Introduction

Ten years ago in 2003, severe acute respiratory syndrome coronavirus (SARS-CoV) shocked the world by its high virulence and efficient transmissibility among humans (1-3), causing the first large-scale epidemic of the 21st century. More than 8,000 patients were infected worldwide, resulting in 774 deaths. In addition to the substantial burden to the healthcare system, SARS also negatively affected the economy with reduced domestic demands and international travel (4).

Attributable global economic loss due to SARS-CoV has been estimated to be 40 billion dollars (5). Two novel human coronaviruses (HCoV), HCoV-HKU1 and HCoV-NL63, were identified shortly after the discovery of SARS-CoV as common causes of human respiratory tract infections. In 2012, a novel human coronavirus, now called Middle East respiratory syndrome coronavirus (MERS-CoV), has emerged in the Middle East to cause fatal human infections in three continents. MERS-CoV human infection is similar to SARS-CoV in having a high fatality rate and the ability to spread from person to person which resulted in secondary cases among close contacts including healthcare workers without travel history to the Middle East. Both viruses also have close relationships with bat coronaviruses. New cases of MERS-CoV infection in humans continue to occur with the origins of the virus still unknown in many cases. A multifaceted approach is necessary to control this evolving MERS-CoV outbreak. Source identification requires detailed epidemiological studies of the infected patients and enhanced surveillance of MERS-CoV or similar coronaviruses in humans and animals. Early diagnosis of infected patients and appropriate infection control measures will limit the spread in hospitals, while social distancing strategies may be necessary to control the outbreak in communities if it remained uncontrolled as in the SARS epidemic.

KEY WORDS
Severe acute respiratory syndrome coronavirus (SARS-CoV); novel coronaviruses; Middle East respiratory syndrome coronavirus (MERS-CoV)
are currently classified into four genera based on the differences in their protein sequences (15). HCoV-229E and HCoV-NL63 belong to Alphacoronavirus. HCoV-OC43 and HCoV-HKU1 belong to lineage B. Betacoronavirus, while SARS-CoV and MERS-CoV belong to lineages B and C Betacoronavirus, respectively. Currently, Gammacoronavirus and Deltacoronavirus have not been reported to cause human disease. Both SARS-CoV and MERS-CoV are phylogenetically closely related to coronaviruses in bats (18-20). In this article, we review the clinical aspects of the novel human coronaviruses identified in the past ten years, and discuss the relationship between these human coronaviruses with their ancestral animal coronaviruses (Video 1).

Clinical aspects of human coronavirus infection

Before the emergence of SARS-CoV, the only coronaviruses that were known to cause human respiratory tract infections were HCoV-229E and HCoV-OC43, which accounted for 15-30% of the common cold, with severe disease being rare (21). In November 2002, an unusual atypical pneumonia due to SARS-CoV started to emerge in Foshan, Guangdong Province, Mainland China (2). In February 2003, the infection spread to Hong Kong and then to Vietnam, North America, and Europe. The virus was first isolated from the open lung biopsy of the 65-year-old doctor who traveled to Hong Kong from Guangzhou (3). The first three criteria of the modified Koch’s postulate, namely the isolation of the virus from diseased hosts, cultivation in host cells and proof of filterability were fulfilled in initial clinical and in vitro studies (3,22-24), while the last three criteria including the production of comparable disease in a related species, reisolation of the virus and detection of specific immune response to the virus were fulfilled in studies conducted in cynomolgus macaques shortly after the discovery of the virus (25). SARS-CoV was characterized by rapidly progressive pneumonia (3). The primary mode of transmission of SARS-CoV appeared to be by respiratory droplets (26) although fecal-oral transmission might also be (3). Apart from the super-spreaders, each case was estimated to infect two to four secondary cases (27). The median incubation period was estimated to be four to seven days (28) and the peak viral load was reached on the 10th day of illness (3). All age groups could be affected by SARS-CoV. Healthcare workers and immunocompromised patients were particularly at risk (29). Presentation with fever was universal, and myalgia and malaise were common early symptoms. Cough, dyspnea, tachyypnea, pleurisy, and diarrhea tended to appear as late symptoms (3). Lymphopenia, deranged liver function tests, and elevated creatine kinase were common laboratory abnormalities (3). Thoracic computed tomography (CT) scans frequently showed ground-glass opacifications despite initial normal chest radiographs. Approximately two-thirds of the patients deteriorated in the second week of illness, characterized by persistent fever, increasing dyspnea, and oxygen desaturation. This was correlated by progression to multifocal air-space consolidations on thoracic CT scans with occasional pneumomediastinum (3). Approximately 20-30% of patients subsequently required intensive care and mechanical ventilation. Pathological analysis of lung biopsy at autopsy demonstrated diffuse alveolar damage, desquamation of pneumocytes, and inflammatory infiltrates with hyaline membrane formation (30).

In January 2004, the novel HCoV-HKU1 was first identified in a 71-year-old man with community-acquired pneumonia in Hong Kong (6) and was subsequently found globally. Both children and elderly with underlying illnesses were affected (8,31). In addition to community-acquired pneumonia, HCoV-HKU1 is associated with acute bronchiolitis and asthmatic exacerbation (31). The incidence of febrile seizure is higher in HCoV-HKU1 infection than infections caused by other human coronaviruses (31). HCoV-HKU1 was also found in a patient with meningitis (32). Most cases occurred during winter and spring coinciding with the influenza season (31-34).

HCoV-NL63 was first reported from the Netherlands (7). The first patient was a 7-month-old child hospitalized for fever, coryza and conjunctivitis, with chest radiographic evidence of bronchiolitis. HCoV-NL63 is associated with croup (20,35), and has also been reported in a patient with diabetes mellitus who developed pericarditis and rhabdomyolysis (11). On the other hand, the association between HCoV-NL63 and Kawasaki disease remains controversial (36). The peak incidence occurs during early summer and autumn in tropical and subtropical areas like Hong Kong (31,37), and during winter in European countries including the Netherlands and the United Kingdom (7,32). Co-infections with other respiratory viruses especially

Video 1. From SARS coronavirus to novel animal and human coronaviruses.
MERS-CoV was first identified in 2012, with Koch’s postulate being fulfilled using a macaque model soon afterwards (38). All reported patients were adults, with a median age of 56 (39). The clinical feature resembled closely to that of the SARS outbreak in 2003 (13), characterized by rapidly progressive acute pneumonia. In contrast with SARS, many patients with MERS-CoV also developed acute renal failure (14). One patient presented with fever, diarrhea and abdominal pain without respiratory symptoms (40). Severe complications included acute respiratory distress syndrome, consumptive coagulopathy, and pericarditis. Although most of the laboratory-confirmed cases originated from the Middle East (the Kingdom of Saudi Arabia, Qatar, Jordan, and the United Arab Emirates), imported cases with occasional secondary spread to close contacts have been reported in the United Kingdom, Germany, France, Italy and Tunisia. Person-to-person transmission is suspected in at least six intra-familial or healthcare-related clusters (41,42). The high fatality of MERS-CoV of over 50% in humans is consistent with in vitro experiments showing rapid viral replication and broad human tissue tropism in cell lines (43,44).

Definitive laboratory confirmation of coronavirus infection by viral culture is limited by the relative difficulty of growing most of these viruses in cell culture and the lack of appropriate biosafety facilities in routine clinical laboratories for SARS-CoV and MERS-CoV. For HCoV-HKU1, viral culture has been reported using human ciliated airways epithelial cell cultures, but this is not widely available (45). Other diagnostic methods include direct antigen detection, serology, and RT-PCR. For example, direct antigen detection in respiratory tract specimens using monoclonal antibody against the nucleocapsid protein has been reported for SARS-CoV and HCoV-HKU1 (33,46,47). Serological testing using neutralization assays can compare the acute and convalescent serum samples, with infection confirmed if there is a four-fold increase in the antibody titers. However, testing requires repeated sampling, and cross-reactive antibodies have been reported. For example, although SARS-CoV and MERS-CoV are phylogenetically distinct, cross-reactive neutralizing antibodies against MERS-CoV are found in 60.7% of SARS patients (48). This may be related to the similarity of the heptad repeat-2 region of the spike protein, which is a significant B cell epitope. Further study of the host immunological response to infection by these viruses might facilitate the understanding of this phenomenon.

Currently, there is no specific antiviral for coronavirus infection approved for clinical use. Many compounds have in vitro activity, but none has undergone randomized placebo controlled trials (2). No antiviral or immunomodulator, including convalescent plasma, was found to be efficacious, and possible harmful effects have been reported for ribavirin and corticosteroid (49). For MERS-CoV, in vitro antiviral activity has been demonstrated for cyclosporin A, interferon-α, interferon-α 2b and ribavirin (50,51). Intensive care with mechanical ventilation and extracorporeal membrane oxygenation support remains to be the main modalities of treatment (52).

**Zoonotic origin of human coronaviruses**

Epidemiological studies have found that the initial patients with SARS had contact with game animals. Seroprevalence studies showed that animal traders had a higher prevalence of SARS-CoV IgG than the general population (53). The first animals found to carry SARS-CoV-like viruses were the Himalayan palm civets and a raccoon dog in live animal markets (54). Subsequent searches for the natural animal host in a surveillance study among non-caged animals from wild areas in Hong Kong revealed that a closely related bat coronavirus, SARS-related Rhinolophus bat coronavirus HKU3 (SARSr-Rh-BatCoV HKU3), existed in Chinese horseshoe bats (Rhinolophus sp.) (20). Phylogenetic analysis suggested that HCoV-NL63 may have originated from bat coronaviruses that have diverged 563-822 years ago (55), while HCoV-HKU1 also likely originated from a bat coronavirus (56). Initial phylogenetic analysis of the replicate gene of MERS-CoV showed that the virus was most closely related to bat CoV-HKU4 and CoV-HKU5 (14,15,57). More recently phylogenetic analysis using partial RdRp sequences showed that the MERS-CoV was more closely related to betacoronaviruses in bats from Europe and Africa (58,59). At the time of writing, related coronaviruses have yet to be isolated from animals in the Middle East. Continued search for the natural definitive and intermediate animal hosts may help to halt the ongoing epidemic.

The hunt for novel coronaviruses in bats accelerated after bats were identified as the natural host of SARS-CoV. Bats are the only flying mammals capable of travelling long distances which facilitates transmission of viruses (60,61). In addition to the SARSr-Rh-BatCoV HKU3, Bat coronaviruses identified in Hong Kong include the Rhinolophus bat CoV-HKU2 (62), Tylonycteris bat coronavirus CoV-HKU4 (18), Pipistrellus bat CoV-HKU5 (18), Myotis bat CoV-HKU6 (18), Miniopterus bat CoV-HKU7 (18) and Miniopterus bat CoV-HKU8 (18), Hipposideros and Rousettus bat CoV-HKU10 (63). Other bat coronaviruses have also been found in different parts of the world (64,65). In addition to bats, birds are also an important origin and reservoir of emerging viruses causing human infections such as avian-origin influenza viruses (61). Numerous coronaviruses were also found in a wide range of birds (66). Bird coronaviruses identified in Hong Kong include bulbul CoV-HKU11, thrush CoV-HKU12, munia CoV-HKU13, white-eye CoV-HKU16, sparrow CoV-HKU17, magpie robin CoV-HKU18, night heron CoV-HKU19, wigeon CoV-HKU20, and common moorhen CoV-HKU21. Most of the bat coronaviruses belong to...
**Conclusions**

In the past 16 years, coronaviruses including SARS-CoV and MERS-CoV, together with the avian-origin influenza A viruses H5N1 and H7N9 (72,73), have led to human epidemics with high case-fatality rates ranging from 10-60%. The zoonotic origin has been found for SARS-CoV, influenza A (H5N1) and A (H7N9), while phylogenetic analysis suggests that MERS-CoV has likely originated from bat coronaviruses. Continued surveillance in mammals and birds will allow better understanding of the ecology of coronaviruses and may aid in the prevention of animal-to-human transmissions and outbreaks in the future. Since person-to-person transmission has been reported in multiple clusters of MERS-CoV infection, stringent infection control measures should be implemented in hospitals for suspected or confirmed human infections. Patients with respiratory symptoms and history of travel to affected areas or contact with MERS-CoV patients within ten to twelve days of symptom onset should be isolated and tested early to avoid nosocomial spread of disease. Ventilatory and ECMO appeared to be effective in some severe cases. Development of an effective vaccine and randomized, placebo-controlled clinical trials on potential specific antiviral agents are urgently needed.

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**References**


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