

OPINION

New opportunities for managing acute and chronic lung infections

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Abstract | Lung diseases caused by microbial infections affect hundreds of millions of children and adults throughout the world. In Western populations, the treatment of lung infections is a primary driver of antibiotic resistance. Traditional therapeutic strategies have been based on the premise that the healthy lung is sterile and that infections grow in a pristine environment. As a consequence, rapid advances in our understanding of the composition of the microbiota of the skin and bowel have not yet been matched by studies of the respiratory tree. The recognition that the lungs are as populated with microorganisms as other mucosal surfaces provides the opportunity to reconsider the mechanisms and management of lung infections. Molecular analyses of the lung microbiota are revealing profound adverse responses to widespread antibiotic use, urbanization and globalization. This Opinion article proposes how technologies and concepts flowing from the Human Microbiome Project can transform the diagnosis and treatment of common lung diseases.

The airways and the lung are in a constant state of challenge from environmental microorganisms. A sedentary adult can inhale more than 10,000 litres of air per day¹, which washes over 40–80 m² of lung surface^{2,3}. In cities, this air may contain up to 100,000 bacteria per litre⁴, a proportion of which are able to penetrate into the thoracic airways and beyond. By contrast, the gut has a surface area of 30 m² (REF. 5), and microbial ingress is limited by gastric acid. Thus, it is not surprising that lung infections commonly affect human health. In the United Kingdom alone, 16 million patients of the National Health Service are treated with antibiotics each year for respiratory tract infections that include 260,000 episodes of community-acquired pneumonia (CAP)⁶. Antibiotic prescription rates are higher in many other European countries than in the United Kingdom⁷. In the United States, acute lung infection in the presence of underlying chronic obstructive pulmonary disease (COPD) is the most common reason for administering antimicrobial therapy to adults; this clinical setting plays a substantial role in driving antimicrobial resistance⁸.

Until recently, standard medical teaching held that the healthy lung is sterile⁹ and that pathogens invade an environment that is normally kept free of microorganisms by the host mucosa. However, the thoracic airways, which are the site of many infections, are now known to support a distinctive community with a density of bacteria similar to that of the small bowel^{10,11}. The consideration of how diverse commensal microbial communities resist exogenous infection^{12–14} has had an enormous influence on understanding and treating bowel diseases¹⁵, and it is timely now to reconsider received wisdom for the management of lung infections. Accessing the lower airways for routine microbial analyses typically involves invasive procedures such as fibre-optic bronchoscopy. These practical difficulties, and the low priority given to non-HIV lung disease by the Human Microbiome Project (HMP), mean that the technological and analytical advances of the HMP have been sparsely applied to the airways and the lung.

In this Opinion article, we review the most common pulmonary infective agents, with an emphasis on bacterial pathogens

and their potential interactions with each other and airway commensals. We discuss the emergence of antibiotic multidrug-resistant (MDR) organisms and highlight the potential for the global spread of pathogens through circulation in cities, schools and hospitals. We then suggest novel diagnostic and therapeutic possibilities for mitigating these serious problems. We propose that the opportunities to improve patient care are substantial and that a coordinated and collaborative international Lung Microbiome Project (to consider the collective genomes of the microorganisms that reside in the lung) together with systematic studies of individual organisms (the microbiota) will be of inestimable value to global health.

The burden of lung infections

Overt respiratory infections are the leading cause of death in developing countries and are associated with more than 4 million deaths annually¹⁶. In children under 5 years of age, pneumonia, which is usually associated with a bacterial infection, accounts for 1.3 million deaths annually and 18% of all-cause mortality¹⁷. Moreover, pneumonia kills far more adults than HIV or malaria¹⁶. Globally, lower respiratory infections result in the loss of 103,000 disability-adjusted life years, which means that lung infections are the largest cause of disease burden to humanity.

Acute infections can afflict healthy adults but are more common in the very young or very old or in those with underlying health problems. They usually affect the lung parenchyma (pneumonia), the airways of the lung (bronchitis) or both (bronchopneumonia) (FIG. 1). Less frequently, infections can spread to the linings of the lung, causing pleurisy and empyema. CAP differs from hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP), which occur in patients who are sick or immunocompromised and result in high morbidity, mortality and costs¹⁸.

In contrast to acute infections, chronic lung infections are typically a consequence of underlying disease. For example, bronchiectasis describes dilations in the thoracic airways (FIG. 1), which can be caused by tuberculosis, scarring after

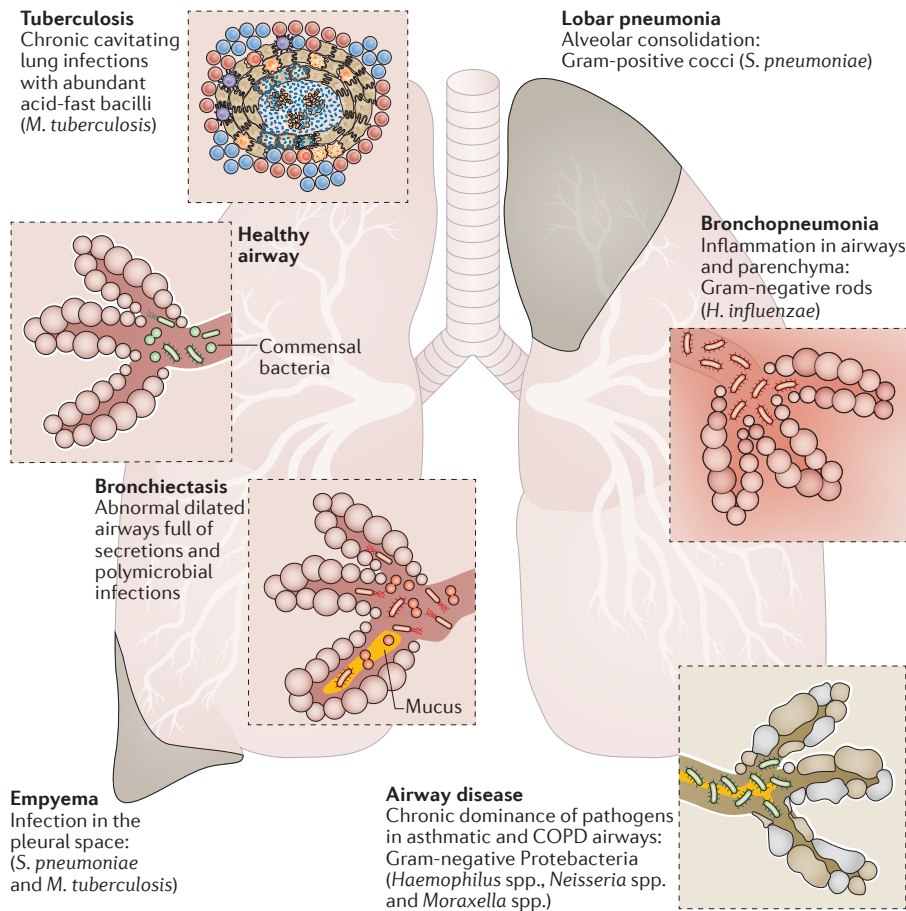


Figure 1 | Pulmonary infections associated with bacterial pathogens. The airways and lung can be affected in a number of different ways by bacterial infection, as shown clockwise starting from the top right: lobar pneumonia affects a whole lobe of the lung and may spill into the surrounding pleura (two thin layers of tissue that protect the lungs and allow them to move during breathing). Lobar pneumonia is typically caused by Gram-positive short chains of *Streptococcus pneumoniae*. Bronchopneumonia is centred in the bronchial tree and spreads into adjoining lung tissues; it is most commonly caused by the Gram-negative rods of *Haemophilus influenzae*. Asthma and chronic obstructive pulmonary disease (COPD) are diseases of the small airways, characterized by overgrowth of Proteobacteria (such as *H. influenzae* and *Neisseria* spp.) and the loss of normal commensal species. Empyema is an infection of the pleural space and is most commonly caused by *S. pneumoniae*. The chronic lung diseases bronchiectasis and cystic fibrosis are associated with chronic resistant infections with abnormal bronchial morphology; these infections are caused by *H. influenzae*, *S. pneumoniae* and opportunistic pathogens such as *Staphylococcus aureus* (a Gram-positive coccus) and *Pseudomonas aeruginosa* (a Gram-negative flagellate bacillus). *Mycobacterium tuberculosis* causes chronic consolidation and cavitation with invasion into draining lymph nodes and eventual systematic spread.

acute infection, primary ciliary dyskinesia, immune deficiencies and cystic fibrosis (in populations of European descent). Bronchiectasis from all causes results in the progressive production of thick, purulent secretions (sputum) and an advancing destruction of the airways and lung parenchyma.

Another underlying disorder that predisposes to infection is COPD, which is typified by inelastic constricted airways with or without loss of lung parenchyma (emphysema) and is usually the result of

cigarette smoking. Chronic lung diseases such as COPD are all accompanied by periods of acute worsening of respiratory symptoms, known as exacerbations. The aetiology of exacerbations encompasses host immune responses as well as complex changes in the viral, fungal and bacterial communities¹⁹. In COPD, acute infective exacerbations cause airway inflammation and hasten disease progression²⁰.

In other conditions, the relationship with infection is more complex. Asthma is a syndrome of intermittent airway

inflammation that causes wheezing and shortness of breath. Notably, asthma affects 250–300 million individuals of all ages, races and ethnicities globally, and its prevalence is increasing alarmingly in the developing world²¹. Urbanization, with the loss of the microbial environment from traditional rural lifestyles, strongly increases susceptibility to disease²². Exacerbations are often triggered by viral infections²³. Asthmatic airways typically contain considerable numbers of bacterial pathogens^{24–28} (FIG. 1) and may also carry as yet unexplored fungi. However, a causal link between these organisms and disease is yet to be established.

The microbiota of healthy lungs

Pulmonary infections with pathogens take place in the presence of commensal lower-airway microbial communities (the microbiota; reviewed in REF. 29). In brief, the sequencing of variable regions of the 16S rRNA gene has shown that healthy airways contain in the region of 100 operational taxonomic units (OTUs; as defined by sequence similarity), which are dominated by the Firmicutes (primarily *Streptococcus* spp. and *Veillonella* spp. OTUs) but also contain Bacteroidetes (mostly *Prevotella* spp.), Fusobacteria, Actinobacteria and Proteobacteria^{10,24–28}. Many of these OTUs may represent facultative anaerobes that are recalcitrant to routine culture. The limited number of phyla and the consistency of the oropharyngeal and lower-airway microbiota between multiple individuals and across studies^{10,30–32} indicate that the airways are not simply passive recipients of inhaled microbiota and provide strong evidence for host selection. These microbial communities are present in infants³⁰, and airways seem likely to be colonized in a way similar to other body surfaces³³ during the weeks after birth.

In health, the oropharynx, the thoracic airways and the fluids from bronchoalveolar lavage (BAL; fluid washings obtained through a bronchoscope wedged into the subsegmental airways) contain similar microbiota^{10,11}, and the extent to which the lower airways are populated by aspiration from the upper respiratory tract is debated³⁴. However, in disease, the presence and abundance of pathogens in the lung are not consistently reflected in the oropharynx^{10,34}, which emphasizes that direct sampling of the lower airways and BAL are desirable in studies of the pulmonary microbiota³⁴, with strict controls for contamination during bronchoscopy¹¹ and laboratory analyses³⁵.

Pulmonary infectious agents

Pulmonary bacterial pathogens have been discovered almost entirely through microbial culture. Historically, the most important lung pathogen has been *Mycobacterium tuberculosis*. Between the 17th and 19th centuries, it accounted for one-fifth of all deaths in Europe and North America³⁶. It may lie latent for years before causing a chronic cavitating lung infection that produces highly infective sputum (FIG. 1). After a marked decline in prevalence, due primarily to improvements in public health, *M. tuberculosis* infections are now increasing in incidence, with MDR strains spreading in many populations.

Another important pathogen is *Streptococcus pneumoniae*, the most frequently detected bacterial cause of acute pneumonia, which typically presents with high fever, lobar consolidation and pleurisy (FIG. 1). In addition, *Haemophilus influenzae* infection is common in patients with smoking-related lung disease, causing bronchial inflammation with or without patchy infiltration into the surrounding lung (bronchopneumonia). *H. influenzae* is underdetected by standard clinical culture methods³⁷. Other Proteobacteria that less commonly cause acute lung infections include *Moraxella* spp. and *Neisseria* spp. Unencapsulated non-typeable *H. influenzae* (NTHI) strains are the main drivers of recurrent airway infection in COPD, together with *Moraxella catarrhalis* and *S. pneumoniae*³⁸. Asthmatic airways appear to be similarly dominated by Proteobacteria (which may be *Neisseria* spp. rather than *H. influenzae*) and Firmicutes (potentially *S. pneumoniae*)^{24–28}, although asthma-associated OTUs have yet to be defined by isolated culture.

The microorganisms described above have the ability to adhere to and invade other airway mucosal surfaces. In addition, *S. pneumoniae* and capsulated forms of *H. influenzae* and *Neisseria meningitidis* are the principal causes of bacterial meningitis, and *S. pneumoniae*, NTHI and *M. catarrhalis* also cause chronic infections of the middle ear (otitis media). All these organisms are commonly carried in the nose and oropharynx by healthy children (BOX 1), and extensive vaccination programmes are changing the prevalence of their capsulated forms. However, it is unencapsulated NTHI strains (which are difficult to vaccinate against) that drive recurrent airway infection in COPD³⁸, and colonization with these strains is also associated with wheeze in infants²⁴. In addition, unencapsulated

strains of *S. pneumoniae* are carried by approximately 10% of children and cause otitis media³⁹. Their role in respiratory disease has not been assessed. The carriage of *S. pneumoniae* and *H. influenzae* by a substantial proportion of healthy children indicates that colonization per se does not cause disease. The important factors that may cause the shift from colonization to invasion and overt disease (BOX 1) have not been extensively studied but may include concomitant viral infections, exposure to cigarette smoke and environmental pollution and progressive growth over the host microbiota⁴⁰.

Interestingly, different bacterial pathogens are associated with the chronic infections that accompany cystic fibrosis and bronchiectasis. *Pseudomonas aeruginosa*, a facultative aerobic proteobacterium that causes opportunistic infection and is associated with antibiotic resistance, is an important pathogen, as are *Burkholderia cepacia*, *Staphylococcus aureus* and nontuberculous mycobacteria (BOX 1).

Notably, the microbiota of those with chronic lung diseases can contain abundant other potential pathogens; *H. influenzae* and *S. pneumoniae* most frequently dominate the sputum and airway microbiota of both patients with cystic fibrosis and patients with bronchiectasis. Common pathogens isolated by culture from patients with HAP and VAP include *Pseudomonas* spp. and other Gram-negative bacilli, staphylococci, streptococci and *Haemophilus* spp.⁴¹. Mixed or polymicrobial infections occur in patients who are immunocompromised or suffering from chronic lung infections, posing further therapeutic challenges⁴².

In addition to bacterial pulmonary disease, viral respiratory infections are common⁴³, and their ability to spread rapidly and cause epidemics has a major global impact. Although viral infections are not the subject of this review, it should be recognized that they do not act alone. *H. influenzae* was first discovered in 1892 during an influenza epidemic⁴⁴, and bacterial pneumonia was the principal cause of death in the 1918–1919 Spanish flu pandemic⁴⁵. Viral infections often trigger exacerbations of asthma and COPD, and multiple interactions can occur between viruses and common bacterial pathogens during acute infection⁴⁶. HIV infection, which suppresses the adaptive immune system, increases the risk of pneumococcal pneumonia 20-fold⁴⁷. Mechanistically, influenza infection promotes adherence and invasion by *S. pneumoniae*⁴⁸. In addition, interactions

between rhinoviruses and *H. influenzae* augment rises in inflammatory markers and exacerbation severity in COPD⁴⁹.

Finally, fungi form symbiotic as well as pathogenic relationships with bacterial and eukaryotic communities. Some fungal organisms can cause distinct syndromes of lung infection⁵⁰. Furthermore, substantial fungal growths may be present in the microbiota of patients with cystic fibrosis and bronchiectasis⁵¹. Thus, important interactions seem likely between fungal and bacterial pathogens⁵². Invasive fungal disease associated with emergent organisms is increasing in incidence in immunocompromised hosts⁵³. In addition, fungal pathogens may be important in HAP but have been incompletely studied¹⁸. The difficulty in isolating fungi by culture almost certainly means that detection rates do not reflect the prevalence of potentially pathogenic species in clinical specimens. Fungi can cause disease by varied mechanisms. Direct invasion of immunocompromised tissues may, at times, be less important than overexuberant host responses to the presence of the fungus. For example, in allergic bronchopulmonary aspergillosis, colonization of the airways by *Aspergillus fumigatus* is accompanied by elevated blood levels of immunoglobulin E and eosinophils. Treatment of the immune response with corticosteroids is currently given priority over fungal eradication.

Current therapeutic strategies

Although the aim of this article is to suggest improvements and new directions to the management of lung infections, it is worthwhile first to summarize contemporary diagnosis and treatment of these illnesses, with an emphasis on the problems that are faced.

Current diagnosis. Bacteriological diagnosis for lung infections in most clinical laboratories depends on isolating pathogens from patient samples by microbial culture. The technology for culture on agar plates was invented in 1880 in Berlin⁵⁴. Plate culture can be effective in isolating pathogens, followed by testing for microbial sensitivities and identification by molecular techniques such as mass spectrometry⁵⁵. However, microbial culture has many subjective elements, beginning with pathogen differentiation from commensals by colony morphology. Failure to identify causal organisms occurs in 50% of patients who have been hospitalized with pneumonia in intensive care units⁵⁶, with

similar low microbial diagnostic rates in pneumonia following immunosuppressive therapy or bone marrow transplants^{57,58}. The administration of antibiotics before specimen collection is an omnipresent problem. Furthermore, the use of specialized culture media or anaerobic culture can stretch the capacity of routine clinical laboratories, and protocols may be erratically guided by clinical suspicion.

Deconvoluting the complex interactions between the microbiota and different pathogens in acute exacerbations or in the

presence of polymicrobial infections is often beyond current diagnostic capabilities, which can make it difficult to make rational clinical decisions about antimicrobial therapy.

Current treatments. Although it is universally accepted that widespread use of antimicrobial agents is causing a global crisis in antibiotic resistance⁵⁹, it has not been sufficiently recognized that antibiotic prescription for lung infections is a primary contributor to this problem^{6,8}. In 2017, the WHO listed 12 antibiotic-resistant ‘priority

pathogens’ that pose the greatest threat to human health; of these, four affect the lungs (*P. aeruginosa*, *S. pneumoniae*, *H. influenzae* and *S. aureus*).

Antibiotics for respiratory infections are usually given orally, with β -lactams often the drugs of first choice. Although the upper airway microbiota appears somewhat resilient and returns to the preantibiotic state quickly, in the gut, substantial selection for antibiotic-resistant organisms can be detected many months after treatment⁶⁰. Because early treatment for lung infections

Box 1 | The mobility and evolution of the lung microbiota

Respiratory pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria* spp. and *Moraxella* spp. are all commonly present in healthy individuals yet lead to disease in only some. They require human contact to spread, and the extent of the contact differs in many environments (see the figure). These bacteria may be carried in the nose and nasopharynx but cause disease in the lower airways and lung parenchyma. Factors that can trigger the switch between colonization and infection include the number of bacteria in vulnerable sites, synergism and nutrient competition with commensals¹²², intercurrent viral infections, cigarette smoking and air pollution. By contrast, bacteria that cause opportunistic infections of damaged lungs (for example, in patients with cystic fibrosis), such as *Pseudomonas aeruginosa* and nontuberculous mycobacteria, follow the exemplar of many enteropathogenic bacteria by having an environmental reservoir¹²³ where they can persist before infecting susceptible hosts.

The potential for viruses to mutate and spread pandemically is universally feared. Less attention has been given to transmission of bacterial and fungal pathogens, although international travel, high population densities and selective pressures for pathogenicity provide fertile grounds for such events. For example, whole-genome sequencing of *Mycobacterium tuberculosis* isolates shows that this exclusively human pathogen emerged as a chronic infection 70,000 years ago, following modern humans out of Africa and evolving traits of virulence and transmissibility to take advantage of progressive human overcrowding¹²⁴. *M. tuberculosis* sequencing can now trace where modern outbreaks arise and how they spread¹²⁵.

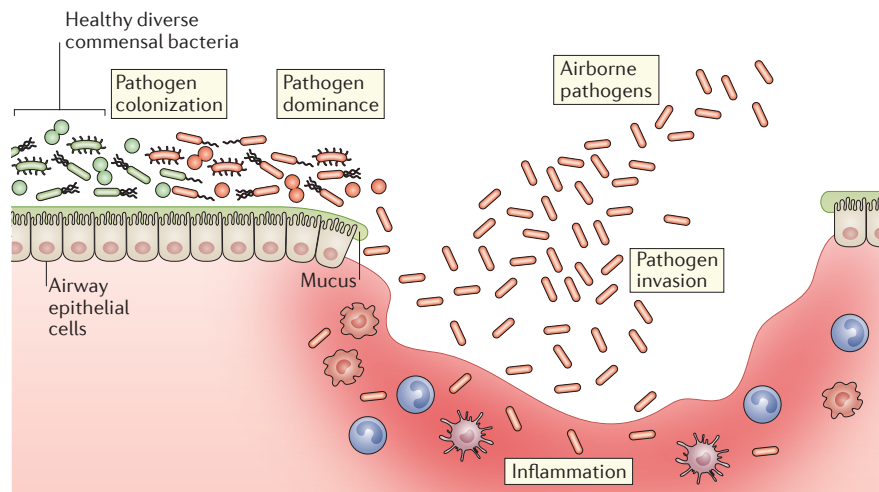
The movement of pathogens within hospitals was first shown by whole-genome sequencing during an outbreak of lung infections with carbapenem-resistant *Klebsiella pneumoniae*¹²⁶. Children with cystic fibrosis are highly prone to chronic lung infections and are thus subject to multiple antibiotic administrations, often within hospitals and their associated clinics. Patient-to-patient transmission of

virulent multidrug-resistant (MDR) *Burkholderia cepacia* was discovered in 1990 (REF. 127), before detection of transmission of MDR strains of *P. aeruginosa*¹²⁸ and *Mycobacterium abscessus*¹²⁹. Alarming, many *M. abscessus* infections are caused by dominant clones that have spread internationally¹³⁰. Knowledge of these risks to patients with cystic fibrosis has led to aggressive measures to control transmission of MDR strains in hospitals and clinics¹³¹.

The spotlight has now turned to emerging fungal pathogens in cystic fibrosis¹³², exemplified by *Scedosporium* spp.¹³³. In patients without cystic fibrosis hospitalized for a long time with lines or tubes entering their body, a recently emergent MDR *Candida auris* is

causing serious invasive infections with a mortality approaching 60%¹³⁴. *C. auris* infections were first reported in Japan, followed by reports from South Korea, India, Kuwait, South Africa, Pakistan and the United Kingdom and then South America and the United States¹³⁴.

These studies show how cosmopolitan MDR strains spread nationally and internationally in a highly connected world, giving new meaning to the ‘everything is everywhere’ hypothesis¹³⁵. The next decade promises to yield much more information about virulent strains of more mundane pathogens, such as *H. influenzae*. It is almost certain that measures to control the spread of organisms from all clinical circumstances will need to be greatly changed.



- Rural populations**
- Rich microbial environment
 - Small pathogen pool
 - Low mixing



- Cities**
- Large pathogen pool
 - Much mixing
 - Selection for AMR
 - More virulent strains in circulation



- Schools and nurseries**
- Naive immunity
 - Selection for transmission



- Hospitals and clinics**
- Intense mixing
 - Strong selection for virulence
 - Strong selection for AMR

Intensity of mixing

AMR, antimicrobial resistance.

improves outcomes, most patients are given antibiotics without a microbial diagnosis, and British Thoracic Society guidelines urge empirical antibiotic use in patients treated for CAP in primary care⁶¹ (FIG. 2). In addition to patients' toxic and allergic responses to antibiotics, antibiotic damage to resident microbial populations considerably increases susceptibility to bowel infections⁶². Similar processes are possible in the lung, and the balance between the beneficial and detrimental effects of antibiotics could profitably be investigated in COPD, cystic fibrosis and bronchiectasis.

The therapeutic strategy in patients with pneumonia of unknown aetiology is to escalate the complexity and intensity of the antibiotic regimen, often with aminoglycosides, with the associated risks of selecting for and disseminating MDR (FIG. 2). The microbiological causes of HAP and VAP vary by location, and MDR to these organisms is almost universal. Broad-spectrum antibiotic therapy for HAP and VAP is given at the onset of disease⁴¹. De-escalation of antibiotics is recommended once culture data are available⁴¹, but this is unlikely to prevent selection for antimicrobial resistance (AMR) in the gut⁶⁰.

Antibiotic prophylaxis is widely used in the management of bronchiectasis, as exemplified by children with cystic fibrosis⁶³. The isolation of *S. aureus* and *P. aeruginosa* by microbial culture is related to worse outcomes, but antibiotic prophylaxis against staphylococci has no clear benefit and may increase carriage of *P. aeruginosa*⁶⁴. Attempting an early, targeted eradication of *P. aeruginosa*, similarly, has no definite beneficial effect on mortality or morbidity⁶⁵.

Evolving therapies

Many of the mutations that confer AMR come with a fitness cost to the bacteria that carry them⁶⁶, which offers the hope that finishing a course of antibiotics may eventually be followed by reversion of the microbiota towards its pre-antibiotic state. Continued efforts to prescribe fewer antibiotics are essential, but other innovative approaches to therapy are now possible. They are summarized in FIGS 2, 3 and discussed in more detail below.

Improved diagnostics. Modern sequencing technology offers a variety of alternative diagnostic approaches to traditional culture-based methods (BOX 2). An immediate benefit of sequence-based detection of the microbiota in clinical

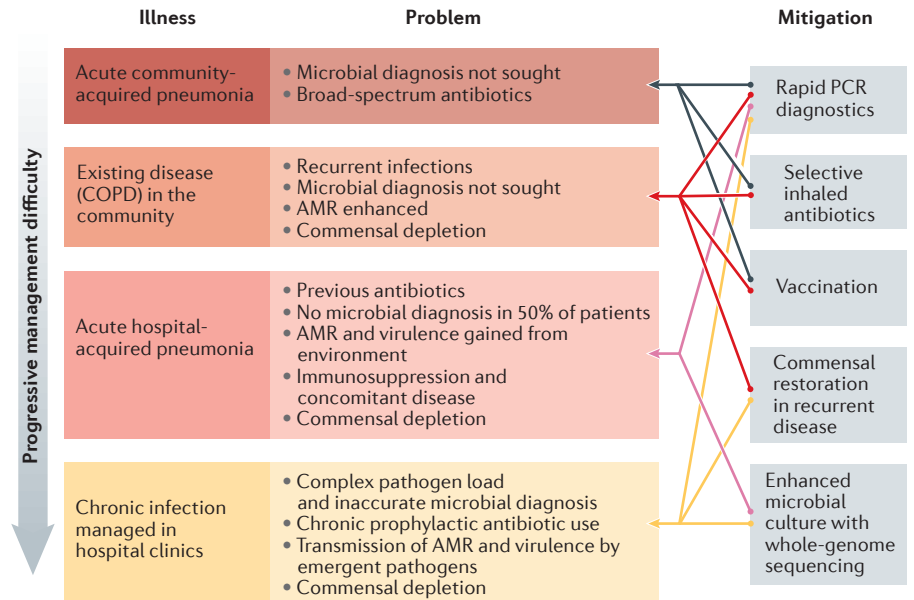


Figure 2 | **Current therapeutic strategies for lung infections.** The flow chart details problems with the current diagnosis and treatment strategies for various lung infections, from simple community-acquired pneumonia to the therapy of chronic bronchial infections. Potential generic improvements to disease management are shown on the right. AMR, antimicrobial resistance; COPD, chronic obstructive pulmonary disease.

samples is the prospect of improved accuracy of clinical diagnostics (FIG. 2). For example, 16S rRNA gene sequence analysis of DNA isolated from clinical specimens can better identify poorly described, rarely isolated or phenotypically aberrant strains. It can be routinely used for identification of mycobacteria (which are difficult to identify by culture-based methods) and can lead to the recognition of novel pathogens and bacteria that are resistant to standard clinical culture^{67–69} (BOX 2). By and large, 16S rRNA gene sequencing from bacterial isolates has been envisaged for microbial identification^{67–69}. However, 16S rRNA gene analysis of the whole bacterial community from biological specimens such as sputum and samples collected from throat swabs may provide direct information of the presence of pathogens without the need for culture (BOX 2).

In contrast to open-ended sequence analysis of 16S rRNA gene amplicons, specific quantitative PCR (qPCR) assays for common pathogens are already possible and may deliver results in a fraction of the time of standard clinical cultures. Additionally, qPCR with general primers can measure the overall number of all bacterial genomes, which can be combined with species-specific assays to interpret the relative dominance of a particular pathogen in the microbiota (as an organism accounting for 50% of the microbiota carries a different

interpretation than one accounting for 2%). Similar diagnostic possibilities exist for fungal pathogens by use of, for example, high-throughput sequencing of the amplified internal transcribed spacer 2 (ITS2) region⁷⁰. Diagnostic fungal cultures are technically difficult and currently take 2–4 weeks of incubation⁷¹, so there are considerable possible speed and accuracy benefits in switching to sequence-based methods of detection⁷².

Problems with diagnosis by the 16S rRNA gene and ITS2 sequencing derive from the accuracy of sequences in databases as well as those generated by the diagnostic laboratory and may result in an uncertain definition of species when based only on sequence information instead of on the traditional phenotypic classification of cultured microbial isolates^{67,68}. In addition, multiple copies of the 16S rRNA gene may be present and show sequence variation in some bacterial species⁶⁹. Limited 16S rRNA gene sequence variation makes discrimination difficult between different *Streptococcus* spp., including *S. pneumoniae*, which may be overcome by sequencing other polymorphic loci⁷³.

In the clinical context, rigorous control is necessary to prevent microbial nucleic acid contamination of swabs, containers of human fomites, PCR kits, sequencing kits and reagents³⁵. Mixtures of bacteria that have a different resistance to lysis⁷⁴ can be overcome

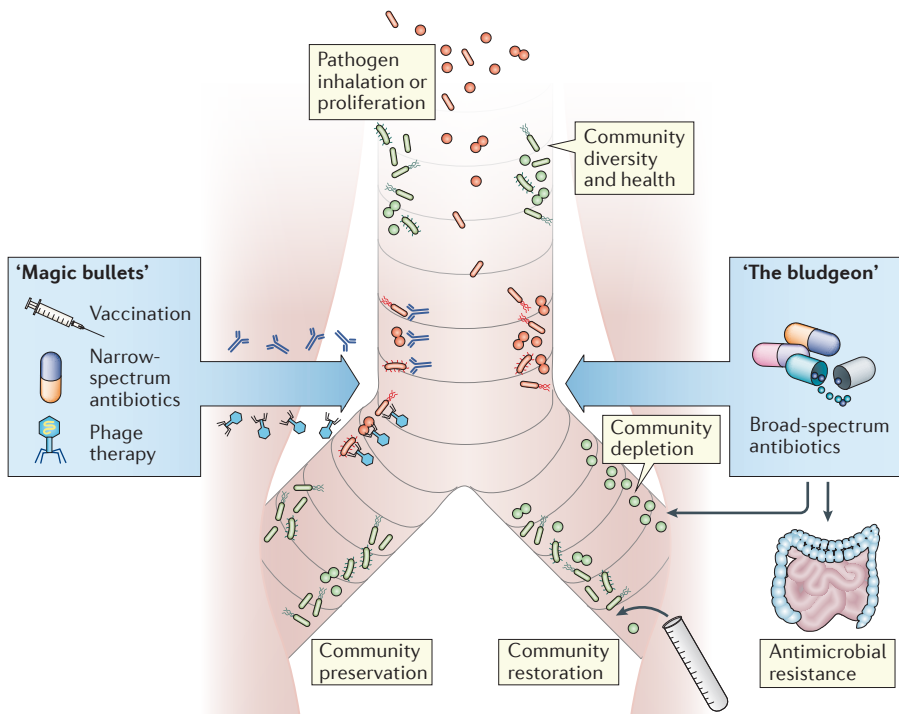


Figure 3 | New therapeutic strategies for lung infections. Healthy airways contain a diverse mixture of commensals. Lung infections with pathogens (red) are typically treated with systemic broad-spectrum antibiotics (the bludgeon) that may deplete healthy commensals (green) and leave antibiotic-resistant organisms in the bowel as well as the lung. New therapeutic approaches (magic bullets) include vaccination, inhaled narrow-spectrum antibiotics with primary effects on particular pathogens and phage therapy. Commensal community restoration may replenish a depleted microbiota and improve resistance to subsequent reinfections.

by standardized methods, such as bead beating (high-energy mixing with grinding beads), with well-understood performances for important organisms. An important consideration is that DNA sequences will not distinguish between live and quiescent microorganisms or extracellular DNA from dead microorganisms.

Other than pathogens, most of the pulmonary microbiota have not been isolated or characterized in any detail²⁹. Studies of the bowel microbiota have shown how improved culture followed by whole-genome sequencing can permit sophisticated analyses of human microbial communities in health and in disease⁷⁵. The diagnostic utility of the sequencing of strain-specific loci or of metagenomes will depend on future systematic culture and genomic sequencing of all the lung microbiota. The perception that human indigenous bacteria are largely recalcitrant to culture is inaccurate⁷⁵, and simple measures may improve isolation of organisms from the lung. Anaerobic culture, for example, has not routinely been used for pulmonary specimens, despite the high numbers of anaerobic OTUs detected by 16S RNA gene sequencing. Mucins

may actively support the presence of some commensals⁷⁶, and the addition of mucins to culture media may provide essential nutrients for colony growth.

Whole-genome sequencing of bacterial isolates has numerous applications to the problem of antibiotic resistance, including the development of novel antibiotics and diagnostic tests, the stewardship of available antibiotics to prevent AMR and the elucidation of factors that generate and perpetuate resistance^{77,78}. Proof-of-principle studies for the value of whole-genome sequencing have come from investigations of *M. tuberculosis*^{79,80} and *P. aeruginosa* infections⁸¹. This systematic approach is easily extended to other pathogens such as *S. pneumoniae* and *Haemophilus* spp. isolated from patients with lung infections. A publicly accessible culture collection for the lung microbiota will encourage the development of model systems to investigate interactions between hosts, pathogens and commensals with straightforward replication of important results.

Metagenomic shotgun sequencing of bowel samples has delivered a mass of information about microbial processes

in health and disease. Sputum, lung biopsy samples and brushings, unlike stool samples, are problematic for microbial metagenomics because the ratio of human to microbial DNA may be higher than 99:1. Strategies to enrich for microbial DNA before making sequencing libraries are technologically possible⁸² but not yet effective in practice. Promisingly, next-generation sequencing of RNA from BAL samples has proved effective in diagnosing viral and bacterial infections⁸³.

Improved antibiotic regimens. The emergence of AMR in pathogens shares many factors in common with the emergence of drug-resistant malignant cells during chemotherapy for cancer. The management of AMR may be improved by strategies that are similar to those used for cancer chemotherapy, where the quantification of resistance mechanisms has been followed by specific countertherapies⁸⁴. This approach has been exemplified by the development of β -lactamase inhibitors to counter penicillin resistance.

Extraordinarily, the experimental and theoretical basis that underlies antibiotic dosage regimens is limited and the results contentious⁸⁵. High-dose antimicrobial chemotherapy has long been considered the best means of controlling drug resistance, but recent studies have shown that this can encourage the appearance of resistant strains at the same time as it releases them from ecological competition⁸⁶. The effectiveness of the common use of combinations of antibiotics has also been questioned, and it has been suggested that in many circumstances, such combinations will actually increase pathogen load⁸⁷. These findings, and the rising tide of AMR, indicate a need for an experimental re-examination of antibiotic regimens in common clinical states. To help this process, elements important in antibiotic resistance are increasingly accessible through genomic sequencing of pathogen isolates and also from partial-genome metagenomic sequence data⁸⁸, which allow a better anticipation of the response to antibiotics. Microbial sequencing can also be used to make decisions about undesirable consequences of antibiotic treatment of (as yet unidentified) keystone commensal organisms as well as about AMR emergence.

Inhaled antibiotics. The administration of oral antibiotics can result in long-lasting adverse alteration of gut microbial

communities⁶⁰, which suggests that benefits will follow from reducing or avoiding exposure of gut organisms to antimicrobials (FIG. 3). Inhalations have been used medically for two millennia, and a wide range of aerosolized medications are now in use or in development for many diseases⁸⁹. The pulmonary delivery of antibiotics (often as powders) for lung infections has shown success, with reduced dosing required and reductions in side effects⁹⁰. Aerosolized (inhaled) antibiotics may be more effective than their systemic counterparts in treating pneumonia⁹¹. When used to treat persistent airway infection in cystic fibrosis, inhaled antibiotics appear more effective than oral or parenteral therapy⁹². It has been suggested that inhaled antibiotics do not promote AMR, and a meta-analysis of studies in cystic fibrosis did not observe significant development of antibiotic-resistant organisms^{90,91}.

A limited range of antibiotics is currently marketed for inhalation and approved for use in cystic fibrosis by the FDA and the European Medicines Agency⁹³. These include tobramycin, colistin and nebulized aztreonam⁹³. Although not approved for other diseases, injectable formulations of gentamicin, tobramycin, amikacin, ceftazidime and amphotericin are currently nebulized 'off-label' to manage non-cystic fibrosis bronchiectasis, drug-resistant nontuberculous mycobacterial infections, VAP and airway infections occurring after transplant⁹³.

The advantages of inhaled therapy encourage a search, beyond the treatment of chronic infections in cystic fibrosis⁹³, for novel antibiotics with chemical characteristics that encourage retention in the lung (FIG. 3). A more selective antibiotic specificity (narrow spectrum) towards major pathogens such as *H. influenzae* is also desirable (FIG. 2). Patients with COPD who suffer frequent lower respiratory tract infections, which contribute enormously to AMR in the community⁸, would be an ideal group for the comparison of inhaled and systemic antibiotic therapies.

Phage therapy. In 1917, bacteriophages were discovered to cause epizootic infections of bacteria and were used for antibacterial therapy and prophylaxis⁹⁴. However, a poor understanding of their biology and the advent of antibiotics curtailed research after 1945 (REF. 94). The early literature suggests that phage therapy is very effective⁹⁴, and there has been a modern renewal of interest in response to

Box 2 | Complex clinical uses of microbial community analyses

DNA and RNA sequence analysis of specimens from the lung may be of direct clinical benefit to the management of lung infections. However, there are obstacles yet to be overcome.

- Simple PCR assays can be used to quantify known pathogens. However, not enough is yet known about many of the unencapsulated Proteobacteria, including non-typeable *Haemophilus influenzae* and *Neisseria* spp. that dominate in chronic obstructive pulmonary disease and asthma. The systematic isolation and sequencing of these organisms is desirable for the successful development of rapid diagnostic assays.
- In contrast to culture techniques, a simple presence or absence of a particular bacterial operational taxonomic unit may not be sufficient to guide therapy. Absolute counts (by quantitative PCR or digital PCR) will be more helpful. Criteria are needed to differentiate between asymptomatic carriage of common pathogens and the presence of disease. Concomitant measure of the total bacterial load can be used to estimate the degree of pathogen dominance.
- Sequence analysis also allows consideration of synergistic effects between members of a microbial community, avoiding the misleading assumption of 'one pathogen, one disease' (REF. 136).
- It will be desirable to develop measures of the health of a patient's microbiome, identify optimal patterns of diversity and resilience and establish the presence or absence of keystone organisms associated with health. These parameters will permit public health promotion of infection resistance in otherwise susceptible individuals and environments.
- The effects of antibiotics will be assessed by their impact on whole microbial communities so that antimicrobial actions may be balanced between commensals and pathogens.
- Mixed infections are common, particularly in the presence of underlying lung disease. The identification of all pathogens in an infection and a knowledge of pathogen synergies will improve treatments.
- Metagenomic transcriptomic analysis (RNA) of bacterial gene expression may identify targets for therapy in particular infections. Metagenomic analysis (DNA) of lung samples is currently severely hampered by high concentrations of human DNA. Single-cell transcriptomics of bacteria purified from samples may be helpful.
- Antibiotic sensitivities can currently be predicted by sequence analysis of bacterial isolates. The application of this technique to complex microbial mixtures may give a measure of general community resistance. Pathogen-specific signatures may be discerned by unique sequences within the community. Alternatively, single-cell sequencing may identify the antimicrobial resistance profile of individual organisms.
- Epidemiological surveys of the transmission and community carriage of common respiratory pathogens, such as non-typeable *H. influenzae*, will underpin strategies to reduce transmission and prevent colonization.

increasing AMR⁹⁵. Phages can be modified genetically to induce desirable biological and therapeutic properties⁹⁶. Importantly, phage infection leads to only transient changes in the microbial communities that harbour targeted pathogens⁹⁷. Despite these advantages, phage therapy is not proving easy to develop within the stringent regulatory requirements for new treatments⁹⁸. Nevertheless, it has shown encouraging results for the treatment of *P. aeruginosa* infections in patients with cystic fibrosis^{99,100} and has major potential for targeted treatment of more common pathogens, such as *H. influenzae* (FIG. 3).

Restoring bacterial communities. Manipulation of the microbiota confers colonization resistance to intestinal infections^{101–103}, and faecal microbiota transplantation is markedly effective in treating the severe illnesses associated with persistent *Clostridium difficile* infection¹⁰⁴

and inflammatory bowel disease¹⁰⁵. There is good reason to consider similar possibilities in the lungs (FIG. 3).

Disordered microbial communities that have a loss of commensal diversity are a feature of asthma^{10,106}, which may be a reasonable pulmonary target for interventional trials. Community restoration might also be of benefit after long-term antibiotic use in children with cystic fibrosis or primary ciliary dyskinesia or in adults with COPD. However, in contrast to faecal microbiota transplantation, healthy individuals do not spontaneously provide airway microbiota in a form suitable for transplantation; in addition, little is known about the characteristics of lower-airway commensals, and pulmonary pathogens commonly colonize the airways without causing disease.

A structured approach to identifying bowel protective organisms has been exemplified for *C. difficile* infections¹⁰².

Similar strategies for the airway microbiota may identify the minimum number of commensal organisms that might provide protection against colonization with pathogenic Proteobacteria and *Streptococcus* spp. or could be used to replace a microbial community with particularly high levels of AMR. The organisms to be used may vary for different diseases and different individuals. The dose of bacteria as well as the species to be introduced is an important consideration. In an analogy of restoring a garden, therapy with antimicrobials to remove pathogens (weeds) before treatment may be helpful. As with other novel therapies, an ethical framework will need to be established around early-stage clinical trials to examine safety before therapies can be explored.

Vaccination. The potential for vaccination to have an impact on common lung infections (FIG. 3) is exemplified by invasive infections with *S. pneumoniae*¹⁰⁷. Although vaccination in the first years of life does not confer long-lasting immunity¹⁰⁸, prevention of invasive disease by *S. pneumoniae* with AMR is achievable¹⁰⁹. The rate of antibiotic-resistant, invasive pneumococcal infections has decreased in young children and older adults with the widespread use of pneumococcal conjugate vaccines¹¹⁰, and vaccination is effective in preventing pneumonia in children and adults following splenectomy¹¹¹.

Respiratory infections caused by NTHI result in enormous global morbidity³⁸. Effective *H. influenzae* vaccines have been directed against capsular antigens and are given routinely in childhood for meningitis and otitis media in most Western societies. However, the unencapsulated NTHI strains that commonly cause lung diseases are not influenced by current vaccines^{112–114}. A vaccine against NTHI would prevent disease and reduce antibiotic use⁸, most clearly in the context of COPD exacerbations^{115,116} but also in CAP, cystic fibrosis and chronic bronchitis. An ideal vaccine antigen should be conserved among strains, have abundant epitopes on the bacterial surface and induce protective immune responses⁸. A conjugate vaccine against protein D (a highly conserved and stable protein in the outer membrane of encapsulated and unencapsulated *H. influenzae* strains), although only partially effective, has demonstrated the feasibility of vaccination for NTHI^{8,114,117}.

Characterization of the asthmatic airway microbiome is at an early stage, but there is a consensus that there is an overgrowth

of *Neisseria* spp. and *Moraxella* spp. as well as of *Haemophilus* spp. and *Streptococcus* spp.^{10,28,30,118,119}. In addition, *S. pneumoniae* has a high reported carriage rate (45%) in children with asthma¹⁰⁸. Should these bacteria be shown definitively to be causing airway inflammation, the success of vaccination against such organisms leads to the intriguing possibility of eventual vaccination to protect against childhood asthma. Inherent in a vaccination strategy would be the recognition that many of the organisms associated with asthma and COPD are likely to be unencapsulated strains. This further emphasizes the need for a concerted attempt to isolate and sequence the lower-airway microbiota.

Vaccines against *P. aeruginosa* are not effective in preventing infections in patients with cystic fibrosis¹²⁰, perhaps suggesting that vaccinations have less value in patients with infections that flourish in luminal secretions, which may be at a distance from mucosal defence mechanisms.

A better understanding of the population distributions of potentially pathogenic Proteobacteria as well as *H. influenzae* and *S. pneumoniae* in the healthy population, as well as in patients with asthma and patients with COPD, is important to help develop rational vaccination strategies. Effective vaccination is likely to be followed by the evolution of population serotypes, which would impart a need for continuous vaccine development¹²¹. However, the global numbers of children and adults at risk suggest that the effort would be worthwhile.

Conclusions

Lung infections have enormous effects on human health, but current treatments are imperfect and fuel a global engine for AMR. Genome sequencing is fundamentally changing our understanding of the airway microbiome in health and disease, not least by revealing the profound pressures that drive the evolution and spread of pathogenic microorganisms. Genomic studies of opportunistic pathogens that accompany cystic fibrosis show how easily hyper-resistant and virulent clones can disseminate from clinic to clinic across the world. These studies provide an exemplar for investigation of the epidemiological suspicion that the evolution and transmission of apparently mundane pathogens may be common. Sequence-based techniques are ready to transform the microbial diagnosis of common lung infections and are the first priority for improving management

of and minimizing AMR. A systematic understanding of the healthy pulmonary microbiota is a necessary platform from which to build new therapies that may include novel inhaled antibiotics, phages, vaccination and techniques to restore healthy microbial communities.

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Author contributions

W.O.C.M.C. carried out research, wrote the article and contributed to discussions, review and editing of the manuscript. M.J.C. and M.F.M. carried out research and contributed to discussions, review and editing of the manuscript. The authors gratefully acknowledge the considerable input of two anonymous reviewers.

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Inserting proteins into the bacterial cytoplasmic membrane using the Sec and YidC translocases

Kun Xie and Ross E. Dalbey

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CORRIGENDUM

Archaea and the origin of eukaryotes

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On pages 714–715 of this article, in the first paragraph of the section *What do we currently know about LECA?*, the sentence “Phylogenomic and comparative genomic analyses have led to the hypothesis that LECA, estimated to have lived ~1–1.9 billion years ago⁵⁴, already was a fully fledged eukaryote and possessed a large number of features that are uniquely found in modern eukaryotes^{55,56}.” should have read “Phylogenomic and comparative genomic analyses have led to the hypothesis that LECA, estimated to have lived ~1–1.9 billion years ago⁵⁴, already was a fully fledged eukaryote and possessed a large number of features that are uniquely found in modern eukaryotes^{55,56}.” This has been corrected in the online version of the article. The authors apologize to the readers for any misunderstanding caused.