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Review Articles

Middle East Respiratory Syndrome Coronavirus (MERS CoV): An Emerging Pathogen

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Abstract:

The Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel coronavirus (nCoV) first reported on 24 September 2012 on ProMED-mail by Egyptian virologist Dr. Ali Mohamed Zaki in Jeddah, Saudi Arabia. He isolated and identified a previously unknown coronavirus from the lungs of a 60-year-old male patient with acute pneumonia and acute renal failure. MERS-CoV is the sixth new type of coronavirus like SARS (but still distinct from it and from the common-cold coronavirus). Until 23 May 2013, MERS-CoV had frequently been referred to as a SARS-like virus, or simply the novel coronavirus, and colloquially on messageboards as "Saudi SARS". These respiratory viruses are an emerging threat to global health security and have led to worldwide epidemics with substantial morbidity, mortality, and economic consequences. Currently confirmatory testing requires molecular diagnostics including either a positive PCR on at least two specific genomic targets or a single positive target with sequencing on a second. However, the interim recommendations for laboratory testing for MERS-CoV should be consulted for the most recent standard for laboratory confirmation. Hajj and Umrah draws some of the largest crowds in the world, and the large crowds bring some health and safety risks. The virus can spread from person to person when people are touching or very near each other, so pilgrims in crowds may be at risk. Symptoms of MERS include fever, cough, and shortness of breath. Most people infected with MERS have had severe illness and pneumonia, and about half of them have died. If anyone develops a fever and cough or difficulty in breathing within 14 days after returning from trip, must seek medical care. The Embassy of Saudi Arabia recommends that the following groups should postpone their plans for Hajj and Umrah in 2013: the elderly, the terminally ill, pregnant women, and children.

Key words: *Middle East Respiratory Syndrome coronavirus, World Health Organization, CDC, Infection*

Introduction:

Middle East Respiratory Syndrome coronavirus (MERS CoV) came to attention as an emerging pathogen causing severe respiratory illness in patients from the Middle East in September 2012.[1] Two cases of rapidly progressive acute respiratory infection in adults associated with a novel coronavirus have generated an international public health response. The two infections were acquired three months apart, probably in Saudi Arabia and Qatar. A novel beta-coronavirus was isolated in Saudi Arabia andsequenced at the Erasmus Medical Centre (EMC) in Rotterdam, the Netherlands[2].MERS CoV is a beta-coronavirus, in the same family as SARS-CoV, and shares a probable origin from bats. In September 2012, the United Kingdom's Health Protection Agency (HPA) named it the London1_novel CoV 2012 and produced the virus' preliminary phylogenetic tree, the genetic sequence of the virusbased on the virus's RNA obtained from the Qatari case.[2] On 25 September 2012, the World Health Organization (WHO) announced that it is "engaged in further characterizing the novel coronavirus" and that it has "immediately alerted all its Member States about the virus and has been leading the coordination and providing guidance to health authorities and technical health agencies."[1]

In May 2013, the Coronavirus Study Group of the International Committee on Taxonomy of Viruses adopted the official designation, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV),[1]which was adopted by the World Health Organization to "provide uniformity and facilitate communication about the disease."[3]Prior to the designation, WHO had used the non-specific designation 'Novel coronavirus 2012' or simply 'the novel coronavirus'.[1]

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Real-time polymerase chain reaction (RT-PCR) was used to test for distinguishing features of a number of known coronaviruses (such as OC43, 229R, NL63, and SARS-CoV), as well as for RNA-dependent RNA polymerase (RdRp), a gene conserved in all coronaviruses known to infect humans. While the screens for known coronaviruses were all negative, the RdRp screen was positive.

Hajj and Umrah draws some of the largest crowds in the world, and the large crowds bring some health and safety risks. The virus can spread from person to person when people are touching or very near each other, so pilgrims in crowds may be at risk. Symptoms of MERS include fever, cough, and shortness of breath. Pilgrims can help protect themselves by washing their hands often with soap and water, avoiding contact with sick people, and not touching their mouth, nose, or eyes. About Tens of thousands of Muslims of *Bangladesh* travel to Saudi Arabia each year to perform Hajj. So, we should pay attention to our health when traveling in the Arabian Peninsula.

Clustersof patients with severe acute respiratory illness (e.g., fever and pneumonia requiring hospitalization) should be evaluated for common respiratory pathogens. If the illnesses remain unexplained, providers should consider testing for MERS-CoV, in consultation with state and local health departments. Therefore, aims of this reviewis to increase the awareness among the physicians and health workers about clinical spectrum of disease caused by MERS-CoV , identify the key components in the surveillance case definition for a patient under investigation, identify specimens to be obtained and the appropriate laboratory test to diagnose a patient with MERS-CoV , and measures appropriate for control of MERS-CoV.

Epidemiology:

As of 28 August 2013, 102 laboratory-confirmed cases had been reported world-wide since the first cases were reported in September 2012.[1] The first cases had onsets in March and April 2012, while the majority of cases occurred between April and June 2013. Most cases have been in residents of Saudi Arabia (81 cases). Forty-nine cases have died, and the case fatality rate is 48%.[1] As of 28 August 2013, at least 58% of confirmed cases had underlying conditions that may make them more susceptible to respiratory conditions, in some cases, multiple underlying conditions.[1,5]Cases with severe symptoms have tended to be older, male and with underlying conditions, whilst mild and asymptomatic cases have tended to be of a range of ages, including children, and without underlying conditions.[3] The median age of all cases with a known age is 50 years. There is currently no evidence indicating transmission of MERS-CoV from asymptomatic infected individuals and no evidence of ongoing, low-prevalence, mildly symptomatic illness in the community.[4]

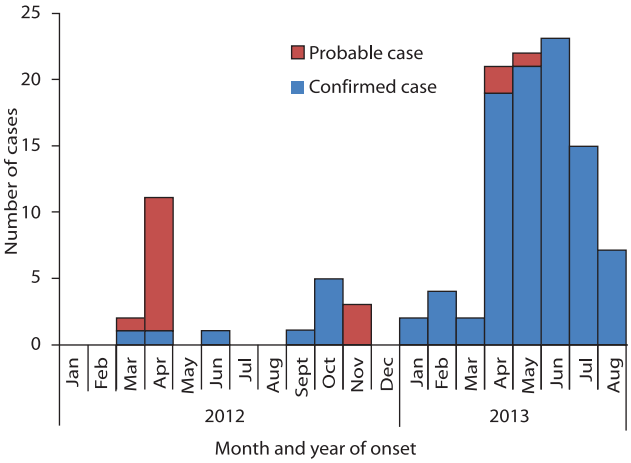

Fig: *WHO-confirmed cases and probable cases of MERS-CoV, by month and year of onset (or month of reporting if the onset date is unknown).*[1, 3,5]

All cases have a history of residence in or travel to the Middle East, or contact with travelers returning from these areas.The infection has occurred in the community (sporadic cases with unknown exposure), in families (contact with infected family members) and in health care facilities (patients and healthcare workers, multiple generations of transmission) from whence the majority of cases have been reported. In one instance, the infection was transmitted to a co-worker.

Pathogenesis:

The closest phylogenetic neighbors to MERS-CoV are putative bat coronaviruses in China (BtCoV-HKU4 and BtCoV-HKU5),[2]the Netherlands (BtCoV/VM314/2008}[6], and a recently discovered isolate from South Africa. All four of these bat coronaviruses have been sequenced only from bat samples and have never been isolated as live viruses from either bats or the environment. The natural reservoir of MERS-CoV has not been identified, although its similarity to these other four viruses suggests that it is of bat origin. Importantly, SARS-CoV emerged from bats as well.[7] Anecdotal evidence suggests that MERS-CoV may have been transmitted to humans via livestock (camels or goats); however, there is no scientific data yet to support this theory.Given the similarities in emergence and apparent zoonotic origins between MERS-CoV and SARS-CoV, initial experiments on MERS-CoV focused on direct comparison with the known molecular biology of SARS-CoV. Infection experiments in cell culture showed that MERS-CoV does not use the SARS-CoV receptor, angiotensin converting enzyme

2 (ACE2), for entry, and that MERS-CoV has a much broader host range than the epidemic isolate of SARS-CoV. [8-14]The genome structure of MERS-CoV is similar to other coronaviruses, with the 52 two-thirds of the genome encoding the non-structural proteins (NSPs) required for viral genome replication, the remaining 32 third of the genome encoding the structural genes that make up the virion (spike, envelope, membrane, and nucleocapsid proteins), and four accessory genes interspersed within the structural gene region.[2]One additional similarity between MERS-CoV and SARS-CoV is their abilities to inhibit a robust type I interferon (IFN) response in infected cells. However, MERS-CoV has been shown to be much more sensitive to exogenous type I IFN treatment compared to SARS-CoV, which may be important for pathogenesis8.[11,14,15]Several SARS-CoV-encoded proteins have demonstrated innate immune signaling antagonism[16]and MERS-CoV encodes several IFN antagonists as well .

MERS-CoV has been shown to infect a range of human, primate, porcine, and bat cell lines.[11]*Ex-vivo* infections of human lungs and human airway epithelial cell cultures identified type II alveolar cells and non-ciliated lung epithelial cells (Clara cells) as the targets of infection, rather than the ACE2-expressing ciliated epithelial cells that SARS-CoV targets [9,15]. Interestingly, in at least one case, endothelial cells were infected as well, showing a distinct difference between the biology of SARS-CoV and MERS-CoV, as SARS-CoV specifically infects ciliated epithelial cells in the lung.[17,18] The receptor for MERS-CoV was recently identified as dipeptidyl peptidase 4 (DDP4) by mass spectrometry analysis of Huh7 cell protein bound to the MERS-CoV Spike protein *in vitro*.[10] Transfection and localization experiments demonstrated that DPP4 is indeed the receptor for MERS-CoV and is necessary for infection of a non-permissive cell line. DPP4 has many diverse functions in glucose homeostasis, T-cell activation, neurotransmitter function, and modulation of cardiac signaling.[19] ACE2 does not require enzymatic function in order to act as a receptor for SARS-CoV entry, but the enzymatic function of ACE2 has been linked to severity of the disease following SARS-CoV infection.[20]Similarly, inhibition of the enzymatic function of DPP4 did not affect virus entry *in vitro*; however, the role of DDP4 enzymatic activity has not been investigated *in vivo*.[10]

Transcriptional analysis of MERS-CoV infected cells has identified several pathways specifically modulated during infection. [9] MERS-CoV is shown to modulate the innate immune response, antigen presentation, mitogen-activated protein kinase (MAPK), and apoptosis pathways. Inhibition of the MAPK pathway showed reduction in viral replication in culture, pointing to potential therapeutics. Importantly, several studies show that MERS-CoV, similar to SARS-CoV, does not induce an early type I IFN response, suggesting that MERS-CoV may encode proteins that inhibit sensing of the viral RNA during infection. [8,11,14,15] The modulation of these pathways may explain the increased lethality of MERS-CoV.

Rhesus macaques infected with MERS-CoV display pneumonia, reduced appetite, significant lung pathology, and inflammatory infiltrates. [21] However, MERS-CoV does not replicate in BALB/c, C57B/6, 129SvEv, or STAT1 knockout mice on the 129SvEv background (Coleman et al, submitted). Interestingly, mouse DPP4 is highly similar to the human DPP4, varying at only 62 positions out of 767 amino acids residues total (92% similarity). However, the differences tend to be on surface-exposed residues which, therefore, could affect binding of viral spike protein to mouse DPP4. Future structural and functional interaction experiments are needed to identify if the mouse DPP4 interacts differently with MERS-CoV spike, as compared to human DPP4, and if the known mutations allowing for this binding could be used for the development of a mouse model of MERS-CoV.

Incubation period

The incubation period for MERS-CoV is estimated to be up to two weeks, but likely shorter in most cases, however more data are required to refine the estimates. The estimation of the incubation period is complicated by a number of factors; community acquired cases have been infected from unknown sources, clusters are complicated by multiple exposures to the index case, and there is the possibility of tertiary cases. The presence of respiratory infections other than MERS-CoV may also complicate the estimation of incubation period, where the date of onset for MERS-CoV, as opposed to symptoms due to the co-infection is unclear.

For a hospital-associated cluster in Saudi Arabia, the incubation period of confirmed cases ranged between a possible one day and 12 days, and was estimated[1]to have been 5.2 days, with a 95% confidence interval of 1.9 to 14.7 days.[21]Other data support an incubation period of up to 12 days, particularly based on a patient who acquired the infection in a hospital room in Lille, France, for whom the incubation period was estimated to have been between 9 and 12 days.[22,23]

At risk patient

There is an increasing trend of severe cases of MERS-CoV infection in older patients with underlying conditions, and milder cases in their contacts who do not have underlying conditions.

In the first 47 cases of MERS-CoV infection in Saudi Arabia, 60% of cases had any underlying co-morbidity, most frequently; diabetes (32 cases, 68%), chronic kidney disease (23 cases, 49%), chronic heart disease (13 cases, 28%), hypertension (16 cases, 34%) chronic lung disease (12 cases 26%).[2] UK case 2 had an underlying malignancy that was likely to have resulted in immunosuppression.[6] Saudi case 2 had diabetes and cardiovascular disease, and the index case in France was a renal transplant recipient.[9] None of the 11 mild or asymptomatic cases in June and July 2013 for whom information is available were reported to have underlying conditions.[1]In the cluster in the UK, the index case was infected with influenza, and the two secondary cases with parainfluenza type 2.[6] It is unclear whether these infections may influence susceptibility, transmissibility or the clinical course of the disease.

The high case-fatality rate may reflect the vulnerability of patients with underlying conditions to respiratory infection (as observed in Canada during the SARS outbreak where co-morbid conditions were independently associated with severe outcomes[11]) and the difficulties in detecting milder cases that may be occurring in the community.

Clinical Presentation:

Most confirmed cases have presented with, or later developed, acute, serious respiratory tract disease. Typical symptoms have included fever, cough and breathing difficulties. The most frequently reported symptoms were; fever (46 cases, 98%), cough (39 cases, 83%), shortness of breath (34 cases, 72%), myalgia (15 cases, 32%) diarrhea (12 cases, 26%), nausea (10 cases, 21%) and vomiting (10 cases, 21%).[2] A range of other symptoms were reported, each with a frequency of 17% or less.[2] A case that was transferred from the UAE to Germany in March 2013 had initial symptoms of rapid onset of ILI and a non-productive cough, progressing to pneumonia on day 2, thrombocytopaenia and on day 14, renal insufficiency requiring dialysis, and death on day 18 was from septic shock.[5]Of the first 47 cases in Saudi Arabia, 89% (42 cases) required management in intensive care, and 72% (34 cases) required mechanical ventilation.[2] All cases had abnormal chest radiographs.[2]

The number of reported cases who are asymptomatic cases or have mild symptoms has increased recently. There have been at least 15 such cases identified through contact tracing in late June and in July 2013, including four health-care workers in Saudi Arabia.[1, 3]Earlier in the outbreak, a case in the UK had only a mild, self-limiting illness.[6] A probable case in a family cluster in Saudi Arabia had only a mild self-limiting illness, but upper respiratory tract swabs were negative for the infection.[7] Milder ILI was also reported in probable secondary cases in the cluster centred on a hospital in Saudi Arabia.[8]An immunocompromised patient in France presented with atypical symptoms; fever and diarrhoea in the absence of respiratory symptoms, but with radiological evidence of pneumonitis.[9]

Renal insufficiency or failure has been noted for many MERS patients; however, it is unclear whether the renal insufficiency or failure is due to the infection, or may be contributed to by anti microbial agents and other medications used in treatment, or whether perhaps it influences disease progression, or some combination of these. Four of the first eight reported cases in Saudi Arabia were reported to have had renal failure in addition to respiratory failure, and both of the patients in France were reported to have had renal failure as was a case transferred to Germany from the UAE.[5, 9, 10] The cluster in a hospital in Saudi Arabia was centred on a dialysis unit, and 13/25 probable and confirmed cases had end stage renal disease.[8]

Diagnosis

Laboratory confirmation of MERS-CoV infections to date has largely been by real-time reverse transcription polymerase chain reaction (rRT-PCR) of lower respiratory tract specimens. Viral loads for MERS-CoV in analysed lower respiratory tract secretions for a patient treated in Germany in March 2013 were high 12 days after onset (the first samples available), and appeared to decline over the following six days, but still well above detection limits.[5] Fresh bronchoalveolar aspirates appeared to be the most reliable sample for MERS-CoV rRT-PCR .[5] In France, repeated assays and sampling showed the that there is the possibility of negative or inconclusive results on upper respiratory tract specimens with low viral loads compared with sputum collected at the same time point which may test positive, with very high viral loads.[9] Throat swabs appeared to be unreliable in a hospital associated outbreak in Saudi Arabia.[8]Upper respiratory tract specimens may not be as useful for confirming the infection by rRT-PCR, however two cases in the UK were PCR positive on throat/ nasopharyngeal swabs.[24]The United States Centers for Disease Control and Prevention recommends that lower respiratory tract specimens be a priority for collection and PCR testing, and that samples should be collected at a range of times post-onset.[25]

Stool samples were widely used during SARS for diagnosis,[26]but to date, there is only limited data on the utility of stool samples for diagnosing MERS. Stool samples collected on days 12 and 16 for a patient transferred to Germany in March 2013, were PCR positive, but the RNA concentrations were close to the lowest limit of detection.[5]

It is unclear whether the patient in Germany had diarrhoea at the time of collection for samples that were PCR positive. Stool samples collected on day 23 from a patient transferred to the UK were negative by rRT-PCR.[24] Whilst diarrhoea has been listed in the clinical picture for other cases, including in two separate clusters in Saudi Arabia,[7,8] no testing has been undertaken. Urine samples from a patient transferred to Germany in March 2013 were rRT PCR positive on days 12 and 13, but not on day 14.[5] RNA concentrations were higher in urine than in stool for at least one sample.

Immunofluorescence assays appeared to be useful to detect antibody to MERS-CoV in both acute and convalescent sera from a patient in Germany and from an experimentally infected macaque.[27] Whilst there were no false positives in a limited number of people who were not thought to have been exposed, further work is needed to validate the assay.[28]The assay detected antibodies when the patient still had virus detectable by rRT-PCR in respiratory secretions. A Western blot was used for confirmation, with good correlation between the results of these two methods.[27] Contacts of the case of MERS-CoV that was imported to Germany were screened using a two-stage testing; indirect immunofluorescence assay, followed by serum neutralisation tests. While one of 85 blood samples was reactive for IgM and another for IgG at low dilutions, specific novel coronavirus antibodies were ruled out by immunofluorescence assay.[15]

On 7 June 2013, the WHO reported that 8 of the 124 specimens collected during investigation of the 2012 outbreak in Jordan were positive by serology (enzyme immunoassay and immunofluorescence assay).[29] Six of these eight were considered probable cases in the outbreak investigation in April 2012. These are not included within the number of confirmed cases being reported by the WHO.

Revised interim case definition for reporting to WHO – Middle East respiratory syndrome coronavirus (MERS-CoV) [30]

These case definitions have been revised based on new information collected since the previous definitions were published. WHO will continue to review and update them as new information becomes available.

Probable case

Three combinations of clinical, epidemiological and laboratory criteria can define a probable case:

- A person with a febrile acute respiratory illness with clinical, radiological, or histopathological evidence of pulmonary parenchymal disease (e.g. pneumonia or Acute Respiratory Distress Syndrome)

  AND

  Testing for MERS-CoV is unavailable or negative on a single inadequate specimen[1]

  AND

  The patient has a direct epidemiologic-link with a confirmed MERS-CoV case[2].

- A person with a febrile acute respiratory illness with clinical, radiological, or histopathological evidence of pulmonary parenchymal disease (e.g. pneumonia or Acute Respiratory Distress Syndrome)

  AND

  An inconclusive MERS-CoV laboratory test (that is, a positive screening test without confirmation)

  AND

  A resident of or traveler to Middle Eastern countries where MERS-CoV virus is believed to be circulating in the 14 days before onset of illness.

- A person with an acute febrile respiratory illness of any severity

  AND

  An inconclusive MERS-CoV laboratory test (that is, a positive screening test without confirmation)

  AND

  The patient has a direct epidemiologic-link with a confirmed MERS-CoV case.

Confirmed case

A person with laboratory confirmation of MERS-CoV infection.

Notes

Inconclusive testing: Patients with an inconclusive initial testing should undergo additional virologic and serologic testing to determine if the patient can be classified as a confirmed MERS-CoV case. It is strongly advised that lower respiratory specimens such as sputum, endotracheal aspirate, or bronchoalveolar lavage fluid be used when possible. If patients do not have signs or symptoms of lower respiratory tract infection and lower track specimens are not available or clinically indicated, both nasopharyngeal and oropharyngeal swab specimens should be collected. If initial testing of a nasopharyngeal swab is negative in a patient who is strongly suspected to have MERS-CoV infection, patients should be retested using a lower respiratory specimen tract or a repeat nasopharyngeal specimen with additional oropharyngeal specimen if lower respiratory tract specimens are not possible, and paired acute and convalescent sera.

Notes:

Inconclusive testing:

- Patients with an inconclusive initial testing should undergo additional virologic and serologic testing to determine if the patient can be classified as a confirmed MERS-CoV case.
- It is strongly advised that lower respiratory specimens such as sputum, endotracheal aspirate, or broncho-alveolar lavage fluid be used when possible.
- If patients do not have signs or symptoms of lower respiratory tract infection and lower tract specimens are not available or clinically indicated, both nasopharyngeal and oropharyngeal swab specimens should be collected.
- If initial testing of a nasopharyngeal swab is negative in a patient who is strongly suspected to have MERS-CoV infection, patients should be retested using a lower respiratory specimen tract or a repeat nasopharyngeal specimen with additional oropharyngeal specimen if lower respiratory tract specimens are not possible, and paired acute and convalescent sera.

Inconclusive tests may include:

- A positive screening test without further confirmation such as testing positive on a single PCR target
- A serological assay considered positive by the testing laboratory.

Currently confirmatory testing requires molecular diagnostics including either a positive PCR on at least two specific genomic targets or a single positive target with sequencing on a second. However, the interim recommendations for laboratory testing for MERS-CoV should be consulted for the most recent standard for laboratory confirmation.

An inadequate specimen would include a nasopharyngeal swab without an accompanying lower respiratory specimen, a specimen that has had improper handling, is judged to be of poor quality by the testing laboratory, or was taken too late in the course of illness.

Asymptomatic cases:

- The demonstration of asymptomatic infection is useful for epidemiological investigations and should be pursued as part of case investigations, however, the burden of proof must be higher due to the risk misclassification because of false positive tests due to laboratory contamination.
- Generally, in most viral infections, an immunological response such as development of specific antibodies would be expected even with mild or asymptomatic infection and as such serological testing may be useful as additional confirmation of the diagnosis.
- Additional steps to reconfirm asymptomatic cases, or any case in which the diagnosis is suspect, could include re-extraction of RNA from the original clinical specimen and testing for different virus target genes, ideally in an independent laboratory.

Treatment

There is no current treatment or vaccination available for MERS-CoV, but, with the continuation of the outbreak, identification of therapeutics is a top priority. Several manuscripts have demonstrated that a variety of therapeutics inhibit MERS-CoV replication in cell culture[9,15]. None have been tested *in vivo*, in part due to the lack of a small animal model, as described above. One promising avenue is to use the knowledge of SARS-CoV and compare it to MERS-CoV. IFNá was shown in multiple models to protect against SARS-CoV-induced disease. MERS-CoV is also sensitive to IFNâ treatment *in vitro*[15]. Ribavirin, a known inhibitor of RNA viruses, has also been demonstrated to inhibit MERS-CoV replication, and together they can inhibit MERS-CoV at nanomolar levels [22]. In the case of MERS-CoV infections, interferon-alpha 3b and ribavirin may work primarily by reducing damaging inflammation of the lung and promoting healing by altering the host response, rather than directly targeting the virus.

Other inhibitors were shown to affect specific pathways, specifically the MAPK pathway. The MAPK inhibitor SB203580 was shown to inhibit MERS-CoV replication in VerE6 cells [9]. Additional therapeutics and vaccinations are in development, with a focus on FDA compounds already in use.

Conclusion:

CDC is working with partners to better understand the risks of this virus, including the source, how it spreads, and how infections might be prevented. CDC has provided information for travelers and is working with health departments, hospitals, and other partners to prepare for possible cases in the United States. Healthcare professionals should evaluate patients for MERS-CoV infection if they develop fever and pneumonia within 14 days after traveling from countries in or near the Arabian Peninsula. They should also evaluate patients for MERS-CoV infection if they have had close contact with a symptomatic recent traveler from this area who has fever and acute respiratory illness. Testing

for MERS-CoV and other respiratory pathogens can be done simultaneously. Positive results for another respiratory pathogen should not necessarily preclude testing for MERS-CoV.

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