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19 Respiratory Viruses and Atypical Bacteria

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KEY POINTS

- Viral and atypical pathogens can cause most clinical manifestations of respiratory disease, but some are associated with particular clinical conditions.
- Clinical disease from the common cold to severe lower respiratory tract infection with systemic dissemination.
- Pathogens generally have a global distribution but prevalence and disease burden often vary seasonally.
- Spectrum of pathogens from established endemic human-adapted viruses to emerging highly pathogenic viruses from animal reservoirs with pandemic threat.
- Severe disease can occur in all age groups but young children, the elderly and the immunocompromised are generally more at risk for severe disease.
- Bacterial and viral co-infection is increasingly being recognized as we test for more pathogens.
- There is a real clinical need to develop vaccines against the commonest viral and atypical bacterial pathogens.

Introduction

Acute respiratory infections are the most frequently occurring illnesses in all age groups globally. Although infections are usually limited to the upper respiratory tract and generally cause mild, self-limiting illnesses, a small percentage progress to the lower respiratory tract, where they can cause potentially life-threatening conditions, such as bronchiolitis and pneumonia.

Annually, 450 million cases of pneumonia are recorded, of whom 4.2 million die (7% of the world's yearly total annual deaths). Young children and the elderly are at particular risk, especially in developing countries: 151 million of 156 million reported episodes of childhood pneumonia are in the developing world. Pneumonia is responsible for about 17% of all deaths in children aged less than 5 years, of which more than 70% occur in sub-Saharan Africa and South-east Asia. In 2008, 1.6 million children under 5 died of pneumonia.[1–3]

Pneumonia is also a major cause of morbidity and mortality at the other end of the age spectrum. The annual incidence of pneumonia in elderly, non-institutionalized patients is between 25 and 44 per 1000 population; up to four times that of patients younger than 65.[4]

The most important aetiological agents of severe lower respiratory illness include bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type b, and viruses such as respiratory syncytial virus and influenza virus. Bacteria are the main causes of pneumonia, and generally have a higher case fatality rate, although this may change with the widespread introduction of *H. influenzae* type b and pneumococcal conjugate vaccines, which could lead to viruses becoming much more prominent causes of respiratory disease. Viruses are the predominant cause of bronchiolitis and exacerbations of asthma and episodic viral wheeze. These clinical syndromes overlap considerably and are often difficult to tell apart.

The clinical manifestations of infection depend not only on the particular agent but also on the individual patient. Pre-existing structural changes to the respiratory tract caused by congenital malformations or damage from previous episodes of infection or trauma, as well as the circumstances of the individual (immunocompromise, malnutrition, poverty, overcrowding, sanitation, pollution, etc.), profoundly affect outcome.

Aetiology

Confirming the presence of a virus will often identify the cause of an illness, although multiple infections can and do occur and there is evidence of viral shedding in healthy individuals post-infection or asymptomatically. In this section, the commonest viruses associated with acute respiratory infection are described. Also mentioned are some organisms found in the respiratory tracts of clinically normal individuals (especially children), for which clinical relevance is as yet unclear, and likewise those recently discovered viruses that have no associated clinical syndrome.

DNA VIRUSES

Adenoviruses

There are 52 different serotypes of human adenovirus, belonging to six different species (A–F). Only about one-third of these are associated with symptomatic illness: species B, C and E are associated with respiratory tract infections, whereas species D causes large epidemics of infective conjunctivitis and species F (serotype 41–42) is associated with infectious diarrhoea. Infections with species B serotypes 3, 7 and 21 are associated with bronchiolitis obliterans, a post-infectious condition characterized by persistent airways obstruction secondary to peribronchiolar fibrosis.[5]

Adenovirus infections are common, have a worldwide distribution and occur throughout the year. Respiratory infections are frequent during childhood, tend to be self-limiting and induce serotype-specific immunity. Outbreaks of adenoviral respiratory infections can occur in closed communities such as day-care centres, boarding schools and especially, among military recruits.[6] Adenoviruses are unusual in that prolonged asymptomatic carriage (up to 2 years in some cases) may occur in the tonsils of children. Thus, the clinical significance of adenoviruses isolated from the throats of children must be interpreted with caution.

Bocavirus

Human bocavirus, a member of the *Parvoviridae* family, was discovered in 2005 in nasopharyngeal aspirates from children with respiratory tract infections.[7] Although suspected, establishing its role as a respiratory pathogen has been difficult for several reasons. First, human bocavirus is not related to any known human respiratory pathogen. Second, it is commonly detected with other respiratory viruses, which have established pathogenic potential. Third, detection may simply reflect asymptomatic persistence or prolonged viral shedding, since other human parvoviruses also show this capacity. It has been suggested that human bocavirus may be reactivated or produce a transient asymptomatic super-infection triggered by the presence of another replicating respiratory agent.

Several studies have shown an association between human bocavirus detection and acute respiratory symptoms, and viral DNA has been detected in the blood of children with both respiratory symptoms and human bocavirus in respiratory specimens. However, definitively establishing a causal relationship will require further study.[8]

RNA VIRUSES

Orthomyxoviridae – Influenza

The influenza viruses, especially influenza virus A, are the most variable of the respiratory viruses. Their pandemic potential and the unpredictability of their emergence is a continuous cause for concern.

There are three genera: influenza virus A, B and C. Influenza virus C only rarely causes (mild) disease in humans and will not be further discussed. Influenza A viruses are subtyped based on their two surface antigens: hemagglutinin (HA; H1–H16) and neuraminidase (NA; N1–N9), which are responsible for host receptor binding/cell entry and cleavage of the HA-receptor complex to release newly formed viruses, respectively. Key amino acids in these proteins, especially in HA, are associated with host specificity and transmissibility.

Aquatic birds are the natural reservoir of influenza A viruses, harbouring all possible subtypes. A selection of subtypes has established endemicity among a range of land and water mammals (e.g. humans, pigs, horses, seals). Currently, H3N2 and H1N1 are endemic among humans. At the time of writing, circulating H1N1 is the lineage that caused a pandemic of mild influenza in 2009 (see below), called H1N1-pdm09. Influenza virus B is almost exclusively a human pathogen also causing yearly seasonal epidemics, with rare reports of infection in dogs, cats, swine and seals.

Both influenza virus A and B exhibit 'antigenic drift'. This phenomenon occurs when the surface antigens of the virus gradually change, progressively and directionally, to escape immunological pressure from the host species. Yearly epidemics of influenza virus A and B are caused worldwide by these drift variants, and contribute to mortality (an estimated 250–500 000 every year) in the elderly, and in those with pre-existing conditions such as chronic cardiopulmonary or renal disease, diabetes, immunosuppression or severe anaemia ('acute on chronic').

New lineages of influenza virus A emerge every few decades through re-assortment of gene segments in animal hosts infected with two different viruses ('antigenic shift') resulting in global pandemics with varying severity due to the absence of immunity in the human population (1918 Spanish flu: H1N1, 40–100 million deaths; 1957 Asian flu: H2N2, 2 million deaths; 1968 Hong Kong flu: H3N2, 500 000 deaths; 2009 H1N1-pdm09, 15 000 deaths).[9] After such an introduction, the new virus usually becomes the dominant circulating lineage of influenza A. One exception was the pandemic of mild influenza among patients under 20 following the reintroduction (after 20 years of absence when H2N2 became dominant) of H1N1 in the human population in 1977; possibly caused by an escape from a research laboratory. Another exception was the introduction in 2009 of a novel lineage of H1N1 influenza virus A, most likely from pigs, in North America, causing a pandemic of relatively mild influenza (with an estimated 15 000 deaths) and generating massive attention from both the public health communities and the general public, and replacing (only) the 1977 H1N1 lineage. Between the 2009 pandemic and the time of writing, H1N1-pdm09 and H3N2 influenza virus A have been co-circulating with influenza B viruses and continue to cause yearly seasonal epidemics worldwide. The timing, extent and direction of either 'drift' or 'shift' have so far been completely unpredictable. With no animal reservoirs to provide such new antigens, shift does not occur in influenza B and thus major epidemics do not occur.

Sporadic dead-end human infections of animal, especially avian, viruses are known to occur and have caused concern regarding pandemic potential. Seal H7N3 and avian H7N7 and H9N2 viruses have caused conjunctivitis and – mostly – mild influenza-like illness in patients in close contact with infected seals or birds.[10] In 2011 there were reports of sporadic human-to-human transmission of porcine H3N2 viruses causing mild influenza-like illness.[11] In contrast, H5N1 avian influenza viruses have consistently caused severe human respiratory illness, in Asia and North Africa, with a mortality of over 50% (Figure 19.1). Highly pathogenic H5N1 viruses were first detected in birds in 1996 in China. Transmission to 18 humans occurred in Hong Kong, six of which were fatal. During the next 6 years, no human or animal cases were recorded. In 2003, the virus re-emerged in China. Since then it has become panzootic among poultry and wild birds and, at the time of writing, has caused 573 sporadic infections (336 fatal) in humans,[12] most of whom reported close contact with wild birds or domestic poultry. The disease presents as a rapidly progressive viral pneumonia with

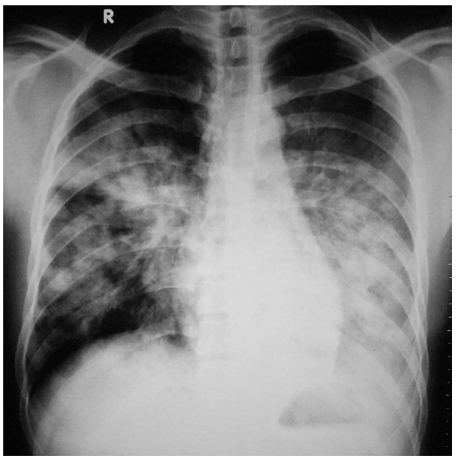

Figure 19.1 A chest X-ray of a 32-year-old man admitted to a Vietnamese hospital on Day 5 of his illness with pneumonia caused by H5N1 avian influenza. Respiratory support on intensive care was initially needed but the patient responded well to oseltamivir.

severe leucopenia and lymphopenia, progressing to ARDS (acute respiratory distress syndrome) and multi-organ dysfunction that fail to respond to standard antibiotic therapy for pneumonia. Early diagnosis and treatment with oseltamivir are associated with a better prognosis.

Despite ferret-transmission models showing that only five mutations are required to enable transmission of virulent virus,[13] their worldwide presence for many years, the huge human–animal interface in Asia and suspected small-scale human-to-human transmission in family clusters, no efficient or sustained human-to-human transmission of H5N1 viruses has yet been recorded.[14]

At the time of writing (April 2013), another avian influenza virus (H7N9) is causing - as yet only - sporadic zoonotic transmission events to humans in China, with no recorded sustained human-to-human transmission. In contrast to H5N1 this virus does not cause disease in wild or domestic birds, making it more difficult to contain. Over a period of 2 months more than 100 cases have been identified. The case fatality rate is around 20 % and elderly people are most affected. This again highlights the unpredictability of these events and the continuously changing threats posed by influenza A viruses. The continued zoonotic transmission of these avian viruses does not imply that they will inevitably lead to another pandemic. However, the unusual severity of H5N1 and H7N9 disease in humans is a continuous cause for concern, because one cannot assume that the acquisition of human-to-human transmissibility (if ever) will be associated with a loss of virulence (as is usual). Irrespective of whether or not this pandemic threat becomes reality, it is clear that avian H5N1 (and H7N9) viruses have already had a significant impact on the global poultry industry and on human economic and social wellbeing and thus ultimately on human health.

Picornaviridae

Rhinoviruses. Virology and clinical textbooks and virtually all web-based information sources describe the 99 serotypes of human rhinovirus (HRVs) as the most frequent cause of the common cold, in both the developed and the developing world. Although the common cold is considered a trivial illness, it is an important disease worldwide in terms of morbidity and economic impact.

In addition to causing the common cold, there is now convincing evidence that HRVs play a significant role in causing lower respiratory symptoms. HRVs can replicate in the lower airways and do appear to play a critical role in causing exacerbations of asthma and other chronic lung diseases. They can also drive the infant immune system towards the asthmatic phenotype, and cause episodes of bronchiolitis and pneumonia that require hospitalization.[15]

Until a few years ago, only two groups of HRVs (A and B) were recognized, but sequencing of HRVs led to the discovery of a third species (HRV-C) in 2006, with distinct structural, biological and possibly also clinical features.[16,17]

Other *Picornaviridae.* The over 100 serotypes of enterovirus (coxsackie A and B viruses, echoviruses and enteroviruses 68–71) are mainly transmitted by the oral–faecal route but can be transmitted by respiratory droplets. Enteroviruses are a major cause of aseptic meningitis in children and adults, but are also associated with common cold, herpangina in children and large epidemics of acute haemorrhagic conjunctivitis and hand, foot and mouth disease. Similarly, parechoviruses are another common cause of aseptic meningitis, but are also implicated as frequent causes of (mild) respiratory illness.

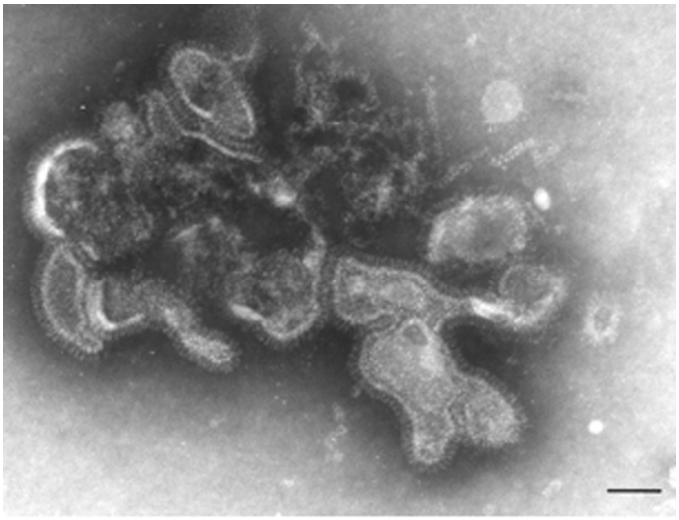

Figure 19.2 Negative stain electron micrograph of human respiratory syncytial virus (bar = 100 nm). *(Original image kindly provided by Professor CA Hart; reproduced from original in British Medical Bulletin; McNamara PS, Smyth RL. The pathogenesis of respiratory syncytial virus disease in childhood. Br Med Bull 2002;61(1):13–28.)*

Paramyxoviridae

Respiratory Syncytial Virus (RSV). This virus is distributed worldwide and is found wherever it has been sought. It is the leading cause of bronchiolitis and the most commonly detected virus in children under 2 years of age hospitalized for lower respiratory infection (Figure 19.2). It is estimated that half of all children are infected during the first year of life, and that by 3 years of age all have experienced at least one infection. Immunity following primary infection does not prevent secondary or subsequent infections, caused by both antigenic differences and failure of RSV to induce (persistent) neutralizing antibodies. In temperate regions, large seasonal epidemics occur annually over cold winter months, but this seasonality is more variable in the tropics (see below). Two subtypes (A and B) have been described and may co-circulate, with one usually predominating in any given year. No obvious differences in disease severity or pathogenesis have been documented between these two subtypes.[18,19]

RSV causes a substantial but variable LRTI disease burden in tropical countries. In a population-based study of infants in Kenya, it was found that RSV was common; approximately 36% of infections led to LTRI, 23% were severe and 3% of infected children were hospitalized.[20] RSV was also the most commonly detected respiratory pathogen in hospitalized children in Vietnam.[21] More recently, it has become clear that RSV causes significant morbidity in the elderly as well as in infants.[22]

Human Metapneumovirus (hMPV). This virus was discovered in 2001.[23] The disease it causes, its distribution and seasonality are very similar to those of RSV. It also has two subtypes and causes infections in children under 2 years old and in the elderly. Retrospective serology, has shown that this is not a new human pathogen, but has been around for a long time.[24]

Parainfluenza Viruses (PIV). There are four species of parainfluenza virus: PIV1–4. PIV1–3 are major causes of lower respiratory tract infections in infants, young children, the immuno-compromised, the chronically ill, and the elderly. PIV1 and 2 typically cause alternating biennial fall epidemics of croup, a high-pitched barking cough in children 2–4 years of age. PIV3 infections occur mainly in the first two years of life. Bronchiolitis and pneumonia are the most common clinical presentations. Only RSV causes more lower respiratory tract infections in neonates and young infants. Both in temperate and tropical regions, there is dissociation between the peaks of activity of PIV3 and RSV/hMPV. Host defence against PIV is mediated largely by humoral immunity to the two surface antigens, but repeated infections are often needed before protection develops.[25]

Rubeola Virus – Measles. There are a number of reasons why measles is often not recognized as a major cause of lower respiratory tract infections. Children with measles may not always be admitted to a general paediatric ward, the aetiology may be attributed to a super infecting pathogen rather than to measles, and some patients with measles (especially when immunocompromised) will fail to develop the typical rash. In patients who do not manifest the typical clinical features, clinical diagnosis of measles is difficult and specific laboratory diagnostics will most likely not be requested and performed. Where the diagnosis has been actively sought in developing countries, measles was found to be a major cause of lower respiratory tract infection, accounting for 6–21% of its morbidity and 8–50% of its mortality.[26] Radiographic evidence of pneumonia is common, also in clinically uncomplicated measles. The effects of the virus on the respiratory tract can be direct (giant cell pneumonitis), in any part of the respiratory tract, or indirect. The latter includes the suppressive effects of the virus on the host immune system, stores of vitamin A and overall nutritional status. All of these may lead to an increased risk of super-infection with other (viral or bacterial) pathogens. Measles pneumonitis can be especially severe in immunocompromised patients.[27]

Nipah Virus. Human Nipah virus infection was first recognized in a large outbreak of 276 reported cases in peninsular Malaysia and Singapore from September 1998 through May 1999. Most patients had contact with sick pigs. Patients presented primarily with encephalitis; 39% died. Large fruit bats of the genus *Pteropus* are the natural reservoir of Nipah virus.

In the 10 years following, no further human cases were noted in Malaysia, but annual human outbreaks have been reported in Bangladesh from May to December. The clinical presentation is dominated by respiratory symptoms and the case fatality has been over 70%.

The most frequently implicated route of infection is ingestion of fresh date palm sap. Date palm sap is harvested from December through March, particularly in west central Bangladesh. A tap is cut into the tree trunk and sap flows slowly overnight into an open clay pot. Infrared camera studies have confirmed that *Pteropus giganteus* bats frequently visit the trees, lick the sap during collection, thus transmitting infection. Humans can also become infected through direct contact with bat secretions, contact with domestic animals that become infected by eating partially eaten bat-saliva-laden fruit or infected date palm sap, or by human-to-human transmission through infected saliva.[28]

Coronaviridae

Coronaviruses. Human coronavirus (HCoV) strains 229E and OC43 have been long recognized as the second main cause of the common cold (10–25%). More recently, two other viruses associated with similar presentation were detected in humans: NL63 and HKU1. These four viruses are ubiquitous and regularly detected in respiratory specimens of a small proportion (1–10%) of children hospitalized with acute respiratory disease in many parts of the world. Infection with these human coronaviruses may present as an upper respiratory tract infection, asthma exacerbation, acute bronchiolitis, pneumonia, febrile seizures and also as croup (especially NL63). Reinfection is common due to rapidly decreasing antibody levels.

SARS-Coronavirus. In 2002–2003, a novel severe form of pneumonia of unknown aetiology emerged in Guangdong, China and was named severe acute respiratory syndrome (SARS). After smouldering for several months, the disease then spread to Hong Kong and rapidly across the world, facilitated by international air travel and a few so-called 'super-spreaders', with most notable outbreaks in Hong Kong and Toronto, Canada. Of affected cases, 21% were healthcare workers.

The rapidly identified culprit, SARS coronavirus, is thought to have jumped to humans in live animal markets in Guangdong. The precursor virus is present in wild *Rhinolophus* bats.[29] Civet cats and other small mammals sold as delicacies in wet markets provided a reservoir and amplifier for the virus and the opportunity for adaptation to humans.

The epidemic of SARS with 8096 cases and 744 deaths in 29 countries across five continents started in November 2002 and came to an end in July 2003. Few sporadic community- and laboratory-acquired infections including limited person-to-person transmission have been recorded since.[30]

SARS was characterized by fever and myalgia rapidly progressing to a respiratory syndrome of cough, dyspnoea followed by acute respiratory distress syndrome. Mortality was significantly lower in children.

SARS is primarily spread by the respiratory route, but oral–faecal transmission has also been implicated. Why the SARS epidemic did not continue to spread is subject to much speculation. Explanations may include the fact that SARS is most infectious in a later stage of infection, allowing for timely containment, and an extraordinary worldwide public health effort to control spread.[31]

With the new interest in coronaviruses, more and more closely related coronaviruses from distantly related animals have been discovered, many of which were the result of recent interspecies jumping. Coronaviruses are implicated as a likely candidate for future outbreaks of zoonotic diseases, and - indeed - a novel coronavirus (EMC) is currently associated with sporadic transmission to humans in the middle-east.

New World Hantaviruses

Hantavirus pulmonary syndrome (HPS) is a rare but important cause of severe respiratory illness in the North and South American continents. It was first recognized in May 1993 during an outbreak of severe, and frequently fatal, respiratory disease in the four corners region of the USA, where the four states Arizona, Colorado, New Mexico and Utah abut.[32] The causative agent was found to be a Hantavirus and was later named *Sin Nombre* virus. The natural host was found to be the deer mouse,

Peromyscus maniculatus, the local population of which had recently increased rapidly. The Hantaviruses are transmitted to humans by inhalation of aerosolized dried excreta. Closely related viruses have since been isolated in North (e.g. New York, Bayou, Black Creek Canal viruses) and South (e.g. Andes virus) Americas, with different rodent hosts but all associated with HPS. These viruses all belong to the same Hantavirus genus as those causing haemorrhagic fever with renal syndrome (HFRS) in the Old World: Hantaan, Seoul and Puumala viruses. Both HFRS and HPS have a similar febrile prodrome with thrombocytopenia and leucocytosis. In HPS, the key differences are that the capillary leakage which follows is localized to the lungs and that, with *Sin Nombre* virus, renal dysfunction is minimal. There was no evidence of human-to-human transmission in this outbreak, but there is evidence that some of the South American Hantaviruses causing HPS may be transmitted between humans in a nosocomial setting.[33]

Herpesviruses

Varicella pneumonitis can occur as a severe complication of chickenpox or in the absence of classical symptoms. It occurs more commonly in adults, an estimated 1:400, and can be life-threatening if it occurs during pregnancy or in immunocompromised patients. Although relatively rare, radiographical abnormalities of the lungs without respiratory symptoms are reported in more than 15% of adults with chickenpox.[34]

Pneumonitis can also rarely occur as a complication of cytomegalovirus or Epstein–Barr virus mononucleosis.[27] Cytomegalovirus is an opportunist pathogen in immunocompromised patients in whom it can cause serious or even fatal respiratory complications. It is more important as an opportunist pathogen of transplant recipients (especially bone marrow transplants) than those immunocompromised through AIDS. Perinatal cytomegalovirus infection may occasionally present as pneumonitis in the newborn.

Atypical Bacteria Associated with the Virology Laboratory

The diagnosis of atypical bacteria has traditionally been undertaken in virology laboratories, because these agents cause syndromes that overlap partially with viral respiratory infection and they were diagnosed serologically before the polymerase chain reaction was added to our diagnostic arsenal (see below). They do not cause the typical clinical picture of lobar pneumonia caused by *Streptococcus pneumoniae* and other bacteria, hence the name 'atypical'. These include: *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae* and *psittaci* and *Coxiella burnetii*. The related *Legionella* bacteria are also discussed here.

Mycoplasma pneumoniae is an important cause of upper respiratory tract infection and bronchitis/pneumonia, usually as sporadic infections or outbreaks through human-to-human transmission among families or in closed environments. In the developed world, it has the highest attack rates among 5–20-year-olds and it is one of the major causes of pneumonia in young adults. A wide spectrum of extra-pulmonary manifestations and post-infectious syndromes has been described, but fall beyond the scope of this book. The chest radiographical patterns of *Mycoplasma* pneumonia are nonspecific and variable and can be indistinguishable from those of bacterial and viral pneumonia.[35] Systematic data from tropical areas are scarce, but its distribution is worldwide.[36]

Chlamydophila are obligate intracellular bacteria. *Chlamydophila pneumoniae* is a common human respiratory pathogen and causes a similar clinical picture to *Mycoplasma*. It is frequently isolated from animals, but their role in transmission is unclear and spread is thought to be human to human. Serosurveys also show worldwide distribution for this bacterium.[37] *Chlamydophila psittaci* is the causative agent of psittacosis, a (rare) zoonotic infection related to exposure to birds which usually presents with fever, headache and myalgia and sometimes causes pneumonia.[38]

Coxiella burnetii causes Q-fever. It is an obligate intracellular bacterium with a spore stage, making it highly resistant to environmental conditions. Humans are infected by aerosol inhalation and develop an acute febrile illness that is either self-limiting or develops into pneumonia or less frequently, endocarditis or a systemic chronic syndrome. Cattle, sheep and goats are the main reservoirs and when infected, shed bacteria in urine, faeces, milk and especially when giving birth. Placentas contain high concentrations of bacteria and people exposed to these are at risk. Given the environmental resilience of this organism, less direct contact with infected animals/placentas may also cause disease. There is no human-to-human spread.[39]

Legionella bacteria are naturally occurring aquatic bacteria. These may grow to high concentrations in warm water, e.g. in cooling towers, heaters and drinking water plumbing, especially when associated with free-living amoebae. Aerosolization and inhalation of *Legionella pneumophila* may lead to (outbreaks of) a self-limited febrile illness called Pontiac fever or a more severe systemic illness with pneumonia called Legionnaires' disease.[40] *Legionella* and *Coxiella* belong to the same family of bacteria, and both are associated with a syndrome of long-lasting post-infectious fatigue.

These bacteria are under-reported in most parts of the world. However, as they are usually susceptible to macrolides/azalides, tetracyclines and fluoroquinolones, it is important that they are diagnosed and treated. The methods of choice to detect these pathogens revolve around nucleic acid amplification techniques on respiratory specimens. A rapid urinary antigen test is available for detection of *Legionella pneumophila*. Detection of *Mycoplasma* specific IgM is useful in acute settings. *Mycoplasma* and *Legionella* can also be cultured, but this may take 1–2 weeks. Detection of seroconversion may provide a diagnosis retrospectively or in chronic disease, and may be useful for epidemiologic purposes.

Empiric treatment protocols for uncomplicated pneumonia usually consist of penicillins, such as amoxicillin with or without clavulanic acid, and do not cover these bacteria. However, most severe pneumonia treatment protocols do tend to include antimicrobial agents such as macrolides that cover these organisms. For any pneumonia of unknown aetiology that does not respond to empiric treatment, adding antimicrobial agents that cover atypical bacteria, is recommended.

CO-INFECTION

With advanced molecular diagnostic techniques (see below), it is now possible to rapidly and simultaneously detect multiple pathogens within biological samples. In recent years, multiplex reverse-transcription PCR has changed our understanding of the viral causes of respiratory illness, and doubtless newer technologies such as 16S rRNA gene sequencing will have a similar effect on how we view bacterial causes over the coming years.

The challenge with these technological advances will be in interpreting the data in a meaningful way for clinicians.

Viral Co-infection

Over the last 5 years, there has been an increase in the number of studies using multiplex RT-PCR to investigate pathogen prevalence in acute respiratory infection. Most of these have been in pre-school children in whom getting respiratory samples (particularly nasopharyngeal aspirates) is routine and relatively easy. In some studies, viruses and atypical bacteria have been detected in over 80% of samples, detection rates much higher than in studies using traditional culture techniques.[41] However, as has been mentioned, these studies should be interpreted with some caution. Pathogens such as Bocavirus and Adenovirus persist for weeks and sometimes months after acute infection and thus their significance during an acute episode, especially when detected with other pathogens, may be uncertain.

This increase in reported prevalence has been mirrored by an increase in the rates of viral co-infection (or probably more accurately, co-detection). In recent studies, two or more viral pathogens have been found in up to 44% of upper respiratory samples from young children with acute respiratory infection.[21,41–45] Co-infection rates appear to vary depending on age, country and living conditions, as well as the number of pathogens tested (Table 19.1). Some of the highest viral detection and co-infection rates have been reported in pre-school children from low-income families living in Brazil, Vietnam and Jordan.[21,41,42] A number of groups has suggested co-infection as a risk factor for severe disease. However, there is no real consensus in the literature supporting this premise, which is perhaps not surprising given that most of the published studies have investigated very different populations and looked for different numbers of pathogens (Table 19.1).

Bacterial Co-infection

For some conditions, a link between viral and bacterial infection is well established. For instance, data reassessed from the influenza pandemics of 1918, 1957 and 1968 suggest that most deaths during these periods were due to secondary bacterial pneumonia, approximately four weeks after the acute infection.[47] Overall, however, the significance of contemporaneous bacterial and viral co-infection in acute respiratory infection is difficult to assess, particularly if analysis is limited to upper airway secretions. While it is often inferred that viral pathogens detected in the upper airways are also in the lower airways of children with chest symptoms, the same cannot be said for bacteria where upper airway detection is as likely to be due to asymptomatic nasopharyngeal carriage.

However, in studies where lung aspirates have been obtained from children with clinical signs of severe pneumonia, both viral and bacterial pathogens can be detected. In a study from the Gambia on 74 children with community-acquired pneumonia, one-third of the 45 with pneumococcal pneumonia, had RSV infection.[48] In contrast, in children from Malawi with a high prevalence of HIV, only 9% of those with pneumococcal infection had a viral co-infection (the commonest being with adenovirus).[49] It is also worth considering, that in the UK, one-fifth of infants with severe RSV bronchiolitis have bacteria in their lower airways at the time of intubation and length of time spent ventilated correlates to bacterial carriage.[50]

Clinical Syndromes

Most viruses and atypical bacteria can cause most clinical manifestations of acute respiratory infection, but some viruses are associated with particular clinical presentations; RSV with bronchiolitis, Adenovirus B3, 7 and 21 with bronchiolitis obliterans, and PIV1-2 with croup.

Infections confined to the upper respiratory tract cause symptoms such as rhinitis, coryza and cough and are for the most part mild, with the exception of croup, which although uncomfortable and distressing, is more severe but not normally fatal. In practice, acute lower respiratory tract infection accounts for most of the serious disease burden. Infections normally start in the upper respiratory tract and then spread to the lower airways, where the effects can be extensive and are rarely confined to one lobe or even one lung. This is in marked contrast with pneumococcal pneumonia. Common lower respiratory tract manifestations of viral or atypical bacterial infections are: bronchiolitis, pneumonia and asthma/episodic viral wheeze.

TABLE 19.1 Viral Atypical Bacterial Prevalence Detected Using Multiplex PCR in Children Presenting to Hospital with Acute Respiratory Infection

	India[44]	Jordan[42]	Hong Kong[46]	Nepal[45]	Brazil[41]	Vietnam[21]	Kenya[43]
Year published	2007	2008	2009	2009	2011	2011	2010
Age (years)	<5	<5	<5	<3	<5	<13	<12
Total number patients in study	301	326	475	629	407	309	759
In-patient (%)	45%	100%	100%	100%	52%	100%	100%
Co-infection rate	7	25	4	1	40	20	7
hRSV	20	43	8	14	37	24	34
hRV	–	11	4	–	19	4	–
AdV	–	37	5	–	25	5	4
Influenza A and B	3	1	11	7	3	17	6
PIV	16	0	9	10	8	7	8
CoV	–	1	4	–	3	8	10
Human Metapneumovirus	4	3	1.5	1	10	7	3
HBoV	–	18	–	–	19	16	2
Mycoplasma Pneumoniae	–	0	2	–	10	–	–
Chlamydophila Pneumoniae	–	5	0	–	1	–	–
PCR negative	65	22	53	70	15	28	44

BRONCHIOLITIS

RSV is the commonest cause of bronchiolitis and is detected in 43–74% of cases.[51] Upper respiratory tract symptoms usually precede lower respiratory tract involvement by a few days. Dyspnoea, subcostal recession and feeding difficulties characterize lower respiratory tract infection. In bronchiolitis, wheeze may be present with a prolonged expiratory phase and crackles.[52] Air trapping results in a rapid respiratory rate, a palpable spleen and liver and a typical radiographic pattern of hyperinflation with diffuse interstitial markings and peribronchial thickening. Segmental atelectasis is often seen. Bronchiolitis may lead to acute respiratory failure with severe bronchospasm, moderate to severe hypoxia and carbon dioxide retention. Apnoea tends to occur in infants under 2 months of age and often in those born prematurely. Supportive measures are still the mainstay of treatment for bronchiolitis. Symptoms associated with clinically significant bronchiolitis (particularly RSV) are not limited to the acute episode, with many children wheezing for years afterwards.

Two key risk factors for getting bronchiolitis are age (<6 months) and exposure to tobacco smoke (particularly antenatally).[52] Other factors increasing the risk of getting more severe disease include male gender, prematurity, an underlying heart or lung condition and never having been breast-fed. Also, poverty, living in a crowded environment and having siblings who attend school or child-care increase the risk of getting bronchiolitis.

PNEUMONIA

Cases of viral pneumonia generally occur during seasonal epidemics of influenza and RSV, and are normally preceded by upper respiratory tract infection.[53] Whereas symptoms of bacterial pneumonia include a rapid onset of fever, rigors, malaise, cough and dyspnoea, symptoms of viral pneumonia tend to be of slower onset and while including cough and dyspnoea, are just as likely to present with rhinitis and wheezing. Biomarkers of sepsis such as white-blood cell count, C-reactive protein and procalcitonin are often normal, and chest radiograph findings likely to show bilateral interstitial infiltrates rather than lobar changes. Children with viral pneumonia respond slowly or not at all to antibiotics in marked contrast to those with bacterial pneumonia. Similar to bronchiolitis, risk factors for severe disease include household smoking and cessation of breast-feeding before 6 months, poverty, malnutrition, HIV and other immunosuppressive disease.

ASTHMA/EPISODIC VIRAL WHEEZE

The asthma epidemic experienced by developed countries over the past three decades is now being mirrored in developing countries as they become more urbanized. Environmental factors appear to be a key factor behind these changes in prevalence.[54] Global associations which positively correlate with asthma symptom prevalence include Gross National Product, *trans*-fatty acids, paracetamol, and women smoking, whereas inverse associations include a diet with food of plant origin, pollen, immunizations, tuberculosis notifications, air pollution, and men smoking.[54]

There is clinical overlap between childhood asthma and episodic viral wheeze. In both conditions, cases tend to be preceded by a viral URTI 1–2 days before wheeze develops.[55] Episodic viral wheeze is a series of discreet episodes of respiratory distress characterized by wheeze occurring in pre-school children, whereas asthma later develops into a chronic disease with exacerbations of sudden deterioration in airway function, day-to-day variation in airway function and fixed or persistent airway obstruction.[56] In developed countries, one-third of children with episodic viral wheeze as a young child go on to develop atopic asthma.[57] Rhinovirus appears to be a particularly common viral cause of asthma exacerbations (especially Rhinovirus C) but other viruses commonly isolated include RSV, hMPV and PIVs.

Epidemiology

Overall, the viruses that cause acute respiratory infection worldwide are the same (Table 19.1), but there are local variations, particularly during outbreaks of unusual organisms (Nipah virus in Bangladesh and India). In temperate parts of the world, there is a clear seasonal pattern in the prevalence of viral respiratory tract infection, with peaks occurring over the cold, winter months. In tropical regions, where average temperature is higher and seasonal temperature change less, variations in prevalence are still seen. This has been best demonstrated for RSV. Strong RSV seasonal patterns, which correlate with rainfall, have been found in Vietnam,[21] India,[58] Papua New Guinea,[59] North-East Brazil,[41] Kenya and the Gambia.[60,61] A possible explanation for this is that children tend to be kept indoors during the rainy season, and the resultant crowding in damp, humid environments prolongs viral survival and encourages spread. This is probably not the whole story however, as in some parts of the world, RSV activity shows no pattern at all (Taiwan[62]), or peaks during the summer months when ambient temperatures are highest (Hong Kong[63]).

Similar patterns are found for influenza virus infection.[64] Thus, for studies large enough to detect such variations, influenza activity peaks in India, North-East Brazil and Senegal, during the months with the highest rainfall and humidity. Similar seasonal variations have also been described for hMPV and PIVs. In contrast, rhinovirus, adenovirus and bocavirus infection appears, for the most part, to be endemic throughout the year. One recent study in North-East Brazil reported peaks in *Mycoplasma pneumoniae* infection that were unrelated to the rainy season.[41] In this study, the authors found that at its peak *Mycoplasma pneumoniae* infection accounted for 17% of hospitalized pneumonia cases in pre-school children, a finding with clear implications for management, given that current treatment guidelines for pneumonia in this age group do not include macrolide/quinolone antibiotics.

Diagnoses

Epidemiological characteristics, patient history, clinical features and accompanying signs and symptoms may give some clues in establishing the diagnosis of specific viral agents, but clinical syndromes are nonspecific and overlap considerably. Aetiological diagnosis can only reliably be made by detection of live virus, viral antigens or nucleic acids in respiratory or other specimens and to a lesser extent by (retrospective) detection of (a rise in) specific antibodies.

There are three main reasons for providing an aetiological diagnosis of viral respiratory infections: to aid clinical

management (specific therapy, stopping antibiotic therapy, infection control); to monitor routine virus activity in the community (epidemiology, e.g. vaccine strain selection for influenza); or for research purposes.

Rapid diagnosis of viral respiratory infections (i.e. in less than 3 hours) has been shown to reduce antibiotic use and to be cost-effective.[65] In addition, such confirmation of the cause is useful in hospital infection control (e.g. in cohorting similar cases) and, occasionally, in deciding whether to use antiviral drugs in selected high-risk patients (see below). Equally, making a rapid diagnosis in an outbreak situation (e.g. of influenza), may justify the use of antiviral medications to limit disease spread.

Diagnosis for some respiratory infections is achievable within 2–3 hours using techniques such as antigen detection (see below). However, these techniques are not universally available, even in hospitals in the developed world, mainly because they are labour- and expertise-intensive. Commercially available point-of-care diagnostic tests, usually lateral flow assays in 'kit format', are available for the diagnosis of influenza virus A and B and for RSV. They are however, expensive, and while they have adequate positive and negative predictive value of infection during influenza epidemics, they can have poor predictive values during periods of low influenza activity.[66]

The increasing need to accurately detect avian influenza H5N1 is best done by sensitive molecular methods (e.g. RT-PCR) because other techniques are less sensitive and virus culture necessitates biosafety level 3 facilities. This is driving reference laboratories, especially in developing countries where H5N1 disease sporadically occurs, to invest in these newer technologies. In time, this will hopefully allow molecular tests for multiple respiratory pathogens (Multiplex PCR) to be undertaken in these same laboratories. However, these methods remain resource- and expertise-intensive and need regular quality control exercises. Microarray techniques that have the potential to detect multiple pathogens in a single test are in development. These methods have enormous potential to increase our understanding of viral (and bacterial) epidemiology.

METHODS OF VIRAL DIAGNOSIS

Laboratory diagnosis of respiratory virus infections depends on the demonstration of either virus or viral components in the patient at the acute stage of the illness, or subsequently an immune (serological) response to the virus.

Demonstration of Virus

There are several approaches to this that include demonstration of: (1) viral antigens by immunofluorescence or enzyme immunoassays;[67] (2) viral infectivity by growth in cell culture; or (3) viral nucleic acid by various techniques. Details of the techniques are not given here, but the advantages and disadvantages of each are indicated in Table 19.2. When setting up a diagnostic laboratory, its purpose should be clearly thought out. If the catchment population is very large, the number of specimens may also be large and the advantages of automation (e.g. in machine-based nucleic acid amplification or enzyme immunoassays) may be significant. Most specimens are likely to come from hospitalized patients because of the practical difficulties in collecting and delivering specimens from the community. Virology specimens are perishable and must be delivered to the laboratory without delay. The quality of the specimen is paramount to obtaining meaningful results. It is easier to take a bad specimen than a good one, and close cooperation with the laboratory will help to raise the positivity rate.

Demonstration of an Immune Response

This, at present, means demonstrating an antibody response in the serum to the stimulus provided by the virus. Seeking responses in cellular immunity or antibody in other body fluids remain research techniques only.

For a valid diagnosis, a convalescent specimen of serum (usually taken after at least 2 weeks) is needed but may

TABLE 19.2 Advantages and Disadvantages of Various Techniques of Virus Diagnosis

Technique	Advantages	Disadvantages
Immunofluorescence	Rapid, i.e. same day Allows assessment of specimen quality Sensitive and specific in experienced hands	Labour-intensive Requires experienced observer(s) Requires high-quality reagents Obtaining good specimens requires skill, determination and persistence evidence of shedding of some viruses in healthy
Enzyme immunoassay	Rapid, available in point-of-care format (~30 min) for some viruses Suitable for large numbers Can be semi-automated Detects incomplete virus particles	No feedback on specimen quality Requires high-quality reagents Automated equipment expensive Difficult to assess results at threshold of positivity Relevance of detection of viral antigens not always clear
Culture	Provides more virus for further analysis Confirms presence of replicating/infective virus Generally regarded as the gold standard	Expensive and a continuing expense Labour-intensive Not as sensitive as nucleic acid amplification, some viruses difficult to isolate or cannot be cultured Mixed infections pose problems Requires high-quality reagents to identify isolates
Detection of nucleic acid by amplification ([RT-]PCR and others)	Sensitive and specific Can detect virus in the presence of antibody Allows assessment of specimen quality Allows for multiplexed assays, random PCR-based array tests in development	Expensive Requires vigilance against false-positive results Labour- and skill-intensive Relevance of detection of viral nucleic acids not always clear

be difficult to collect. This is particularly true with children. Nevertheless, unless an antibody response can be demonstrated (seroconversion or a rising titre) some uncertainty over the validity of the result will remain. An alternative is to demonstrate an IgM-class response but this suffers from the twin disadvantages that IgM antibodies (and thus the corresponding results), are relatively non-specific compared to IgG, and such tests are not routinely available for all viruses.

Treatment

Supportive care with fluids and oxygen remain the mainstay of treatment for most respiratory viral infections. For some individuals, respiratory support on high-dependency units or intensive care is needed. Extracorporeal membrane oxygenation (ECMO) is sometimes required to maintain oxygen saturation in individuals with severe viral pneumonia.

The Adamantanes (amantadine and rimantadine) were options for prevention (in outbreaks within closed communities of high-risk individuals) and less convincingly, for treatment of influenza virus A infections.[68] However, these therapies have now become obsolete as H3N2 resistance to them has been increasing since 2003, and H1N1-pdm09 and most avian H5N1 viruses are completely resistant.

Neuraminidase inhibitors such as oseltamivir (Tamiflu), zanamivir (Relenza) and peramivir (Rapiacta), which inhibit the viral enzyme neuraminidase in both influenza virus A and B, are effective for treatment and prophylaxis of influenza. In the 2007/2008 influenza season an influenza virus A H1N1 lineage emerged that was naturally resistant against oseltamivir, and soon became the dominant lineage worldwide. This was a cause for serious concern, but in 2009 the oseltamivir-susceptible H1N1-pdm09 virus replaced the resistant lineage.

For treatment of uncomplicated influenza, neuraminidase inhibitors have to be given within 48 hours of onset for apparent clinical benefit. For severe influenza, influenza in the immuno-compromised, and avian influenza, benefit can still be found if these drugs are administered after 48 hours and so treatment should not be withheld in these cases. They are expensive and are best used on those most at risk of serious illness – those at the extreme ends of life. While both zanamivir (given by inhaler) and oseltamivir (given orally) can be used for prophylaxis against H5N1 influenza disease, the systemically active oseltamivir is the preferred option for treatment given the potential for dissemination of H5N1 beyond the respiratory tract. However, experience with human cases of H5N1 avian influenza has shown that resistance may develop rapidly and may be a major problem in widespread prophylactic or therapeutic use.

Ribavirin is a purine nucleoside analogue that is believed to interfere with viral nucleic acid function. It is expensive, difficult to deliver and teratogenic (therefore potentially toxic to both patient and treating team). Systematic reviews have failed to show any convincing evidence for its use in either acute bronchiolitis or in more severe disease.[69] Given the high cost, safety concerns, challenges in delivery and weakness of trial data, ribavirin is generally reserved for use with immunocompromised children in a PICU setting. Ribavirin may have some effect in influenza but evidence to support its use is minimal. It has also been used in Hantavirus pulmonary syndrome but, again, the evidence of efficacy is minimal.

Cidofovir is available for severe adenovirus infections, but causes severe side-effects and needs to be administered simultaneously with probenecid.

Intravenous acyclovir and oral valacyclovir are effective in the treatment of varicella or herpes simplex infections of the respiratory tract in the immunocompromised patient. It should also be used in an immunocompetent patient (usually an adult) with varicella pneumonia. Ganciclovir and foscarnet are useful in cytomegalovirus infection in the immunosuppressed, but a detailed discussion of this problem is beyond the scope of this chapter.

In many parts of the world (especially South-east Asia), antibiotics are extensively used to treat any form of mild respiratory illness. Although there may be some benefit to the use of antibiotics in preventing secondary bacterial sinusitis, otitis media or pneumonia, over-the-counter availability of antibiotics, self-medication or medication by untrained pharmacy workers should be strongly discouraged because of the selection and subsequent spread of resistant pathogens and of (multidrug) resistant commensal bacteria in oral and intestinal flora.

Prevention

With the cells of the target organ immediately accessible to viruses, it is proving difficult to produce effective vaccines to respiratory tract viruses.[70] Other than in measles, which has a systemic phase, vaccines have had only limited success. In the tropics, even the measles vaccine has limitations as much of this virus' impact is during infancy, when existing vaccines lack effectiveness at inducing immunity in the presence of passive maternal antibody.

Yearly influenza vaccination is recommended in many countries for persons at high risk (e.g. patients with underlying heart, respiratory or immunocompromising diseases, patients on dialysis, the elderly) and contains antigens from two current influenza A virus subtypes (H3N2 and H1N1-pdm09) and from influenza virus B. The constituents are modified annually for each hemisphere as the prevalent strains 'drift and shift' (see above). The conventional influenza vaccine is formalin-killed egg-grown virus and has provided useful protection, particularly in the elderly and those with pre-existing lung damage in whom even minimal protection may be enough to prevent death. An alternative approach of a live attenuated vaccine containing cold-adapted influenza strains has shown some efficacy and such vaccines are also available.

The possible emergence of an H5N1 avian strain adapted to man has stimulated research into new ways to produce vaccines (e.g. using reverse genetics or a disabled adenovirus as a vector for influenza antigens) and for new antiviral drugs. Currently (at time of writing), H5N1 vaccines are licensed for use in the USA and the EU, but these are not available in endemic countries.

Earlier attempts at production of formaldehyde inactivated RSV vaccine were associated with more severe forms of disease in vaccinees. Prevention of RSV, severe measles and varicella in susceptible (immunocompromised or severely malnourished) contacts may also be achieved by passive immunization. Monoclonal antibodies such as Palivizumab provide some protection to RSV infection in vulnerable infants (e.g. premature babies), but it is very expensive and administration is in the form of monthly intramuscular injections over the RSV season.[71]

Normal human gamma globulin is effective in preventing/attenuating measles if administered within 3 days of contact. For the prophylaxis of varicella, high-titre varicella-zoster human immune globulin (ZIG) must be used. Maximum protection (from severe disease, but not from infection) follows administration within 48 hours of contact, but some benefit may accrue if given within 10 days of exposure.

Summary

Respiratory viral disease, like diarrhoeal disease, is a major cause of morbidity and mortality in the developing world and has significant economic consequences. Much respiratory disease, particularly in childhood, is either totally due to viruses or is virus-initiated, with similar organisms found in tropical and temperate regions. Viral and atypical bacterial epidemiological data are incomplete everywhere (but more so for the poorer parts of the world) and come mostly from hospitalized patients.

RSV is a universal childhood pathogen found everywhere. The numbers of virologically confirmed diagnoses each year in hospitalized children in the Newcastle and Tyneside area in the UK (population about 1 million) and from Hong Kong island (population about 0.7 million) are remarkably similar: 500–600 and 500–700 cases, respectively. There are likely to be many more RSV cases in the larger cities of Asia and South America, in overcrowded environments where the potential for severe disease will be exacerbated by malnutrition, air pollution, poor sanitation, minimal medical care, etc.

A new pandemic of influenza A (similar to the one that swept the world in 1918/1919) could be a major health problem of the future, but when and if this will happen is unknown. We have learnt from the 2009 pandemic that pandemics can be mild, but there is still fear of H5N1 crossing the species barrier and causing a pandemic with a high case fatality rate. Current preparative measures, such as 'stockpiling' oseltamivir and vaccine development, may help to reduce its impact.

There is a very real clinical and economic need to develop effective treatments and vaccines against the common causes of viral and atypical bacterial respiratory disease but this will require an enormous commitment of resources.

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