at least one bacterium of the *Oribacterium* genus in a respiratory sample of the patient, preferably in an endotracheal aspirate (ETA) of the patient,

wherein when the relative abundance of said at least one bacterium of the *Oribacterium* genus is determined to be lower than the third predetermined threshold value, such as, for example lower than 0.0003, in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient, then a high risk for HAP outcome is concluded for the patient.

[0140] In some embodiments, wherein the respiratory complication is HAP, the method comprises a step of determining the relative abundance of at least one bacterium of the *Veillonella* genus in a respiratory sample of the patient, preferably in an endotracheal aspirate (ETA) of the patient,

wherein when the relative abundance of said at least one bacterium of the *Veillonella* genus is determined to be lower than the fourth predetermined threshold value, such as, for example lower than 0,04 or than 0.004, in the respiratory sample of the patient,

preferably in the endotracheal aspirate (ETA) of the patient, then a high risk for HAP outcome is concluded for the patient.

[0141] In some embodiments, wherein the respiratory complication is HAP, the method comprises a first step of determining the relative abundance of at least one bacterium of the *Streptococcus* genus in a respiratory sample of the patient, preferably in an endotracheal aspirate (ETA) of the patient,

wherein when the relative abundance of said at least one bacterium of the *Streptococcus* genus is determined to be lower than the first predetermined threshold value, such as, for example lower than 0.01 or than 0,125, in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient, then a high risk for HAP outcome is concluded for the patient,

wherein when the relative abundance of said at least one bacterium of the *Streptococcus* genus is determined to be equal to or greater than the first predetermined threshold value, such as, for example greater than 0.01 or 0.125, in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient, then

the method comprises a second step of determining the relative abundance of at least one bacterium of the *Atopobium* genus in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient,

wherein when the relative abundance of said at least one bacterium of the *Atopobium* genus is determined to be lower than the second predetermined threshold value, notably lower than 0.005, in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient, then a high risk for HAP outcome is concluded for the patient,

wherein when the relative abundance of said at least one bacterium of the *Atopobium* genus is determined to be equal to or greater than the second predetermined threshold value, notably greater than 0.005, in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient, then

the method comprises a third step of determining the relative abundance of at least one bacterium of the *Oribacterium* genus in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient,

wherein when the relative abundance of said at least one bacterium of the *Oribacterium* genus is determined to be lower than the third predetermined threshold value, notably lower than 0.0003, in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient, then a high risk for HAP outcome is concluded for the patient,

wherein when the relative abundance of said at least one bacterium of the *Oribacterium* genus is determined to be equal to or greater than the third predetermined threshold value, notably greater than 0.0003, in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient, then

the method comprises a fourth step of determining the relative abundance of at least one bacterium of the *Veillonella* genus in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient,

wherein when the relative abundance of said at least one bacterium of the *Veillonella* genus is determined to be lower than the fourth predetermined threshold value, notably lower than 0.004, in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient, then a high risk for HAP outcome is concluded for the patient, and

wherein when said relative abundance of said at least one bacterium of the *Veillonella* genus is determined to be equal to or greater than the fourth predetermined threshold value, notably greater than 0.004, in the respiratory sample of the patient, preferably in the endotracheal aspirate (ETA) of the patient, then a low risk for HAP outcome is concluded for the patient.

[0142] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of determining the presence or the abundance of at least one bacterium of a genus selected from *Achromobacter, Acidovorax, Acinetobacter, Actinomyces, Afipia, Anaerococcus, Anaeroplasma, Arcobacter, Atopobium, Bacillus, Bacteroides, Bdellovibrio, Bifidobacterium, Bulleidia, Campylobacter, Caulobacter, Corynebacterium, Devosia, Dialister, Enterococcus, Fimbriimonas, Finegoldia, Flavobacterium, Fluviicola, Fusobacterium, Gardnerella, Gemmata, Granulicatella, Lachnoanaerobaculum, Leptotrichia, Methylobacterium, Microbacterium, Moryella, Neisseria, Novosphingobium, Opitutus, Oribacterium,*

Paracoccus, Parvimonas, Pediococcus, Pedobacter, Pelomonas, Peptostreptococcus, Planctomyces, Porphyromonas, Prevotella, Propionibacterium, Prosthecobacter, Pseudomonas, Ralstonia, Reyranella, Rothia, Scardovia, Sediminibacterium, Selenomonas, Serratia, Staphylococcus, Stenotrophomonas, Streptococcus, Treponema, Veillonella in a respiratory sample of the patient.

[0143] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of determining the presence or the abundance of at least one bacterium of a genus selected from *Ralstonia, Acidovorax, Afipia, Scardovia, Achromobacter, Bacteroides, Paracoccus, Reyranella, Microbacterium, Enterococcus, Bifidobacterium, Pelomonas, Fimbriimonas, Gardnerella, Sediminibacterium, Rothia, Anaerococcus, Serratia, Finegoldia, Caulobacter, Corynebacterium, Staphylococcus, Acinetobacter, Actinomyces, Bulleidia, Streptococcus, Moryella, Pseudomonas, Lachnoanaerobaculum, Methylobacterium* in a respiratory sample of the patient, preferably in an endotracheal aspirate (ETA) of the patient.

[0144] In one embodiment, the method of the present invention further comprises a step of mathematically combining the abundance (preferably the relative abundance) detected for the bacteria listed hereinabove, thereby obtaining a score.

[0145] In one embodiment, the method of the invention comprises summing the abundance (preferably the relative abundance) of at least one bacterium of a genus selected from *Reyranella, Microbacterium, Fimbriimonas, Sediminibacterium, Serratia, Caulobacter, Bulleidia, Moryella, Methylobacterium, Actinomyces, Streptococcus, Pseudomonas* and/or subtracting the abundance of at least one bacterium of a genus selected from *Ralstonia, Acidovorax, Afipia, Scardovia, Achromobacter, Bacteroides, Paracoccus, Enterococcus, Bifidobacterium, Pelomonas, Gardnerella, Rothia, Anaerococcus, Finegoldia, Corynebacterium, Staphylococcus, Acinetobacter, Actinomyces, Streptococcus, Lachnoanaerobaculum.*

[0146] In one embodiment, the method of the invention comprises summing the abundance (preferably the relative abundance) of bacteria of a genus selected from

Reyranella, Microbacterium, Fimbriimonas, Sediminibacterium, Serratia, Caulobacter, Bulleidia, Moryella, Methylobacterium, Actinomyces, Streptococcus, Pseudomonas and subtracting the abundance of bacteria of a genus selected from *Ralstonia, Acidovorax, Afipia, Scardovia, Achromobacter, Bacteroides, Paracoccus, Enterococcus, Bifidobacterium, Pelomonas, Gardnerella, Rothia, Anaerococcus, Finegoldia, Corynebacterium, Staphylococcus, Acinetobacter, Actinomyces, Streptococcus, Lachnoanaerobaculum.*

[0147] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of determining the presence or the abundance of at least one bacterium of a genus selected from *Staphylococcus, Gardnerella, Ralstonia, Achromobacter, Bulleidia, Actinomyces, Streptococcus, Bifidobacterium, Lachnoanaerobaculum, Rothia* in a respiratory sample of the patient, preferably in endotracheal aspirate (ETA) of the patient.

[0148] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of determining the presence or the abundance of at least one bacterium of a genus selected from *Novosphingobium, Propionibacterium, Ralstonia, Pediococcus, Neisseria, Devosia, Fusobacterium, Fluviicola, Opitutus, Selenomonas, Actinomyces, Prevotella, Peptostreptococcus, Gemmata, Bacteroides, Bdellovibrio, Campylobacter, Sediminibacterium, Oribacterium, Atopobium, Treponema, Dialister, Arcobacter, Leptotrichia, Anaeroplasma, Corynebacterium, Porphyromonas, Pedobacter, Parvimonas, Granulicatella, Bacillus, Stenotrophomonas, Flavobacterium, Prosthecobacter, Veillonella, Planctomyces, Rothia* in a respiratory sample of the patient, preferably in bronchoalveolar lavage (BAL) of the patient.

[0149] In one embodiment, the method of the present invention further comprises a step of mathematically combining the abundance (preferably the relative abundance) detected for the bacteria listed hereinabove, thereby obtaining a score.

[0150] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of determining the presence or the abundance of at least one bacterium of a genus selected from *Flavobacterium, Veillonella,*

Propionibacterium, Prevotella in a respiratory sample of the patient, preferably in bronchoalveolar lavage (BAL) of the patient.

[0151] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of determining the presence or the abundance of at least one bacterium of a genus selected from *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella* and *Prosthecobacter* in a respiratory sample of the patient, such as, for example, in an endotracheal aspirate (ETA) or a bronchoalveolar lavage (BAL) of the patient. In some embodiments, the presence or abundance of at least one bacterium of the following genus is determined: *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella* and *Prosthecobacter.*

[0152] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of a genus selected from *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella* and *Prosthecobacter* in a respiratory sample of the patient. In some embodiments, the presence or abundance of at least one bacterium of the following genus is detected or determined: *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella* and *Prosthecobacter.*

[0153] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of a genus selected from *Staphylococcus, Ralstonia* and *Enterococcus* in a respiratory sample of the patient, wherein the respiratory sample of the patient is an endotracheal aspirate of the patient. In some embodiments, the presence or abundance of at least one bacterium of the following genus is determined: *Staphylococcus, Ralstonia, Enterococcus.*

[0154] In one embodiment, the method of the invention comprises summing the abundance (preferably the relative abundance) of at least one (preferably of the 3) bacterium of a genus selected from *Staphylococcus, Ralstonia* and *Enterococcus.*

[0155] In one embodiment, a high score is indicative of a low risk of developing ARDS or ARDS related death. In one embodiment, a score inferior or equal to -0.1 is indicative of a high risk of developing ARDS or ARDS related death.

[0156] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Staphylococcus* genus in a respiratory sample of the patient.

[0157] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Staphylococcus* genus in an endotracheal aspirate (ETA) of the patient.

[0158] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Staphylococcus* genus in a respiratory sample of the patient,

wherein when the presence at least one bacterium of the *Staphylococcus* genus is detected in the respiratory sample of the patient, then a high risk for ARDS outcome is concluded for the patient.

[0159] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Staphylococcus* genus in an endotracheal aspirate (ETA) of the patient,

wherein when the presence at least one bacterium of the *Staphylococcus* genus is detected in the endotracheal aspirate (ETA) of the patient, then a high risk for ARDS outcome is concluded for the patient.

[0160] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the

abundance of at least one bacterium of the *Ralstonia* genus in a respiratory sample of the patient,

[0161] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Ralstonia* genus in an endotracheal aspirate (ETA) of the patient.

[0162] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Ralstonia* genus in a respiratory sample of the patient,

wherein when the presence at least one bacterium of the *Ralstonia* genus is detected in the respiratory sample of the patient, then a high risk for ARDS outcome is concluded for the patient.

[0163] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Ralstonia* genus in an endotracheal aspirate (ETA) of the patient,

wherein when the presence at least one bacterium of the *Ralstonia* genus is detected in the endotracheal aspirate (ETA) of the patient, then a high risk for ARDS outcome is concluded for the patient.

[0164] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Enterococcus* genus in a respiratory sample of the patient.

[0165] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the

abundance of at least one bacterium of the *Enterococcus* genus in an endotracheal aspirate of the patient.

[0166] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Enterococcus* genus in a respiratory sample of the patient,

wherein when the presence at least one bacterium of the *Enterococcus* genus is detected in the respiratory sample of the patient, then a high risk for ARDS outcome is concluded for the patient.

[0167] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Enterococcus* genus in an endotracheal aspirate (ETA) of the patient,

wherein when the presence at least one bacterium of the *Enterococcus* genus is detected in the endotracheal aspirate (ETA) of the patient, then a high risk for ARDS outcome is concluded for the patient.

[0168] In one embodiment, the respiratory complication is ARDS or ARDS related death and the method of the present invention comprises a first step of detecting the presence or determining the abundance of at least one bacterium of the *Staphylococcus* genus in a respiratory sample of the patient, a second optional step of determining the presence or abundance of at least one bacterium of the *Ralstonia* genus in the respiratory sample of the patient, and a third optional step of determining the presence or abundance of at least one bacterium of the *Enterococcus* genus in the respiratory sample of the patient.

[0169] In one embodiment, the respiratory complication is ARDS or ARDS related death and the method of the present invention comprises a first step of detecting the presence or determining the abundance of at least one bacterium of the *Staphylococcus* genus in an endotracheal aspirate of the patient, a second optional step of determining the presence or abundance of at least one bacterium of the *Ralstonia* genus in the endotracheal aspirate

of the patient and a third optional step of determining the presence or abundance of at least one bacterium of the *Enterococcus* genus in the endotracheal aspirate of the patient.

[0170] In one embodiment, the respiratory complication is ARDS or ARDS related death and the method of the present invention comprises a first step of determining the presence of at least one bacterium of the *Staphylococcus* genus in a respiratory sample of the patient, a second optional step of determining the presence of at least one bacterium of the *Ralstonia* genus in the respiratory sample of the patient, and a third optional step of determining the presence of at least one bacterium of the *Enterococcus* genus in the respiratory sample of the patient.

[0171] In one embodiment, the respiratory complication is ARDS or ARDS related death and the method of the present invention comprises a first step of determining the presence of at least one bacterium of the *Staphylococcus* genus in an endotracheal aspirate (ETA) of the patient, a second optional step of determining the presence of at least one bacterium of the *Ralstonia* genus in the endotracheal aspirate (ETA) of the patient, and a third optional step of determining the presence of at least one bacterium of the *Enterococcus* genus in the endotracheal aspirate (ETA) of the patient.

[0172] In one embodiment, wherein the respiratory complication is ARDS or ARDS related death, the second step is performed if the presence of said at least one bacterium of the *Staphylococcus* genus is not detected in the respiratory sample of the patient, notably if the presence of said at least one bacterium of the *Staphylococcus* genus is not detected in the endotracheal aspirate (ETA) of the patient.

[0173] In one embodiment, wherein the respiratory complication is ARDS or ARDS related death, the third step is performed if the presence of said at least one bacterium of the *Ralstonia* genus is not detected in the respiratory sample of the patient, notably if the presence of said at least one bacterium of the *Ralstonia* genus is not detected in the endotracheal aspirate (ETA) of the patient.

[0174] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence of at least one bacterium of the *Staphylococcus* genus in an endotracheal aspirate of the patient,
wherein when the presence of said at least one bacterium of the *Staphylococcus* genus is detected in the endotracheal aspirate of the patient, then a high risk for ARDS outcome is concluded for the patient,
wherein when the presence of said at least one bacterium of the *Staphylococcus* genus is not detected in the endotracheal aspirate of the patient, then
the method comprises a step of detecting the presence of at least one bacterium of the *Ralstonia* genus in the endotracheal aspirate of the patient,
wherein when the presence of said at least one bacterium of the *Ralstonia* genus is detected in the endotracheal aspirate of the patient, then a high risk for ARDS outcome is concluded for the patient,
wherein when the presence of said at least one bacterium of the *Ralstonia* genus is not detected in the endotracheal aspirate of the patient, then
the method comprises a step of detecting the presence of at least one bacterium of the *Enterococcus* genus in the endotracheal aspirate of the patient,
wherein when the presence of said at least one bacterium of the *Enterococcus* genus is detected in the endotracheal aspirate of the patient, then a high risk for ARDS outcome is concluded for the patient, and
wherein when the presence of said at least one bacterium of the *Enterococcus* genus is not detected in the endotracheal aspirate of the patient, then a low risk for ARDS outcome is concluded for the patient.

[0175] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of a genus selected from *Flavobacterium, Veillonella* and *Prosthecobacter* in a respiratory sample of the patient.

[0176] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the

abundance of at least one bacterium of a genus selected from *Flavobacterium, Veillonella* and *Prosthecobacter* in a bronchoalveolar lavage (BAL) of the patient.

[0177] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Flavobacterium* genus in a respiratory sample of the patient.

[0178] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Flavobacterium* genus in an endotracheal aspirate (ETA) of the patient.

[0179] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of determining the presence or abundance of at least one bacterium of the *Flavobacterium* genus in a respiratory sample of the patient, wherein when the presence at least one bacterium of the *Flavobacterium* genus is detected in the respiratory sample of the patient, then a low risk for ARDS outcome is concluded for the patient.

[0180] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of determining the presence or abundance of at least one bacterium of the *Flavobacterium* genus in a bronchoalveolar lavage (BAL) of the patient,
wherein when the presence at least one bacterium of the *Flavobacterium* genus is detected in the bronchoalveolar lavage (BAL) of the patient, then a low risk for ARDS outcome is concluded for the patient.

[0181] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Veillonella* genus in a respiratory sample of the patient.

[0182] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Veillonella* genus in an endotracheal aspirate (ETA) of the patient.

[0183] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of determining the presence or abundance of at least one bacterium of the *Veillonella* genus in a respiratory sample of the patient, wherein when the presence at least one bacterium of the *Veillonella* genus is detected in the respiratory sample of the patient, then a low risk for ARDS outcome is concluded for the patient.

[0184] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of determining the presence or abundance of at least one bacterium of the *Veillonella* genus in a bronchoalveolar lavage (BAL) of the patient,
wherein when the presence at least one bacterium of the *Veillonella* genus is detected in the bronchoalveolar lavage (BAL) of the patient, then a low risk for ARDS outcome is concluded for the patient.

[0185] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Prosthecobacter* genus in a respiratory sample of the patient.

[0186] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the *Prosthecobacter* genus in an endotracheal aspirate (ETA) of the patient.

[0187] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of determining the presence or abundance of at least one bacterium of the *Prosthecobacter* genus in a respiratory sample of the patient,

wherein when the presence at least one bacterium of the *Prosthecobacter* genus is detected in the respiratory sample of the patient, then a low risk for ARDS outcome is concluded for the patient.

[0188] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of determining the presence or abundance of at least one bacterium of the *Prosthecobacter* genus in a bronchoalveolar lavage (BAL) of the patient,
wherein when the presence at least one bacterium of the *Prosthecobacter* genus is detected in the bronchoalveolar lavage (BAL) of the patient, then a low risk for ARDS outcome is concluded for the patient.

[0189] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or abundance of at least one bacterium of the genus *Flavobacterium,* the presence or abundance of at least one bacterium of the genus *Veillonella* and the presence or abundance of at least one bacterium of the genus *Prosthecobacter* in a bronchoalveolar lavage of the patient, wherein a low risk for ARDS outcome is concluded for the patient when at least one bacterium of the genus *Flavobacterium*, at least one bacterium of the genus *Veillonella* and at least one bacterium of the genus *Prosthecobacter* are detected in the bronchoalveolar lavage of the patient.

[0190] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of the genus *Flavobacterium,* detecting the presence or determining the abundance of at least one bacterium of the genus *Veillonella* and detecting the presence or determining the abundance of at least one bacterium of the genus *Prosthecobacter* in a bronchoalveolar lavage of the patient, wherein a high risk for ARDS outcome is concluded for the patient when no bacterium of the genus *Flavobacterium*, no bacterium of the genus *Veillonella* and no bacterium of the genus *Prosthecobacter* are detected in the bronchoalveolar lavage of the patient.

[0191] In one embodiment, the respiratory complication is ARDS or ARDS related death and the method of the present invention comprises a first step of determining the presence or abundance of at least one bacterium of the *Flavobacterium* genus in a respiratory sample of the patient, a second optional step of determining the presence or abundance of at least one bacterium of the *Veillonella* genus in the respiratory sample of the patient, and a third optional step of determining the presence or abundance of at least one bacterium of the *Prosthecobacter* genus in the respiratory sample of the patient. In one embodiment, the respiratory sample is a bronchoalveolar lavage of the patient.

[0192] In one embodiment, the respiratory complication is ARDS or ARDS related death and the method of the present invention comprises a first step of determining the presence of at least one bacterium of the *Flavobacterium* genus in a respiratory sample of the patient, a second optional step of determining the presence of at least one bacterium of the *Veillonella* genus in the respiratory sample of the patient, and a third optional step of determining the presence of at least one bacterium of the *Prosthecobacter* genus in the respiratory sample of the patient.

[0193] In one embodiment, wherein the respiratory complication is ARDS or ARDS related death, the second step is performed if the presence of said at least one bacterium of the *Flavobacterium* genus is not detected in the respiratory sample of the patient.

[0194] In one embodiment, wherein the respiratory complication is ARDS or ARDS related death, the third step is performed if the presence of said at least one bacterium of the *Veillonella* genus is not detected in the respiratory sample of the patient.

[0195] In one embodiment, the respiratory complication is ARDS or ARDS related death and the method of the present invention comprises a first step of determining the presence of at least one bacterium of the *Flavobacterium* genus in a bronchoalveolar lavage (BAL) of the patient, a second optional step of determining the presence of at least one bacterium of the *Veillonella* genus in the bronchoalveolar lavage (BAL) of the patient, and a third optional step of determining the presence of at least one bacterium of the *Prosthecobacter* genus in the bronchoalveolar lavage (BAL) of the patient.

[0196] In one embodiment, wherein the respiratory complication is ARDS or ARDS related death, the second step is performed if the presence of said at least one bacterium of the *Flavobacterium* genus is not detected in the bronchoalveolar lavage of the patient.

[0197] In one embodiment, wherein the respiratory complication is ARDS or ARDS related death, the third step is performed if the presence of said at least one bacterium of the *Veillonella* genus is not detected in the bronchoalveolar lavage of the patient.

[0198] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence of at least one bacterium of the *Flavobacterium* genus in a bronchoalveolar lavage of the patient, wherein when the presence of said at least one bacterium of the *Flavobacterium* genus is detected in the bronchoalveolar lavage of the patient, then a low risk for ARDS outcome is concluded for the patient.

[0199] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence of at least one bacterium of the *Veillonella* genus in the bronchoalveolar lavage of the patient, wherein when the presence of said at least one bacterium of the *Veillonella* genus is detected in the bronchoalveolar lavage of the patient, then a low risk for ARDS outcome is concluded for the patient.

[0200] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence of at least one bacterium of the *Prosthecobacter* genus in the bronchoalveolar lavage of the patient, wherein when said at least one bacterium of the *Prosthecobacter* genus is detected in the bronchoalveolar lavage of the patient, then a low risk for ARDS outcome is concluded for the patient.

[0201] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence of at least one bacterium of the *Prosthecobacter* genus in the bronchoalveolar lavage of the patient,

wherein when the presence of said at least one bacterium of the *Prosthecobacter* genus is not detected in the bronchoalveolar lavage of the patient, then a high risk for ARDS outcome is concluded for the patient.

[0202] In some embodiments, wherein the respiratory complication is ARDS or ARDS related death, the method comprises a step of detecting the presence of at least one bacterium of the *Flavobacterium* genus in a bronchoalveolar lavage of the patient,
wherein when the presence of said at least one bacterium of the *Flavobacterium* genus is detected in the bronchoalveolar lavage of the patient, then a low risk for ARDS outcome is concluded for the patient,
wherein when said at least one bacterium of the *Flavobacterium* genus is not detected in the bronchoalveolar lavage of the patient, then
the method comprises a step of detecting the presence of at least one bacterium of the *Veillonella* genus in the bronchoalveolar lavage of the patient,
wherein when the presence of said at least one bacterium of the *Veillonella* genus is detected in the bronchoalveolar lavage of the patient, then a low risk for ARDS outcome is concluded for the patient,
wherein when said at least one bacterium of the *Veillonella* genus is not detected in the bronchoalveolar lavage of the patient, then
the method comprises a step of detecting the presence of at least one bacterium of the *Prosthecobacter* genus in the bronchoalveolar lavage of the patient,
wherein when said at least one bacterium of the *Prosthecobacter* genus is detected in the bronchoalveolar lavage of the patient, then a low risk for ARDS outcome is concluded for the patient, and
wherein when the presence of said at least one bacterium of the *Prosthecobacter* genus is not detected in the bronchoalveolar lavage of the patient, then a high risk for ARDS outcome is concluded for the patient.

[0203] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of a genus selected from the *Rothia, unc_Lactobacillales, Streptococcus, Actinomyces, unc_Enterococcaceae,*

unc_Gemellaceae, unc_Gemellales, Haemophilus, Ralstonia, Acinetobacter, unc_Prevotellaceae, unc_Streptococcaceae, Enterococcus, unc_Bacteroidales, Veillonella, Megasphaera, unc_Rhizobiaceae, Granulicatella in a respiratory sample of the patient.

[0204] In one embodiment, the method of the present invention further comprises a step of mathematically combining the abundance (preferably the relative abundance) detected for the bacteria listed hereinabove, thereby obtaining a score.

[0205] In one embodiment, the method of the invention comprises summing the abundance (preferably the relative abundance) of at least one bacterium of a genus selected from *Rothia, unc_Lactobacillales, Streptococcus, Actinomyces, unc_Gemellaceae, Haemophilus, Veillonella, unc_Streptococcaceae* and/or subtracting the abundance of at least one bacterium of a genus selected from *Acinetobacter, Enterococcus, Ralstonia, Megasphaera, unc_Enterococcaceae, unc_Bacteroidales, unc_Prevotellaceae, unc_Gemellales, unc_Rhizobiaceae.*

[0206] In one embodiment, the method of the invention comprises summing the abundance (preferably the relative abundance) of bacteria of a genus selected from *Rothia, unc_Lactobacillales, Streptococcus, Actinomyces, unc_Gemellaceae, Haemophilus, Veillonella, unc_Streptococcaceae* and subtracting the abundance of at least one bacterium of a genus selected from *Acinetobacter, Enterococcus, Ralstonia, Megasphaera, unc_Enterococcaceae, unc_Bacteroidales, unc_Prevotellaceae, unc_Gemellales, unc_Rhizobiaceae.*

[0207] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of a genus selected from the *Rothia* genus, the *Haemophilus* genus and the *unc_Bacteroidales* genus in a respiratory sample of the patient.

[0208] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of detecting the presence or

determining the abundance of at least one bacterium of a genus selected from the *Rothia* genus, the *Streptococcus* genus and the genera of the *Lactobacillales* order in a respiratory sample of the patient. In some embodiments, the presence or abundance of at least one bacterium of the following genus is determined: *Rothia* genus, the *Streptococcus* genus and the genera of the *Lactobacillales* order.

[0209] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of detecting the presence or determining the abundance of at least one bacterium of a genus selected from the *Rothia* genus, the *Streptococcus* genus and the genera of the *Lactobacillales* order in an endotracheal aspirate (ETA) of the patient or in a bronchoalveolar lavage (BAL) of the patient. In some embodiments, the presence or abundance of at least one bacterium of the following genus is determined: *Rothia* genus, the *Streptococcus* genus and the genera of the *Lactobacillales* order.

[0210] In one embodiment, the method of the invention comprises summing the abundance (preferably the relative abundance) of at least one (preferably of the 3) bacterium of a genus selected from *unc_Lactobacillales, Rothia* and *Streptococcus*.

[0211] In one embodiment, a high score is indicative of a low risk of prolonged mechanical ventilation. In one embodiment, a score inferior or equal to about 0.5 is indicative of a high risk of prolonged mechanical ventilation

[0212] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining a relative abundance of at least one bacterium of a genus selected from the *Rothia* genus, the *Streptococcus* genus and the genera of the *Lactobacillales* order, in an endotracheal aspirate (ETA) of the patient or in a bronchoalveolar lavage (BAL) of the patient.

[0213] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative abundance of at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in a respiratory sample of the patient.

[0214] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative abundance of at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in a respiratory sample of the patient,

wherein when the relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the respiratory sample of the patient is greater than a predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the respiratory sample of the patient, then a low risk for prolonged mechanical ventilation (MV) outcome is concluded for the patient.

[0215] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative abundance of at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in an endotracheal aspirate (ETA) or in a bronchoalveolar lavage (BAL) of the patient.

[0216] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative abundance of at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in an endotracheal aspirate (ETA) or in a bronchoalveolar lavage (BAL) of the patient.

wherein when the relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is greater than a predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the endotracheal aspirate (ETA) or in a bronchoalveolar lavage (BAL) of the patient, then a low risk for prolonged mechanical ventilation (MV) outcome is concluded for the patient.

[0217] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the respiratory sample of the patient is determinable and relates to the relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in a respiratory sample of the subjects of a reference population of subjects. The reference population of subjects refers to a group of subjects with shared factors, influences, or condition.

[0218] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the respiratory sample of the patient is less than 0.0005. In some embodiments, wherein the respiratory complication is HAP, the predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the respiratory sample of the patient is 0.00001, 0.00005, 0.00010, 0.00020, 0.00030, 0.00040, or 0.0005. In some embodiments, wherein the respiratory complication is HAP, the predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the respiratory sample of the patient is lower than lower than 0.03, such as, for example, is lower or equal to 0,024.

[0219] In one embodiment, the respiratory complication is prolonged mechanical ventilation (MV) and the predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the respiratory sample of the patient, such as, for example, in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is about 0.00010 or 0.024. In some embodiments, the predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order is 0.00010 or 0.024.

[0220] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative

abundance of at least one bacterium of the *Rothia* genus in a respiratory sample of the patient.

[0221] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative abundance of at least one bacterium of the *Rothia* genus in a respiratory sample of the patient,

wherein when the relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient is lower than a predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient, then a high risk for prolonged mechanical ventilation (MV) outcome is concluded for the patient.

[0222] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative abundance of at least one bacterium of the *Rothia* genus in an endotracheal aspirate (ETA) or in a bronchoalveolar lavage (BAL) of the patient.

[0223] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative abundance of at least one bacterium of the *Rothia* genus in an endotracheal aspirate (ETA) or in a bronchoalveolar lavage (BAL) of the patient,
wherein when the relative abundance of said at least one bacterium of the *Rothia* genus in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is lower than a predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, then a high risk for prolonged mechanical ventilation (MV) outcome is concluded for the patient.

[0224] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient,

notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is determinable and relates to the relative abundance of said at least one bacterium of the *Rothia* genus in a respiratory sample, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the subjects of a reference population of subjects. The reference population of subjects refers to a group of subjects with shared factors, influences, or condition.

[0225] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is less than about 0.03, such as, for example, lower than about 0.01. In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is of about 0.0001, 0.0010, 0.0020, 0.0030, 0.0040, 0.0050, 0.0060, 0.0070, 0.0080, 0.0090, 0.0095 or 0,010. In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is of about 0.027.

[0226] In one embodiment, the respiratory complication is prolonged mechanical ventilation (MV) and the predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient is about 0.006. In some embodiments, the predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient is 0.006.

[0227] In one embodiment, the respiratory complication is prolonged mechanical ventilation (MV) and the predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in in the endotracheal aspirate (ETA) or in the

bronchoalveolar lavage (BAL) of the patient is about 0.006. In some embodiments, the predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient is 0.006.

[0228] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative abundance of at least one bacterium of the *Streptococcus* genus in a respiratory sample of the patient.

[0229] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative abundance of at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient,
wherein when the relative abundance of said at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient, is lower than a predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient, then a high risk for prolonged mechanical ventilation (MV) outcome is concluded for the patient.

[0230] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative abundance of at least one bacterium of the *Streptococcus* genus in an endotracheal aspirate (ETA) or in a bronchoalveolar lavage (BAL) of the patient.

[0231] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative abundance of at least one bacterium of the *Streptococcus* genus in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient,
wherein when the relative abundance of said at least one bacterium of the *Streptococcus* genus in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is lower than a predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus in the endotracheal aspirate (ETA) or in

the bronchoalveolar lavage (BAL) of the patient then a high risk for prolonged mechanical ventilation (MV) outcome is concluded for the patient.

[0232] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is determinable and relates to the relative abundance of said at least one bacterium of the *Streptococcus* genus in a respiratory sample, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the subjects of a reference population of subjects. The reference population of subjects refers to a group of subjects with shared factors, influences, or condition.

[0233] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is less than 0.3, such as, for example, lower than 0.2 or lower than 0.1. In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is 0.001, 0.005, 0.010, 0.020, 0.030, 0.040, 0.050, 0.060, 0.070, 0.080, 0.090, or 0.100 or 0.125.

[0234] In one embodiment, the respiratory complication is prolonged mechanical ventilation (MV) and the predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient, is about 0.03 or 0.125.

[0235] In one embodiment, the respiratory complication is prolonged mechanical ventilation (MV) and the predetermined threshold value of relative abundance of said at

least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient is 0.03 or 0.125.

[0236] In one embodiment, the respiratory complication is prolonged mechanical ventilation (MV) and the predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is about 0.03 or 0.125.

[0237] In one embodiment, the respiratory complication is prolonged mechanical ventilation (MV) and the predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is 0.03 or 0.125.

[0238] In one embodiment, the respiratory complication is prolonged mechanical ventilation (MV) and the method of the present invention comprises a first step of detecting the presence or determining the abundance of at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in a respiratory sample of the patient, a second optional step of detecting the presence or determining the abundance of at least one bacterium of the *Rothia* genus in the respiratory sample of the patient and a third optional step of detecting the presence or determining the abundance of at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient.

[0239] In one embodiment, the second step is performed if the relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the respiratory sample of the patient, is lower than or equal to the first predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the respiratory sample of the patient.

[0240] In one embodiment, the third step is performed if the relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient, is greater than or equal to the second predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient.

[0241] In one embodiment, the respiratory complication is prolonged mechanical ventilation (MV) and the method of the present invention comprises a first step of detecting the presence or determining the abundance of at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in a endotracheal aspirate (ETA) or in a bronchoalveolar lavage (BAL) of the patient, a second optional step of detecting the presence or determining the abundance of at least one bacterium of the *Rothia* genus in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient and a third optional step of detecting the presence or determining the abundance of at least one bacterium of the *Streptococcus* genus in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient.

[0242] In one embodiment, the second step is performed if the relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient is lower than or equal to the first predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient.

[0243] In one embodiment, the third step is performed if the relative abundance of said at least one bacterium of the *Rothia* genus in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is greater than or equal to the second predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient.

[0244] In some embodiment, the respiratory complication is prolonged mechanical ventilation (MV) and the method of the present invention comprises a first step of determining the relative abundance of at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in a respiratory sample of the patient and comparing the relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order to a first predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales*

order, a second optional step of determining the relative abundance of at least one bacterium of the *Rothia* genus in the respiratory sample of the patient and comparing the relative abundance of said at least one bacterium of the *Rothia* genus to a second predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus, and a third optional step of determining the relative abundance of at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient and comparing the relative abundance of said at least one bacterium of the *Streptococcus* genus to a third predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus. In one preferred embodiment, the respiratory sample of the patient is an endotracheal aspirate (ETA) of the patient. In another preferred embodiment, the respiratory sample is a bronchoalveolar lavage (BAL) of the patient.

[0245] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the first predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the respiratory sample of the patient is determinable and relates to the relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in a respiratory sample of the subjects of a reference population of subjects. The reference population of subjects refers to a group of subjects with shared factors, influences, or condition.

[0246] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the first predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the respiratory sample of the patient is less than about 0.03, such as, for example, lower than about 0.0005. In some embodiments, wherein the respiratory complication is HAP, the first predetermined threshold value of relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the respiratory sample of the patient is of about 0.00001, 0.00005, 0.00010, 0.00020, 0.00030, 0.00040, or 0.0005. In some embodiments, wherein the respiratory complication is HAP, the first predetermined threshold value of relative abundance of

said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the respiratory sample of the patient is of about 0.024.

[0247] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the second predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is determinable and relates to the relative abundance of said at least one bacterium of the *Rothia* genus in a respiratory sample, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the subjects of a reference population of subjects. The reference population of subjects refers to a group of subjects with shared factors, influences, or condition.

[0248] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the second predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is less than about 0.03, such as, for example, lower than about 0.01. In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the second predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is 0.0001, 0.0005, 0.0010, 0.0020, 0.0030, 0.0040, 0.0050, 0.0060, 0.0070, 0.0080, 0.0090, or 0,010. In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the second predetermined threshold value of relative abundance of said at least one bacterium of the *Rothia* genus in the respiratory sample of the patient, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is of about 0.027.

[0249] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the third predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient, notably in the endotracheal aspirate (ETA) or in the

bronchoalveolar lavage (BAL) of the patient, is determinable and relates to the relative abundance of said at least one bacterium of the *Streptococcus* genus in a respiratory sample, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the subjects of a reference population of subjects. The reference population of subjects refers to a group of subjects with shared factors, influences or condition.

[0250] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the third predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is less than about 0.2, such as, for example, lower than about 0.15 or lower than about 0.1. In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the third predetermined threshold value of relative abundance of said at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient, notably in the endotracheal aspirate (ETA) or in the bronchoalveolar lavage (BAL) of the patient, is of about 0.001, 0.010, 0.020, 0.030, 0.040, 0.050, 0.060, 0.070, 0.080, 0.090, 0.100 or 0.125.

[0251] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative abundance of at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in a respiratory sample of the patient,

wherein when the relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in a respiratory sample of the patient, is determined to be greater than 0.00001, 0.00005, 0.00010, 0.00020, 0.00030, 0.00040, 0.0005, 0.01, or 0.03 in the respiratory sample of the patient, then a low risk for prolonged mechanical ventilation outcome is concluded for the patient,

wherein when the relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in a respiratory sample of the patient is determined to be lower than or equal to 0.00001, 0.00005, 0 0.00010, 0.00020, 0.00030, 0.00040 0.0005, 0.010, or 0.030 in the respiratory sample of the patient, then

the method comprises a step of determining the relative abundance of at least one bacterium of the *Rothia* genus in the respiratory sample of the patient,

wherein when the relative abundance of said at least one bacterium of the *Rothia* genus is determined to be lower than 0.0001, 0.0010, 0.0020, 0.0030, 0.0040, 0.0050, 0.0060, 0.0070, 0.0080, 0.0090, 0,010 or 0.030 in the respiratory sample of the patient, then a high risk for prolonged mechanical ventilation outcome is concluded for the patient,

wherein when said relative abundance of said at least one bacterium of the *Rothia* genus is determined to be equal or greater than 0.0001, 0.0010, 0.0020, 0.0030, 0.0040, 0.0050, 0.0060, 0.0070, 0.0080, 0.0090,0,010 or 0.030 in the respiratory sample of the patient, then

the method comprises a step of determining the relative abundance of at least one bacterium of the *Streptococcus* genus in the respiratory sample of the patient,

wherein when said relative abundance of said at least one bacterium of the *Streptococcus* genus is determined to be lower than 0.001, 0.005, 0.010, 0.020, 0.030, 0.040, 0.050, 0.060, 0.070, 0.080, 0.090, 0,100 or 0.200 in the respiratory sample of the patient, then a high risk for prolonged mechanical ventilation outcome is concluded for the patient,

wherein when said relative abundance of said at least one bacterium of the *Streptococcus* genus is determined to be equal to or greater than 0.001, 00.005, 0.010, 0.020, 0.030, 0.040, 0.050, 0.060, 0.070, 0.080, 0.090, 0,100 or 0.200 in the respiratory sample of the patient, then a low risk for prolonged mechanical ventilation outcome is concluded for the patient.

[0252] In some embodiments, wherein the respiratory complication is prolonged mechanical ventilation (MV), the method comprises a step of determining the relative abundance of at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in an endotracheal aspirate (ETA) of the patient,

wherein when the relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the endotracheal aspirate (ETA) of the patient, is determined to be greater than 0.0001 in the endotracheal aspirate (ETA) of the patient, then a low risk for prolonged mechanical ventilation outcome is concluded for the patient,

wherein when the relative abundance of said at least one bacterium of a genus selected from the genera of the *Lactobacillales* order in the endotracheal aspirate (ETA) of the patient, is determined to be lower than or equal to 0.0001 in the endotracheal aspirate (ETA) of the patient, then

the method comprises a step of determining the relative abundance of at least one bacterium of the *Rothia* genus in the endotracheal aspirate (ETA) of the patient,

wherein when the relative abundance of said at least one bacterium of the *Rothia* genus is determined to be lower than 0.006 in the endotracheal aspirate (ETA) of the patient, then a high risk for prolonged mechanical ventilation outcome is concluded for the patient,

wherein when said relative abundance of said at least one bacterium of the *Rothia* genus is determined to be equal or greater than 0.006 in the endotracheal aspirate (ETA) of the patient, then

the method comprises a step of determining the relative abundance of at least one bacterium of the *Streptococcus* genus in the endotracheal aspirate (ETA) of the patient,

wherein when said relative abundance of said at least one bacterium of the *Streptococcus* genus is determined to be lower than 0.03 in the endotracheal aspirate (ETA) of the patient, then a high risk for prolonged mechanical ventilation outcome is concluded for the patient,

wherein when said relative abundance of said at least one bacterium of the *Streptococcus* genus is determined to be equal to or greater than 0.03 in endotracheal aspirate (ETA) of the patient, then a low risk for prolonged mechanical ventilation outcome is concluded for the patient.

[0253] The invention also concerns a method of providing an adapted care to a subject at risk of having or developing a respiratory complication, the method comprising the steps of:

- identifying a subject with a risk of having or developing a respiratory complication in a subject by the method according to the invention, and
- providing an adapted care to a subject identified with a risk of having or developing a respiratory complication.

[0254] In some embodiments, the respiratory complication is selected in the group of acute respiratory distress syndrome (ARDS), Hospital Acquired Pneumonia (HAP) and Prolonged Mechanical Ventilation (MV).

[0255] In some embodiments, the respiratory complication is acute respiratory distress syndrome (ARDS). In some embodiments, the respiratory complication is Hospital Acquired Pneumonia (HAP). In some embodiments, the respiratory complication Prolonged Mechanical Ventilation (MV).

[0256] In one embodiment, the adapted care corresponds to a restoration of the lung microbiota to a healthy situation. In one embodiment, the adapted care corresponds to a restoration of Streptococcus spp lung colonization.

[0257] An adapted care can be of any nature.

[0258] In one embodiment, the adapted care corresponds to the administration of a composition, probiotic composition or pharmaceutical composition as described herein.

[0259] In some embodiments, the adapted care comprises or consists of an administration of a therapeutic composition to the subject at risk of having or developing a respiratory complication.

[0260] In some embodiments, the adapted care comprises or consists of an administration of a therapeutic composition to the subject at risk of having acute respiratory distress syndrome (ARDS) or developing acute respiratory distress syndrome (ARDS).

[0261] In some embodiments, the adapted care comprises or consists of an administration of a therapeutic composition to the subject at risk of having Hospital Acquired Pneumonia (HAP) or developing Hospital Acquired Pneumonia (HAP).

[0262] In some embodiments, the adapted care comprises or consists of an administration of a therapeutic composition to the subject at risk of having Prolonged Mechanical Ventilation (MV) or developing Prolonged Mechanical Ventilation (MV).

[0263] In some embodiments, the adapted care comprises or consists of an administration of an antibiotic composition to the subject at risk of having ARDS or to the subject developing ARDS.

[0264] In some embodiments, the adapted care comprises or consists of an administration of an antibiotic composition comprising or consisting of a broad-spectrum antibiotic to the subject at risk of having ARDS or to the subject developing ARDS.

[0265] In some embodiments, the adapted care comprises or consists of an administration of an antibiotic composition comprising or consisting of a broad-spectrum antibiotic to the subject at risk of having ARDS or to the subject developing ARDS without symptoms of ARDS or with only few symptoms of ARDS.

[0266] In some embodiments, the adapted care comprises or consists of an administration of an antibiotic composition comprising or consisting of at least one antibiotic compound selected in the group of cephalosporin and fluoroquinolone to the subject at risk of having ARDS or to the subject developing ARDS. In some embodiments, cephalosporin is a third-generation cephalosporin.

[0267] In some embodiments, the adapted care comprises or consists of an administration of an antibiotic composition to the subject at risk of having Hospital Acquired Pneumonia (HAP) or to the subject developing Hospital Acquired Pneumonia (HAP).

[0268] In some embodiments, the adapted care comprises or consists of an administration of an antibiotic composition comprising or consisting of a broad-spectrum antibiotic to the subject at risk of having HAP or to the subject developing HAP.

[0269] In some embodiments, the adapted care comprises or consists of an administration of an antibiotic composition comprising or consisting of a broad-spectrum antibiotic to the subject at risk of having HAP or to the subject developing HAP with only few symptoms of HAP.

[0270] In some embodiments, the adapted care comprises or consists of an administration of an antibiotic composition comprising or consisting of at least one antibiotic compound selected in the group of cephalosporin and fluoroquinolone to the subject at risk of having HAP or to the subject developing HAP. In some embodiments, cephalosporin is a third-generation cephalosporin.

[0271] In some embodiments, the adapted care comprises or consists of an administration of an antibiotic composition to the subject at risk of having Prolonged Mechanical Ventilation (MV) or developing Prolonged Mechanical Ventilation (MV).

[0272] In some embodiments, the adapted care comprises or consists of an administration of an antibiotic composition comprising or consisting of a broad-spectrum antibiotic to the subject at risk of need of prolonged MV or to the subject in need of prolonged MV.

[0273] In some embodiments, the adapted care comprises or consists of an administration of an antibiotic composition comprising or consisting of a broad-spectrum antibiotic to the subject at risk of need of prolonged MV or to the subject in need of prolonged MV with only few symptoms of need of prolonged MV.

[0274] In some embodiments, the adapted care comprises or consists of an administration of an antibiotic composition comprising or consisting of at least one antibiotic compound selected in the group of cephalosporin and fluoroquinolone to the subject at risk of need of prolonged MV or to the subject in need of prolonged MV. In some embodiments, cephalosporin is a third-generation cephalosporin.

[0275] In some embodiments, the adapted care comprises or consists of a digestive tract selective decontamination.

[0276] In some embodiments, the adapted care comprises or consists of an immunotherapy. In some embodiments, the adapted care comprises or consists of an activation immunotherapy.

[0277] In some embodiments, immunotherapy is designed to elicit and/or amplify an immune response in a subject at risk of having ARDS or to a subject developing ARDS.

[0278] In some embodiments, immunotherapy is designed to elicit and/or amplify an immune response in a subject at risk of having HAP or in a subject developing HAP.

[0279] In some embodiments, immunotherapy is designed to elicit and/or amplify an immune response in a subject at risk of need prolonged MV or in a subject in need of prolonged MV.

[0280] The invention also concerns a composition, such as, for example, a probiotic composition comprising at least one bacterium of the *Flavobacterium* genus, or of the *Veillonella* genus, or of the *Prosthecobacter* genus, or of the *Streptococcus* genus, or of the *Atopobium* genus, or of the *Oribacterium* genus, or of one of the genera of the *Lactobacillales* order, or of the *Rothia* genus, or at least one metabolite thereof, and to the use of this composition in the prevention or treatment of a respiratory complication in a subject.

[0281] In one embodiment, the at least one bacterium is alive, and the composition may thus be referred to as a probiotic composition. In one embodiment, the probiotic composition comprises a physiologically acceptable excipient (*e.g.*, lactose monohydrate, physiological saline, sugars, in particular glucose).

[0282] In one embodiment, the at least one bacterium is dead. In one embodiment, the at least one bacterium is killed by heat shock and/or UV treatment.

[0283] In one embodiment, the composition of the invention comprises at least one metabolite produced by at least one bacterium of the *Flavobacterium* genus, or of the *Veillonella* genus, or of the *Prosthecobacter* genus, or of the *Streptococcus* genus, or of the *Atopobium* genus, or of the *Oribacterium* genus, or of one of the genera of the *Lactobacillales* order, or of the *Rothia* genus. In one embodiment, the at least one metabolite is recovered from the supernatant of a culture medium of at least one of the bacteria listed hereinabove.

[0284] In one embodiment, the composition comprises a mixture of alive bacteria and/or dead bacteria and/or metabolites.

[0285] In one embodiment, the composition of the present invention further comprises at least one prebiotic, i.e., an agent, such as, for example, a sugar, that favors the growth or survival of at least one bacterium listed hereinabove after administration to a subject.

[0286] In one embodiment, the composition, such as, for example, the probiotic composition, is a pharmaceutical composition and further comprises a pharmaceutically acceptable excipient.

[0287] Pharmaceutically acceptable excipients that may be used in the pharmaceutical composition as described herein include, for example, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances (for example sodium carboxymethylcellulose), polyethylene glycol, polyacrylates, waxes, polyethylene- polyoxypropylene- block polymers, polyethylene glycol and wool fat.

[0288] In one embodiment, the composition or probiotic composition comprises at least one bacterium of one of the genera of the *Lactobacillales* orders and at least one bacterium of the *Rothia* genus.

[0289] In one embodiment, the composition or probiotic composition comprises at least one bacterium of the *Streptococcus* genus, such as, for example, *S. mitis*, *S. salivarius*, *S. oralis, S. sanguinis* or *S. parasanguinis*.

[0290] In some embodiments, the composition or probiotic composition is for use in the prevention or treatment of an acute respiratory distress syndrome (ARDS) in a subject at risk of having ARDS or in a subject developing ARDS.

[0291] In some embodiments, the composition or probiotic composition for use in the prevention or treatment of ARDS in a subject at risk of having ARDS or in a subject developing ARDS, comprises or consists of at least one bacterium of at least one of the *Flavobacterium* genus, the *Veillonella* genus and the *Prosthecobacter* genus.

[0292] In some embodiments, the composition or probiotic composition for use in the prevention or treatment of ARDS in a subject at risk of having ARDS or in a subject developing ARDS, comprises or consists of at least one bacterium of the genus *Flavobacterium*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of ARDS in a subject at risk of having ARDS or in a subject developing ARDS, comprises or consists of at least one bacterium of the genus *Veillonella*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of ARDS in a subject at risk of having ARDS or in a subject developing ARDS, comprises or consists of at least one bacterium of the genus *Prosthecobacter*.

[0293] In some embodiments, the composition or probiotic composition for use in the prevention or treatment of ARDS in a subject at risk of having ARDS or in a subject developing ARDS, comprises or consists of at least one bacterium of the genus *Flavobacterium* and at least one bacterium of the genus *Veillonella*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of ARDS in a subject at risk of having ARDS or in a subject developing ARDS, comprises or consists of at least one bacterium of the genus *Flavobacterium* and at least one bacterium of the genus *Prosthecobacter*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of ARDS in a subject at risk of having ARDS or in a subject developing ARDS, comprises or consists of at least one bacterium of the genus *Veillonella* and at least one bacterium of the genus *Prosthecobacter*.

[0294] In some embodiments, the composition or probiotic composition for use in the prevention or treatment of ARDS in a subject at risk of having ARDS or in a subject developing ARDS, comprises or consists of at least one bacterium of the genus

Flavobacterium, at least one bacterium of the genus *Veillonella* and at least one bacterium of the genus *Prosthecobacter*.

[0295] In some embodiments, the composition or probiotic composition is for use in the prevention or treatment of Hospital Acquired Pneumonia (HAP) in a subject at risk of having HAP or in a subject developing HAP.

[0296] In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of at least one of a genus selected in the group of the *Streptococcus* genus, the *Atopobium* genus, the *Oribacterium* genus and the *Veillonella* genus.

[0297] In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Streptococcus*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Atopobium*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Oribacterium*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Veillonella*.

[0298] In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Streptococcus* and at least one bacterium of the genus *Atopobium*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Streptococcus* and at least one bacterium of the

genus *Oribacterium*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Streptococcus* and at least one bacterium of the genus *Veillonella*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Atopobium* and at least one bacterium of the genus *Oribacterium*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Atopobium* and at least one bacterium of the genus *Veillonella*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Oribacterium* and at least one bacterium of the genus *Veillonella*.

[0299] In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Streptococcus*, at least one bacterium of the genus *Atopobium* and at least one bacterium of the genus *Oribacterium*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Streptococcus*, at least one bacterium of the genus *Atopobium* and at least one bacterium of the genus *Veillonella*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Atopobium*, at least one bacterium of the genus *Oribacterium* and at least one bacterium of the genus *Veillonella*. In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of

the genus *Streptococcus*, at least one bacterium of the genus *Atopobium*, at least one bacterium of the genus *Oribacterium* and at least one bacterium of the genus *Veillonella*.

[0300] In some embodiments, the composition or probiotic composition for use in the prevention or treatment of HAP in a subject at risk of having HAP or in a subject developing HAP, comprises or consists of at least one bacterium of the genus *Streptococcus*, at least one bacterium of the genus *Atopobium*, at least one bacterium of the genus *Oribacterium* or at least one bacterium of the genus *Veillonella*.

[0301] In some embodiments, the composition or probiotic composition is for use in the prevention or treatment of Hospital Acquired Pneumonia (HAP) in a subject at risk of having HAP or in a subject developing HAP.

[0302] In some embodiments, the composition or probiotic composition for use in the prevention or treatment in a subject at risk of need of Prolonged Mechanical Ventilation (MV) or in a subject developing a need of Prolonged Mechanical Ventilation (MV), comprises or consists of at least one bacterium selected in a genus of the genera of the *Lactobacillales* order, in the *Rothia* genus or in the *Streptococcus* genus.

[0303] In some embodiments, the composition or probiotic composition for use in the prevention or treatment in a subject at risk of need of MV or in a subject developing a need of MV, comprises or consists of at least one bacterium of a genus selected from the genera of the *Lactobacillales* order. In some embodiments, the composition or probiotic composition for use in the prevention or treatment in a subject at risk of need of MV or in a subject developing a need of MV, comprises or consists of at least one bacterium of the *Rothia* genus. In some embodiments, the composition or probiotic composition for use in the prevention or treatment in a subject at risk of need of MV or in a subject developing a need of MV, comprises or consists of at least one bacterium of the *Streptococus* genus.

[0304] In some embodiments, the composition or probiotic composition for use in the prevention or treatment in a subject at risk of need of MV or in a subject developing a need of MV, comprises or consists of at least one bacterium of a genus selected from the

genera of the *Lactobacillales* order and at least one bacterium of the *Rothia* genus. In some embodiments, the composition or probiotic composition for use in the prevention or treatment in a subject at risk of need of MV or in a subject developing a need of MV, comprises or consists of at least one bacterium of a genus selected from the genera of the *Lactobacillales* order and at least one bacterium of the *Streptococcus* genus. In some embodiments, the composition or probiotic composition for use in the prevention or treatment in a subject at risk of need of MV or in a subject developing a need of MV, comprises or consists of at least one bacterium of the *Rhotia* genus and at least one bacterium of the *Streptococcus* genus.

[0305] In some embodiments, the composition or probiotic composition for use in the prevention or treatment in a subject at risk of need of MV or in a subject developing a need of MV, comprises or consists of at least one bacterium of a genus selected from the genera of the *Lactobacillales* order, at least one bacterium of the *Rhotia* genus and at least one bacterium of the *Streptococcus* genus.

[0306] The invention also concerns the use of a composition or probiotic composition comprising at least one bacterium of the *Flavobacterium* genus, or of the *Veillonella* genus, or of the *Prosthecobacter* genus, or of the *Streptococcus* genus, or of the *Atopobium* genus, or of the *Oribacterium* genus, or of one of the genera of the *Lactobacillales* order, or of the *Rothia* genus, or metabolites thereof for the obtention of a medicament for use in preventing or treating a respiratory complication in a subject.

[0307] The invention also concerns a method for treating or preventing a respiratory complication in a subject in need thereof comprising administrating to the subject a composition or probiotic composition or pharmaceutical composition comprising at least one bacterium of the *Flavobacterium* genus, or of the *Veillonella* genus, or of the *Prosthecobacter* genus, or of the *Streptococcus* genus, or of the *Atopobium* genus, or of the *Oribacterium* genus, or of one of the genera of the *Lactobacillales* order, or of the *Rothia* genus, or metabolites thereof.

[0308] In one embodiment, a therapeutically effective amount of the composition is to be administered to the subject. In some embodiments, the title of bacteria in the probiotic

composition is no more than 10^6 cfu/mL (wherein "cfu" stands for "colony forming unit"). In some embodiment, the title of bacteria in the probiotic composition is in the range of 10^3 to 10^6 cfu/mL.

[0309] In some embodiments, the subject being a subject at risk of having acute respiratory distress syndrome (ARDS) or developing acute respiratory distress syndrome (ARDS), the method comprises or consists of at least two successive distinct or identical administrations of at least one bacterium of the genus *Flavobacterium*, at least one bacterium of the genus *Veillonella* or at least one bacterium of the genus *Prosthecobacter* to the patient.

[0310] In some embodiments, the subject being a subject at risk of having Hospital Acquired Pneumonia (HAP) or developing Hospital Acquired Pneumonia (HAP), the method comprises or consists of at least two successive distinct or identical administrations of at least one bacterium of the genus *Streptococcus*, at least one bacterium of the genus *Atopobium*, at least one bacterium of the genus *Oribacterium* or at least one bacterium of the genus *Veillonella* to the patient.

[0311] In some embodiments, the subject being a subject at risk of need of Prolonged Mechanical Ventilation (MV) or developing a need of Prolonged Mechanical Ventilation (MV), the method comprises or consists of at least two successive distinct or identical administrations of a probiotic composition comprising or consisting of at least one bacterium of a genus selected from the genera of the *Lactobacillales* order, at least one bacterium of the genus *Rhotia* and/or at least one bacterium of the genus *Streptococcus* to the patient.

[0312] In some embodiments, the subject being a subject at risk of having acute respiratory distress syndrome (ARDS) or a subject developing acute respiratory distress syndrome (ARDS), or a subject at risk of having Hospital Acquired Pneumonia (HAP) ora subject developing Hospital Acquired Pneumonia (HAP), or a subject at risk of need of Prolonged Mechanical Ventilation (MV) or a subject developing a need of Prolonged Mechanical Ventilation (MV), the adapted care comprises or consists of one or more administration(s) of a probiotic composition and at least one step of detecting the presence

or determining the abundance of at least one bacterium of at least one genus selected from the group comprising *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella, Prosthecobacter, Streptococcus, Atopobium, Oribacterium,* a genus of the *Lactobacillales* order, and *Rothia* in a respiratory sample of the patient.

[0313] In some embodiments, the administration of a probiotic composition to the subject is maintained as long as the subject is determined to be a subject with a risk of having or developing a respiratory complication.

[0314] In some embodiments, the probiotic composition is administered intratracheally or oropharyngeally.

[0315] The present invention further relates to a method for determining a risk of unfavorable response to treatment in a subject,
wherein said method comprising a step of detecting the presence or abundance of a least one bacterium of a genus selected from the group comprising *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella, Prosthecobacter, Streptococcus, Atopobium, Oribacterium,* a genus of the *Lactobacillales* order, and *Rothia* in a respiratory sample of the patient, and
wherein said subject is a critically ill patient, such as, for example, a critically ill patient in an intensive care unit, preferably receiving mechanical ventilation (MV) or prolonged mechanical ventilation.

[0316] In one embodiment, said treatment is a composition of the present invention, such as, for example, a probiotic composition or pharmaceutical composition as described herein.

[0317] The present invention also relates to a method for monitoring the response of a subject to a treatment,

wherein said method comprises at least two steps of detecting the presence or abundance of a least one bacterium of a genus selected from the group comprising *Staphylococcus*, *Ralstonia*, *Enterococcus*, *Flavobacterium*, *Veillonella*, *Prosthecobacter*, *Streptococcus*,

Atopobium, *Oribacterium*, a genus of the *Lactobacillales* order, and *Rothia* in a respiratory sample of the subject,

wherein said subject is a critically ill patient, such as, for example, a critically ill patient in an intensive care unit, preferably receiving mechanical ventilation (MV) or prolonged mechanical ventilation having received treatment as described hereinabove.

[0318] Examples of treatments include, but are not limited to, antimicrobial therapy, prebiotic, probiotic and/or immune therapy.

[0319] In one embodiment, the presence or abundance of at least one bacterium of the genus listed hereinabove is detected before administration of a treatment, and after administration of a treatment.

[0320] In one embodiment, the method is for determining a favorable response to treatment in a subject.

[0321] In one embodiment, the subject favorably responds to the treatment if a correction of a respiratory microbial dysbiosis detected before treatment is corrected after treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

[0322] **Figure 1** is a combination of graphs showing beta diversity comparisons of the respiratory microbiomes of the samples (including BAL, ETA and OS) collected from ICU patients and healthy controls. Analyses were performed on the 16S rRNA genes. Principal-coordinate analysis of Bray-Curtis distances. The proportion of variance explained by each principal-coordinate axis is denoted in the corresponding axis labels.

[0323] **Figure 2** is a combination of graphs showing the relative abundance of the healthy respiratory microbiome core genus in respiratory samples from healthy controls and critically ill patients. Included are samples collected at BAL, ETA, and OS.

[0324] **Figure 3** is a combination of graphs showing beta diversity comparisons of the respiratory microbiomes of the samples collected from ICU patients, that is, Bronchoalveolar Lavages (BAL) samples, tracheal aspirates (ETA) samples, and oral samples (OS). Analyses were performed on the 16S rRNA genes. Principal-coordinate analysis of Bray-Curtis distances. The proportion of variance explained by each principal-coordinate axis is denoted in the corresponding axis labels.

[0325] **Figure 4** is a graph showing alpha diversity indices in samples collected from ICU patients along the respiratory tract were measured using the number of observed genera. We included samples collected at different sites: BAL, ETA and OS. Statistical significance was assessed by Kruskal-Wallis with Dunn's tests. *P≤0.05, **P≤0.01, ***P<0.001.

[0326] **Figure 5** is a graph showing respiratory microbiome core of critically ill patients. UpSet plot of the core respiratory microbiome in the 2177 samples collected in intensive care unit patients, including BAL, ETA, and OS. The cut-off for prevalence was the presence of the genus in at least 40% of the cohort of patients in each sampling site.

[0327] **Figure 6** is a combination of graphs showing beta diversity comparisons of the respiratory microbiomes of the ETA collected from ICU patients. Analyses were performed on 16S rRNA genes. Principal-coordinate analysis of Bray-Curtis distances. The proportion of variance explained by each principal-coordinate axis is denoted in the corresponding axis labels. Statistical significance was assessed by the Bray-Curtis distance (PERMANOVA) and two-sided Mann-Whitney test for the principal coordinates.

[0328] **Figure 7** is a combination of graphs showing the relative abundance of the main phyla in ETA samples from critically ill patients with or without ARDS.

[0329] **Figure 8** is a representation of a Fast-and-frugal tree-based staging scheme to predict ARDS.

[0330] **Figure 9** is a graph showing Receiver Operating Characteristic (ROC) of Fast-and-frugal tree for ARDS classification applying a ten-repeated 10-fold cross-validation

scheme. The model evaluation displays the cross-validation error as a ROC curve, with a 95% confidence interval shaded in grey.

[0331] **Figure 10** is a graph showing Kaplan-Meier estimates of the duration of mechanical ventilation in patients with ARDS *vs* no ARDS microbiome signature. Statistical significance was assessed by Cox proportional-hazards model.

[0332] **Figure 11** is a combination of graphs showing beta diversity comparisons of the respiratory microbiomes of the BAL collected from ICU patients. Analyses were performed on 16S rRNA genes. Principal-coordinate analysis of Bray-Curtis distances. The proportion of variance explained by each principal-coordinate axis is denoted in the corresponding axis labels.

[0333] **Figure 12** is a combination of graphs showing the relative abundance of the main phyla in BAL samples from critically ill patients with or without ARDS.

[0334] **Figure 13** is a representation of a fast-and-frugal tree-based staging scheme for ICU patients to predict ARDS in BAL samples.

[0335] **Figure 14** is a graph showing Receiver Operating Characteristic (ROC) of the Fast-and-frugal tree for prediction of ARDS applying a ten-repeated 10-fold cross-validation scheme. The model evaluation displays the cross-validation error as a receiver operating characteristic (ROC) curve, with a 95% confidence interval shaded in gray.

[0336] **Figure 15** is a combination of graphs showing beta diversity comparisons of the respiratory microbiomes of the ETA collected from critically ill patients, regarding respiratory microbiome signatures of HAP in endotracheal aspirates. Analyses were performed on 16S rRNA genes. Principal-coordinate analysis of Bray-Curtis distances. The proportion of variance explained by each principal-coordinate axis is denoted in the corresponding axis labels. Statistical significance was assessed by the Bray-Curtis distance (PERMANOVA) and two-sided Mann-Whitney test for the principal coordinates.

[0337] **Figure 16** is a combination of graphs showing the relative abundance of the main phyla in ETA samples from critically ill patients with or without HAP.

[0338] **Figure 17** is a representation of a fast-and-frugal tree-based staging scheme to predict HAP, regarding respiratory microbiome signatures of HAP in endotracheal aspirates (ETA).

[0339] **Figure 18** is a graph showing ROC of Fast-and-frugal tree for HAP classification applying a ten-repeated 10-fold cross-validation scheme. The model evaluation displays the cross-validation error as a receiver operating characteristic (ROC) curve, with a 95% confidence interval shaded in grey.

[0340] **Figure 19** is a graph showing Kaplan-Meier estimates of the duration of mechanical ventilation in patients with HAP vs no HAP microbiome signature. Statistical significance was assessed by Cox proportional-hazards model.

[0341] **Figure 20** is a combination of graphs related to respiratory microbiome signatures of prolonged mechanical ventilation in endotracheal aspirates and summarizing the genera with different relative abundance in ETA of critically ill patients with short vs. prolonged mechanical ventilation. The function computes for each genus the significance using a non-parametric Wilcoxon test and different effect sizes for the association (significance for an FDR corrected p-value <0.05, AUC, and fold change).

[0342] **Figure 21** is a representation of a fast-and-frugal tree-based staging scheme for MV duration classification.

[0343] **Figure 22** is a graph showing ROC of Fast-and-frugal tree for prolonged MV classification applying a ten-repeated 10-fold cross-validation scheme. The model evaluation displays the cross-validation error as a receiver operating characteristic (ROC) curve, with a 95% confidence interval shaded in gray.

[0344] **Figure 23** is a combination of graphs related to external validation of respiratory microbiome signatures in an independent prospective cohort, showing beta diversity comparisons of the respiratory microbiomes of the ETA collected from brain-injured patients with or without ARDS. Principal-coordinate analysis of Bray-Curtis distances. The proportion of variance explained by each principal-coordinate axis is denoted in the corresponding axis labels.

[0345] **Figure 24** is a combination of graphs showing beta diversity comparisons of the respiratory microbiomes of the ETA collected from brain-injured patients with or without HAP. Principal-coordinate analysis of Bray-Curtis distances. The proportion of variance explained by each principal-coordinate axis is denoted in the corresponding axis labels.

[0346] **Figure 25** is a combination of graphs showing beta diversity comparisons of the respiratory microbiomes of the ETA collected from brain-injured patients with short, media or prolonged mechanical ventilation (MV) classification. Principal-coordinate analysis of Bray-Curtis distances. The proportion of variance explained by each principal-coordinate axis is denoted in the corresponding axis labels.

[0347] **Figure 26** is a graph showing ROC of Fast-and-frugal tree for ARDS applying a ten-repeated 10-fold cross-validation scheme. The model evaluation displays the cross-validation error as a receiver operating characteristic (ROC) curve.

[0348] **Figure 27** is a graph showing ROC of Fast-and-frugal tree for HAP applying a ten-repeated 10-fold cross-validation scheme. The model evaluation displays the cross-validation error as a receiver operating characteristic (ROC) curve.

[0349] **Figure 28** is a graph showing Receiver Operating Characteristic (ROC) of fast-and-frugal tree for prolonged mechanical ventilation applying a ten-repeated 10-fold cross-validation scheme. The model evaluation displays the cross-validation error as a receiver operating characteristic (ROC) curve.

[0350] **Figure 29** is a Kaplan-Meier curve showing an estimation of the duration of mechanical ventilation in patients stratified by microbiome score for ARDS. Statistical significance was assessed by Cox proportional-hazards model.

[0351] **Figure 30** is a Kaplan-Meier curve showing an estimation of the duration of mechanical ventilation in patients stratified by microbiome score for HAP. Statistical significance was assessed by Cox proportional-hazards model.

[0352] **Figure 31** is a Kaplan-Meier curve showing an estimation of the duration of mechanical ventilation in patients stratified by microbiome score for prolonged MV. Statistical significance was assessed by Cox proportional-hazards model.

[0353] **Figure 32** is a diagram showing the experimental procedure.

[0354] **Figures 33A-D** is a combination of histological photographs showing lung sections from PBS-instilled mice (sham) (**Figures 33A-B**) and *S. mitis* instilled mice (**Figures 33C-D**) at day 1 (**Figures 33A** and **C**) and day 3 (**Figures 33B** and **D**) post-instillation.

[0355] **Figures 34A-D** is a combination of histograms showing: the percentage of initial weight of PBS-instilled mice (sham), *S. mitis* instilled mice, and *E. coli* instilled mice at day 1 post-instillation (**Figure 34A**), the number of neutrophils in PBS-instilled mice (sham), *S. mitis* instilled mice, and *E. coli* instilled mice at day 1 post-instillation (**Figure 34B**), the number of neutrophils in PBS-instilled mice (sham), *S. mitis* instilled mice, and *E. coli* instilled mice at day 7 post-instillation (**Figure 34C**) and the phagocytic capacity of neutrophils from PBS-instilled mice (sham), and *S. mitis* instilled mice at day 7 post-instillation (**Figure 34D**). Graphs show mean ± SD and are pooled data from 2-3 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001, and ns not significant.

[0356] **Figures 35A-D** is a combination of histograms and flow cytometry plot showing: the count of monocyte-derived inflammatory macrophages (I-MAC) in PBS-instilled mice (sham), *S. mitis* instilled mice, and *E. coli* instilled mice at day 7 post-instillation (**Figure 35A**), a flow cytometry staining of CD11c and CD11b in PBS-instilled mice (sham), and *S. mitis* instilled mice at day 7 post-instillation (**Figure 35B**), the phagocytic capacity of I-MAC from PBS-instilled mice (sham), and *S. mitis* instilled mice at day 7 post-instillation (**Figure 35C**) and the phagocytic capacity of resident alveolar macrophages (R-MAC) from PBS-instilled mice (sham), and *S. mitis* instilled mice at day 7 post-instillation (**Figure 35D**). Graphs show mean ± SD and are pooled data from 2-3 independent experiments. *P < 0.05, and ***P < 0.001.

[0357] **Figures 36A-D** is a combination of histograms and pie chart showing: the count of resident T cells in PBS-instilled mice (sham), *S. mitis* instilled mice, and *E. coli* instilled mice at day 7 post-instillation (**Figure 36A**), the percentage of CD4+, CD8+, TCR γδ+ and other cells in *S. mitis* instilled mice at day 7 post-instillation (**Figure 36B**), the percentage of IL-17 expression in CD4+ T cells in PBS-instilled mice (sham), and *S. mitis* instilled mice at day 7 post-instillation (**Figure 36C**) and the percentage of IL-17 expression in CD4+ resident T cells in PBS-instilled mice (sham), and *S. mitis* instilled mice at day 7 post-instillation (**Figure 36D**). Graphs show mean ± SD and are pooled data from 2-3 independent experiments. *P < 0.05, **P < 0.01, ****P < 0.0001, and ns not significant.

[0358] **Figure 37** is a diagram showing the experimental procedure.

[0359] **Figure 38** is a histogram showing the pulmonary *E. coli* loads 24 hours post pneumonia in PBS-instilled mice infected with *E. coli* (sham), and *S. mitis* instilled mice infected with *E. coli*. Graphs show mean ± SD and are pooled data from 2-3 independent experiments. ns not significant.

[0360] **Figure 39** is a histogram showing the percentage of initial weight at day 0, 1, 2 and 3 post *E. coli* pneumonia infection in PBS-instilled mice infected with *E. coli* (circles), and *S. mitis* instilled mice infected with *E. coli* (squares). Graphs show mean ± SD and are pooled data from 2-3 independent experiments. **P < 0.01, and ***P < 0.001.

[0361] **Figures 40A-G** is a combination of histograms showing: the numbers of lung neutrophils (**Figure 40A**), monocyte-derived inflammatory macrophages (I-MAC) (**Figure 40B**), NK cells (**Figure 40C**), CD4 T cells (**Figure 40D**), γδ T cells (**Figure 40E**), resident CD4 T cells (**Figure 40F**) and resident γδ T cells (**Figure 40G**) in PBS-instilled mice (sham), PBS-instilled mice infected with *E. coli* (E. coli) or *S. mitis* instilled mice infected with *E. coli* (S.mitis.E.coli) at 24 hours post *E. coli* infection. Graphs show mean ± SD and are pooled data from 2-3 independent experiments. *P < 0.05, **P < 0.01, and ***P < 0.001.

[0362] **Figures 41A-D** is a combination of histograms showing: the percentage of TNFα expression (**Figure 41A**) or IL-1β expression (**Figure 41B**) in resident alveolar macrophages, and the percentage of IL-17 expression in CD4 T cells (**Figure 1C**) or CD4 resident T cells (**Figure 1D**) from PBS-instilled mice (sham), PBS-instilled mice infected with *E. coli* (E. coli) or *S. mitis* instilled mice infected with *E. coli* (S.mitis.E.coli) at 24 hours post *E. coli* infection. Graphs show mean ± SD and are pooled data from 2-3 independent experiments. *P < 0.05, ***P < 0.001, ****P < 0.0001, and ns not significant.

[0363] **Figure 42** is a combination of a classification decision tree (**Figure 42A**) and of a ROC (**Figure 42B**) for ARDS classification from an EAT sample.

[0364] **Figure 43** is a combination of a classification decision tree (**Figure 43A**) and of a ROC (**Figure 43B**) for ARDS classification from a BAL sample.

[0365] **Figure 44** is a combination of a classification decision tree (**Figure 44A part 1 and part 2**) and of a ROC (**Figure 44B**) for HAP classification from an ETA sample.

[0366] **Figure 45** is a combination of a classification decision tree (**Figure 45A**) and of a ROC (**Figure 45B**) for the probability of successful extubation.

EXAMPLES

[0367] The present invention is further illustrated by the following examples.

Example 1

Materials and Methods

Search strategy for available respiratory microbiome data.

We followed the recommended Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines to select the articles. We searched in MEDLINE, Web of Science, and Scopus databases for articles published in peer-reviewed journals,

written in English, and indexed up until May 2022. We used several search terms that were combined, including microbiota, microbiome, microbial community, gut microbial composition, respiratory microbiome, lung microbiome, metagenomics, lower respiratory tract, oropharyngeal tract, intensive care unit, mechanical ventilation, acute respiratory distress syndrome, ventilator-associated pneumonia.

Study inclusion criteria in the meta-analyses

[0368] We included observational (cross-sectional, case-control and cohort designs) and interventional studies involving adult critically ill humans (18 years old or older) that reported respiratory microbiome and described ARDS and or HAP occurrence during hospitalization in intensive care unit (ICU). We also included studies reporting the respiratory composition of healthy adult humans to serve as controls.

Outcomes studied

[0369] We assessed differences in the respiratory microbiome in terms of alpha and beta diversity and terms of taxonomy, in critically ill patients hospitalized in ICU compared to healthy individuals; in patients with ARDS compared with patients without ARDS; in patients with HAP, compared with patients without HAP; and in patients with prolonged MV (i.e., with late successful extubation) compared with patients with short MV (i.e., with early successful extubation), defined as the highest versus lowest quartile of MV duration in survivors. When investigating the time to weaning from MV, mortality was treated by censoring the observed duration of MV. ARDS refers to any acute syndrome of acute hypoxemic respiratory failure. HAP refers to any lung inflammation caused by bacteria, viruses and/or parasites developing in patients hospitalized for more than 48 hours. Day 0 was defined as in-ICU admission. Microbiome profiles of patients with HAP and ARDS were independently used to develop both microbiome signatures.

Study selection and data extraction

[0370] All titles and abstracts found by the above-disclosed search strategy were screened for relevance. Titles and abstracts were then retrieved in full and evaluated for inclusion eligibility in the meta-analysis and 17 studies were retained (including IBIS

cohort), as detailed in Tables 1 to 3. Raw sequencing data (*i.e.*, fastq files) were then uploaded from the specified database (https://www.ebi.ac.uk/ and https://www.ncbi.nlm.nih.gov/sra) and metadata were requested from the corresponding authors.

Microbiome analyses

[0371] Sequences were preprocessed, quality-filtered, and analyzed using QIIME 2 (2019.10 release) (https://qiime2.org/) (Bolyen E, Rideout JR, Dillon MR, *et al.*, (2019), Nat Biotechnol; 37: 852–7, Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2.). QIIME 2 computes error-corrected amplicon sequence variants (ASV) for Illumina read sequences. We used QIIME 2 in combination with its Deblur plugin (Amir A, McDonald D, Navas-Molina JA, *et al.*, (2017), Msystems; 2: e00191-16, Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns). Raw reads were imported into a QIIME 2 artifact before merging paired-end reads and quality filtering. Reads were then denoised using the "deblur denoise-16S" command and trimmed based on demux-summary.qzv. Representative sequences and their abundances were extracted by feature-table (McDonald D, Clemente JC, Kuczynski J, *et al.*, (2012), Gigascience; 1: 7, The Biological Observation Matrix (BIOM) format or: how I learned to stop worrying and love the ome-ome). A naive Bayes classifier was fitted with 16S rRNA gene sequences extracted from Greengenes version 13_8 (DeSantis, T. Z. *et al.*, (2006), Appl Environ Microb 72, 5069–5072, Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB). ASVs classified as mitochondria or chloroplasts were excluded from further analysis. Compositions of microbiota communities were summarized by proportions at different taxonomy levels, including genus, family, order, class, and phylum ranks. First, we assessed alpha diversity that summarizes the distribution of ASV in each sample into a single number based on ASV richness and evenness, using the Shannon index and the number of observed genera. We also assessed the beta diversity that describes the differences in composition between samples by calculating the Bray-Curtis distance. Tests for categorical differences in beta diversity were performed using PERMANOVA as implemented in R's vegan package. Alpha diversity measures (observed ASV and

Shannon diversity) were calculated from the ASV tables collapsed at the genus level, using diversity as implemented in R's vegan package.

[0372] We next plotted network correlation plot of the core respiratory microbiome in the respiratory samples collected in critically ill patients, using a cut-off for prevalence (presence of a genus in at least 50% of the cohort) and Spearman's correlation analysis. We compared samples collected in patients with or without ARDS, and samples collected in patients with or without HAP using a Mann-Whitney U test blocked for 'study' and ANCOM-BC with adjusting for 'study'. A p-value with a False Discovery Rate (FDR corrected p-value) of 0.20 was considered significant.

Fast and frugal trees procedure

[0373] To aid clinical decision-making, we fitted a Fast and Frugal Tree (FFT) to predict risk groups accurately (Hafenbrädl, S., Waeger, D., Marewski, J. N. & Gigerenzer, G., (2016), J Appl Res Mem Cogn 5, 215–231, Applied Decision Making With Fast-and-Frugal Heuristics ; Raab, M. & Gigerenzer, G., (2015), Front Psychol 6, 1672, The power of simplicity: a fast-and-frugal heuristics approach to performance science; Phillips, N. D., Neth, H., Woike, J. K. & Gaissmaier, W., (2017), Judgm Decis Mak 12, 344–368, FFTrees: A toolbox to create, visualize, and evaluate fast-and-frugal decision trees). FFTs are simpler versions of decision trees and have been shown to perform competitively with random forests and to facilitate biologic interrogation by decreasing the number of key features. The selected top features associated with ARDS or HAP, found using a Mann-Withney U test blocked for 'study' and ANCOM with adjusting for 'study' and with a p-value with a False Discovery Rate (FDR corrected p-value) of 0.05, were then introduced in the FFT procedure. Data were processed with default settings except when stated otherwise. The most striking feature of FFT is that, unlike decision trees, it is usually simple enough for a human mind to memorize.

Study design and population of the IBIS cohort

Human subjects and human samples

[0374] Bioresources: IBIS (cohort), Nantes, France. Patients were enrolled from 31st

January 2017 to 5th May 2020 in two French Surgical Intensive Care Units of one university hospital (Nantes, France). The collection of human samples has been declared to the French Ministry of Health (Programme de recherche "Immunologie", DC2014-2206, authorization renewed DC-2017-2987), and was approved by the Comite de Protection des Personnes Ouest IV (7/04/2015 and 08/10/2020). Written informed consent from a next-of-kin was required for enrolment. Retrospective consent was obtained from patients when possible.

[0375] Inclusion criteria were brain injury (Glasgow Coma Scale below or equal to 12 and abnormal brain-CT scan) and receiving invasive mechanical ventilation. Exclusion criteria were cancer in the previous five years, immunosuppressive drugs, and pregnancy. All patients were clinically followed up for 28 days. Control samples were collected from matched healthy blood donors (age ± 10 years, sex, race), recruited at the Blood Transfusion Center (Etablissement Français du Sang, Nantes, France).

[0376] Endotracheal aspirates (ETA) were collected on days 0, 4, and 7 after ICU admission, frozen at -80c and stored until sequencing.

Sequencing and analysis of ETA samples from the IBIS cohort

[0377] *Specimen processing*: Specimens were centrifuged (30 minutes at 15,000 G) and the resulting pellet was used for DNA isolation. Pellets were resuspended in 360µl ATL buffer (Qiagen DNeasy Blood & Tissue kit). Sterile laboratory water and AE buffer used in DNA isolation were collected and analyzed as potential sources of contamination, as were extraction controls (empty isolation tubes) and blank sequencing wells.

[0378] *Bacterial DNA isolation*: Genomic DNA was extracted from mini-BAL pellets (Qiagen DNeasy Blood & Tissue kit, Qiagen, Hilden, Germany) using a modified protocol previously demonstrated to isolate bacterial DNA (Mason KL, Erb Downward JR, Mason KD, Falkowski NR, Eaton KA, Kao JY, Huffnagle GB., (2012), Infect Immun: 80(10): 3371-3380, Candida albicans and bacterial microbiota interactions in the cecum during recolonization following broad-spectrum antibiotic therapy). Sterile laboratory water and AE buffer used in DNA isolation were collected and analyzed as potential

sources of contamination. Specimens were processed in a randomized order to minimize the risk of false pattern formation due to reagent contamination (Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Walker AW., (2014), BMC Biol: 12: 87, Reagent and laboratory contamination can critically impact sequence-based microbiome analyses).

[0379] *16s rRNA gene sequencing*: The V4 region of the 16s rRNA gene was amplified using published primers (Forward PCR primer **SEQ ID NO: 25**: 5' GTGCCAGCMGCCGCGGTAA; Reverse PCR primer **SEQ ID NO: 26**: 5' TAATCTWTGGGVHCATCAGG, Read 1 sequencing primer **SEQ ID NO: 27**: 5' ACGTACGTACGGTGTGCCAGCMGCCGCGGTAA and Read 2 sequencing primer **SEQ ID NO: 28**: 5'ATTAGAWACCCBDGTAGTCCGGCTGACTGACT) and the dual-indexing sequencing strategy developed by the laboratory of Patrick D. Schloss (Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD., (2013), Appl Environ Microbiol: 79(17): 5112-5120, Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform). Sequencing was performed using the Illumina MiSeq platform (San Diego, CA), using a MiSeq Reagent Kit V2 (500 cycles), according to the manufacturer's instructions with modifications found in the Schloss SOP (Schloss PD. MiSeq SOP - mothur. 2015 [cited; Available from: http://www.mothur.org/wiki/MiSeq_SOP). Accuprime High Fidelity Taq was used in place of Accuprime Pfx SuperMix. Primary PCR cycling conditions were 95°C for two minutes, followed by 20 cycles of touchdown PCR (95°C 20 seconds, 60°C 20 seconds and decreasing 0.3 degrees each cycle, 72°C 5 minutes), then 20 cycles of standard PCR (95°C for 20 seconds, 55°C for 15 seconds, and 72°C for 5 minutes), and finished with 72°C for 10 minutes.

[0380] *Bacterial DNA quantification*: Bacterial DNA was quantified using a QX200 Droplet Digital PCR System (BioRad, Hercules, CA). Primers were 5'-GCAGGCCTAACACATGCAAGTC-3' (SEQ ID NO: 23) and 5'-CTGCTGCCTCCCGTAGGAGT-3' (SEQ ID NO: 24). The cycling protocol was 1 cycle at 95°C for 5 minutes, 40 cycles at 95°C for 15 seconds and 60°C for 1 minute, 1 cycle

at 4°C for 5 minutes, and 1 cycle at 90°C for 5 minutes all at a ramp rate of 2°C/second. The BioRad C1000 Touch Thermal Cycler was used for PCR cycling. Droplets were quantified using the Bio-Rad Quantisoft software. Two replicates were used per sample. No-template control specimens were used and were run alongside mini-BAL specimens.

[0381] *Statistical analysis*: We used the same method for the prospective cohort analysis as for the meta-analysis. Sequences were preprocessed, quality-filtered, and analyzed using QIIME 2 (2019.10 release) (https://qiime2.org/) (Bolyen E, Rideout JR, Dillon MR, *et al.*, (2019), Nat Biotechnol; 37: 852–7, Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2). QIIME 2 computes error-corrected amplicon sequence variants (ASV) for Illumina read sequences. We used QIIME 2 in combination with its Deblur plugin (Amir A, McDonald D, Navas-Molina JA, *et al.*, (2017), Msystems; 2: e00191-16, Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns). Raw reads were imported into a QIIME 2 artifact before merging paired-end reads and quality filtering. Reads were then denoised using the "deblur denoise-16S" command and trimmed based on demux-summary.qzv. Representative sequences and their abundances were extracted by feature-table (McDonald D, Clemente JC, Kuczynski J, *et al.*, (2012), Gigascience; 1: 7, The Biological Observation Matrix (BIOM) format or: how I learned to stop worrying and love the ome-ome.). A naïve Bayes classifier was fitted with 16S rRNA gene sequences extracted from Greengenes version 13_8 (DeSantis TZ, Hugenholtz P, Larsen N, *et al.*, (2006), Appl Environ Microb; 72: 5069–72, Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB). ASVs classified as mitochondria or chloroplasts were excluded from further analysis. Compositions of microbiota communities were summarized by proportions at different taxonomy levels, including genus, family, order, class, and phylum ranks. Tests for categorical differences in beta diversity were performed using PERMANOVA as implemented in R's vegan package. Alpha diversity measures (observed ASV and Shannon diversity) were calculated from the ASV tables collapsed at the genus level, using diversity as implemented in R's vegan package.

Scripts for the microbiome analysis

[0382] *Alpha diversity:* Alpha diversity measures (observed ASV and Shannon diversity) were calculated from the ASV tables collapsed at the genus level, using diversity as implemented in R's vegan package. Alpha diversity was expressed as the Shannon index for normalized numbers of sequences for each sample. Rarefaction was done by random subsampling with 1043 reads as threshold. Data were processed with default settings except when stated otherwise.

[0383] *Beta diversity*: Tests for categorical differences in beta diversity were performed using PERMANOVA as implemented in R's vegan package (Bray-Curtis distances with permutational analysis of variance [permanova] at 1000 permutations). β diversity was summarised using principal coordinate (PCo) analysis of Bray-Curtis distances, as they represent most features of β diversity.

Correlation network: We plotted network correlation plot of the core respiratory microbiome in the respiratory samples collected in critically ill patients (including samples collected at BAL, ETA, and OS), using a cut-off for prevalence (presence of a genus in at least 50% of the cohort) and Spearman's correlation analysis. We used Cytoscape (Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T., (2003), Genome Res.;13:2498-504, Cytoscape: a software environment for integrated models of biomolecular interaction networks) to plot the results of the spearman correlation, and all correlations between the most prevalent genera were plotted. We plotted all the respiratory microbiome, combining BAL, ETA and OS samples, and also we plotted separately BAL, ETA or OS samples.

[0384] The 3 shared genera *Streptococcus*, *Prevotella*, and *Pseudomonas* were compared assessed by Kruskal-Wallis and Dunn's tests.

[0385] *The upset plot*: UpSetR generates static UpSet plots (Conway JR, Lex A, Gehlenborg N., (2017), Bioinformatics.; 33:2938-2940, UpSetR: an R package for the visualization of intersecting sets and their properties). The UpSet technique visualizes set intersections in a matrix layout and introduces aggregates based on groupings and queries. The matrix layout enables the effective representation of associated data, such as the number of elements in the aggregates and intersections (here features in BAL, ETA and

OS), as well as additional summary statistics derived from subset or element attributes (here for example the common features in BAL, ETA and OS, n = 3).

[0386] *Mann-Withney U test blocked for 'study'*: For the association studies, all timepoints were included. Especially for the propective cohort, the 3 timepoints were used for the analyses.

[0387] *Association plots and ROC curve plot:* Association plots and ROC curve plot were plotted using scripts from siamcat package (Wirbel J, Zych K, Essex M. *et al.*, (2021), Genome Biol; 22:9", Microbiome meta-analysis and cross-disease comparison enabled by the SIAMCAT machine learning toolbox). .

[0388] *Validation of the microbiome signatures*. To assess the value and the interest of a microbiome signature, we built the risk index, calculated using the sum of relative abundances of the taxa that were significantly associated with the occurrence of the outcome minus the sum of the relative abundances of the taxa that were associated the absence of occurrence of the outcome. This risk index is therefore able to summarize all the significant microbiome signatures linked the outcome in a given sample into a single number.

Results

Summary of available data

[0389] We found 17 studies realized in patients hospitalized in intensive care units. We gathered individual data from 2177 respiratory samples, including 310 bronchoalveolar lavages (BAL), 1083 endotracheal aspirates (ETA), and 784 oropharyngeal swabs (OS) issued from 1029 critically ill patients. The median time of the sample collection was day 1 (interquartile 1-5) after ICU admission. Acute respiratory distress syndrome and HAP were recorded in 223 (21.6%) and 271 (26.3%) patients. Study characteristics are described in Tables 1 to 3. Data from 327 bronchoalveolar lavages (BAL) collected in healthy individuals were obtained from four studies (Charlson, E. S. *et al.*, (2011), Am J Resp Crit Care 184, 957–963, Topographical Continuity of Bacterial Populations in the Healthy Human Respiratory Tract.; Dickson, R. P. *et al.*, (2017), Mbio 8, e02287-16,

Bacterial Topography of the Healthy Human Lower Respiratory Tract; Morris, A. *et al.*, (2013), Am J Resp Crit Care 187, 1067–1075, Comparison of the Respiratory Microbiome in Healthy Nonsmokers and Smokers; Bassis, C. M. *et al.*, (2015), Mbio 6, e00037-15, Analysis of the Upper Respiratory Tract Microbiotas as the Source of the Lung and Gastric Microbiotas in Healthy Individuals) .

Table 1. Patients included in the meta-analysis with BAL samples and available ARDS outcomes.

Study	Definition of ARDS	Number of patients included	Analysis	Type of sample	Date of samples	Type of patients
Walsh et al	PaO_2/FiO_2 ratio <300	48 patients, including 24 ARDS patient	16S, V4 region, Illumina MiSeq	Bronchial washings	within 72 hours of hospitalization	Burn and inhalation injury
Schmitt et al	PaO_2/FiO_2 ratio <300	30 patients, including 15 ARDS patient	16S, V4 region, Illumina MiSeq	Bronchoalveolar lavage	In the ARDS group: samples collected at ARDS onset, and 5 and 10 days later. In the control group, only one BAL was collected.	Sepsis-induced ARDS following major abdominal surgery and oesophageal resection serving as controls
Smith et al	Defined as acute respiratory failure	15 patients, including 1 ARDS patient	16S, V4 region, Ion Torrent Sequencing	Bronchoalveolar lavage	Patients that remain ventilated for over 36 hours	surgical ICU
Dickson et al (PMID: 31973575)	Based on the Berlin definition	91 patients, including 17 ARDS patients	16S, V4 region, Illumina MiSeq	Bronchoalveolar lavage	24 hours of ICU admission	ICU
Dickson et al (PMID: 27670109)	Based on the American-European Consensus Conference definition	68 ARDS patients	16S, V4 region, Illumina MiSeq	Bronchoalveolar lavage	within 7 days of meeting ARDS criteria	ICU in three academic medical centers

ARDS: acute respiratory distress syndrome, ICU: intensive care unit.

Walsh et al, PLoS One. 2017 Mar 30;12(3):e0173848. doi: 10.1371/journal.pone.0173848. PMID: 28358811; PMCID: PMC5373524.,

Schmitt et al, J Hosp Infect. 2020 Apr 24:S0195-6701(20)30203-6. Doi: 10.1016/j.jhin.2020.04.028. Epub ahead of print. PMID: 32339614.,

Smith et al, PLoS One. 2016 Nov 29;11(11):e0166313. doi: 10.1371/journal.pone.0166313. PMID: 27898681; PMCID: PMC5127496.

Dickson et al (PMID31973575), Am J Respir Crit Care Med. 2020 Mar 1;201(5):555-563. doi: 10.1164/rccm.201907-1487OC. PMID: 31973575; PMCID: PMC7047465.,

Dickson et al (PMID: 27670109), Nat Microbiol. 2016 Jul 18;1(10):16113. doi: 10.1038/nmicrobiol.2016.113. PMID: 27670109; PMCID: PMC5076472.

Table 2. Patients included in the meta-analysis with ETA samples and available ARDS outcomes.

Study	Definition of ARDS	Number of patients included	Analysis	Type of sample	Date of samples	Type of patients
Baek et al	PaO_2/FiO_2 ratio <300	60 patients, including 8 ARDS patient	16S, V3-V4 region, Illumina MiSeq	Endotracheal tube aspirates	Day 1, Day 3 and Day 7 of ICU admission	Patients who had been placed on mechanical ventilation (Cardiac arrest, Neurological distress, Post-operative status, Respiratory)
Kelly et al	PaO_2/FiO_2 ratio <300	15 patients, including 2 ARDS patient	16S, V1-V2 region, Illumina MiSeq	Endotracheal tube aspirates	within 24 h of intubation and every 48–72 h thereafter	Heterogeneous set of acute indications for mechanical ventilation
Kitsios et al	PaO_2/FiO_2 ratio <300	301 subjects, 72 were diagnosed with ARDS	16S, V3-V4 region, Ion Torrent Sequencing	Endotracheal tube aspirates	Within 72 hours of ICU admission	Mechanically-ventilated patients with acute respiratory failure
Panzer et al	PaO_2/FiO_2 ratio <300	74 patients, including 16 ARDS patients	16S, V4 region, Illumina MiSeq	Endotracheal tube aspirates	ICU admission (n = 74) and at 48 hours after admission (n = 30)	Mechanically ventilated patients admitted to the ICU after severe blunt trauma

ARDS: acute respiratory distress syndrome, ICU: intensive care unit

Baek et al, J Transl Med. 2020 Dec 7;18(1):464. doi: 10.1186/s12967-020-02642-z. PMID: 33287847; PMCID: PMC7720271.

Kelly et al, Microbiome. 2016 Feb 11;4:7. doi: 10.1186/s40168-016-0151-8. PMID: 26865050; PMCID: PMC4750361.

Kitsios et al, Am J Respir Crit Care Med. 2020 Dec 15;202(12):1666-1677. doi: 10.1164/rccm.201912-2441OC. PMID: 32717152; PMCID: PMC7737572.

Panzer et al, Am J Respir Crit Care Med. 2018 Mar 1;197(5):621-631. doi: 10.1164/rccm.201702-0441OC. PMID: 29035085; PMCID: PMC6005235.

Table 3. Patients included in the meta-analysis with ETA samples and available VAP outcomes.

Study	Definition of VAP	Number of patients included	Analysis	Type of sample	Date of samples	Type of patients
Back et al	Clinical and radiological signs of VAP	60 patients (120 samples VAP, 53 samples no VAP)	16S, V3-V4 region, Illumina MiSeq	Endotracheal tube aspirates	Day 1, Day 3 and Day 7 of ICU admission	Patients who had been placed on mechanical ventilation (Cardiac arrest, Neurological distress, Post-operative status, Respiratory)
Kelly et al	Clinical and radiological signs of VAP	15 patients (28 samples VAP, 14 samples non VAP)	16S, V1-V2 region, Illumina MiSeq	Endotracheal tube aspirates	within 24 h of intubation and every 48–72 h thereafter	Heterogeneous set of acute indications for mechanical ventilation
Kitsios et al	Clinical and radiological signs of VAP	301 patients (90 samples VAP, 145 no VAP)	16S, V4 region, Illumina MiSeq	Endotracheal tube aspirates	Within 72 hours of ICU admission	Mechanically-ventilated patients
Emonet et al	Clinical and radiological signs of VAP with a clinical pulmonary infection score (CPIS) > 6 [24] and ≥ 103 bacteria/mL in protected catheter mini-BAL fluid or ≥ 104 bacteria/mL in bronchoscopic BAL fluid.	263 patients (76 samples VAP, 174 no VAP)	16S, V3-V4 region, Illumina MiSeq	Endotracheal tube aspirates	on D0 (day of intubation); D3 (3 days after intubation); DVAP-3 (3 days before VAP); DVAP (day of VAP diagnosis); and DVAP + 3 (3 days after VAP).	Mechanically-ventilated adult patients

Sommerstein et al	Clinical VAP was defined as a patient with a new or progressive pulmonary infiltrate on chest radiography (according to the radiology report). Also, two of the following three criteria were required: body temperature higher than 38 °C, leukocyte count greater than 12 G/L or lower than 4 G/L, and/or purulent respiratory secretions within 48-h before/after the radiography.	10 patients (25 samples VAP, 27 no VAP)	16S, V3-V4 region, Illumina MiSeq	Endotracheal tube aspirates	Within 10 days of ICU admission	Mechanically-ventilated patients with acute respiratory failure
Xiaoling et al	Inclusion criteria of P. aeruginosa VAP patients included [1]: (1) mechanical ventilation > 48 h; (2) satisfied two of the following: body temperature > 38 °C or < 36 °C, leukopenia	54 patients (89 samples VAP, 18 no VAP)	16S, V3-V4 region, Illumina MiSeq	Endotracheal tube aspirates	Initial samples were collected within 24 h post P. aeruginosa VAP diagnosis. Sequential sample collection was performed at day 7 and day 14 post initial sample collection.	Pseudomonas aeruginosa ventilatorassociated pneumonia patients

	or leukocytosis, or purulent secretions; (3) new or progressive chest infiltrates, for patients with underlying pulmonary or cardiac disease, two serial chest radiographs were required for assessment; (4) endotracheal aspiration cultured P. aeruginosa at least + 2 growth using semi-quantitative measurements					
Zakharkina et al	VAP was diagnosed using consensus criteria;15 a new and persistent radiographic infiltrate plus at least two of the following criteria: (a) temperature >38°C or <36°C; (b) leucocytes >10 or <4×103/mm3; (c) purulent tracheal aspirate16 but always needed quantitative microbiological	35 patients (56 samples VAP, 40 no VAP)	16S, 454 Roche	Endotracheal tube aspirates	Within 72 hours of ICU admission	Mechanically-ventilated patients with acute respiratory failure

	confirmation to fulfil the diagnosis of 'VAP' (>103 or >106 colony forming units (CFU)/mL in endotracheal aspirate (ETA)					

[0390] *VAP: Ventilator-associated pneumonia, ICU: intensive care unit*

Baek et al. J Transl Med. 2020 Dec 7;18(1):464. doi: 10.1186/s12967-020-02642-z. PMID: 33287847; PMCID: PMC7720271.

Kelly et al, Microbiome. 2016 Feb 11;4:7. doi: 10.1186/s40168-016-0151-8. PMID: 26865050; PMCID: PMC4750361.

Kitsios et al, Am J Respir Crit Care Med. 2020 Dec 15;202(12):1666-1677. doi: 10.1164/rccm.201912-2441OC. PMID: 32717152; PMCID: PMC7737572.

Emonet et al, Intensive Care Med. 2019 Aug;45(8):1082-1092. Doi: 10.1007/s00134-019-05660-8. Epub 2019 Jun 17. PMID: 31209523; PMCID: PMC6667422.

Sommerstein et al, Antimicrob Resist Infect Control. 2019 May 22;8:81. doi: 10.1186/s13756-019-0530-6. PMID: 31139364; PMCID: PMC6530040.

Qi et al, Respir Res. 2018 Jul 27;19(1):139. doi: 10.1186/s12931-018-0847-3. PMID: 30049266; PMCID: PMC6062970. Zakharkina et al, Thorax. 2017 Sep;72(9):803-810. doi: 10.1136/thoraxjnl-2016-209158. Epub 2017 Jan 18. PMID: 28100714.

Characterization of the dysbiosis along the respiratory airways in critically ill patients

[0391] We first aimed to confirm the state of respiratory dysbiosis in critically ill patients and second to compare the microbiome profiles based on the site of sampling (OS, ETA, and BAL).

[0392] We compared the microbiome composition of the samples collected during ICU hospitalization to those from healthy individuals. We confirmed the significantly different projection in Principal Coordinates Analysis (PCoA) of samples collected in ICU patients compared to samples collected in healthy individuals (p-value = 0.001, r^2 = 0.016; (**Figure 1**). On a genus level, the alpha diversity of the respiratory microbiome composition was lower in critically ill patients than in healthy controls. It was lower in the samples collected after five days of hospitalization than those collected before day 4. In the 2117 samples from critically ill patients, we found that five of the six most prevalent genera belonged to the healthy respiratory microbiome core (**Figure 2**). However, the relative abundance of the healthy respiratory microbiota core was lower in critically ill patients than in healthy controls (34% vs. 43%, respectively, p-value < 0.01, (**Figure 2**). More specifically, the relative abundances of *Fusobacterium*, *Veillonella*, and *Prevotella* were lower in critically ill patients (p=0.03, <0.001, and <0.001, respectively), while *Haemophilus* was significantly more abundant (p<0.001). *Pseudomonas*, which was not observed in healthy subjects, was observed in 53% of critically ill patients, with a relative abundance reaching 5.3% on average (**Table 4**).

Table 4. Core respiratory microbiome in the 2177 samples collected from critically ill patients.

Genus	**Prevalence**	**Detection (mean relative abundance in samples)**
*Streptococcus**	0.87	0.125
*Prevotella**	0.79	0.098
*Veillonella**	0.72	0.040
Actinomyces	0.64	0.011
*Fusobacterium**	0.63	0.018
*Haemophilus**	0.61	0.046
Paraprevotella	0.61	0.020

Campylobacter	0.60	0.005
Rothia	0.57	0.027
Atopobium	0.57	0.005
Unclassified Enterobacteriaceae	0.57	0.059
Neisseria	0.56	0.035
Mycoplasma	0.55	0.034
Porphyromonas	0.55	0.009
Corynebacterium	0.55	0.020
Lactobacillus	0.54	0.024
Pseudomonas	0.53	0.053
Dialister	0.51	0.003
Capnocytophaga	0.50	0.008

Data obtained from BAL, ETA, and OS. The cut-off for prevalence was the presence of the genus in at least 50% of the cohort of patients. (* members of the healthy respiratory microbiome core).

[0393] The relative abundance of *Pseudomonas* in patients was negatively correlated with the relative abundance of the genera of the healthy respiratory core microbiota. This series of analyzes confirmed that critical illnesses induce a state of respiratory dysbiosis mainly characterized by the healthy microbiome core replacement by *Pseudomonas* genera.

[0394] We then questioned if the loss of healthy microbiome core was homogenously observed along the respiratory tract or specific to the site of samplings. To answer this question, we first observed that the three main contributors to the respiratory microbiome architecture were first the 'Study' ($p<0.001$, $R^2=0.12$), second the 'Site of sampling' ($p<0.001$, $R^2=0.008$), and third the medical condition 'healthy vs. critical illness' ($p<0.001$, $R^2=0.007$). We further investigated the effects of the sampling site in this variability and found that bronchoalveolar lavages (BAL), endotracheal aspirates (ETA), and oropharyngeal swabs (OS) had a significantly different architecture (**Figure 3**) and that the alpha diversity decreased from OS to BAL (**Figure 4**). At the genus level, only two members of the healthy microbiome core (*Streptococcus, Prevotella*) were commonly found in BAL, ETA, and OS, and **Tables 5-7**), but with their relative abundances varied with the site of samplings.

Table 5. Core respiratory microbiome in the 334 BAL samples collected in intensive care unit patients.

Genus	Prevalence	Detection (mean relative abundance in samples)
Streptococcus	0.71	0.104
Pseudomonas	0.62	0.054
Prevotella	0.50	0.059
Haemophilus	0.48	0.046

The cut-off for prevalence was the presence of the genus in at least 40% of the cohort of the patients.

Table 6. Core respiratory microbiome in the 1139 tracheal aspirates samples collected in intensive care unit patients.

Genus	Prevalence	Detection (mean relative abundance in samples)
Streptococcus	0.88	0.15
Prevotella	0.78	0.09
Veillonella	0.71	0.033
Fusobacterium	0.62	0.016
Haemophilus	0.62	0.063
Actinomyces	0.61	0.011
Rothia	0.58	0.020
Neisseria	0.57	0.034
Corynebacterium	0.56	0.022
Pseudomonas	0.56	0.076
Campylobacter	0.54	0.003
Porphyromonas	0.52	0.007
Mycoplasma	0.50	0.025
Atopobium	0.52	0.004

The cut-off for prevalence was the presence of the genus in at least 50% of the cohort of the patients.

Table 7. Core respiratory microbiome in the 792 oral samples collected in intensive care unit patients.

Genus	prevalence	detection (mean relative abundance in samples)

Prevotella	*0.95*	*0.131*
Streptococcus	*0.94*	*0.093*
Veillonella	*0.91*	*0.062*
Actinomyces	*0.86*	*0.014*
Campylobacter	*0.83*	*0.008*
Fusobacterium	*0.82*	*0.024*
Atopobium	*0.80*	*0.006*
Mycoplasma	*0.74*	*0.055*
Dialister	*0.73*	*0.005*
Rothia	*0.72*	*0.045*
Porphyromonas	*0.71*	*0.013*
Haemophilus	*0.71*	*0.023*
Capnocytophaga	*0.69*	*0.014*
Lactobacillus	*0.69*	*0.041*
Parvimonas	*0.69*	*0.005*
Neisseria	*0.67*	*0.042*
Corynebacterium	*0.67*	*0.023*
Selenomonas	*0.65*	*0.003*
Oribacterium	*0.65*	*0.003*
Treponema	*0.65*	*0.004*
Moryella	*0.58*	*0.002*
Megasphaera	*0.57*	*0.008*
Tannerella	*0.57*	*0.001*
Mogibacterium	*0.56*	*0.001*
Bulleidia	*0.55*	*0.001*
Pseudomonas	*0.52*	*0.031*
Leptotrichia	*0.51*	*0.004*
Peptostreptococcus	*0.50*	*0.003*

The cut-off for prevalence was the presence of the genus in at least 50% of the cohort of the patients.

[0395] The replacement of the healthy core microbiome by *Pseudomonas* genera increased from the upper airways (OS) to the alveolar spaces (BAL).

[0396] We then aimed to determine which site offers the best compromise between clinical accessibility and accuracy. We found that the richness of the ICU respiratory microbiome core, as represented by the set size, was higher in OS and ETA than in BAL, but this signature, as represented by the intersection size, was more frequently observed

in BAL and ETA than in OS (**Figure 5**). We thus prioritized the development of dysbiosis signatures in ETA samples.

ARDS microbiome score in ETA samples

[0397] To develop a respiratory microbiome signature for ARDS, we selected participants with one or more ETA samples collected during the first week of ICU hospitalization and ARDS outcome available (number of samples analyzed: 551 ETA samples). Out of the 450 patients included in four studies, 98 (22%) had ARDS at baseline or during the follow-up. We found that ETA samples from patients with ARDS had a significantly different overall architecture than others (**Figure 6**). Since 'study' was the main contributor to the architecture of the respiratory microbiome, the study effect was treated as a blocking factor for all the analyses. The alpha diversity of ETA was not statistically different between ARDS and no ARDS patients. When comparing patients with extreme Shannon index values (first quartile vs last quartile values), the rate of ARDS was 21% in patients with low alpha diversity and 20% in those with preserved alpha diversity. The composition of the tracheal respiratory microbiome was different at the phylum and genus levels between patients with or without ARDS (**Figure 7**). *Bacteroidetes* relative abundance was higher in critically ill patients than in healthy controls (26% vs. 12%, $p<0.001$), and the proportion of *Firmicutes* was lower (28% vs. 42%, $p<0.001$) (Figure S2C). Firmicutes and Proteobacteria relative abundances were higher in patients with ARDS than without ARDS (respectively 45% vs. 37%, and 35% vs 25%). While *Streptococcus* and *Prevotella* were negatively correlated with *Haemophilus* and *Staphylococcus* in patients without ARDS, but not in patients with ARDS. Notably, the high relative abundance of *Streptococcus* correlated with the low abundance of *Staphylococcus* in patients without ARDS.

[0398] Thus, we selected the top features associated with ARDS using a Mann-Withney U test blocked for 'study' and ANCOM with adjusting for 'study.' With a p-value with a False Discovery Rate (FDR corrected p-value) of 0.20, we identified a panel of 30 genera with different relative abundance in ARDS and no ARDS microbiomes. We notably observed that ARDS was associated with high relative abundances of *Staphylococcus*, *Ralstonia*, and *Enterococcus*. We then employed a stacked machine learning model

consisting of spectral clustering and the Fast and Frugal Tree (FFT) technique to define a parsimonious predictive score using a minimal number of criteria. FFT-based modeling offered a simple, 3-factor decision tree that can predict the individual risk of ARDS (**Figure 8**). We applied a ten-repeated 10-fold cross-validation scheme to test the accuracy of the FFT-based model and calculated a mean-classification AUC of 0.751 (**Figure 9**). Patients with ARDS signature defined by the FFT model had a longer duration of mechanical ventilation than patients with no ARDS signature (Hazard Ratio (HR) 1.338, 95%CI 1.032-1.734, P=0.026, **(Figure 10**). The mortality rate in patients with ARDS signature was 73% vs 26% in patients with no ARDS microbiome signature (p=0.028).

[0399] As an exploratory analysis, we questioned the effect of the sampling time on the accuracy of the ARDS signature. Thus, we built a risk index by calculating the sum of the relative abundances of taxa associated with no ARDS minus the sum of the relative abundances associated with ARDS. The ARDS risk index decreased when the relative abundance of ARDS-associated taxa increased. We observed that the risk index values were not significantly different between early and late samples. We also observed that the AUC of the FFT-based model was 0.72 for samples collected during the first week, 0.78 during the second week, and 0.73 afterwards. These series of analyses suggested that the developed ARDS microbiome signature was robust for samples collected during the first three weeks of ICU hospitalization.

[0400] We also tested a score built by Classification Decision Tree, which can improve the model's accuracy and interpretability, but usually uses more variables than FFT. The Classification Decision Tree-based model included 10 factors but did not have significantly better accuracy than the parsimonious FFT-based score (AUC = 0.789, sensitivity = 87.5%, specificity = 66.5%) **(Figure 42A** and **42B).**

Respiratory microbiome signature of ARDS in bronchoalveolar lavages did not significantly out perform the ETA signature

[0401] We tested the ETA-based FFT model in the BAL sample dataset and observed a decreased accuracy for ARDS classification (AUC = 0.68). We thus aimed to develop a

BAL-specific microbiome score associated with ARDS and thus repeated the approach described in **Figures 7** to **10** in the cohort of 252 participants from five studies with at least one BAL obtained during the first week of ICU hospitalization (number of samples analyzed = 283, number of ARDS patients = 125/252, 50%). Even after treating the study as a blocking factor for all the analyses, we observed a statistically different significant architecture of the respiratory microbiome between patients with or without ARDS (PC2: crude p-value <0.001, p-value=0.04 after adjustment for the study effect, **(Figure 11**). The alpha diversity was lower in samples from ARDS patients compared to no ARDS patients, and the composition of the BAL respiratory microbiome was different at the phylum and genus levels between patients with or without ARDS (**Figure 12**). The top features selection using a Mann-Withney U test blocked for 'study' and ANCOM with adjusting for 'study' identified a panel of 37 microbes that were differentially abundant between samples from ARDS and no ARDS patients, and as expected different from those selected in ETA. The FFT-based modeling offered a 3-factor decision tree based on the relative abundances of *Flavobacterium*, *Veillonella* and *Prosthecobacter*, that predicts individual risk of ARDS from the BAL microbiome with good accuracy (mean AUC of 0.81, **Figures 13 and 14**). The model's overall accuracy was not significantly improved when using the Classification Decision Tree method (AUC = 0.79, sensitivity = 75%, specificity = 83%, **Figure 43A** and **43B**). The accuracy of the BAL-specific microbiome signature for ARDS was not significantly higher than the ETA-specific microbiome signature (p=0.514 for mean AUCs comparisons), supporting the development of respiratory microbiome signatures in ETA samples which are easily accessible. Following the same strategy, we developed a microbiome score in the OS dataset, which reached a low accuracy (AUC 0.65, data not shown), further strengthening our decision to favor ETA samples for developing respiratory microbiome signatures.

HAP microbiome score

[0402] We then developed a microbiome signature associated with HAP in a cohort of 738 participants with at least one ETA sample and with HAP outcome available (number of samples = 955 from 9 studies, patients with HAP on baseline or during follow-up = 484/738 (65.6%) patients). Based on a PCoA analysis, we observed that ETA samples

from critically ill patients with HAP had a significantly different overall architecture than ETA from those without HAP, even after adjustment on the study effect (**Figure 15**) for the variability across the studies). The microbiome alpha diversity was lower in HAP than in no-HAP samples collected in critically ill patients. When comparing patients with extreme Shannon index values (first versus last quartile values), the rate of HAP was 57% in patients with low alpha diversity (first quartile) and 42% in those with preserved alpha diversity (fourth quartile, P=0.001). The composition of the tracheal respiratory microbiome was different at the phylum and genus levels between patients with or without HAP, with an increased relative abundance of *Proteobacteria* in HAP patients (**Figure 16**). *Streptococcus* and *Veillonella* relative frequencies were negatively correlated with the relative frequency of *Pseudomonas*.

[0403] The top feature selection approach identified a panel of 66 microbes that were differentially abundant between samples from HAP and no HAP samples. FFT-based modelling offered a 4-factor decision tree associated with HAP classification. Notably, HAP was associated with low relative abundances of *Streptococcus* and *Veillonella*, two genera belonging to the healthy respiratory microbiome core. FFT-based modelling offered a 4-factor decision tree that is associated with HAP classification (**Figure 17**). We applied a ten-repeated 10-fold cross-validation scheme to test the accuracy of the FFT-based model for HAP classification and calculated a mean AUC of 0.72 (**Figure 18**). Patients with HAP signature defined by the FFT model had a longer duration of mechanical ventilation than patients classified as no-HAP (HR 1.505, 95%CI 1.16-1.952, P=0.019, (**Figure 19**). The mortality rate in patients with a HAP signature was 32% (68/213 patients) vs 29% in those with no HAP microbiome signature (p=0.49). Finally, we tested this model in BAL and observed a decreased accuracy for HAP classification (AUC = 0.61) which did not support the extrapolation of this score in distal samples.

[0404] As an exploratory analysis, the accuracy of the HAP microbiome score estimated by the Classification Decision Tree was 0.67 (sensitivity = 56%, specificity = 71%) **(Figure 44A** and **44B).** We also questioned the effect of the sampling time on the accuracy of the HAP signature. Thus, we built a risk index for HAP by calculating the sum of the relative abundances of taxa associated with no HAP minus the sum of the

relative abundances associated with HAP. The HAP risk index decreased over time (ANOVA p-value = 0.002), demonstrating that the total relative abundance of taxa associated with HAP increased after two weeks in ICU. In a subgroup analysis based on sample timing, the AUC of the FFT-based model for HAP was 0.72 during the first week, 0.72 during the second week, and 0.66 after, demonstrating that the HAP signature accuracy decreased after two weeks in ICU.

Prolonged mechanical ventilation signature

[0405] The diagnosis of ARDS and HAP is challenging in clinical practice, and false positive or negative diagnoses are frequent (Bellani, G. *et al.*, (2016), Jama 315, 788–800, Epidemiology, Patterns of Care, and Mortality for Patients With Acute Respiratory Distress Syndrome in Intensive Care Units in 50 Countries; Luyt, C.-E. *et al.*, (2020), Intens Care Med 46, 2168–2183, Pulmonary infections complicating ARDS). A main medical aim is to individually predict an unfavourable outcome characterized by prolonged mechanical ventilation. Thus, we tested whether it was possible to define a microbiome signature associated with prolonged mechanical ventilation or with time-to-successful extubation in critically ill patients. Among the 700 patients with ETA samples, the median duration of mechanical ventilation was 6 (4-11) days. All the samples were collected before the weaning from mechanical ventilation and the median time to successful extubation was 10 (4-11) days since ICU admission. We thus developed a score to predict short (< 4 days, first quartile) and prolonged (>10 days, third quartile) mechanical ventilation and found 17 features associated with this outcome (**Figure 20**). The FFT-based modelling offered a 3-factor decision tree for classification as prolonged mechanical ventilation based on the relative abundances of *Lactobacillales*, *Rothia* and *Streptococcus* (**Figure 21**). We applied a ten-repeated 10-fold cross-validation scheme to test the accuracy of the FFT-based prediction model for HAP and calculated a mean-prediction AUC of 0.727 (**Figure 22**). The mortality of patients classified as prolonged mechanical ventilation based on the FFT classification was higher than in patients classified as short mechanical ventilation (64% vs 36%; P = 0.001). We tested the ETA-based FFT model in BAL sample datasets and observed a similar accuracy for the duration of mechanical ventilation classification (AUC 0.71). Further supporting the medical relevance of this score, the mortality rate in patients with an early successful

extubation microbiome signature was 23% (49/217 patients) vs 36% (85/233 patients) in those with late extubation microbiome signature (p=0.001).

[0406] As an exploratory analysis, the Classification Decision Tree-based model also included three factors (*Lactobacilliales*, *Rothia*, and *Haemophilus*) with increased accuracy (AUC 0.882, sensitivity = 82%, specificity = 90%) (**Figure 45A** and **45B**). To test the impact of the early successful extubation definition, we built a risk index by calculating the sum of the relative abundances of taxa associated with early extubation minus the sum of the relative abundances associated with prolonged MV. The risk index thus varied from -1 (samples rich in taxa associated with prolonged MV) to +1 (samples rich in taxa associated with early extubation). We observed that the risk index of samples collected during the first week of hospitalization was higher than those collected during the second week or after. This observation suggests that the early successful extubation signature can be useful in ICU populations with a median duration of MV of close to one week, routinely reported in ARDS (median 8 days) 2 and HAP (median 7 days) 3 patients.

External validation of the signatures of ARDS, HAP, and prolonged mechanical ventilation

[0407] Finally, we aimed to validate the accuracy of these scores in a prospective independent cohort of ICU patients. We analyzed 277 ETA samples collected on days 0, 4, and 7 after ICU admission in a cohort of 136 brain-injured patients requiring invasive mechanical ventilation hospitalized in one French hospital. The study population characteristics are described in Table 8. The rates of ARDS and HAP were 38% and 51%, respectively, and all cases of ARDS were related to severe HAP.

Table 8. Demographic characteristics of the validation cohort

	Total population	**HAP, yes**	**HAP, no**
Number of patients, *n*	136	70	66
Age, *years*	44 (18)	43 (18)	46 (17)
Male *n (%)*	104 (76%)	60 (86%)	44 (67%)
Pathology, *n (%)* Traumatic brain injury Stroke	 122 (90%) 14 (16%)	 62 (89%) 8 (11%)	 60 (91%) 6 (9%)
SAPS-II	44 (15)	42 (15)	47 (13)
Acute respiratory distress syndrome *n (%)*	25 (38%)	25 (100%)	0

Mechanical ventilation duration, days*	18 (15)	25 (16)	10 (9)
ICU length of stay, days*	25 (20)	34 (21)	15 (13)
In-ICU mortality	34 (25%)	13 (19%)	21 (32%)

[0408] We confirmed that the respiratory microbiome architecture differed between patients with or without ARDS (**Figure 23**), patients with or without HAP (**Figure 24**), and patients with or without prolonged mechanical ventilation (**Figure 25**). The ETA-based microbiome FFT scores for ARDS (**Figure 26**), HAP (**Figure 27**), and prolonged mechanical ventilation (**Figure 28**) performed fairly in this independent cohort of severe brain-injured patients (AUC= 0.75, 0.70, and 0.68, respectively, **Figures 26, 27, 28**).

[0409] Finally, we tested if the microbiome-based FFT classifications were associated with prolonged mechanical ventilation and risk of death. We observed that patients with ARDS, HAP or prolonged MV microbiome signatures had longer times to liberation from mechanical ventilation than patients with no signature (Hazard Ratio 1.584 (95CI% 1.11-2.259), 1.831 (95%CI 1.519-2.398) and 1.468 (95%CI 1.082-1.692), respectively) (**Figures 29, 30 and 31**). The mortality rates at day 90 were not significantly different between patients with or without ARDS signatures (25% (20/79 patients) vs. 22% (10/45), p=0.76), with HAP vs. without HAP signatures (27% (20/72 patients) vs. 19% (10/52), p=0.38), or with prolonged vs. short MV signatures (23% (23/102 patients) vs. 18% (4/22), p=0.78).

Example 2

Materials and Methods

[0410] As shown on the diagram depicting the experimental procedure (**Figure 32**), C57BL/6JRj mice were intra-tracheally instilled with either PBS (negative control), *Streptococcus mitis* (*S. mitis*) (ATCC 49456) or *Escherichia coli* (*E. coli*) K-12.

[0411] One day after the first instillation, half of the mice were euthanized for histological staining of lung sections; counting of pulmonary neutrophils; and weight measurement.

[0412] On day 3, the remaining mice were instilled again with either PBS (negative control), or *S. mitis*.

[0413] On day 7, mice were euthanized for histological staining of lung sections; counting of pulmonary neutrophils, monocyte-derived inflammatory macrophages (I-MAC), resident alveolar macrophages (R-MAC), interstitial macrophages (IM), and resident T cells; cytokine measure; and evaluation of neutrophil, I-MAC and R-MAC phagocytosis function.

Results

[0414] As shown on **Figures 33A-D**, a single (**Figure 33C**) or repeated (**Figure 33D**) *S. mitis* intra-tracheal instillations did not induce disorganization of the lung parenchyma, as compared to single (**Figure 33A**) or repeated (**Figure 33B**) intra-tracheal instillations with PBS. As shown on the histogram of **Figure 34A**, mice instilled with *S.mitis* exhibited the same weight as mice instilled with PBS, as opposed to mice instilled with *E. coli*, which shows that *S. mitis* administration is well tolerated.

[0415] A significant influx of neutrophils was observed into the lungs 24 hours post-instillation with *S. mitis*, but of lesser magnitude than in the case of an *E. coli* pneumonia (**Figure 34B**). At day 7, lung neutrophils returned to baseline, as shown by the similar neutrophil count in all conditions (**Figure 34C**). In addition, the phagocytic function of neutrophils at day 7 was similar between PBS-instilled mice and *S. mitis*-instilled mice (**Figure 34D**), which demonstrates that neutrophil phagocytic function is not impaired in *S. mitis*-instilled mice.

[0416] *S. mitis* induces immunological changes in the respiratory tract of colonized mice at day 7. As shown on **Figures 35A** and **35B**, mice instilled with *S. mitis* exhibited a significant increase of the number of monocyte-derived inflammatory macrophages (I-MAC), as compared to mice instilled with PBS. In addition, those I-MAC possessed a significant phagocytic capacity, as compared to I-MAC from PBS-instilled mice (**Figure 35C**). Furthermore, *S. mitis* induced phenotypic adaptations in resident alveolar macrophages (R-MAC), such as a higher CD11b expression (**Figure 35B**) and a defective phagocytosis, as compared to PBS-instilled mice (**Figure 35D**).

[0417] NK cells, T cells, and resident T cells counts were not significantly modified in *S. mitis*-instilled mice (**Figures 36A** and **36B**). However, *S. mitis*-instilled mice showed

a significant increase of the percentage of IL-17 expression in CD4+ T cells (**Figure 36C**), as well as a significant increase of the percentage of IL-17 expression in the subgroup of CD4+ resident T cells (**Figure 36D**), as compared to PBS-instilled mice.

Example 3

Materials and Methods

[0418] As shown on the diagram depicting the experimental procedure (**Figure 37**), C57BL/6JRj mice were intra-tracheally instilled with either PBS (negative control), or *S. mitis* (ATCC 49456).

[0419] On day 3 after the first instillation, mice were instilled again with either PBS, or *S. mitis*.

[0420] On day 7, both PBS and *S. mitis* treated mice were intra-tracheally instilled with *E. coli* K-12.

[0421] Mice were weighted on day 7, 8, 9 and 10. On day 8, some of the mice were euthanized and pulmonary *E. coli* loads were measured; lung neutrophils, monocyte-derived inflammatory macrophages (I-MAC), NK cells, CD4 T cells, γδ T cells, resident CD4 T cells and resident γδ T cells were counted; and cytokine production was measured.

Results

[0422] Pulmonary *E. coli* loads at 24 hours post-infection, were not significantly lower in *S. mitis* colonized mice than in the uncolonized group (**Figure 38**). Mice weight recovery after the *E. coli* pneumonia was significantly improved in the *S. mitis* colonized mice, as shown by the significant increase in weight at day 2 and 3 post *E. coli* pneumonia (**Figure 39**). At 24 hours post *E. coli* pneumonia, the numbers of lung neutrophils (**Figure 40A**), monocyte-derived inflammatory macrophages (I-MAC) (**Figure 40B**), NK cells (**Figure 40C**), CD4 T cells (**Figure 40D**), γδ T cells (**Figure 40E**), resident CD4 T cells (**Figure 40F**) and resident γδ T cells (**Figure 40G**) were significantly higher in the *S. mitis* colonized mice, as compared to PBS-instilled mice (sham), and PBS-instilled mice infected with *E. coli* (E. coli). A trend towards reduced TNF-α (**Figure 41A**) and IL-1β

(**Figure 41B**) cytokine expression was observed for resident alveolar macrophages in response to *E. coli* pneumonia after *S. mitis* colonization, as compared to PBS-instilled mice infected with *E. coli.* On the other hand, an increase in the percentage of IL-17 expression was observed in CD4 T cells (**Figure 41C**) and CD4 resident T cells (**Figure 41D**) from *S. mitis* instilled mice infected with *E. coli*, as compared to PBS-instilled mice infected with *E. coli*.

CLAIMS

1. A method for determining a risk of developing a respiratory complication in a subject or for identifying a subject at risk of developing a respiratory complication, said method comprising a step of detecting the presence or abundance of a least one bacterium of a genus selected from the group comprising *Staphylococcus, Streptococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella, Prosthecobacter, Atopobium, Oribacterium,* a genus of the *Lactobacillales* order, and *Rothia* in a respiratory sample of the patient.

2. The method according to claim **1**, wherein the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus *Staphylococcus* in the respiratory sample, **and** of detecting the presence or abundance of at least one bacterium of a genus *Ralstonia* or *Enteroccus* in the respiratory sample.

3. The method according to claim **1**, wherein the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus *Staphylococcus* in the respiratory sample, **and** of detecting the presence or abundance of at least one bacterium of a genus *Ralstonia* in the respiratory sample, **and** of detecting the presence or abundance of at least one bacterium of a genus *Enteroccus* in the respiratory sample.

4. The method according to claim **1**, wherein the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus *Streptococcus* in the respiratory sample, **and** of detecting the presence or abundance of at least one bacterium of a genus *Atopobium*, *Oribacterium* and *Veillonella* in the respiratory sample.

5. The method according to claim **1**, wherein the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus *Streptococcus* in the respiratory sample, **and** of detecting the presence or abundance of at least one bacterium of a genus *Veillonella* in the respiratory sample.

6. The method according to claim **1**, or claim **2**, or claim **3**, or claim **4**, or claim **5**, wherein the subject is critically ill patient.

7. The method according to claim **1** or claim **2**, or claim **3**, or claim **4**, or claim **5**, or claim **6**, wherein the respiratory sample is selected from the group comprising, or consisting of, an endotracheal aspirate (ETA), a bronchoalveolar lavage (BAL), and an oropharyngeal swab (OS).

8. The method according to any one of claims **1** to **7**, wherein the presence or abundance of the at least one bacterium is assessed through the detection of sequences specific of said at least one bacterium, preferably by PCR or RT-PCR.

9. The method according to any one of claims **1** to **8**, wherein the respiratory complication is selected from the group comprising, or consisting of, hospital acquired pneumonia (HAP), acute respiratory distress syndrome (ARDS), ARDS related death and prolonged mechanical ventilation (MV).

10. The method according to any one of claims **1** to **9**, wherein the respiratory complication is HAP, and wherein the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus selected from *Streptococcus, Atopobium, Oribacterium* and *Veillonella* in a respiratory sample of the patient, preferably an endotracheal aspirate of the patient.

11. The method according to any one of claims **1** to **10**, wherein the respiratory complication is ARDS or ARDS related death, and wherein the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus selected from *Staphylococcus, Ralstonia, Enterococcus, Flavobacterium, Veillonella* and *Prosthecobacter* in a respiratory sample of the patient.

12. The method according to claim **11**, wherein the respiratory complication is ARDS or ARDS related death, and wherein the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus selected from *Staphylococcus, Ralstonia* and *Enterococcus* in an endotracheal aspirate of the patient.

13. The method according to claim **12**, wherein the respiratory complication is ARDS or ARDS related death, and wherein the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus selected from *Flavobacterium, Veillonella* and *Prosthecobacter* in a bronchoalveolar lavage of the patient.

14. The method according to any one of claims **1** to **13**, wherein the respiratory complication is prolonged mechanical ventilation, and wherein the method comprises a step of detecting the presence or abundance of at least one bacterium of a genus selected from a genus of the *Lactobacillales* order, *Rothia* and *Streptococcus* in a respiratory sample of the patient, preferably in an endotracheal aspirate or in a bronchoalveolar lavage of the patient, more preferably in an endotracheal aspirate of the patient.

15. A method of providing an adapted care to a subject at risk of having or developing a respiratory complication, the method comprising the steps of:

- identifying a subject with a risk of having or developing a respiratory complication in a subject by the method according to any one of claims **1** to **14**, and
- providing an adapted care to a subject identified with a risk of having or developing a respiratory complication.

16. A composition comprising at least one bacterium of the *Flavobacterium* genus, or of the *Veillonella* genus, or of the *Prosthecobacter* genus, or of the *Streptococcus* genus, or of the *Atopobium* genus, or of the *Oribacterium* genus, or of one of the genera of the *Lactobacillales* order, or of the *Rothia* genus or metabolites thereof, for use in the prevention or treatment of a respiratory complications in a subject.

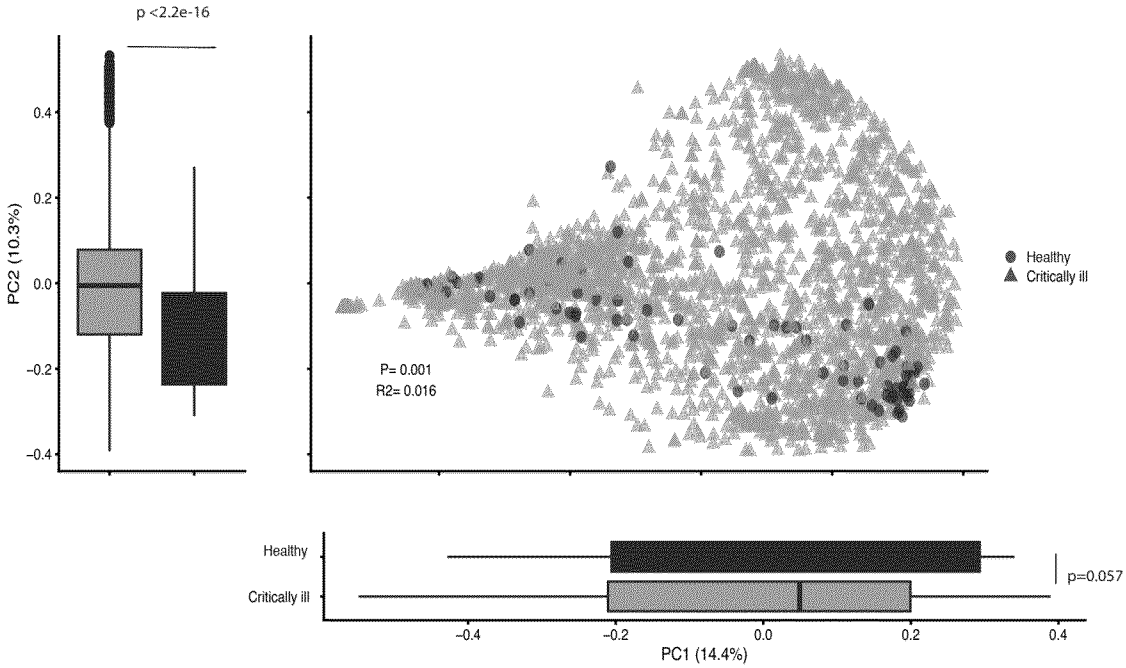

p <2.2e-16
PC2 (10.3%)
0.4
0.2
0.0
−0.2
−0.4
P= 0.001
R2= 0.016
Healthy
Critically ill
Healthy
Critically ill
p=0.057
−0.4
−0.2
0.0
0.2
0.4
PC1 (14.4%)

FIG. 1

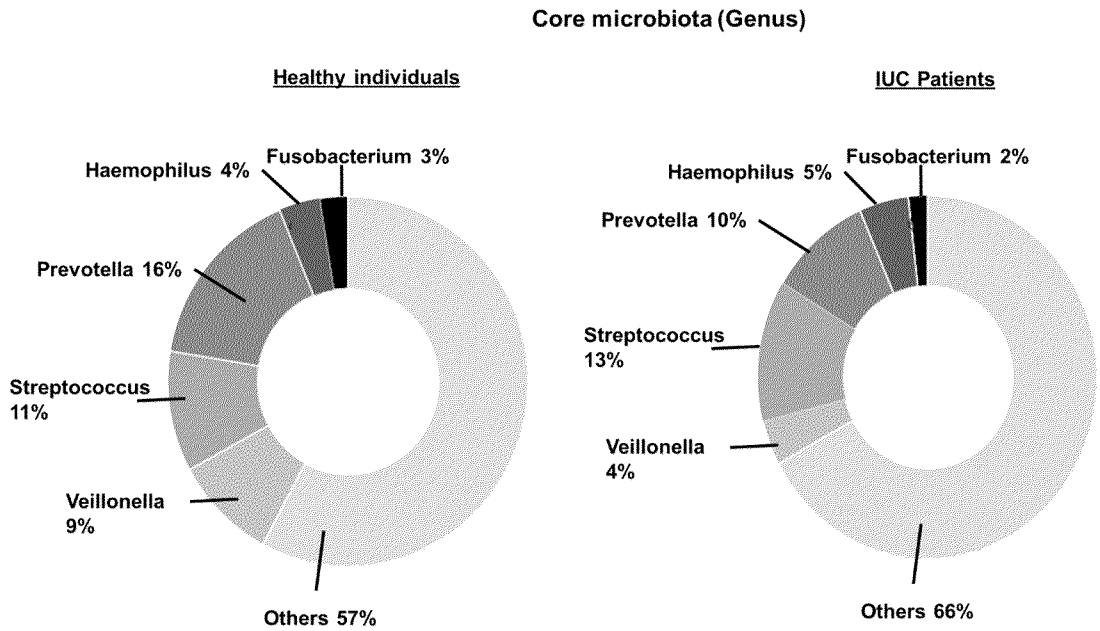

Core microbiota (Genus)
Healthy individuals
Haemophilus 4%
Fusobacterium 3%
Prevotella 16%
Streptococcus 11%
Veillonella 9%
Others 57%
IUC Patients
Haemophilus 5%
Fusobacterium 2%
Prevotella 10%
Streptococcus 13%
Veillonella 4%
Others 66%

FIG. 2

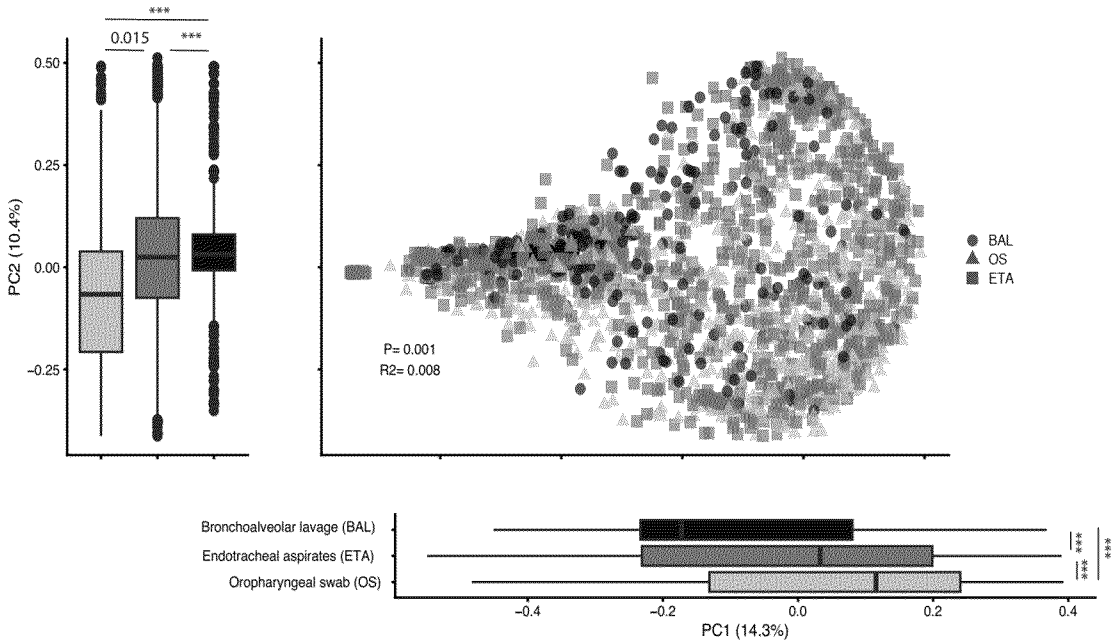

***
0.015
***
0.50
0.25
0.00
−0.25
PC2 (10.4%)
P= 0.001
R2= 0.008
BAL
OS
ETA
Bronchoalveolar lavage (BAL)
Endotracheal aspirates (ETA)
Oropharyngeal swab (OS)
***
***
***
−0.4
−0.2
0.0
0.2
0.4
PC1 (14.3%)

FIG. 3

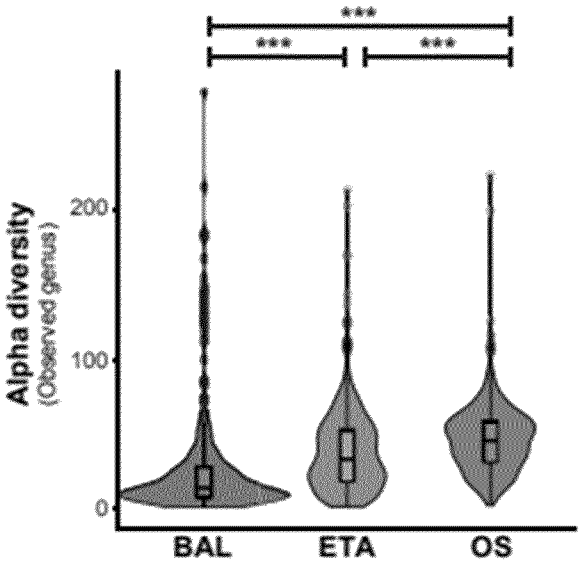

***
***
***
Alpha diversity
(Observed genus)
200
100
0
BAL
ETA
OS

FIG. 4

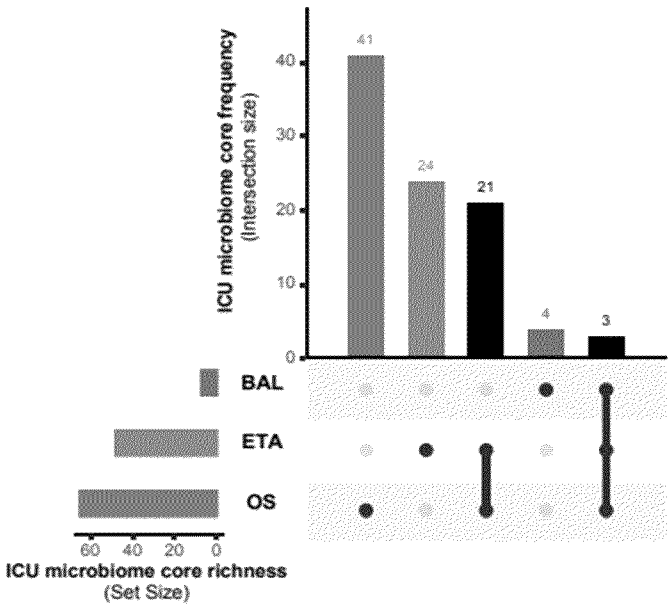

ICU microbiome core frequency
(Intersection size)
41
24
21
4
3
40
30
20
10
0
BAL
ETA
OS
60 40 20 0
ICU microbiome core richness
(Set Size)

FIG. 5

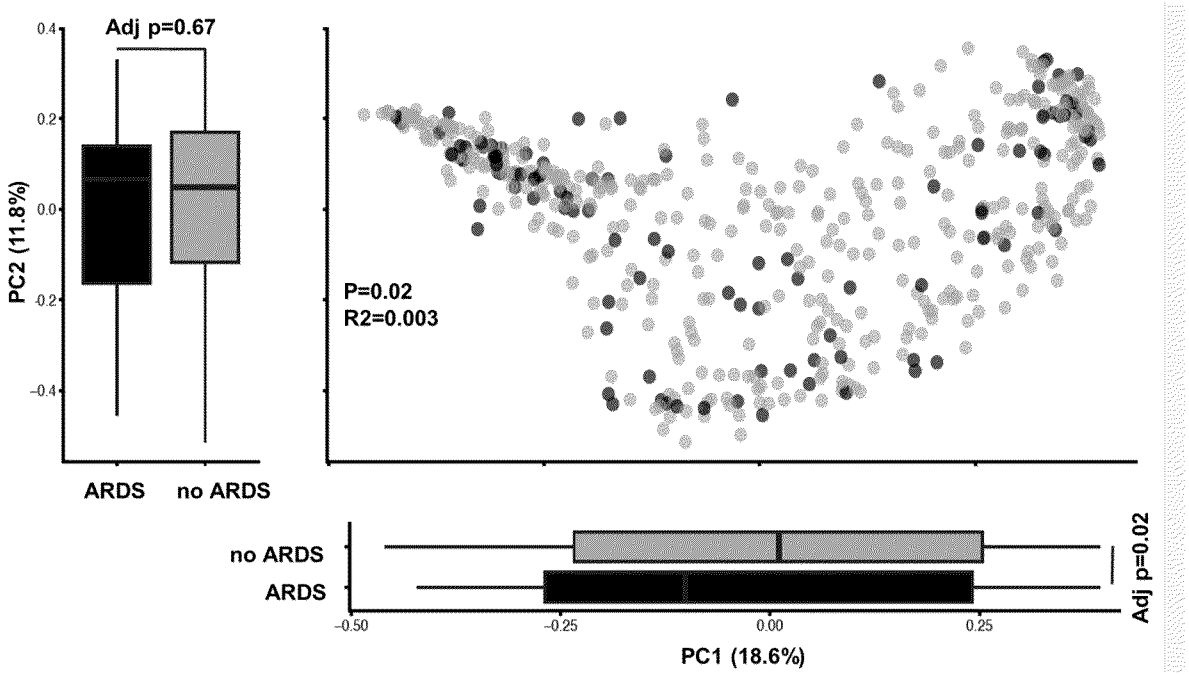

Adj p=0.67
PC2 (11.8%)
0.4
0.2
0.0
-0.2
-0.4
ARDS
no ARDS
P=0.02
R2=0.003
no ARDS
ARDS
-0.50
-0.25
0.00
0.25
PC1 (18.6%)
Adj p=0.02

FIG. 6

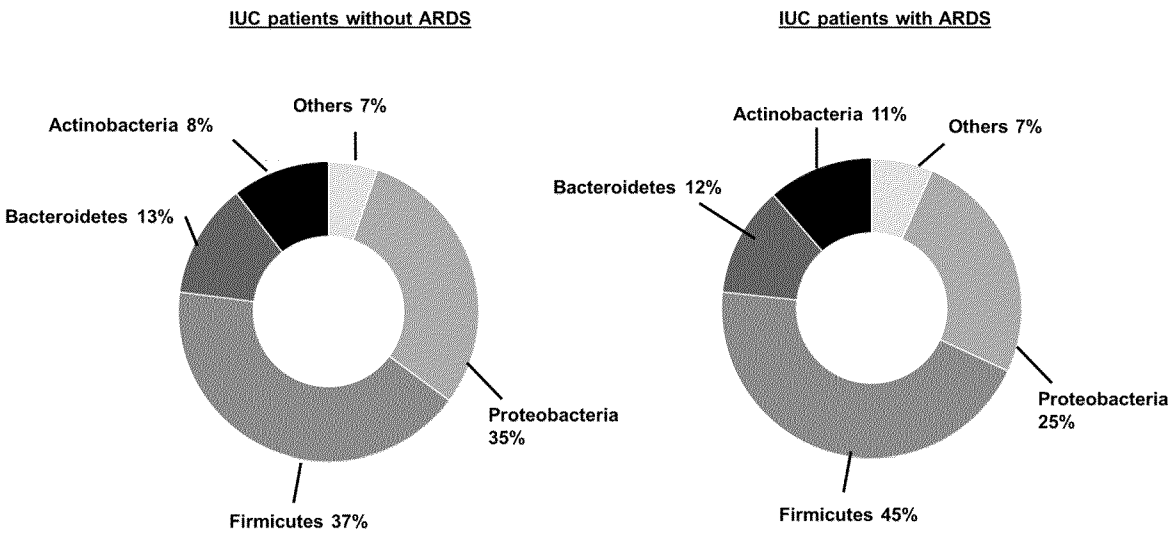

IUC patients without ARDS
Others 7%
Actinobacteria 8%
Bacteroidetes 13%
Proteobacteria 35%
Firmicutes 37%
IUC patients with ARDS
Actinobacteria 11%
Others 7%
Bacteroidetes 12%
Proteobacteria 25%
Firmicutes 45%

FIG. 7

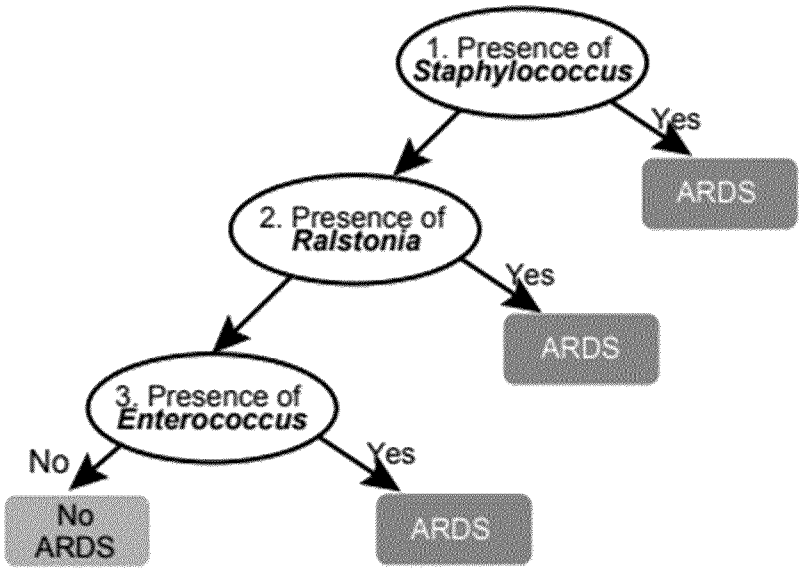

1. Presence of Staphylococcus
Yes
ARDS
2. Presence of Ralstonia
Yes
ARDS
3. Presence of Enterococcus
No
No ARDS
Yes
ARDS

FIG. 8

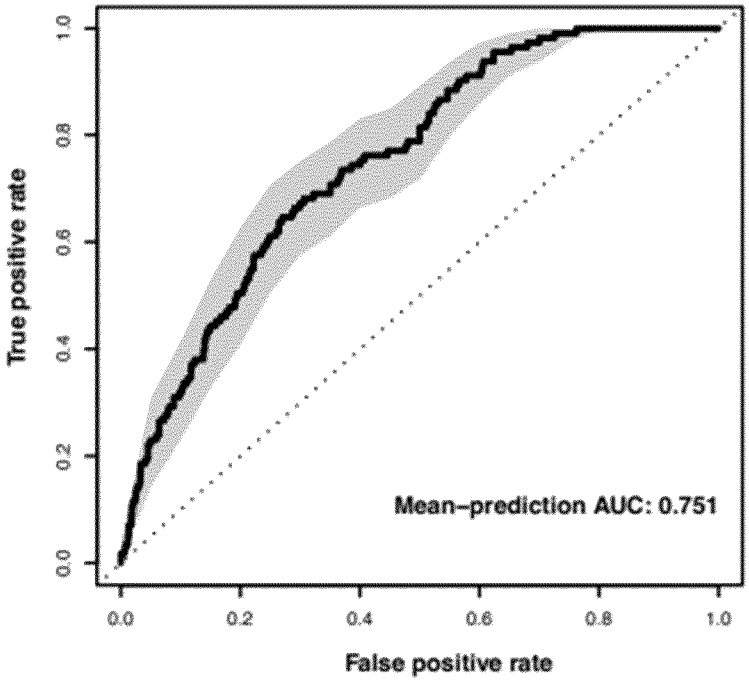

True positive rate
False positive rate
Mean–prediction AUC: 0.751
0.0
0.2
0.4
0.6
0.8
1.0

FIG. 9

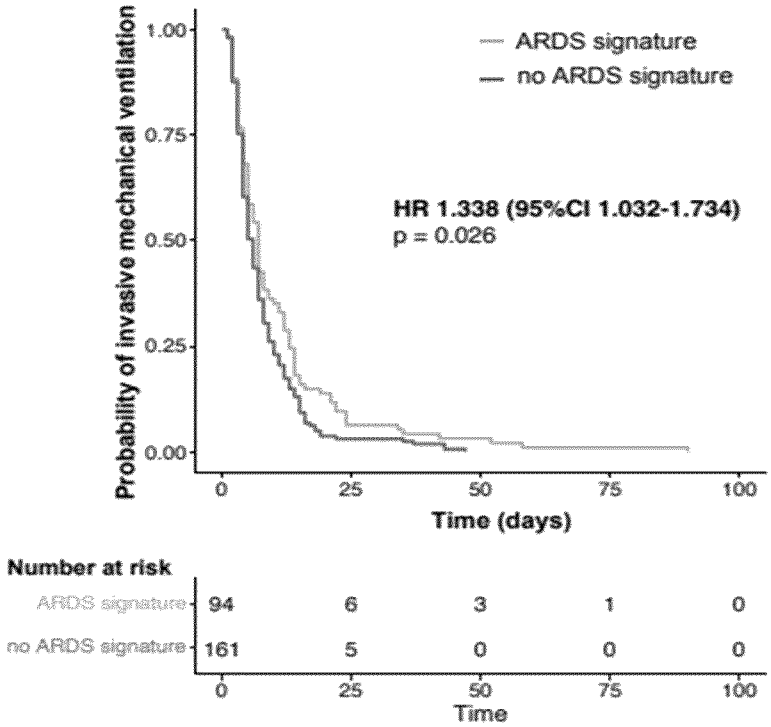

Probability of invasive mechanical ventilation
ARDS signature
no ARDS signature
HR 1.338 (95%CI 1.032-1.734)
p = 0.026
Time (days)
Number at risk
ARDS signature 94 6 3 1 0
no ARDS signature 161 5 0 0 0
Time

FIG. 10

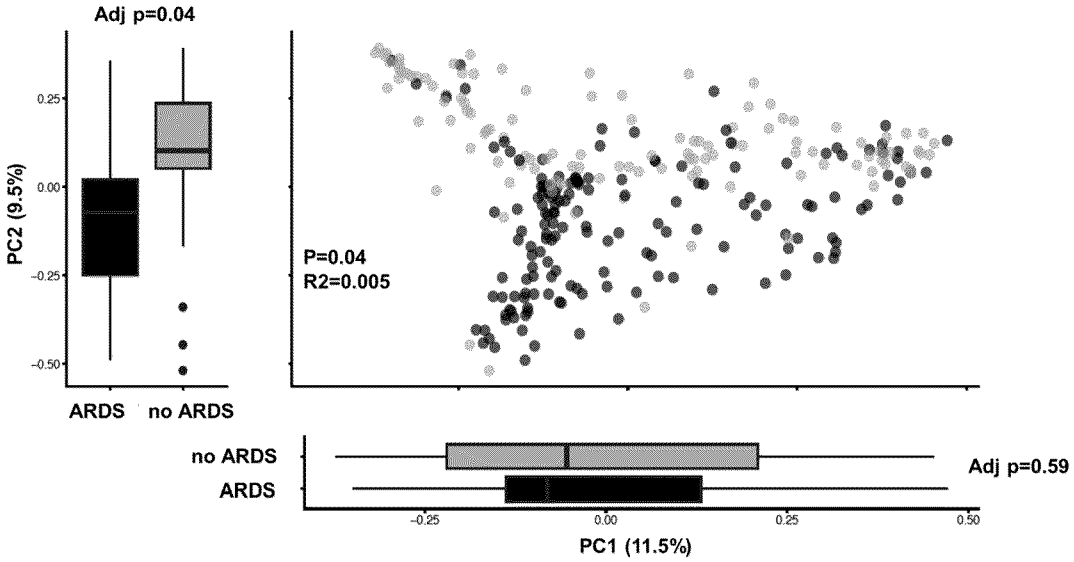

Adj p=0.04
PC2 (9.5%)
0.25
0.00
−0.25
−0.50
ARDS
no ARDS
P=0.04
R2=0.005
no ARDS
ARDS
Adj p=0.59
−0.25
0.00
0.25
0.50
PC1 (11.5%)

FIG. 11

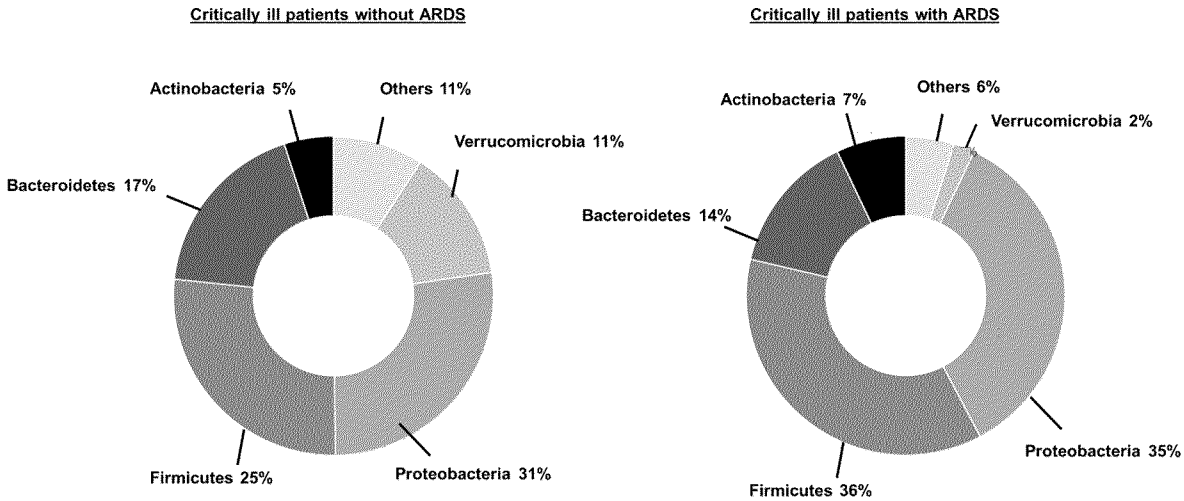

Critically ill patients without ARDS
Actinobacteria 5%
Others 11%
Verrucomicrobia 11%
Bacteroidetes 17%
Firmicutes 25%
Proteobacteria 31%
Critically ill patients with ARDS
Actinobacteria 7%
Others 6%
Verrucomicrobia 2%
Bacteroidetes 14%
Proteobacteria 35%
Firmicutes 36%

FIG. 12

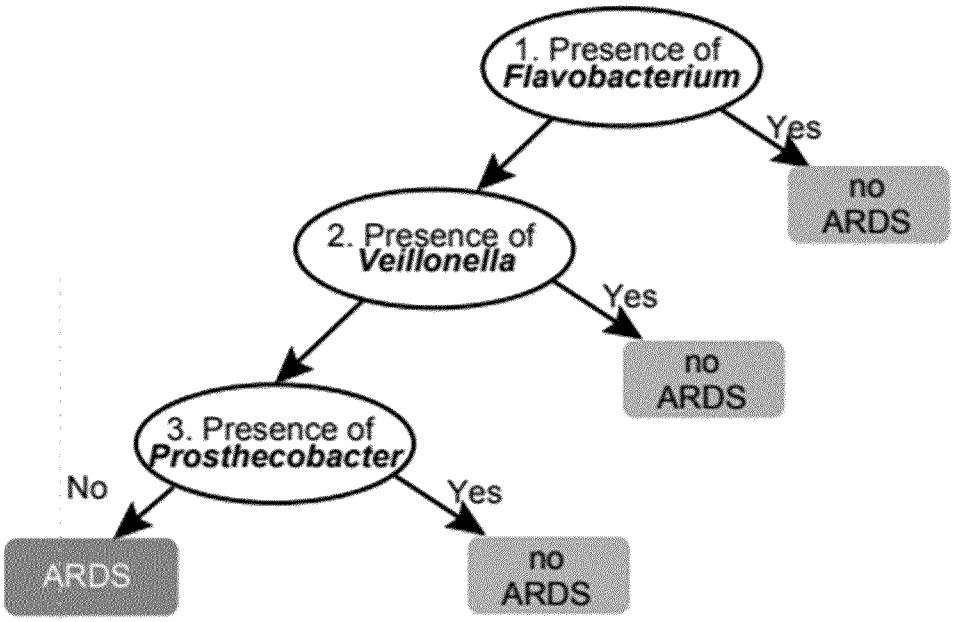

1. Presence of
Flavobacterium
Yes
no
ARDS
2. Presence of
Veillonella
Yes
no
ARDS
3. Presence of
Prosthecobacter
No
Yes
ARDS
no
ARDS

FIG. 13

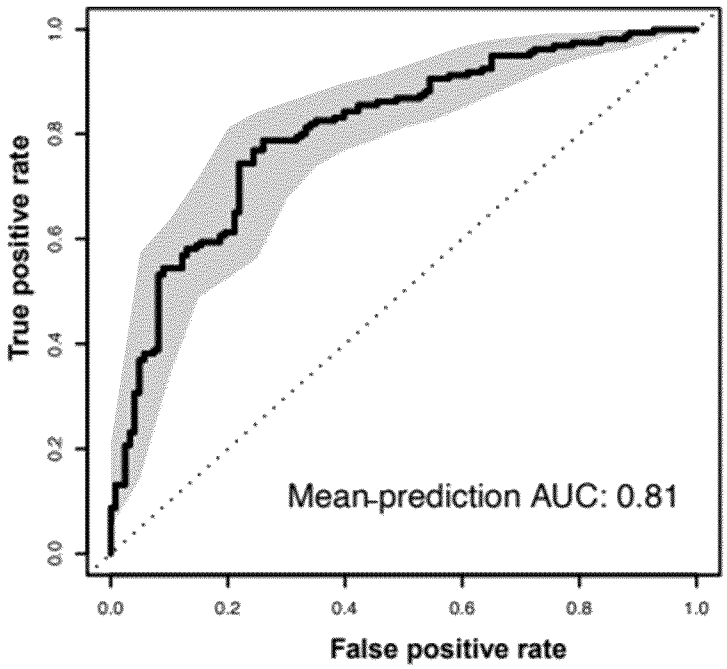

True positive rate
False positive rate
Mean-prediction AUC: 0.81
0.0
0.2
0.4
0.6
0.8
1.0

FIG. 14

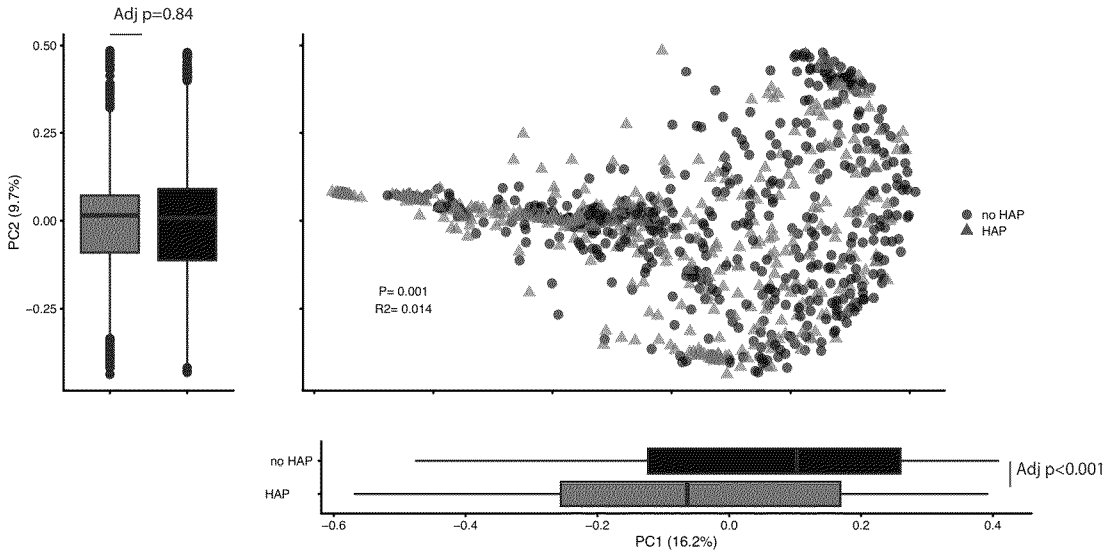

Adj p=0.84
0.50
0.25
0.00
−0.25
PC2 (9.7%)
P= 0.001
R2= 0.014
no HAP
HAP
no HAP
HAP
Adj p<0.001
−0.6
−0.4
−0.2
0.0
0.2
0.4
PC1 (16.2%)

FIG. 15

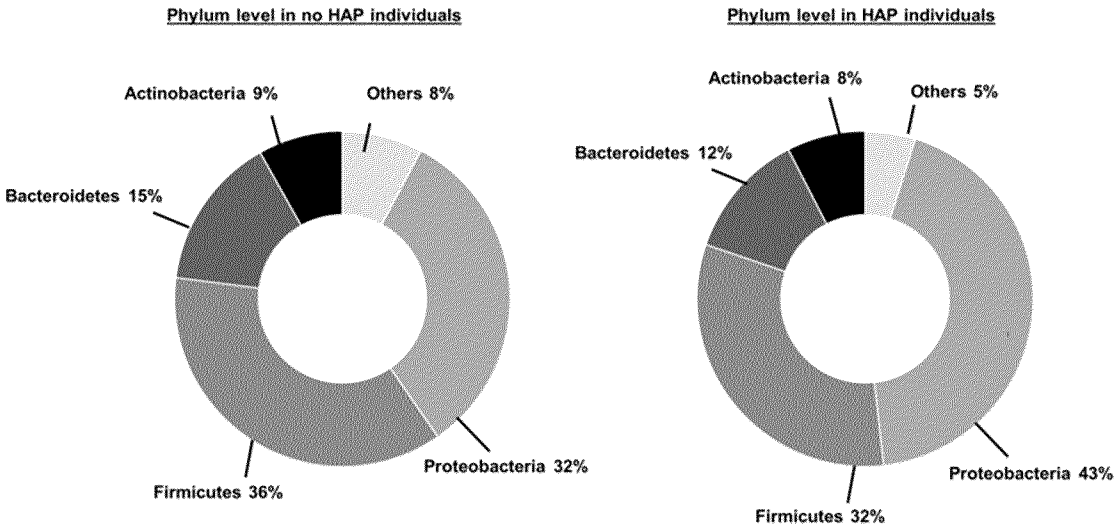

Phylum level in no HAP individuals
Actinobacteria 9%
Others 8%
Bacteroidetes 15%
Proteobacteria 32%
Firmicutes 36%
Phylum level in HAP individuals
Actinobacteria 8%
Others 5%
Bacteroidetes 12%
Proteobacteria 43%
Firmicutes 32%

FIG. 16

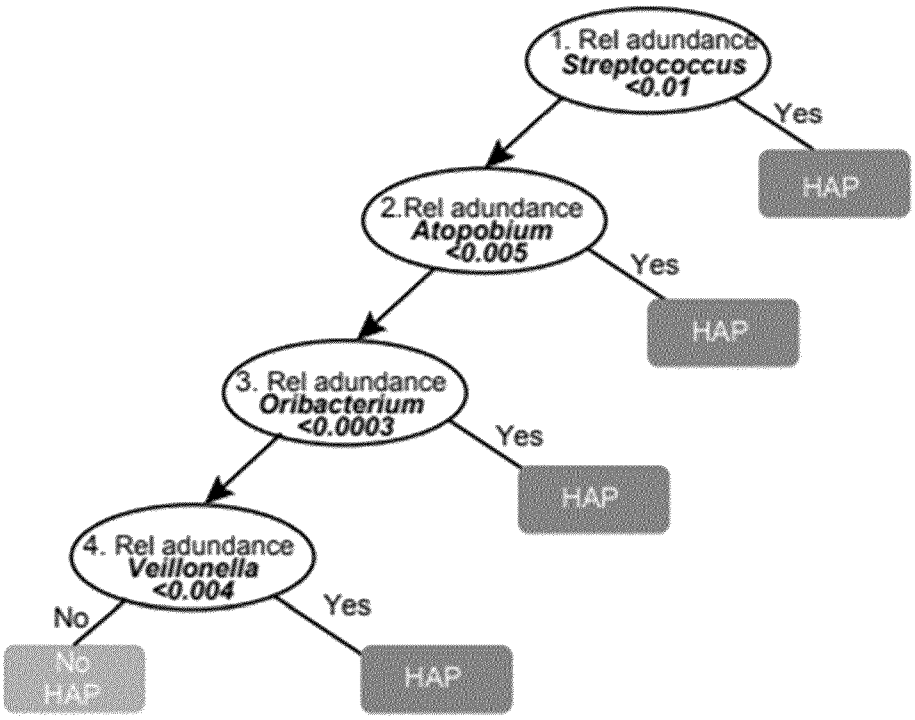

1. Rel adundance
Streptococcus
<0.01
Yes
HAP
2.Rel adundance
Atopobium
<0.005
Yes
HAP
3. Rel adundance
Oribacterium
<0.0003
Yes
HAP
4. Rel adundance
Veillonella
<0.004
No
Yes
No
HAP
HAP

FIG. 17

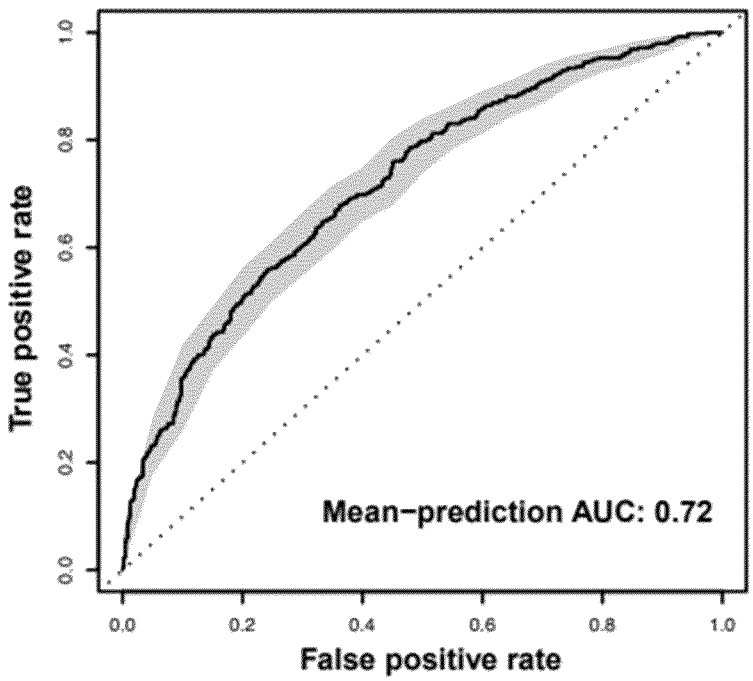

True positive rate
False positive rate
Mean−prediction AUC: 0.72
0.0
0.2
0.4
0.6
0.8
1.0

FIG. 18

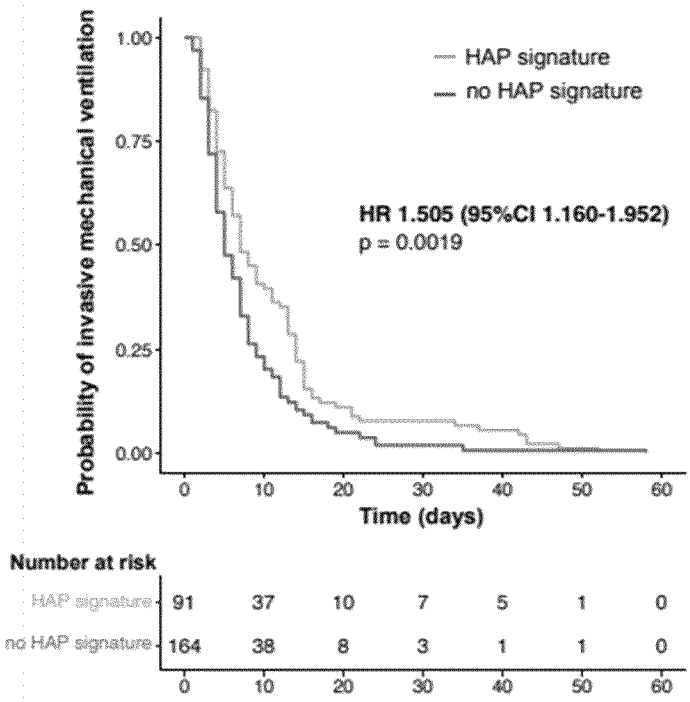

Probability of invasive mechanical ventilation
1.00
0.75
0.50
0.25
0.00
HAP signature
no HAP signature
HR 1.505 (95%CI 1.160-1.952)
p = 0.0019
0
10
20
30
40
50
60
Time (days)
Number at risk
HAP signature 91 37 10 7 5 1 0
no HAP signature 164 38 8 3 1 1 0

FIG. 19

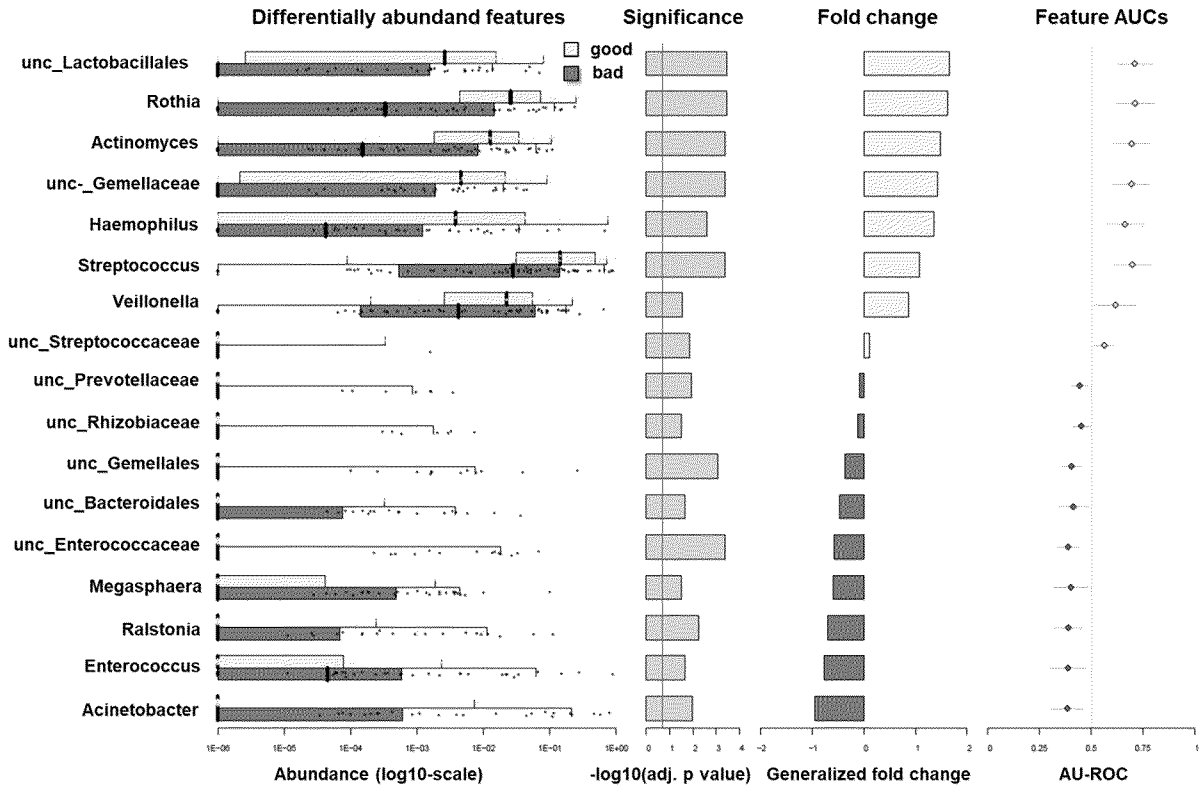

Differentially abundand features
Significance
Fold change
Feature AUCs
good
bad
unc_Lactobacillales
Rothia
Actinomyces
unc-_Gemellaceae
Haemophilus
Streptococcus
Veillonella
unc_Streptococcaceae
unc_Prevotellaceae
unc_Rhizobiaceae
unc_Gemellales
unc_Bacteroidales
unc_Enterococcaceae
Megasphaera
Ralstonia
Enterococcus
Acinetobacter
Abundance (log10-scale)
-log10(adj. p value)
Generalized fold change
AU-ROC

FIG. 20

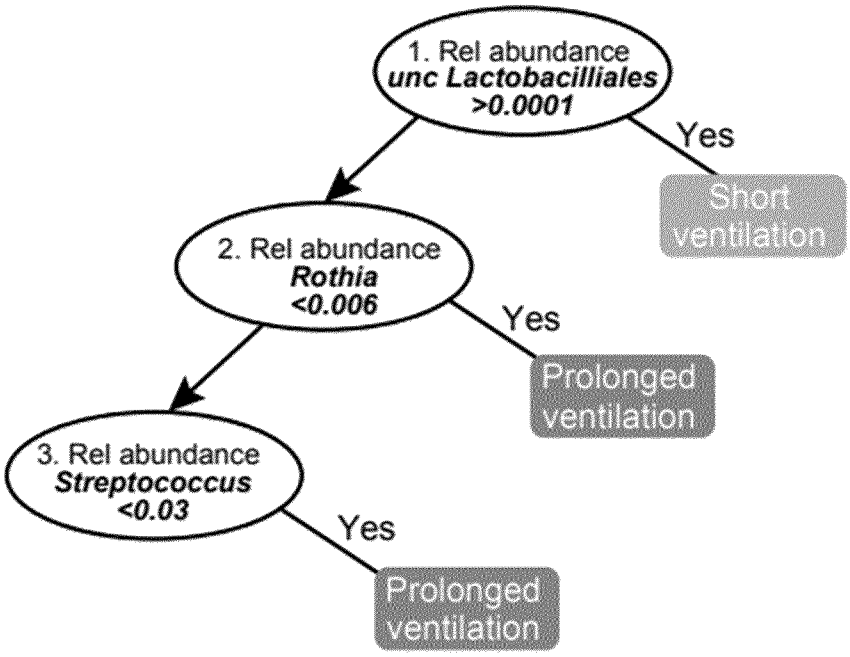

1. Rel abundance
unc Lactobacilliales
>0.0001
Yes
Short ventilation
2. Rel abundance
Rothia
<0.006
Yes
Prolonged ventilation
3. Rel abundance
Streptococcus
<0.03
Yes
Prolonged ventilation

FIG. 21

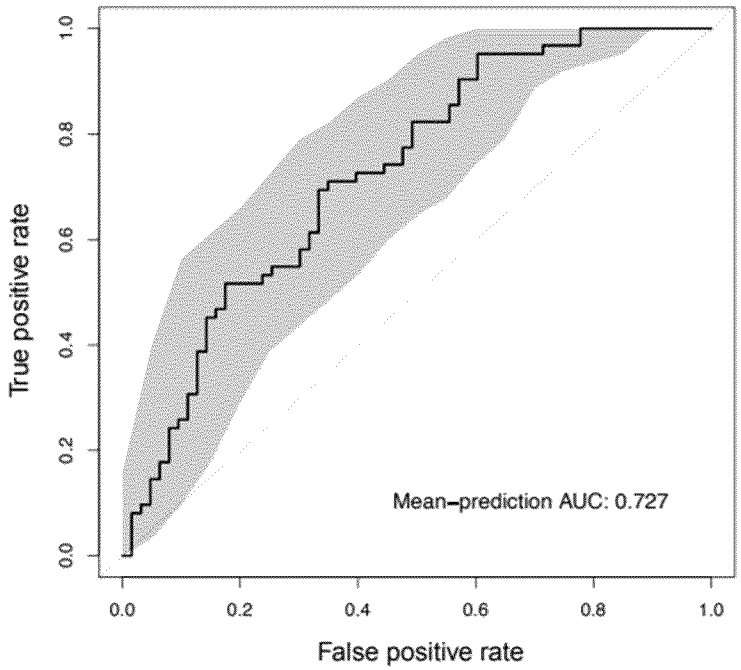

True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0
Mean–prediction AUC: 0.727

FIG. 22

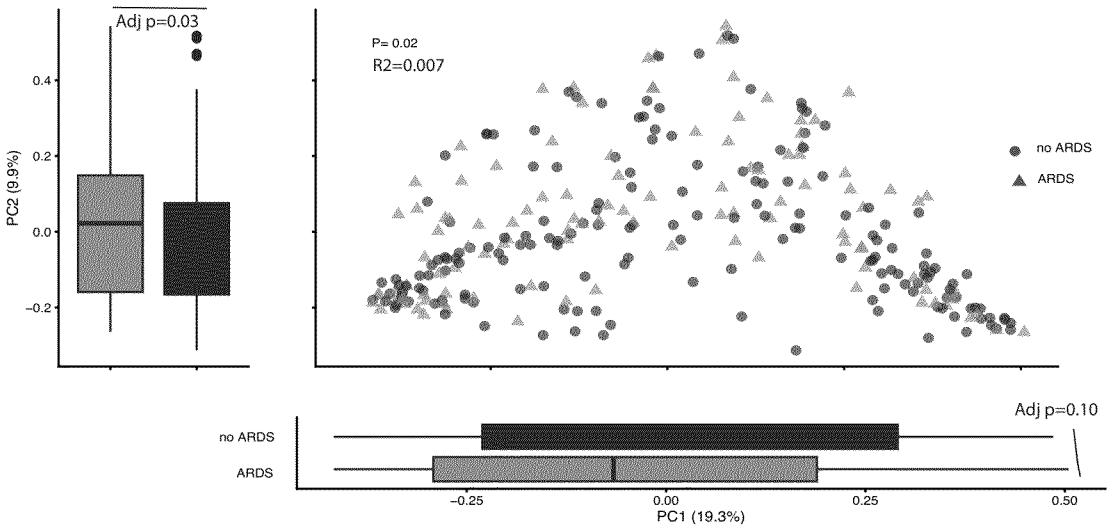

Adj p=0.03
PC2 (9.9%)
0.4
0.2
0.0
−0.2
P= 0.02
R2=0.007
no ARDS
ARDS
Adj p=0.10
no ARDS
ARDS
−0.25
0.00
0.25
0.50
PC1 (19.3%)

FIG. 23

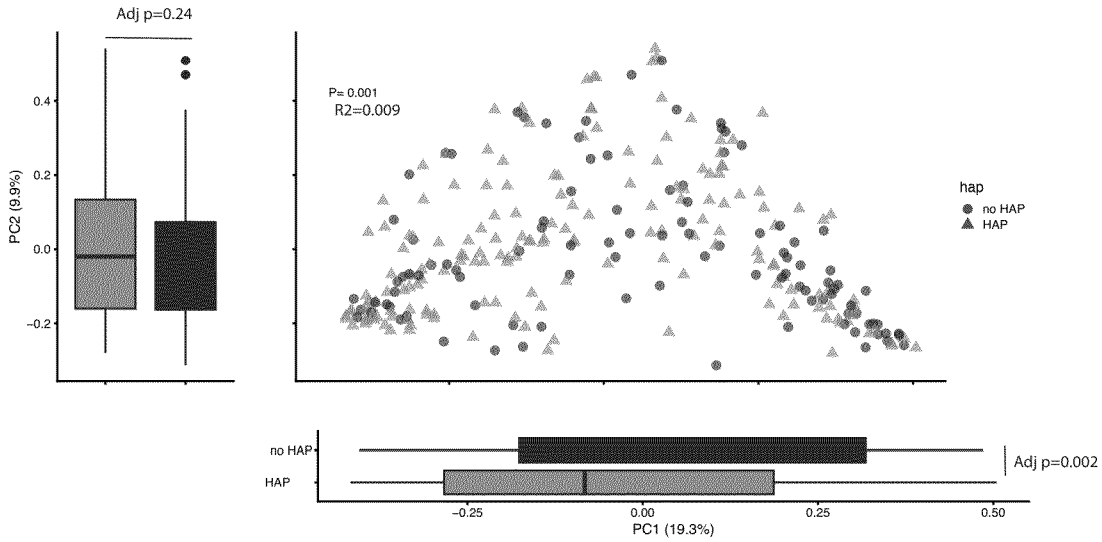

Adj p=0.24
PC2 (9.9%)
0.4
0.2
0.0
−0.2
P= 0.001
R2=0.009
hap
no HAP
HAP
no HAP
HAP
Adj p=0.002
−0.25
0.00
0.25
0.50
PC1 (19.3%)

FIG. 24

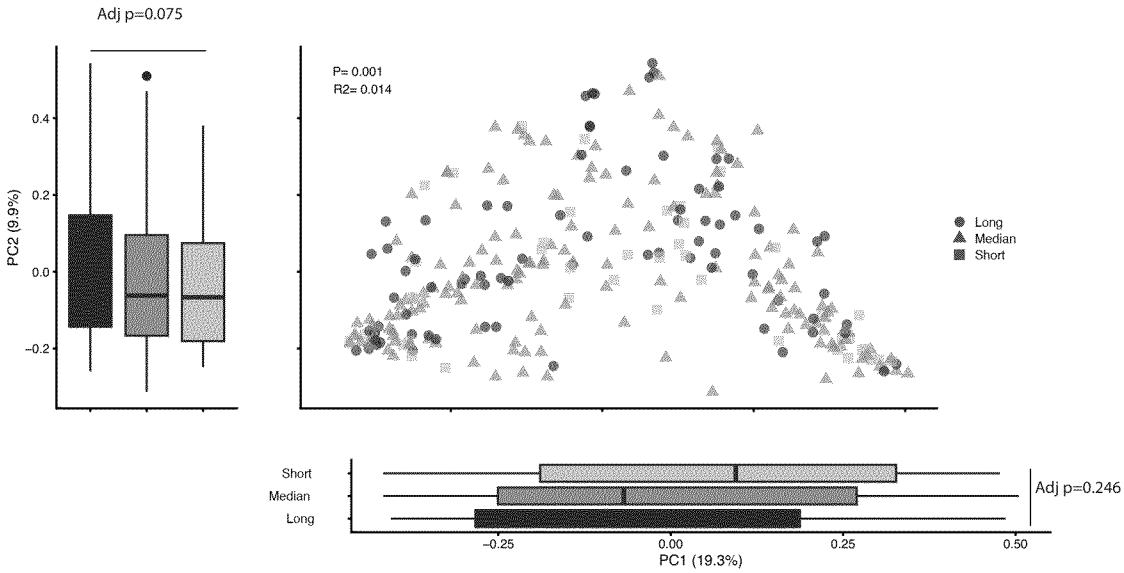

Adj p=0.075
PC2 (9.9%)
0.4
0.2
0.0
−0.2
P= 0.001
R2= 0.014
Long
Median
Short
Short
Median
Long
Adj p=0.246
−0.25
0.00
0.25
0.50
PC1 (19.3%)

FIG. 25

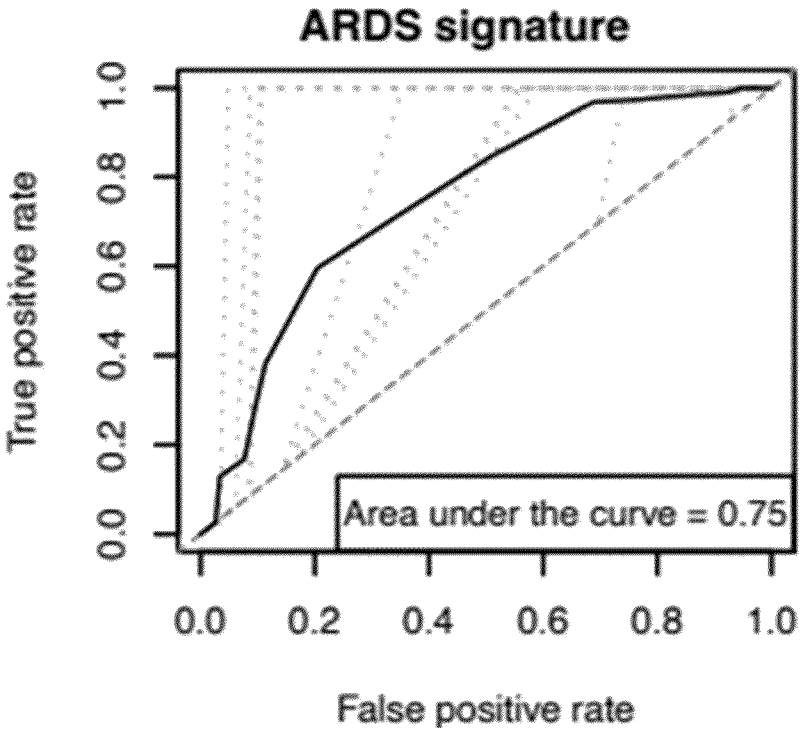

ARDS signature
True positive rate
0.0
0.2
0.4
0.6
0.8
1.0
Area under the curve = 0.75
False positive rate

FIG. 26

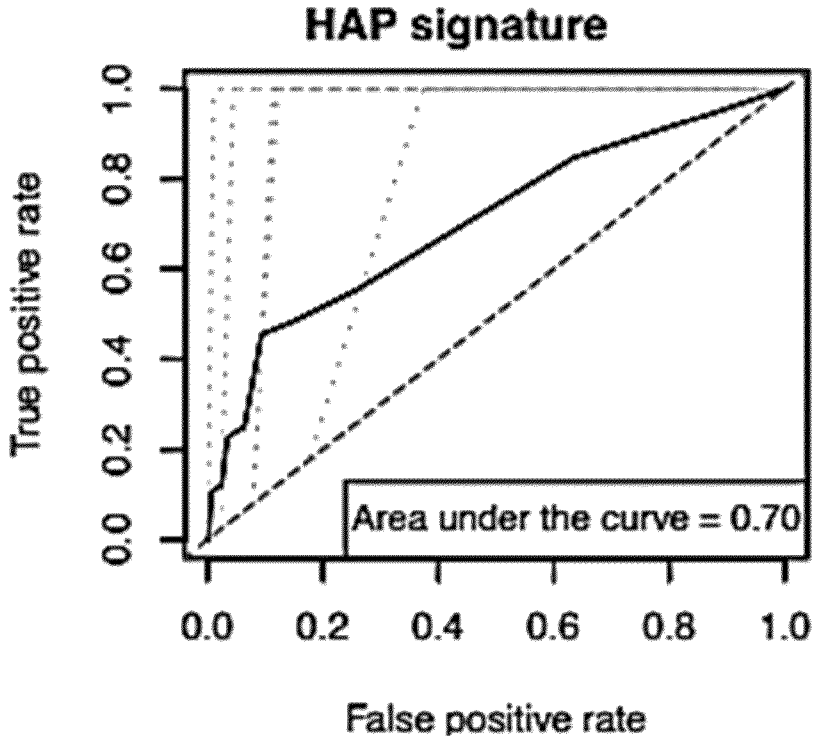

HAP signature
True positive rate
0.0
0.2
0.4
0.6
0.8
1.0
Area under the curve = 0.70
False positive rate

FIG. 27

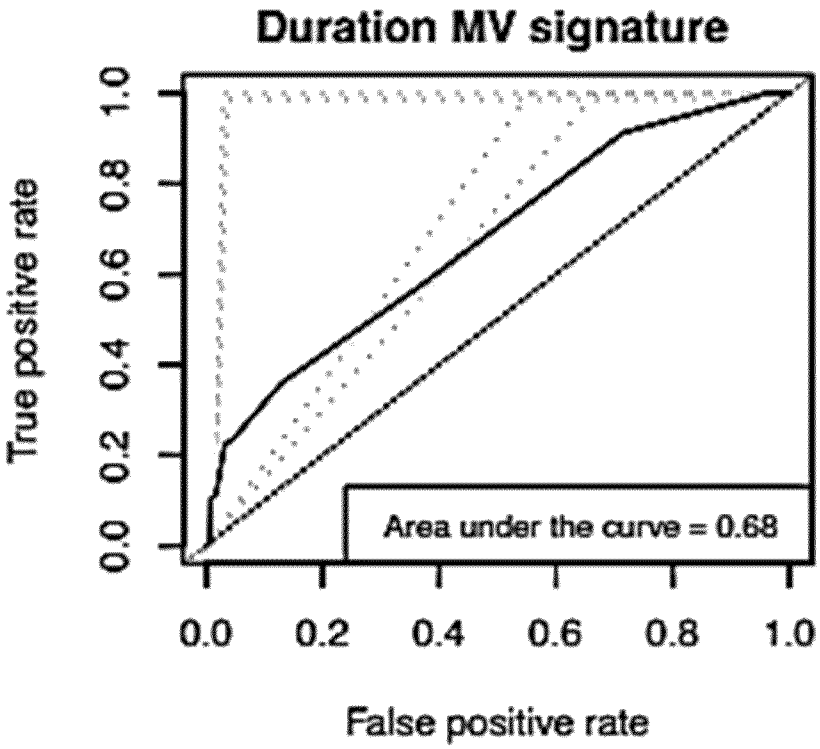

Duration MV signature
True positive rate
0.0
0.2
0.4
0.6
0.8
1.0
Area under the curve = 0.68
False positive rate

FIG. 28

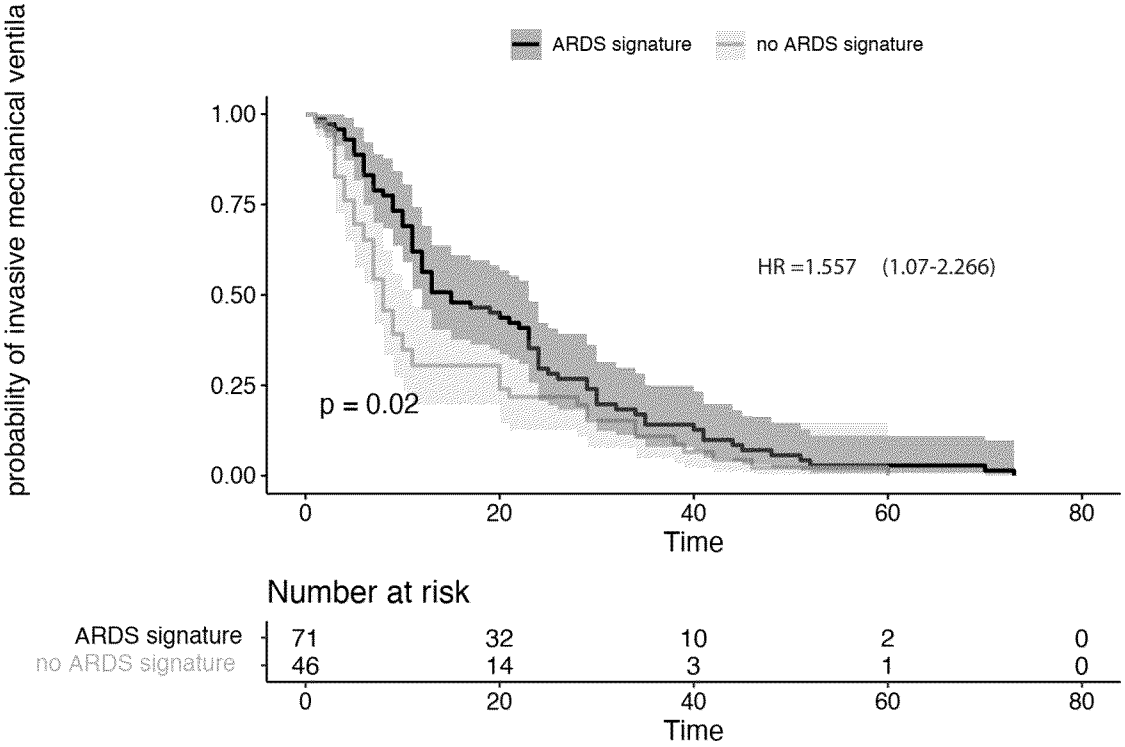

probability of invasive mechanical ventila
ARDS signature
no ARDS signature
1.00
0.75
0.50
0.25
0.00
HR =1.557 (1.07-2.266)
p = 0.02
0
20
40
60
80
Time
Number at risk
ARDS signature 71 32 10 2 0
no ARDS signature 46 14 3 1 0
0
20
40
60
80
Time

FIG. 29

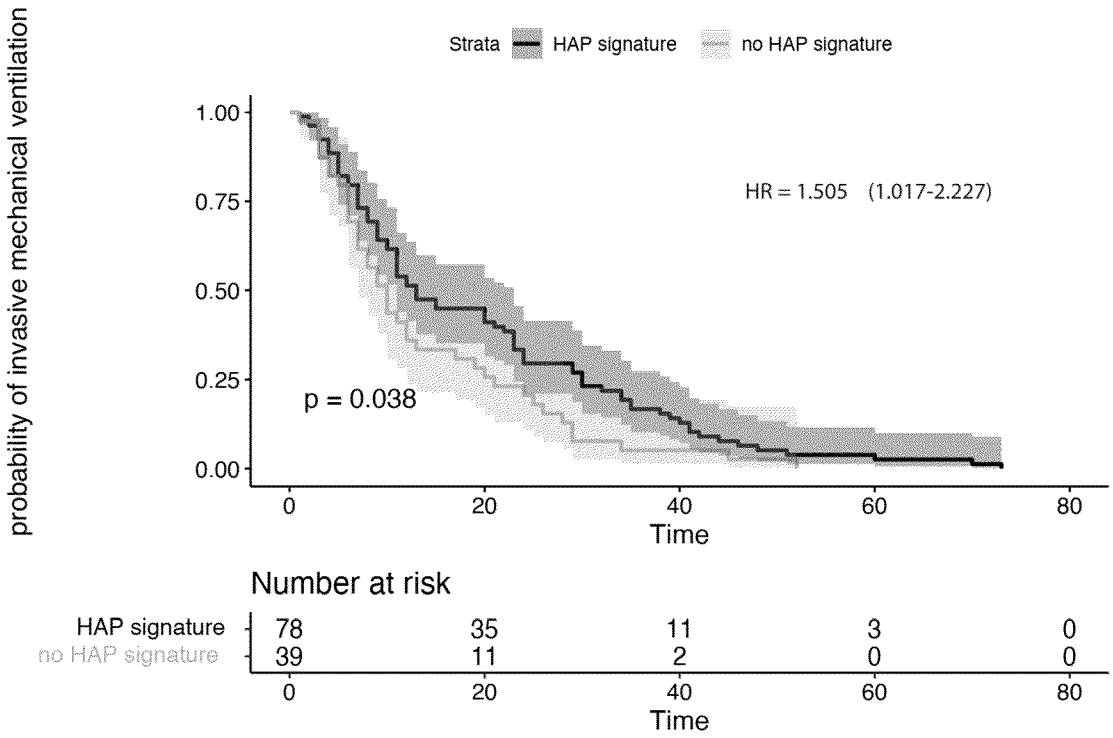

probability of invasive mechanical ventilation
Strata
HAP signature
no HAP signature
1.00
0.75
0.50
0.25
0.00
HR = 1.505 (1.017-2.227)
p = 0.038
0
20
40
60
80
Time
Number at risk
HAP signature 78 35 11 3 0
no HAP signature 39 11 2 0 0
0
20
40
60
80
Time

FIG. 30

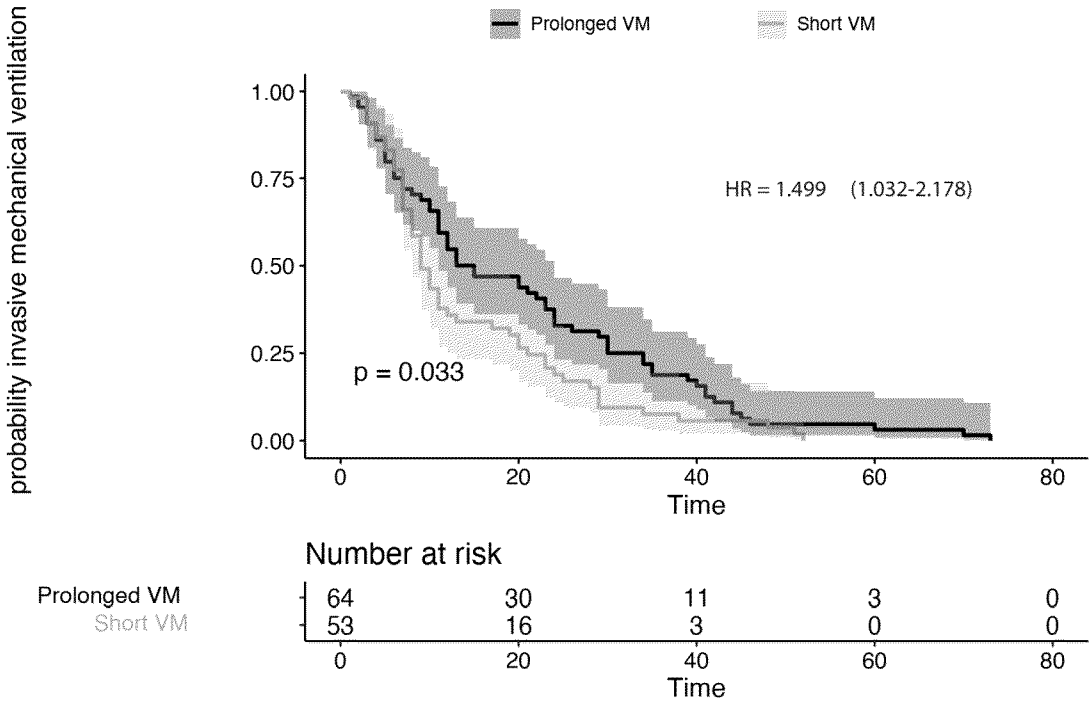

Prolonged VM
Short VM
probability invasive mechanical ventilation
1.00
0.75
0.50
0.25
0.00
HR = 1.499 (1.032-2.178)
p = 0.033
0
20
40
60
80
Time
Number at risk
Prolonged VM
Short VM
64 30 11 3 0
53 16 3 0 0
0
20
40
60
80
Time

FIG. 31

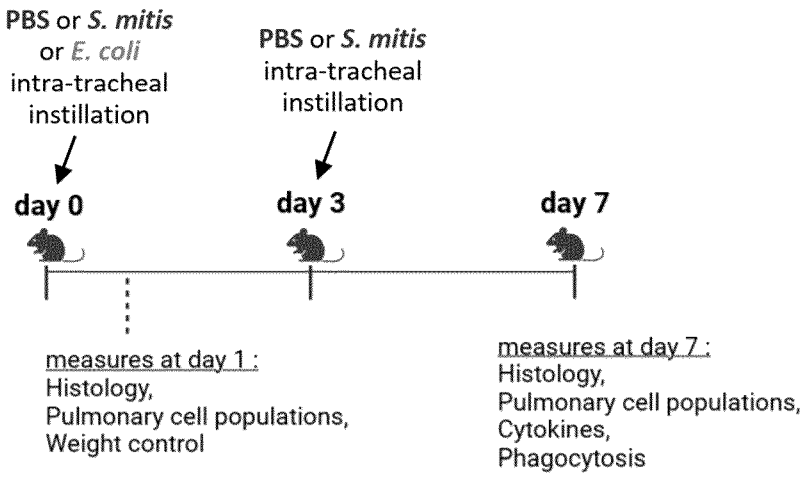


FIG. 32

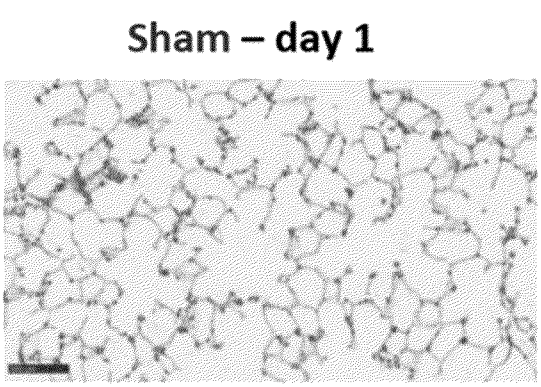


FIG. 33A

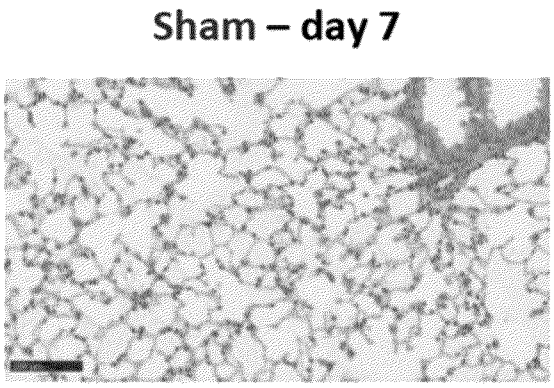


FIG. 33B

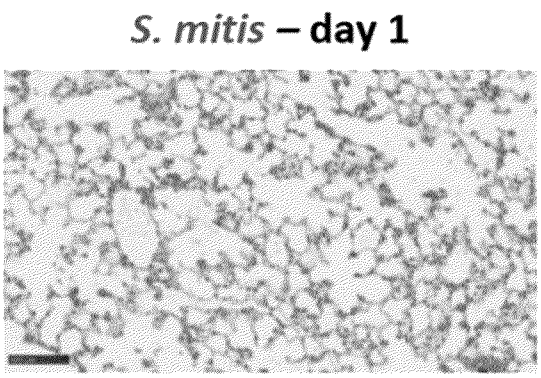


FIG. 33C

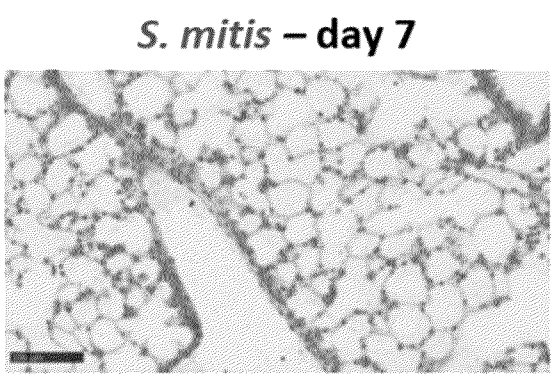


FIG. 33D

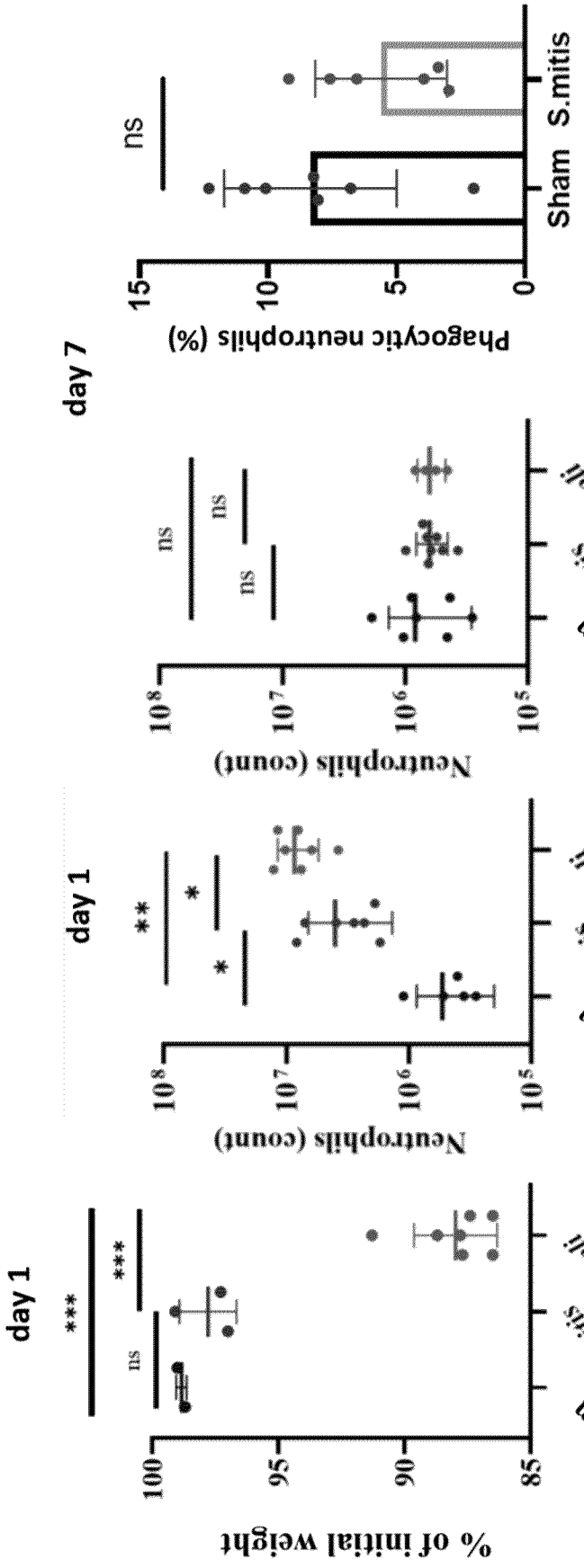
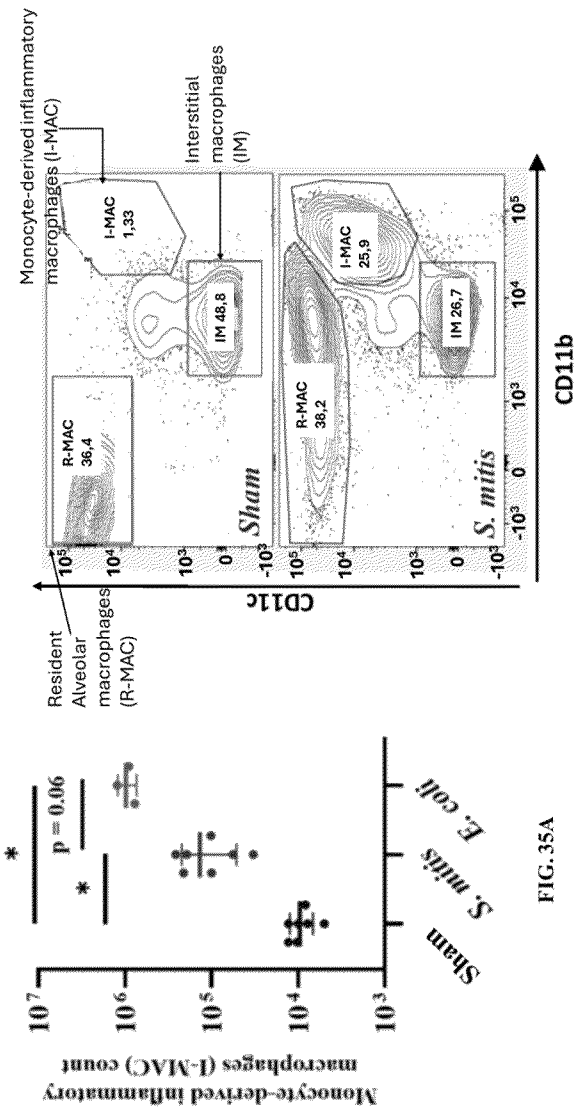


FIG. 34A

FIG. 34B

FIG. 34C

FIG. 34D


Monocyte-derived inflammatory macrophages (I-MAC) count
p = 0.06
Sham
S. mitis
E. coli
FIG. 35A
Monocyte-derived inflammatory macrophages (I-MAC)
Interstitial macrophages (IM)
Resident Alveolar macrophages (R-MAC)
R-MAC 36,4
I-MAC 1,33
IM 48,8
Sham
R-MAC 38,2
I-MAC 25,9
IM 26,7
S. mitis
CD11c
CD11b
FIG. 35B
Phagocytic I-MAC (%)
Sham
S.mitis
FIG. 35C
Phagocytic R-MAC (%)
Sham
S.mitis
FIG. 35D

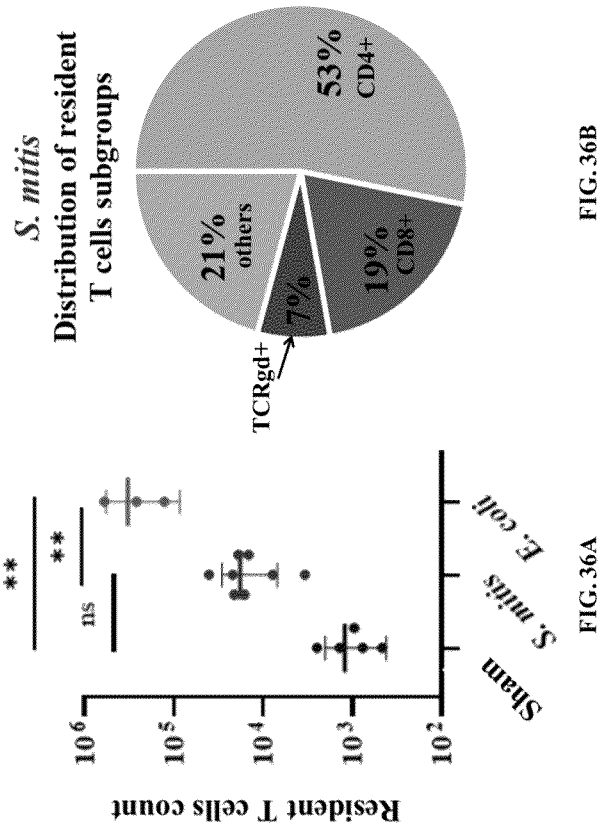


FIG. 36A

FIG. 36B

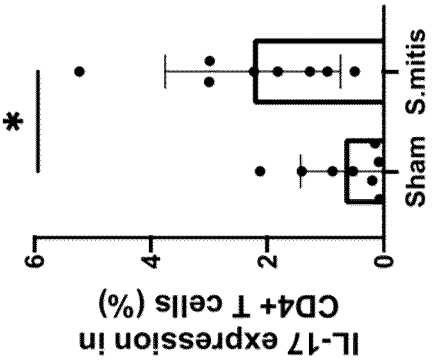


FIG. 36C

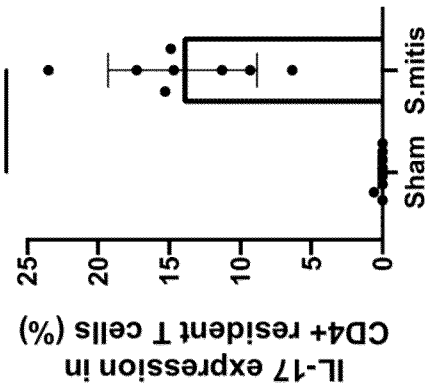


FIG. 36D

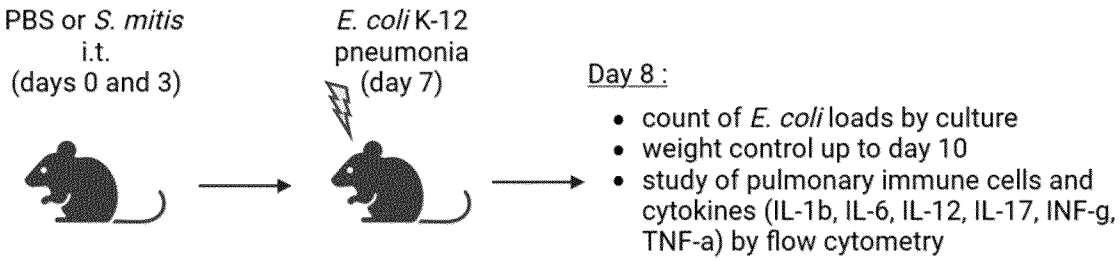


FIG. 37

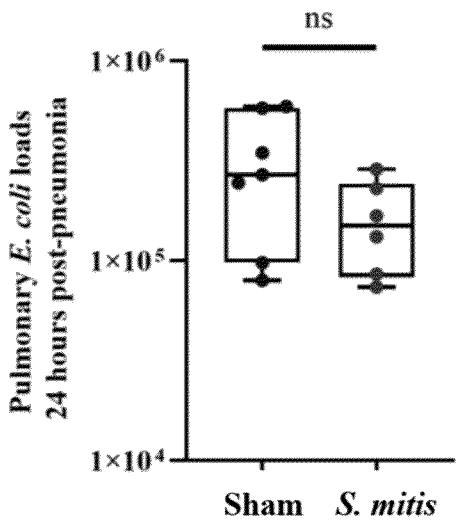


FIG. 38

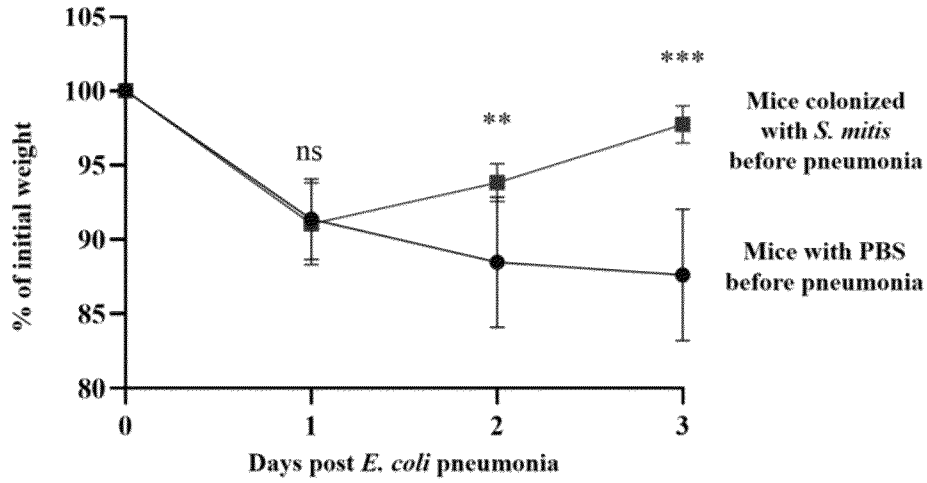


FIG. 39

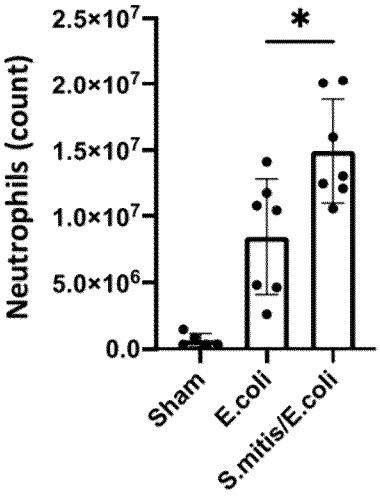


FIG. 40A

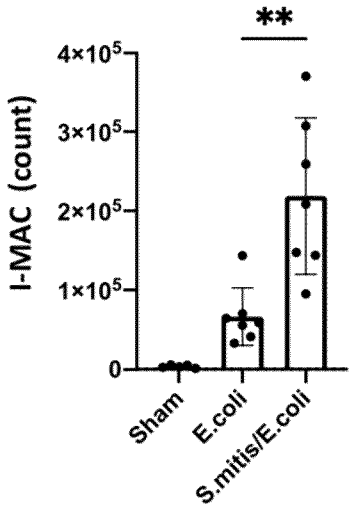


FIG. 40B

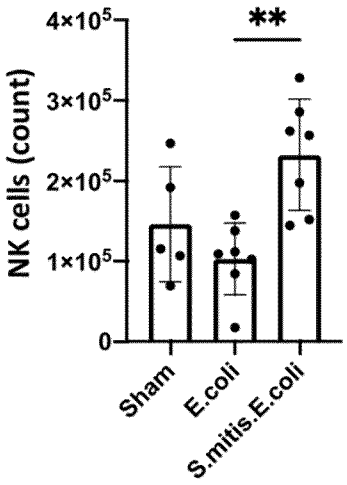


FIG. 40C

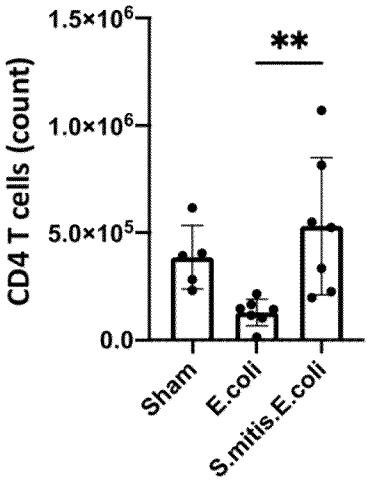


FIG. 40D

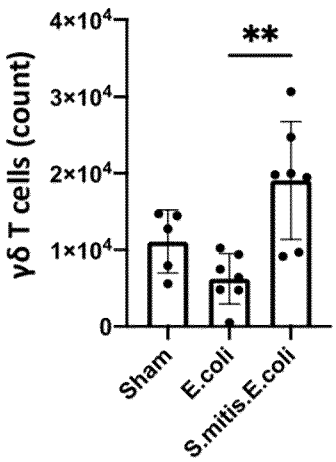


FIG. 40E

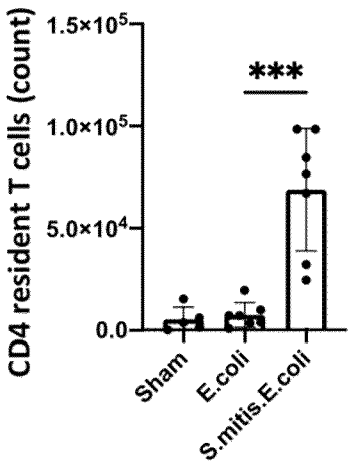


FIG. 40F

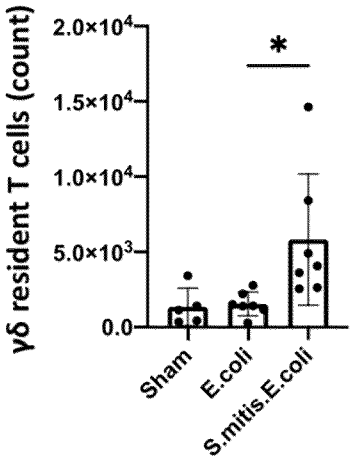


FIG. 40G

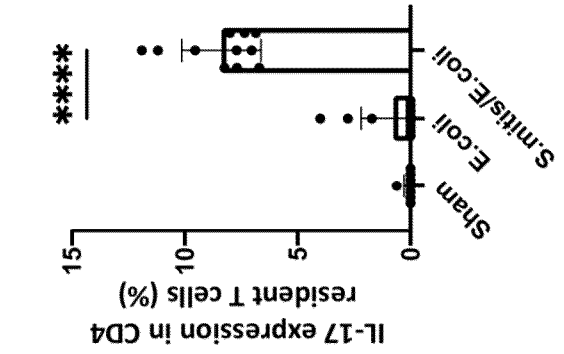


FIG. 41A

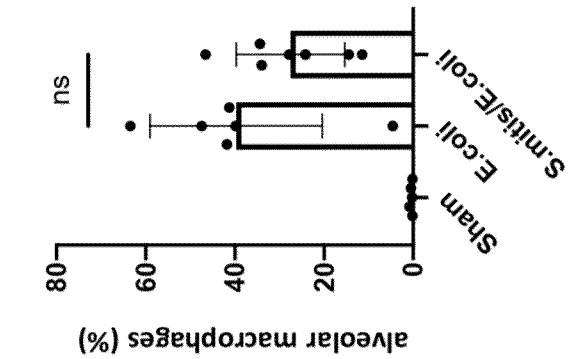


FIG. 41B

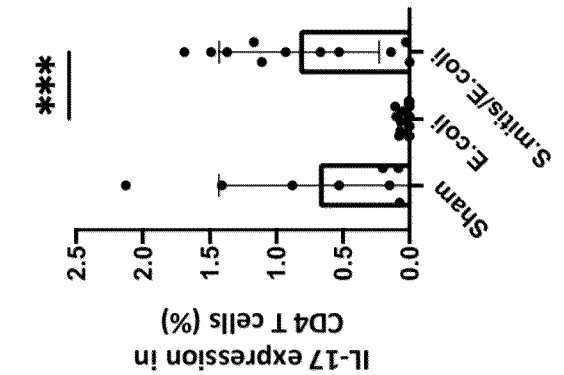


FIG. 41C

FIG. 41D

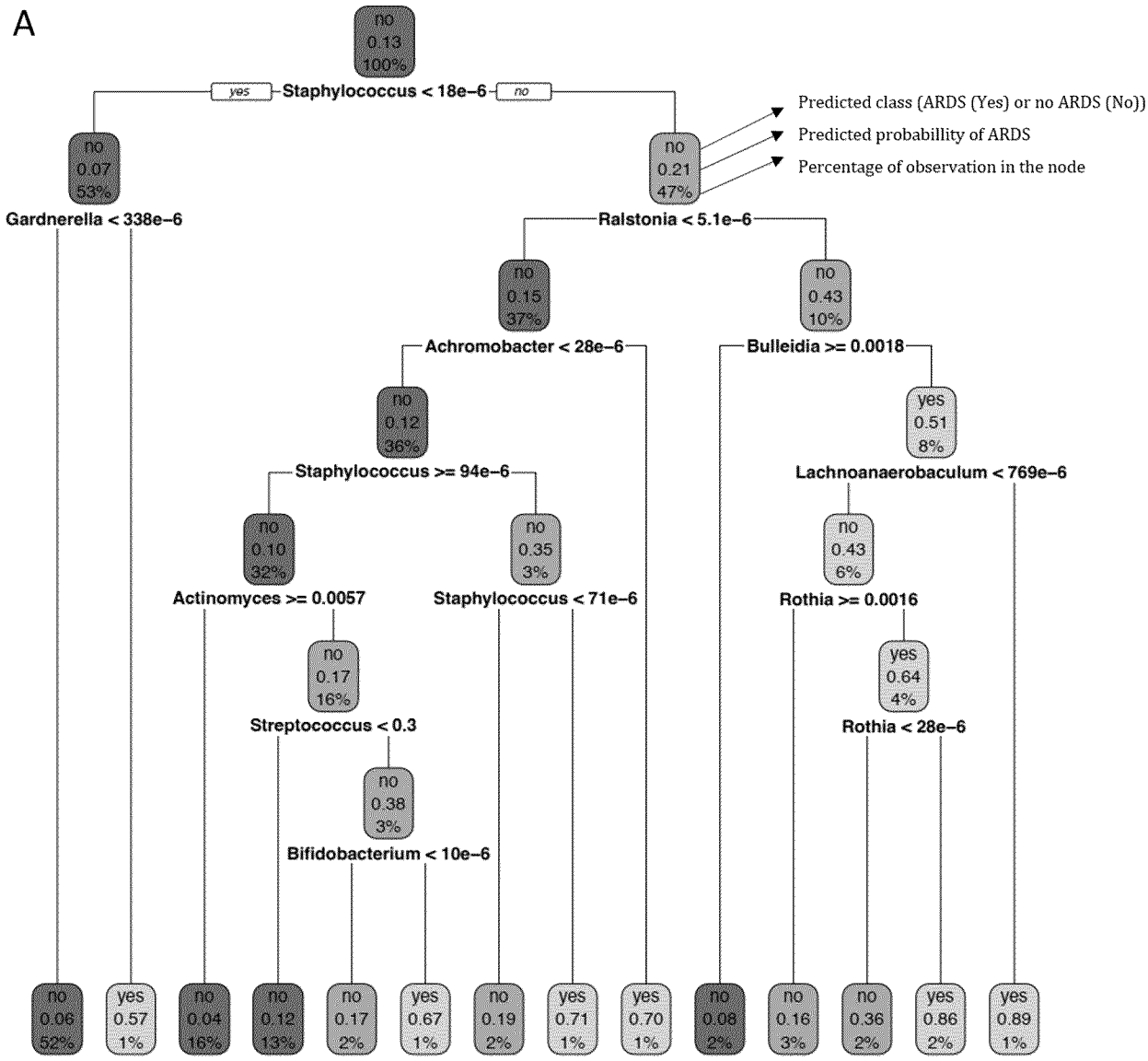

A
no
0.13
100%
yes
Staphylococcus < 18e−6
no
Predicted class (ARDS (Yes) or no ARDS (No))
Predicted probabillity of ARDS
Percentage of observation in the node
no
0.07
53%
no
0.21
47%
Gardnerella < 338e−6
Ralstonia < 5.1e−6
no
0.15
37%
no
0.43
10%
Achromobacter < 28e−6
Bulleidia >= 0.0018
no
0.12
36%
yes
0.51
8%
Staphylococcus >= 94e−6
Lachnoanaerobaculum < 769e−6
no
0.10
32%
no
0.35
3%
no
0.43
6%
Actinomyces >= 0.0057
Staphylococcus < 71e−6
Rothia >= 0.0016
no
0.17
16%
yes
0.64
4%
Streptococcus < 0.3
Rothia < 28e−6
no
0.38
3%
Bifidobacterium < 10e−6
no
0.06
52%
yes
0.57
1%
no
0.04
16%
no
0.12
13%
no
0.17
2%
yes
0.67
1%
no
0.19
2%
yes
0.71
1%
yes
0.70
1%
no
0.08
2%
no
0.16
3%
no
0.36
2%
yes
0.86
2%
yes
0.89
1%

FIG. 42A

B

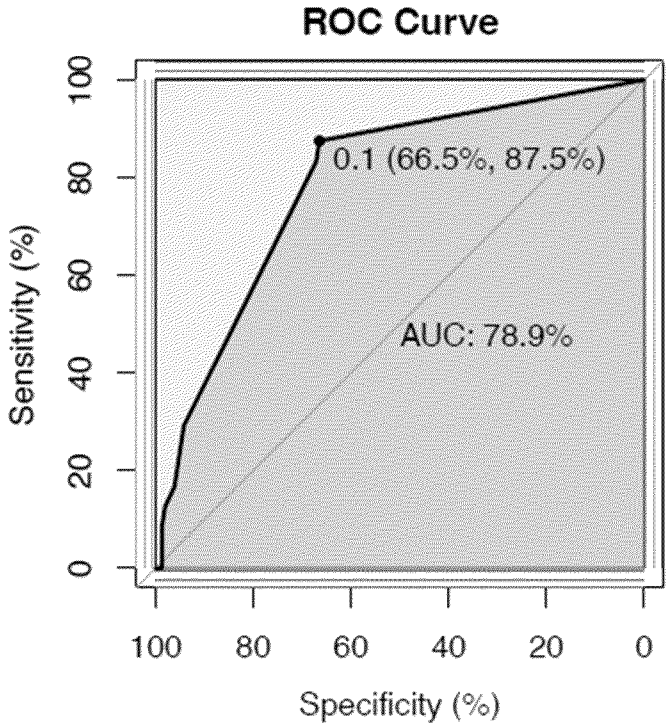
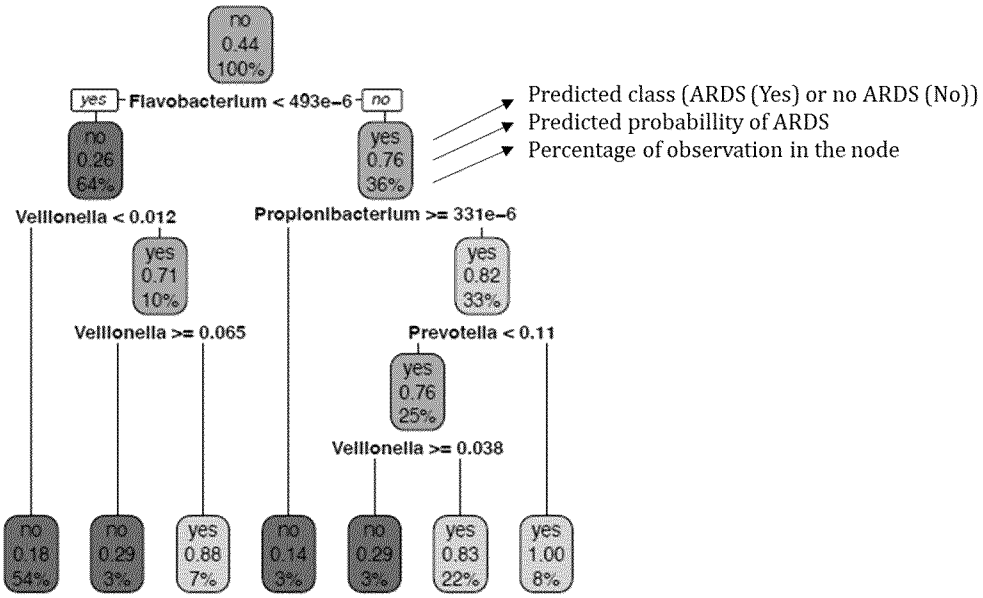

ROC Curve
Sensitivity (%)
100
80
60
40
20
0
0.1 (66.5%, 87.5%)
AUC: 78.9%
100
80
60
40
20
0
Specificity (%)

FIG. 42B

A



B

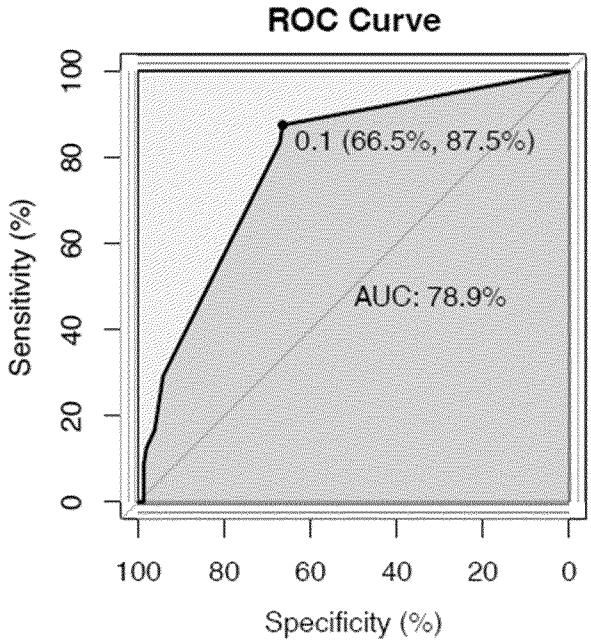


FIG. 43A and 43B

A (part1)

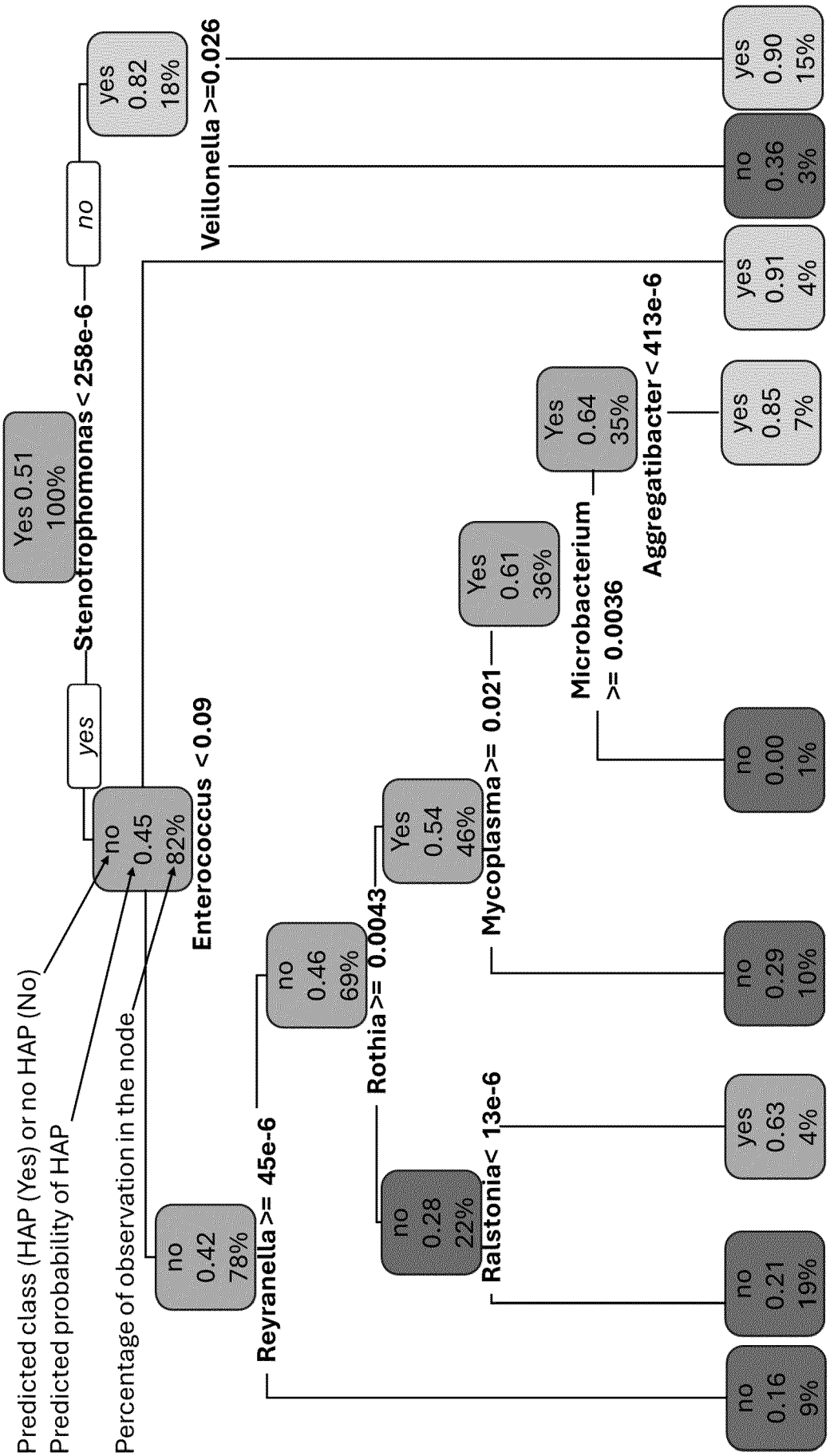


FIG. 44A (part 1)

A (part2)

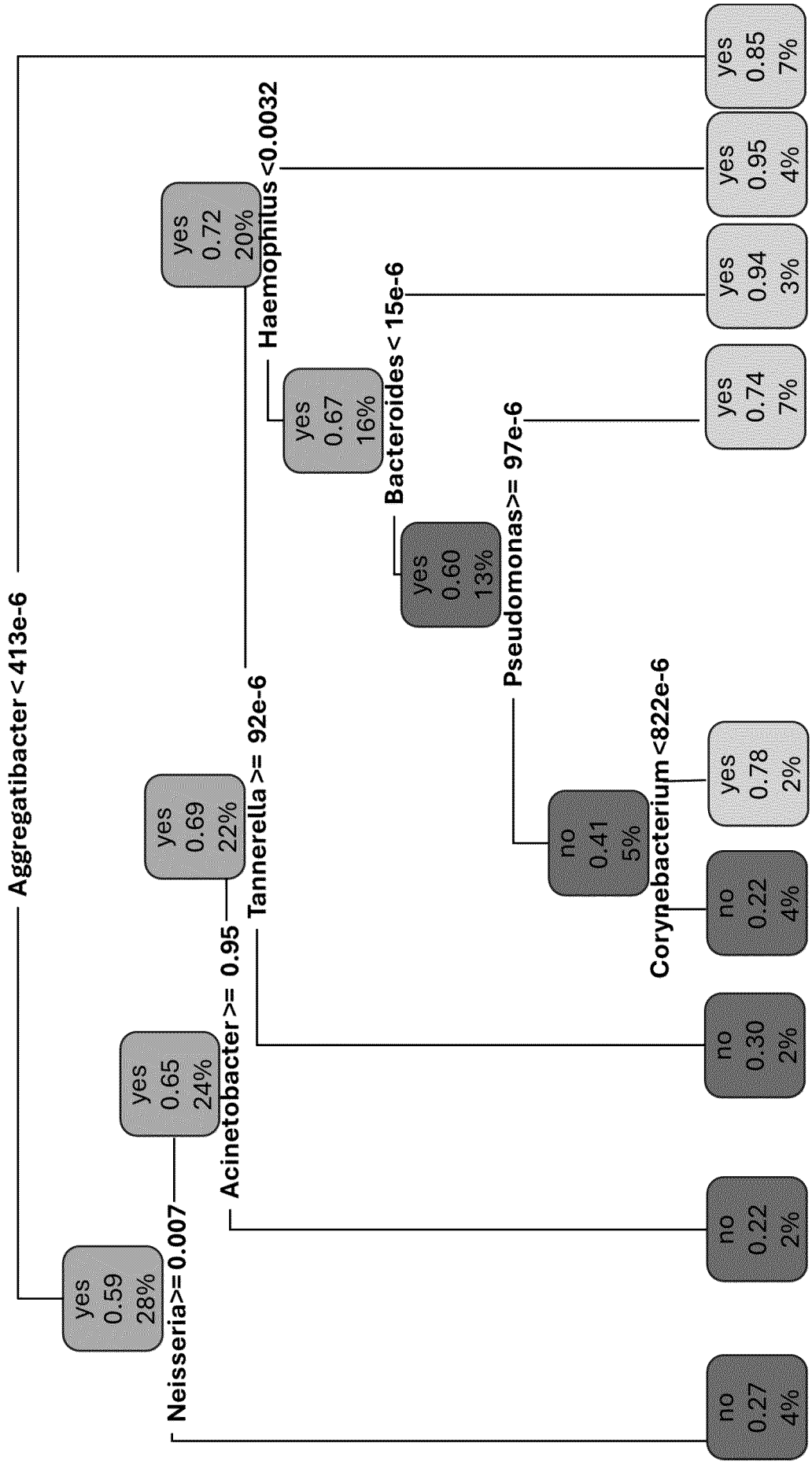


FIG. 44A (part 2)

B

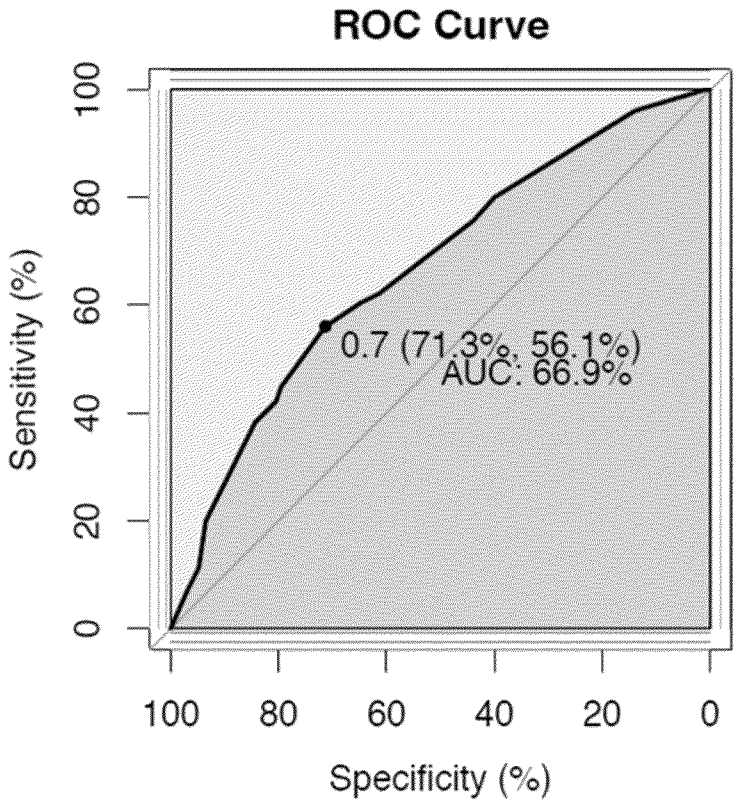

ROC Curve
Sensitivity (%)
100
80
60
40
20
0
0.7 (71.3%, 56.1%)
AUC: 66.9%
100
80
60
40
20
0
Specificity (%)

FIG. 44B

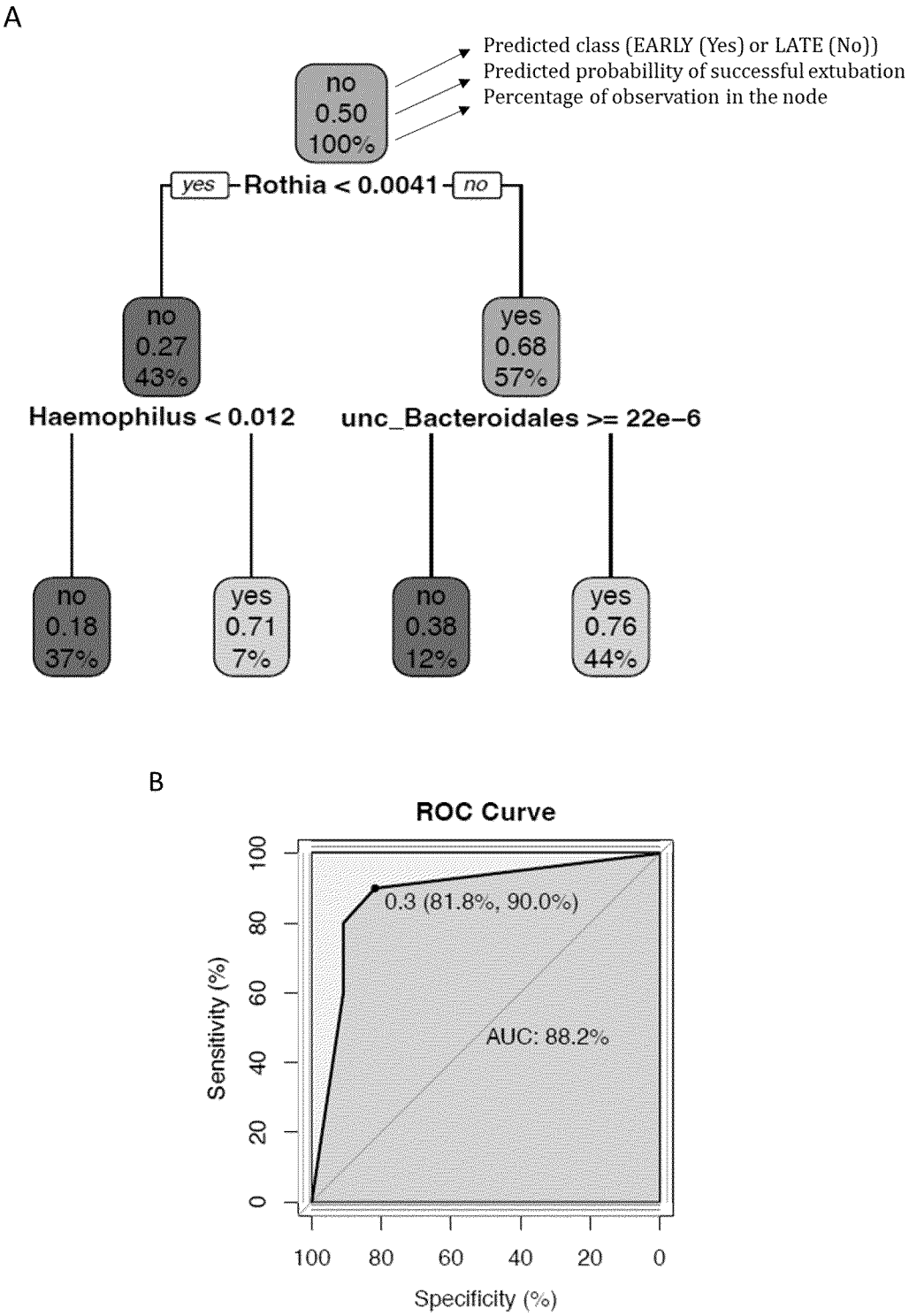


FIG. 45A and 45B