

Repertoire of bacterial species cultured from the human oral cavity and respiratory tract

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While the gut microbiota is currently in the spotlight, the airway microbiome has been recently associated with several pulmonary diseases and carcinogenesis. As there are several biases associated with high-throughput sequencing methods, cultivation techniques are crucial for the investigation of the human microbiome. We thus aimed to build an exhaustive database, including a list of microbes isolated by culture from respiratory specimens, by performing a review of the literature. Herein, we have listed a total of 756 species cultured from the human respiratory tract. This represents 27.23% of the overall bacterial richness captured from human being by culture methods. This repertoire could be valuable for the elucidation of the interactions between the respiratory microbiome and human health.

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Gaining knowledge about the composition of the human microbiome is one of the most exciting challenges of the 21st century in human health, and this knowledge could contribute to the development of therapies for metabolic diseases [1,2], infections [3] and cancer [4]. Recently, based on the intersection of large datasets and universal scaling laws, Locey *et al.* demonstrated that the Earth could contain nearly one trillion species, with only 0.001% identified to date [5]. The number of identified bacterial species with standing in nomenclature is currently 15,974 [6,7]. In 2018, Bilen *et al.* listed 2776 bacterial species that have been isolated from human beings by culture-based methods [8]. Over the last few years, advances in high-throughput sequencing have opened a window into the microbiome [9], and the renewal of bacterial culture methods has greatly contributed to the establishment of the prokaryotic repertoire associated with humans [10,11]. As a matter of fact, it was estimated in 2018 that 2776 species were cultured from human beings, this spectacular increase being the result of the high-throughput culture techniques, in particular culturomics [8]. Culturomics is the method allowing the description of the microbial composition by high-throughput culture [12]. If the microbes from the gut remain the most studied human prokaryotes isolated by culture [13,14,15], intensive research is currently dedicated to the characterization of complex communities that inhabit environments such as the lungs, the skin [16], human urine [17] or the female genital tract [18].

The respiratory tract and oral cavity are constantly exposed to microbiota, either via inhalation or by subclinical microaspiration from birth [19,20], and constitute a warm environment exposed to the diverse microbes present in the air [21]. Historically, the lung environment has been considered as sterile, and this belief has persisted in contemporary medicine [19]. During the 20th century, this belief has led to the development of culture protocols whose purpose was to identify clinically significant pathogens [19] and has led to falsely conclude that the presence of bacteria known to be part of the upper respiratory tract microbiota in the lungs represents a contamination [22]. The understanding of how the lungs and the microbiota interact and exist was improved by the advent of molecular methods and high-throughput sequencing [23,24]. New knowledge has indicated that the lung is not sterile [22] and, in fact, harbors an abundance of diverse interacting microbiota. Thus, in addition to the gut microbiota, the airway

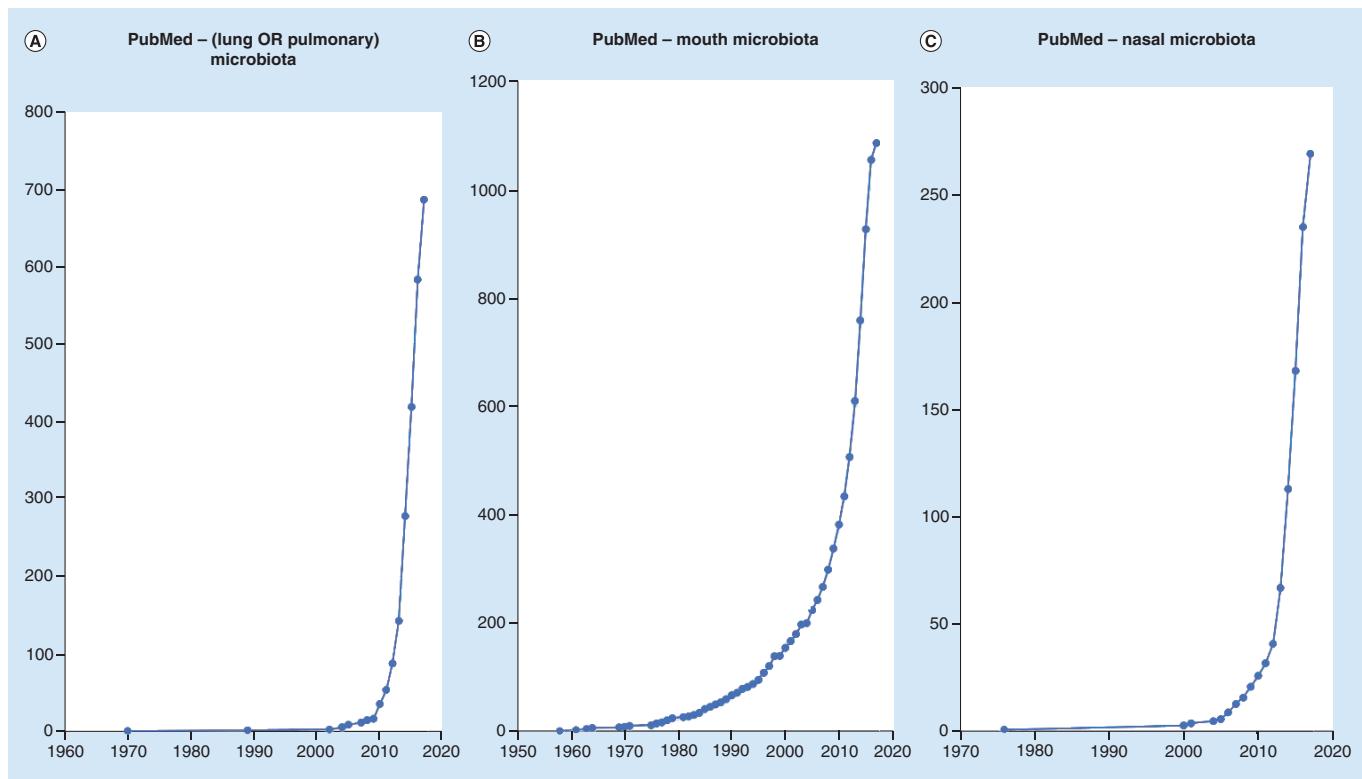


Figure 1. Number of items found in the PubMed database using the keywords 'nasal microbiota', '(lung or pulmonary) microbiota' and 'mouth microbiota'.

microbiome is also the subject of intensive research, as evidenced by the growing number of items retrieved from PubMed, especially since 2010, and by the advent of high-throughput sequencing methods (Figure 1).

In this review, we provide an exhaustive and specific database that lists all bacterial species from the respiratory tract and oral cavity that have been cultured at least once.

Importance of the field

The advent of high-throughput techniques enabled to depict a landscape of oral and respiratory microbiota in different situations. Thus, alterations of the low respiratory tract microbiota have been to date linked with several chronic lung diseases including asthma [25], chronic obstructive pulmonary disease [26], cystic fibrosis [27], bronchiectasis [28], idiopathic pulmonary fibrosis [29] but also with acute respiratory distress syndrome [30], bronchitis [31] or bronchopulmonary dysplasia [32]. More importantly, perturbations of the airway microbiota were recently associated with lung cancer [33], which echoes recent studies of medical importance highlighting the significant contribution of gut microbiota in response to anticancer therapy [4]. These works underline the importance of establishing a comprehensive repertoire of bacteria associated with a specific anatomical site and the need to make available bacterial strains for *in vitro* experiments.

Thus, great efforts allow creating and updating the database of all the bacteria identified in the oral and respiratory microbiome, which are made available for the scientific community. For instance, the Human Oral Microbiome Database includes a total of 770 microbial species of which 57% are officially named, 13% unnamed but cultivated and 30% are known only as uncultivated phylotypes [34]. The Oral Microbiome Bank of China, which contains 289 bacterial strains, was also created in China because the Human Oral Microbiome Database does not reflect the Chinese oral microbial status [35].

However, such a repertoire of strains has not been established for the entire human respiratory tract to the best of our knowledge, motivating the creation of a repertoire of cultured prokaryotes from the human airway microbiota in the present study.

Table 1. Query patterns.

Query patterns	Syntax
QP1	(#3[tiab] OR #3[MeSH] OR #5[tiab]) AND ('Trachea'[Mesh] OR 'Respiratory Mucosa'[Mesh:noexp] OR 'Pharynx'[Mesh] OR 'Larynx'[Mesh] OR 'Lung'[Mesh] OR 'Tracheal Diseases'[Mesh] OR 'Laryngeal Neoplasms'[Mesh] OR 'Lung Neoplasms'[Mesh] OR 'Tracheal Neoplasms'[Mesh] OR 'Respiratory Tract Infections'[Mesh] OR 'Asthma'[Mesh] OR 'Lung Diseases'[Mesh] OR 'Laryngeal Diseases'[Mesh] OR 'Bronchial Diseases'[Mesh] OR 'Bronchoalveolar Lavage'[Mesh] OR 'Bronchoscopy'[Mesh] OR 'Laryngoscopy'[Mesh] OR 'Sputum'[Mesh]) AND 'humans'[MeSH]
QP2	(#3[tiab] OR #3[MeSH] OR #5[tiab]) AND ('microbiology'[SH] OR 'isolation and purification'[SH] OR 'Bacteriological Techniques'[MeSH] OR 'Culture Media'[Mesh]) AND ('Trachea'[Mesh] OR 'Respiratory Mucosa'[Mesh:noexp] OR 'Pharynx'[Mesh] OR 'Larynx'[Mesh] OR 'Lung'[Mesh] OR 'Tracheal Diseases'[Mesh] OR 'Laryngeal Neoplasms'[Mesh] OR 'Lung Neoplasms'[Mesh] OR 'Tracheal Neoplasms'[Mesh] OR 'Respiratory Tract Infections'[Mesh] OR 'Asthma'[Mesh] OR 'Lung Diseases'[Mesh] OR 'Laryngeal Diseases'[Mesh] OR 'Bronchial Diseases'[Mesh] OR 'Bronchoalveolar Lavage'[Mesh] OR 'Bronchoscopy'[Mesh] OR 'Laryngoscopy'[Mesh] OR 'Sputum'[Mesh]) AND 'humans'[MeSH]
#3 and #5 were replaced dynamically (during program execution) for each species by the following: #3 = Bacterial species name or National Center for Biotechnology Information taxonomy synonym(s), if any. #5 = Abbreviations of bacterial species name (e.g., ' <i>E. coli</i> ' or ' <i>E. coli</i> ').	

Bibliographical methods

We first used a list of validated prokaryotic names, which contained 16,194 species with standing in nomenclature on 16 May 2016 [36]. We identified the 'keywords' that characterize the respiratory tract [37,38,39], the oral cavity, the specimens collected [40,41,42,43] from these locations and the culture-based method [43,44,45]. Based on our knowledge of the MEDLINE indexing method and with the help of the 'keywords' list, we established a list of medical subject headings (MeSHs) terms and subheadings relevant for our search focused on species identified in the human respiratory tract in MEDLINE [46]. The MeSH tree was considered to retain the terms of the highest level in the MeSH hierarchy. The MeSH terms, subheadings, species names and abbreviations were combined to establish two query patterns (Table 1). We have developed a computer program capable of querying PubMed/MEDLINE for each of the bacterial species mentioned above. This program, previously used by Hugon *et al.* [13], uses 'Entrez Programming Utilities' provided by National Center for Biotechnology Information [47]. For each bacterium, the program provides the number of bibliographic records (n1 and n2) retrieved by query patterns QP1 and QP2, respectively (due to the syntactical construction of queries: n1 >= n2). The bibliographic records found were analyzed 'manually' (reading titles, abstracts and full texts if needed) to confirm that the record indeed identified a bacterium found in the upper respiratory tract, low respiratory tract and/or oral cavity. For this analysis, we proceeded in steps, beginning with the records retrieved by QP2 (the most specific query). If the keywords 'respiratory tract', 'oral cavity' and 'culture-based technique' were not found, we then analyzed the QP1 records (the most sensitive application). Based on the National Center for Biotechnology Information taxonomy website [36], we classified prokaryotes by phylum, family and genus. We also used 'list of prokaryotes according to their aerotolerant or obligate anaerobic metabolism' [48] to check the tolerance to oxygen of each species or genus. The risk-group classifications were obtained according to the German technical rules for biological agents. The bacteria were classified into three groups: risk group 1 (organisms that do not cause disease in healthy adult humans), risk group 2 (organisms that can cause disease in humans, but the disease is treatable or preventable) and risk group 3 (organisms that cause serious disease in humans). Other information on the bacterial origin were found in the list of prokaryotic names with standing in nomenclature [6,7]. The list of cultured prokaryotes from humans previously established by Bilen *et al.* in 2018 was used to compare species isolated from the respiratory tract with those cultured from the entire human being. Graphical tools used are detailed in supplementary data (Supplementary Figure 1, extra methodologies).

Anatomical approach & specimens for culturing bacteria from the airway microbiome

Respiratory tract '*sensu stricto*'

The respiratory tract is the path of airways from the nose to the lungs. Conventionally, the respiratory tract is divided into two sections: the upper respiratory tract (URT) and the lower respiratory tract (LRT); the larynx is the boundary between these two sections [38,49] (Supplementary Figure 2). The LRT extends from the larynx to the alveoli and includes the trachea, bronchi, bronchioles and lungs [39,49]. While sputum is the most common specimen obtained for microbiological analysis of the LRT [50], contamination of the sputum by oral flora led to the development of invasive procedures for sampling. Bronchoscopy has been widely advocated. Samples that can be obtained by bronchoscopy include bronchial brushings, bronchial washings, bronchoalveolar lavage fluid

and transbronchial biopsy specimens [51]. Sputum, obtain by noninvasive techniques, is limited by the difficulty of obtaining high-quality specimens. However, it has been recently shown that sputum is suitable for standard microbiological analysis when compared with bronchoalveolar lavage fluid [52].

The URT comprises an interconnected system extending from the external nares to the larynx, including the anterior nares, nasal cavity, nasopharynx, sinuses, eustachian tube, middle ear cavity, oropharynx and larynx, forming the interface between the external environment and the lower respiratory and GI tracts [37,53]. To access the microbiota of the URT, most studies in the field, to date, have used the following noninvasive sampling techniques: nasal aspiration, nasopharyngeal aspiration, nasopharyngeal flocked swabs, nasal flocked swabs, nasopharyngeal swabs, nasal washes, nasal swabs, oropharyngeal swabs, pernasal flocked swabs and throat washes [42,43].

Oral cavity

Due to its anatomical configuration, the human oral cavity provides a unique opportunity to study the oral microbiome as the collection of specimens is easy. These specimens are saliva, mouthwash samples [41], supragingival and subgingival plaque samples, dental plaque, tongue dorsum samples, noma lesion samples or pus samples from patients with acute dentoalveolar abscesses.

Evolution of microbiological methods to culture prokaryotes from the respiratory tract

After empirically developing culture media using environmental components such as nutrients, Koch observed the growth of pathogenic organisms in the form of colonies [54], initiating the culture of human pathogens. Among other key discoveries, intense research was dedicated to the isolation of *Treponema* species as they are recognized as etiologic agents of human oral disease [55,56]. Strategies such as the use of membrane filters and incorporation of the antibiotics rifampin were successful [57] and were followed by the design of specific media [58,59]. In a similar fashion, after the discovery of *Mycobacterium tuberculosis* in 1882 by Koch [60], the Mycobacteriaceae now include a high number of species that were isolated through different approaches [61,62].

The advent of Matrix-assisted laser desorption/ionization Time-of-Flight Mass Spectrometry (MALDI-TOF) MS in clinical microbiology also significantly contributed to the knowledge about the respiratory microbiota [63,64] and facilitated the design of large projects, including microbial culturomic studies [11]. When the latter was applied to the respiratory tract, eight totally new species were cultured for the first time [65,66].

In parallel with culture-based methods, a major turning point in the research of the respiratory microbiota was the incorporation of high-throughput techniques [67]. However, more studies have demonstrated that culture-dependent and culture-independent studies are complementary [68,69]. For example, Bousbia *et al.* identified a wide variety of pneumonia-causing agents in intensive care units using both molecular and culture methods [70]. Nevertheless, the rebirth of culture methods is ongoing, and microbes not cultured yet but identified by sequencing methods can now be grown [10,11,71].

The repertoire of bacterial species cultured from the human oral cavity & respiratory tract

Bacteria cultured from the respiratory tract

Using a literature search, we identified 577 species from the human respiratory tract that have been isolated by culture at least once. The microbiota of the LRT is richer as it contains 514 species, whereas 202 species were isolated from the URT, with 138 species in common (Figure 2, Supplementary Table 1).

More specifically, the most represented phyla in the LRT were Actinobacteria (42.0%) and Proteobacteria (36.2%) (Figure 3). These phyla contained 121 genera, of which the most represented were *Mycobacterium* (21.8%) followed by *Nocardia* (6.0%) (Table 2, Figure 4). Only 42 species (8.2%) were strictly anaerobic (Figure 3, Supplementary Table 2), mostly belonging to the genera *Prevotella* (eight species; 19.0%), *Clostridium* (seven species; 16.7%) and *Bacteroides* (four species; 9.5%).

Most of these bacteria were first cultured from a variety of sites in the human body (325 species; 63.2%), of which 146 (44.9%) species were from the LRT specimen (Figure 3, Supplementary Table 2). Of these, 95 bacteria (65.5%) were surprisingly first isolated after the year 2000, and only 24 species (16.6%) were first isolated before 1980 (Figure 5, Supplementary Table 2). Other microorganisms were isolated for the first time from the environment (77 species; 15.0%) and from animals (31 species; 6.0%), plants (16 species; 3.1%) and food (ten species; 1.9%) (Figure 3, Supplementary Table 2).

More than half of the total LRT species (293/514 species; 57.0%) were first cultured at 37°C, and five species were cultured at temperatures above 37°C (i.e., 45 and 55°C). Among the 146 species first isolated from the LRT

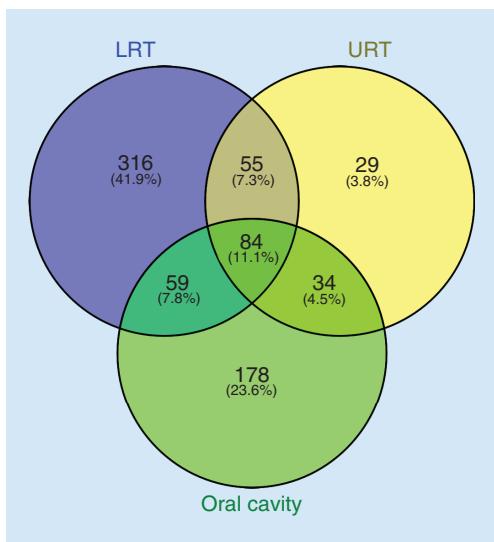


Figure 2. Venn diagram approach for the identification of bacteria of the oral cavity, upper respiratory tract and lower respiratory tract.

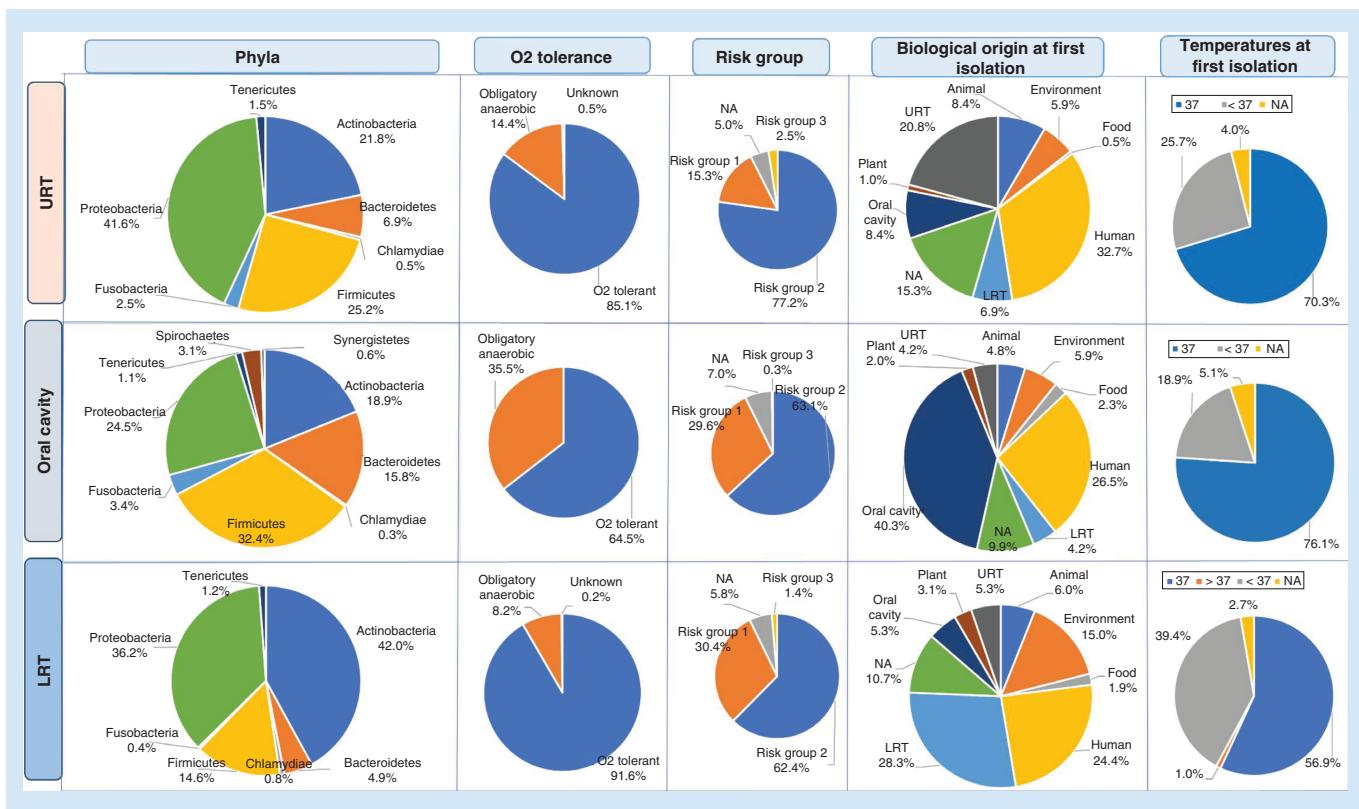


Figure 3. Proportion of bacterial species from the oral cavity, upper respiratory tract and lower respiratory tract, listed according to their phylum, oxygen tolerance, risk group, biological origin and temperature upon first isolation.

specimen, 43.8% were incubated below 37°C (i.e., 28, 30 and 35°C). Interestingly, one species, *Kroppenstedtia pulmonis*, was cultured for the first time at 45°C from an LRT specimen (Figure 3, Supplementary Table 2) [72].

The total of 202 species isolated from the URT were also grouped in the same seven bacterial phyla, but in this case, Proteobacteria was the most represented (41.6%) followed by Firmicutes (25.2%) (Table 2, Figure 3). The URT repertoire comprised 76 different genera, of which 58 included two species or less. *Corynebacterium* (7.9%) and *Streptococcus* (7.9%) were the most represented genera (Table 2, Figure 4B). Among them, 29 bacterial species

Table 2. Summary table of all the phyla and 15 genera with the largest number of species of all the bacteria identified.

	Oral cavity			URT			LRT			ALL		
	N	Rank	%	N	Rank	%	N	Rank	%	N	Rank	%
Number of phyla	9			7			7			9		
Number of genera	122			76			121			180		
Number of species	355			202			514			756		
Phylum												
Actinobacteria	67	3	18.9	44	3	21.8	216	1	42.0	255	1	33.7
Proteobacteria	87	2	24.5	84	1	41.6	186	2	36.2	239	2	31.6
Firmicutes	115	1	32.4	51	2	25.2	75	3	14.6	153	3	20.2
Bacteroidetes	56	4	15.8	14	4	6.9	25	4	4.9	71	4	9.4
Fusobacteria	12	5	3.4	5	5	2.5	2	7	0.4	12	5	1.6
Tenericutes	4	7	1.1	3	6	1.5	6	5	1.2	11	6	1.5
Spirochaetes	11	6	3.1	0	/	/	0	/	/	8	7	1.1
Chlamydiae	1	9	0.3	1	7	0.5	4	6	0.8	4	8	0.5
Synergistetes	2	8	0.6	0	/	/	0	/	/	2	9	0.3
Genera												
<i>Mycobacterium</i>	9	8	2.5	13	4	6.4	112	1	21.8	112	1	14.8
<i>Prevotella</i>	34	1	9.6	8	6	4.0	8	13	1.6	34	2	4.5
<i>Nocardia</i>	2	36	0.6	2	19	1.0	31	2	6.0	31	3	4.1
<i>Streptococcus</i>	25	2	7.0	16	1	7.9	22	3	4.3	31	4	4.1
<i>Corynebacterium</i>	9	8	2.5	16	1	7.9	12	6	1.2	23	5	3.0
<i>Burkholderia</i>	1	53	0.3	5	8	2.5	20	4	3.9	20	6	2.6
<i>Legionella</i>	1	53	0.3	1	33	0.5	17	5	3.3	18	7	2.4
<i>Neisseria</i>	10	7	2.8	13	4	6.4	11	8	1.6	18	8	2.4
<i>Staphylococcus</i>	12	3	3.4	14	3	6.9	6	19	1.2	17	9	2.2
<i>Actinomyces</i>	12	3	3.4	4	12	2.0	10	10	1.9	16	10	2.1
<i>Lactobacillus</i>	11	5	3.1	0	/	/	11	8	1.6	15	11	2.0
<i>Pseudomonas</i>	9	8	2.5	4	12	2.0	10	10	1.9	14	12	1.9
<i>Achromobacter</i>	1	53	0.3	3	15	1.5	12	6	1.2	12	13	1.6
<i>Bordetella</i>	1	53	0.3	6	7	3.0	10	10	1.9	11	14	1.5
<i>Treponema</i>	11	5	3.1	0	/	/	0	/	/	11	15	1.5

The bold numbers in front of number of species should be written as usual. The other bold numbers represents the most represented taxa in each anatomical site. We showed the proportion of bacterial species from the oral cavity, upper respiratory tract and lower respiratory tract according to their phylum and genera.
N = Number of bacterial species.
% = Percentages. It was obtained between the number of species of bacteria identified for each phylum or genus and the total number of species of bacteria in each of the sites.
ALL = Overall view of the bacteria isolated at least once by culture methods from the oral cavity, URT and LRT.
LRT: Lower respiratory tract; URT: Upper respiratory tract.

(14.4%) were obligate anaerobes, mostly grouped in the *Prevotella* and *Fusobacterium* genera (five species; 17.2% each).

Culture growth at 37°C was the main contributor to the URT repertoire (142 species; 70.3%), but incubation below 37°C enabled the recovery of, mainly, *Proteobacteria* (42/52 species) (Figure 3, Supplementary Table 1). Close to 3/4 of the URT repertoire (144 species; 71.3%) was discovered before 1980; 41 species (20.3%) were discovered between 1980 and 1999; and 17 species (8.4%) were discovered after the year 2000. The trend is similar for species primarily isolated from the URT (Figure 5, Supplementary Table 2).

Among the bacteria belonging to risk group 3, *Mycobacterium xenopi*, *Mycobacterium microti* and *Orientia tsutsugamushi* were cultured from the LRT but not from the URT, whereas *Bacillus anthracis* was never, to the best of our knowledge, recovered from the LRT specimen but was isolated from URT samples (Supplementary Table 2).

Bacteria cultured from the oral cavity

As culture of bacteria from the oral cavity is a subject of intense research, we have purposefully separated data recovered from the oral cavity from that regarding other anatomical sites. Consequently, we found 355 bacterial species from the oral cavity that had been cultured at least once. These species were distributed among nine taxonomic phyla: Firmicutes (32.4%) and Proteobacteria (24.5%) were the most represented (Table 2 & Figure 3).

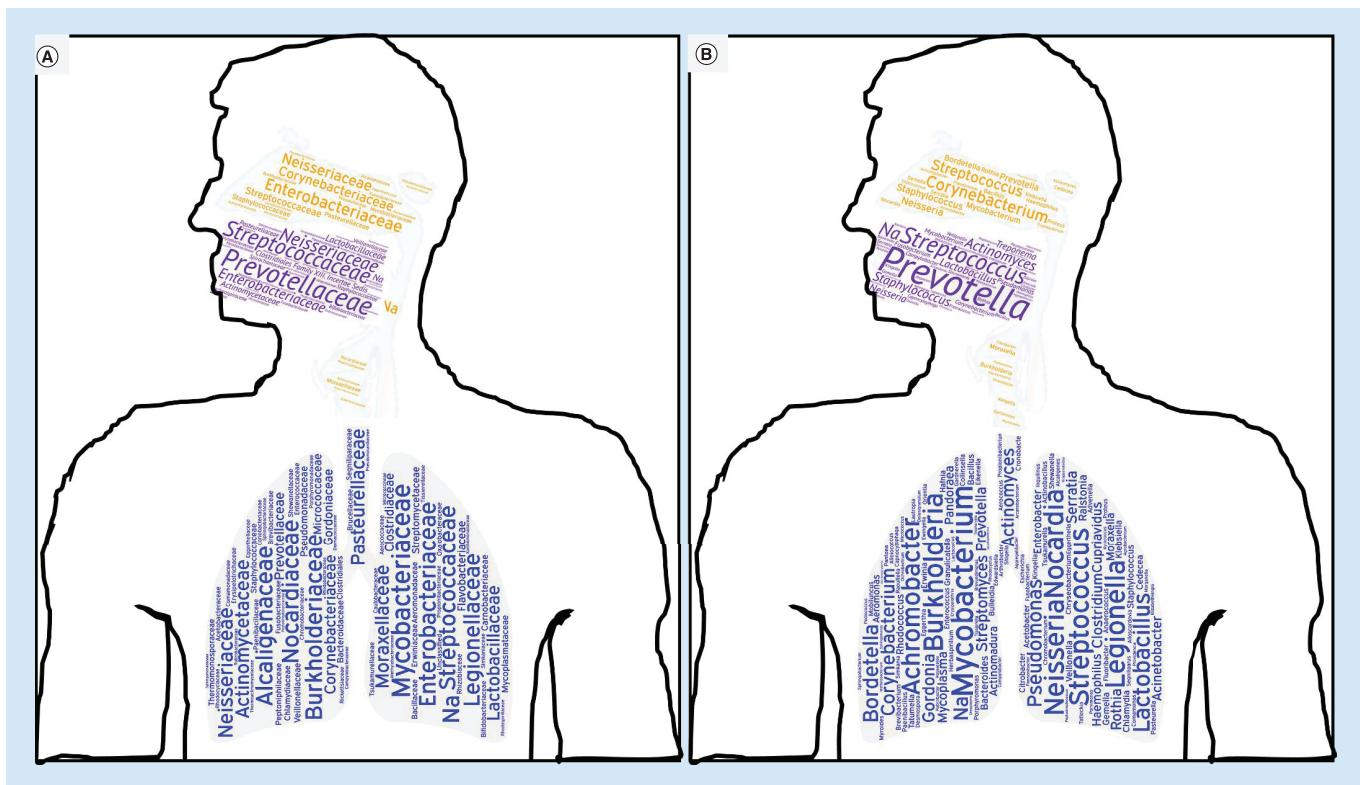


Figure 4. Most represented taxa from the respiratory tract. (A) Word cloud showing the most represented families from the oral cavity, upper respiratory tract and lower respiratory tract. (B) Word cloud showing the most represented genera from the oral cavity, upper respiratory tract and lower respiratory tract.

These phyla comprised 122 different genera, and 71.3% of these genera were composed of one or two species. *Prevotella* and *Streptococcus* (25 species, 7.0%) were those containing the highest number of species (Table 2 & Figure 4). Among these genera, 126 bacteria (35.5%) were obligate anaerobes and distributed among 18 families, of which *Prevotellaceae* (27 species, 29.4%) was the most represented (Figure 3 & Supplementary Table 2).

Of these 355 bacterial species, 143 (40.2%) were first isolated from oral specimens, of which a large majority were isolated when incubation at 37°C was performed (131 species, 91.6%). On the other hand, 267 (75.1%) species were first cultured from other human samples (Figure 3 & Supplementary Table 2). A large part of this repertoire had already been identified before 1980 (162 species, 45.6%), whereas species primarily cultured from oral samples were mainly discovered between 1980 and 2000 (67/143 species, 46.9%) (Figure 5 & Supplementary Table 2).

Comparison between bacterial species cultured from the human oral cavity, upper respiratory tract & lower respiratory tract

In this study, we identified 756 different bacterial species from the airway microbiome with different bacterial richness according to each site of the respiratory tract. Thus, the LRT harbors the highest number of different species followed by the oral cavity (355 species) and the URT (202 species). Overall, 83 species were isolated from all three sites. The URT appears to be the less specific as it shares 58.42 and 68.32% of its species with the oral cavity and LRT, respectively. On the other hand, the oral cavity and in particular, the LRT harbors 179 and 316 unique species, respectively (Figure 2 & Supplementary Table 1).

Actinobacteria is the most represented phylum in the LRT, as well as *Mycobacterium* at the genus level. In the URT, Proteobacteria at the phylum level and *Corynebacterium* and *Streptococcus* at the genus level are the most represented taxa. Similarly, Firmicutes, Streptococcus and *Prevotella* comprise the highest number of species cultured from the human oral cavity. Members of the phyla Spirochaetes (11 species) and Synergistetes (two species) were only isolated from the oral cavity (Figures 3 & 4).

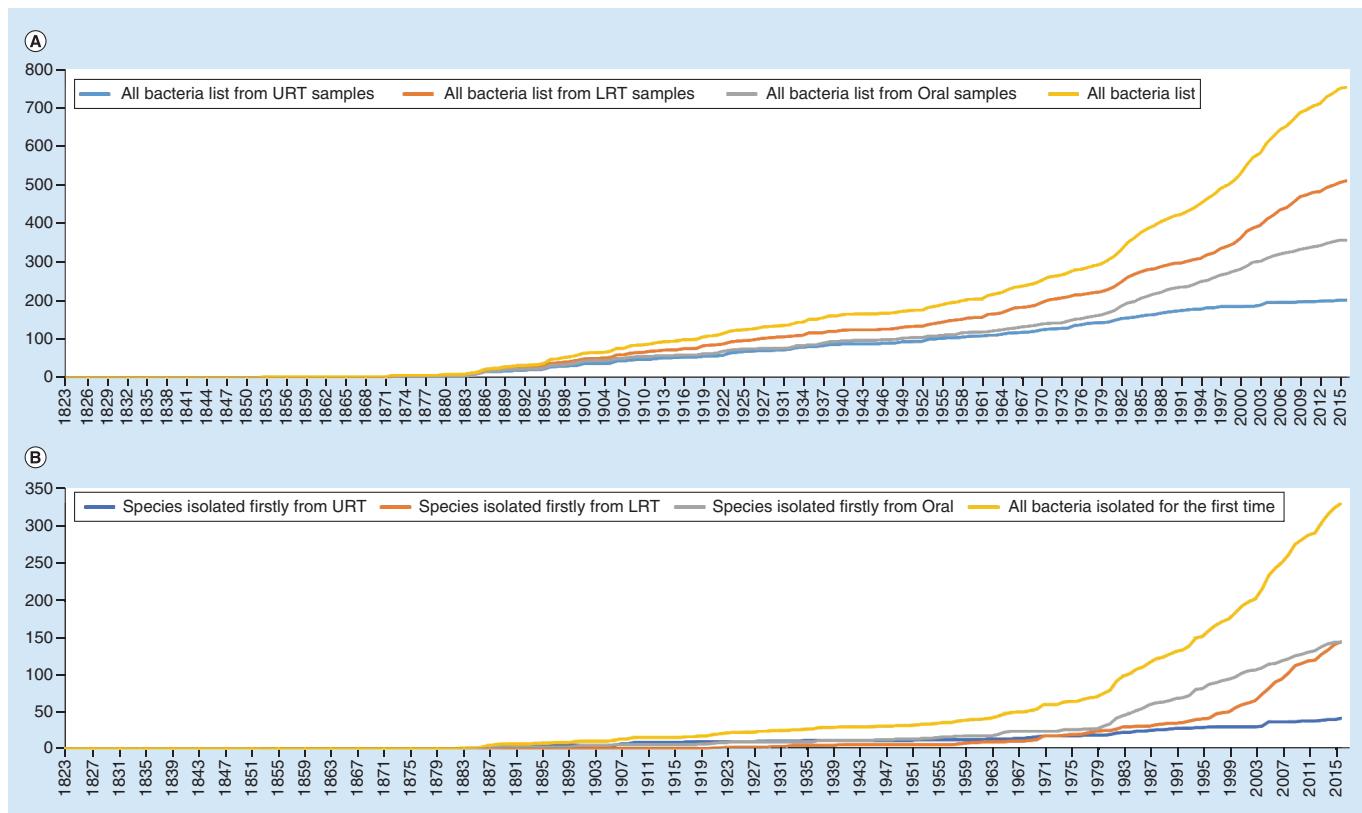


Figure 5. Evolution over time of the bacterial species recovered by culture from the respiratory tract. (A) Evolution curve of the total number of bacterial species isolated at least once per culture in the oral cavity, the lower and upper respiratory tract. **(B)** Evolution curve of all species of bacteria isolated for the first time from a sample of the oral cavity, upper and lower respiratory tract. Both graphs are based on the date of the first identification of the bacteria.

The ecosystem harboring the highest number of anaerobes is the oral cavity (35.5%) followed by URT (14.4%) and LRT (8.2%). Among anaerobes, the Prevotellaceae family has always been the most represented family (Figure 3 & Supplementary Table 2).

Assessing pathogenicity through literature

We tried to make estimations based on the literature by analyzing the frequency of case reports involving each bacterium within the URT, oral cavity and LRT. These data were then compared with the common respiratory tract pathogenic bacteria found in three sources (i.e., Dubourg *et al.* 2015 [52]; Mandell, Douglas and Bennett's *Principles and Practice of Infectious Diseases (5th Edition)* [73] and *Infectious Diseases (4th Edition)* by Cohen *et al.* [74,75,76,77,78,79]). Total 60 % of the bacteria included in this repertoire were reported as potential respiratory pathogens in humans (Figure 6A).

LRT infections reported were mainly pneumonia, lung abscesses, tuberculosis, tracheitis and bronchitis (Supplementary Table 4). Considering the URT, the most reported infections were sinusitis, epiglottitis, pharyngitis/tonsillitis and pharyngeal abscesses (Supplementary Table 5). We have listed the most frequent bacteria according to the number of verified case reports found (Figure 6). Almost all LRT bacteria identified in (b) and (c) with respectively 90 and 80% also belong to the top 20 bacteria identified herein, with the highest number of case reports associated with the LRT. However, the overlap decreased to 50% when compared with (a), because the latter only includes isolates from standard cultures performed routinely in the microbiology laboratory and have thus excluded fastidious bacteria such as nontuberculous mycobacteria or *Legionella* (Supplementary Table 7A). The latter are however less common. Their presence in the top 20 identified herein could thus be due to a publication bias. With respect to URT bacteria, 15 of the 20 bacteria (75%) with the most case reports have been identified as URT pathogens and are found in (b) and (c) (Supplementary Table 7B).

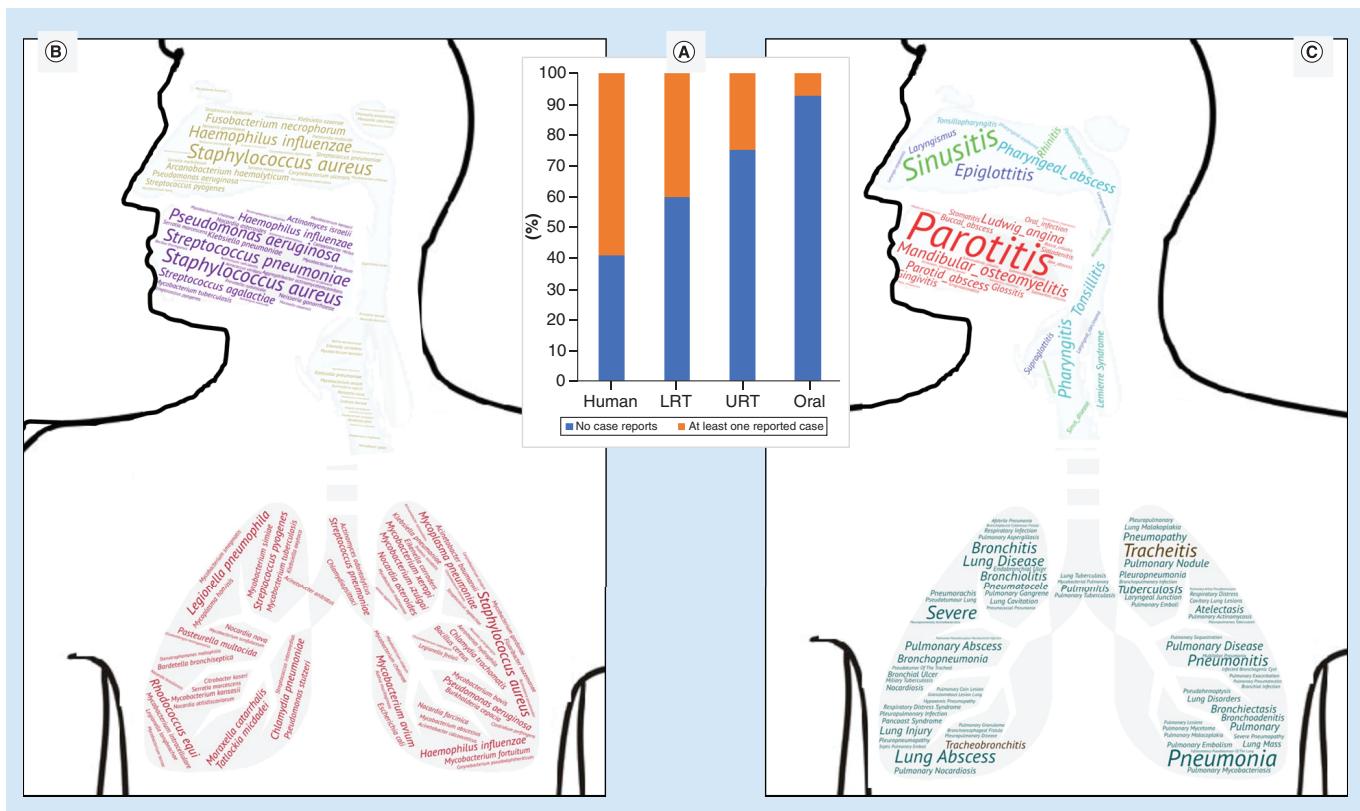


Figure 6. Most represented bacteria from respiratory involved in human infections according to literature. (A) Proportion of bacterial species involved in infections of the human oral cavity, the URT, the LRT and human body in general, when analyzing the case reports found in the PubMed database using the keywords ‘bacteria name and their synonyms’ AND ‘humans’ [MeSH] AND Case Reports[ptyp]. (B) Word cloud showing the most abundant bacterial species in the human oral cavity, URT, the LRT according to the number of verified case reports found in the PubMed database using the keywords ‘bacteria name and their synonyms’ AND ‘humans’ [MeSH] AND Case Reports[ptyp]. (C) Word cloud showing the most frequent infections reported in the oral cavity, URT and the LRT according to the number of verified case reports found in the PubMed database using the keywords ‘bacteria name and their synonyms’ AND ‘humans’ [MeSH] AND Case Reports[ptyp].

LRT: Lower respiratory tract; URT: Upper respiratory tract.

We found discrepancies between bacteria associated with the highest number of case reports published and those found in three different databases derived from clinical microbiological sources. Over publication of rare cases, such as for *Rhodococcus equi*, which was an emerging microbe linked with immunosuppression, could partially explain these inconsistencies. However, the incidence of some microorganisms could be underestimated as they are not often searched for in clinical microbiology or because they can be misidentified, as is the case for *Fusobacterium necrophorum* and *Klebsiella ozoneae*.

Conclusion

This work enabled the first establishment of the repertoire of the airway microbiome of human beings. We list 756 species, which represents 27.23% of the overall bacterial richness found in humans by culture methods when compared with Bilen *et al.* 2018. We are nevertheless aware that such systematic search comprises several biases. Indeed, we found in this work a higher number of species cultured from the oral cavity than that from upper respiratory tract, and this finding reflects the intense research dedicated to the elucidation of periodontal diseases and dental caries. Also, the species variety within a family or a genus is not predictive of their prevalence in the human respiratory tract, since *Mycobacterium* is the genus comprising the highest number of species isolated from lungs, while its frequency among human beings is lower than that of respiratory commensals such as *Streptococci*. These findings could be explained by different taxonomic criteria to delineate new species according to the phylogenetically closest neighbor. In a similar fashion, the number of case reports involving specific species does not reflect their incidence in respiratory infections as microbes such as pneumococci or MTB are not found among the top ten

published pathogens. In a similar fashion, *B. anthracis* was not recovered from the respiratory tract by culture as it is typically cultured from the lymph nodes but clearly passes through the lungs [72]. The presence of *Mycobacterium gordonae* is also still debated as it is considered as a contaminant by some physicians [80]. However, this list could be of medical interest to address potential pathogens to seek after elimination of the most common ones. Moreover, it could be used to confirm isolation of unusual bacteria from respiratory specimens.

Future perspective

Finally, we believe that the establishment of a repertoire of cultured bacteria from the respiratory tract is crucial to identify the main players in the respiratory tract homeostasis for further studies. The detection of bacterial DNA from respiratory specimens is made easy with high-throughput sequencing methods but remains difficult to interpret. Indeed, respiratory tract is constantly exposed to various bacterial contents from the air, and the different anatomical sections are interconnected. Thus, culture methods ensure that prokaryotes isolated are viable and allow establishing a robust repertoire potentially relevant for future research on the microbiome.

The fact that most species first isolated from the LRT were mostly recovered after 2000 highlights the need to pursue efforts for culturing microbes from airway specimens (Figure 5B). Indeed, this observation could reflect the substantial advances made in identification methods, especially the advent of MALDI-TOF MS. Interestingly, a substantial proportion of these species was previously cultured from other human body areas (Figure 3 & Figure 5A), highlighting the need to comprehensively culture samples from different sites. The latter shares many bacterial species and comprehensive identification of each microbiota could allow understanding how bacteria colonize human surfaces. Combination of high-scale culture methods such as culturomics, proteomics and metagenomics will help elucidate the interactions between the airway microbiota and healthy and diseased hosts [81].

Executive summary

Importance of the field

- Alterations of the lower respiratory tract microbiota have been to date linked with several chronic lung diseases.
- Airway commensals were recently suggested to play a role in lung cancer carcinogenesis.
- If several collections of oral bacterial strains exist, no repertoire of cultured prokaryotes from the human respiratory tract has been established yet.

Evolution of culture methods

- Targeted strategies were developed over time to isolate specific bacteria from the respiratory tract such as for *Mycobacterium* spp.
- Implementation of MALDI-TOF MS in microbiology laboratories contributed to identify additional species from the respiratory tract.
- Preliminary culturomic studies allowed to identify new microbes from respiratory specimens.

The repertoire of bacterial species cultured from the human oral cavity & respiratory tract

- A total of 756 prokaryotes were identified in this repertoire.
- We found 577 species from the human respiratory tract that have been isolated by culture at least once.
- The microbiota of the lower respiratory tract (LRT) is richer as it contains 514 species, whereas 202 species were isolated from the upper respiratory tract, with 138 species in common.
- A total of 355 bacterial species from the oral cavity had been cultured at least once, reflecting intense research dedicated to periodontal diseases.
- Actinobacteria, Proteobacteria and Firmicutes were the most represented phyla of the LRT, upper respiratory tract and oral microbiota, respectively.

Assessing pathogenicity through literature

- Systematic bibliographical search did not allow obtaining a relevant classification of respiratory pathogens when measuring the frequency of reported cases.

Perspective

- The 756 species identified in this study represents 27.23% of the overall bacterial variety found in humans by culture methods.
- As detecting bacterial DNA from respiratory specimens is very easy using high-throughput sequencing methods, the establishment of a robust repertoire is crucial for further microbiome research.
- The fact that most species first isolated from the LRT were mostly discovered after 2000 highlights the need to pursue efforts for culturing microbes from the respiratory tract.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/fmb-2018-0181

Author contributions

MD Mbogning Fonkou analyzed the data, create figures and wrote the review. J-C Dufour performed the literature searches on Medline and revised bibliographical methods. G Dubourg analyzed the data and revised the review. D Raoult designed and wrote the review.

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