Review on Worldwide Distribution and Molecular Detection of Middle East Respiratory Syndrome Corona Virus (MERS-COV)

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Abstract: Middle East Respiratory Syndrome coronavirus infection (MERS-CoV) became an evolving worldwide health concern. It was to inlight (highlight) the current epidemiological distribution and to gate update data about the molecular characteristics of MERS-COV. Most of the MERS-CoV infections have occurred in the Arabian Peninsula, but additional cases have been reported from other countries. In Ethiopia, there was high seroprevalence of MERS-CoV evident in Afar, Oromia, Amhara, Somalia and Tigray between camels. The clinical spectrum of Middle East Respiratory Syndrome coronavirus infection ranges from no symptoms (asymptomatic) or mild respiratory symptoms to severe acute respiratory disease and death. Transmission of the disease was mainly by personal contact or by the infected bat or dromedary camels which were seen in most of the cases. The genome sequence an assay targeting upstream of the envelope gene (upE), followed by confirmation with assays targeting either the open reading frame 1b (ORF 1b) or 1a (ORF 1a) of the virus had identified and real-time reverse transcriptase-polymerase chain reaction (real-time PCR). Special prevention as, the WHO advised people at risk of MERS-CoV infection to avoid contact with camels, practice good personal hygiene and avoid drinking raw milk or eating contaminated food peeled or cooked. MERS-CoV is a zoonotic emerging disease with bats and dromedary camels as the important animals mainly involved as sources for its emergence, outbreak and epidemiological pattern.

Key words: Coronavirus · Epidemiology · Molecular Characterization · MERS-COV

INTRODUCTION

Corona Viruses are species in the genera of viruses belonging to the subfamily Corona virinae in the family Corona viridae. Coronavirus are enveloped viruses with a positive-sense RNA genome which is characterized by nucleocapsid of helical symmetry. The genomic size of Coronavirus ranges from approximately 26 to 32 kilobases, extraordinarily large for an RNA virus. With its characteristic surface, the virions appear as a crown like image under the electron microscope and so the viruses are named after the Latin word corona, meaning crown [1].

Corona viruses are recognized causes of mild respiratory tract infections in humans, first identified in the 1960s. These large RNA viruses affect a wide range of animals including domestic and companion animals and bats. Limited surveillance data show that bats are the host of greatest diversity of corona viruses, varying by region and species. It suggests that bats may be the natural reservoir of the virus [2].

Most of the MERS-CoV infections have occurred in the Arabian Peninsula (Kuwait, Bahrain, Qatar, the United Arab Emirates, Oman, Yemen and Saudi Arabia), but additional cases have been reported from countries in North Africa, Europe, North America and Asia due to movement of infected individuals [3].

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection is an evolving worldwide health crisis. Since the country has much population of camels and cultures of stay with them, it is important to indicate the early diagnosis, control, prevention and management of disease [4].

Therefore, the aim of this review is:

- To inlight (highlight) the current epidemiological distribution.
- To gate update data about their molecular characteristics of MERS-COV.
Etiologic Agent of MERS: Middle East respiratory syndrome is caused by the newly identified MERS corona virus (MERS-CoV), with single-stranded RNA belonging to the genus beta corona virus [5]. There are three main sub-groupings of Corona viruses: alpha, beta and gamma and a fourth provisionally-assigned new group called delta Coronaviruses. Alpha Co-V: – Human examples: HCoV-220E, HCoV-NL63 – Pig, dog and cat CoVs. Beta Co-V: – HCoV-OC43, HCoV-HKU1, HCoV-SARS – MHV, rat, pig and cow CoVs – MERS-CoV. Gamma Co-V: – Chicken and turkey CoVs Delta Co-V: – Bird CoVs [1].

Taxonomical Classification:

![Taxonomical Classification of corona virus][1]

Structural Features of Novel MERS-CoV: Electron microscope image of Middle East Respiratory Syndrome virus particles, colorized in yellow. Here the virus of MERS-CoV contains mainly spike like structures mainly consists of glycoprotein for mainly receptor binding and antigenic activity. It also contains nucleocapsid phosphoprotein for RNA-binding, Membrane glycoprotein for triple membrane spanning and positive strand RNA (+)ssRNA. The glycoprotein spike serves as the shape of crown for the virus so called as corona virus [6].

Epidemiology

Distribution: Most of the MERS-CoV infections have occurred in the Arabian Peninsula (Kuwait, Bahrain, Qatar, the United Arab Emirates, Oman, Yemen and Saudi Arabia), but additional cases have been reported from countries in North Africa, Europe, North America and Asia due to movement of infected individuals. The outbreak in the Republic of Korea in May 2015 was the largest MERS-CoV outbreak ever recorded outside Saudi Arabia and resulted in 185 laboratories confirmed human infections in Korea and one in China, with 36 deaths [3].
Corona viruses infect many different animals and cause them to have respiratory, gastrointestinal, liver and neurologic diseases. Most of these Corona viruses usually infect only one animal species or, at most, a small number of closely related species [7].

Natural Reservoir: Recent phylogenetic analysis of viral isolates from humans, camels and bats revealed that bats may have been the original primary reservoir of the virus and they may have initially transmitted the virus to camels [8].

Modes of Transmission
Animal to Human: Animal and genetic studies provide some evidence that MERS-CoV might be a zoonotic disease, but it is still not clear how the disease transmits from animals to humans. The possibility of transmitting virus from bat excreta to humans has been discussed for other viruses such as bat rabies or Hantavirus, where humans can be infected by inhaling infectious viral particles present in dust [1].

Human-to-Human Transmission: The transmission of MERS is defined as sporadic, between family members; often occurs in health care settings and requires close and prolonged contact. Human-to-human of MERS-CoV requires close contact and can occur among relatives in households and among patients and health care workers in health care settings (nosocomial infection) [3]. It is clear that the disease can be passed on from person to person, probably through by close contacts or via health care facilities [9].

Nosocomial transmission has been a hallmark of MERS-CoV. A large outbreak was documented in Al-Ahsa, Saudi Arabia: 23 confirmed and 11 probable cases were diagnosed as part of a single outbreak that involved four healthcare facilities in 2013. The majority of cases were patients, but five family members and two healthcare workers were also affected. The hemodialysis unit was the most heavily affected, with nine confirmed cases, but transmission also occurred in the intensive care unit and the medical ward. Despite a thorough investigation, questions remain, including whether person-to-person transmission occurred through airborne, respiratory droplets, or direct contact. Based on data, airborne transmission of MERS-CoV cannot be excluded, but there is no indication that it plays an important role in the transmission of the virus. Once strict infection control measures were implemented, the outbreak was contained [10].

Pathogenesis: MERS-CoV uses the receptor dipeptidyl-peptidase-4 (DPP-4) to enter its host cell. Sequence comparison between the receptor-binding domain of the MERS-CoV spike protein and several mammalian DPP-4 sequences showed a higher percentage identity in the amino acid residues critical for virus entry between human and horse DPP-4 than between human and dromedary DPP-4. It has been shown that MERS-CoV can use horse DPP-4 expressed on non susceptible cells, but no data are available on susceptibility of primary horse cells [8].

The replication of Coronavirus occurs in host cell cytoplasm. The viruses primarily bind to the receptor on the cell surface via the spike (S) protein. When S protein is bound to the receptor, a conformational structure occurs in the structure and the process of entry into the virus cell begins. This process with endocytosis is dependent of pH through the receptor. After entering the cytoplasm, the virus particle releases the RNA genome [6].
Structure of the Spike: The coronavirus spike protein is a type I glycoprotein that forms the peplomers on coronavirus particles. Some coronaviruses spikes (most from group II and III viruses) are cleaved into two subunits by a furin-like enzymatic activity during processing in the Golgi. The prototype MHV spike is 180 kDa; for most MHV strains, it is cleaved into two noncovalently associated subunits of about 90 kDa (294). The amino-terminal S1 subunit, which is believed to form the globular head of the mature protein, contains a receptor binding domain (RBD) within the first 340 amino acids (163). The RBDs of HCoV-229E spikes are also found in S1, although not at the amino termini. S1 of MHV contains, downstream of the RBD, a “hypervariable domain” (HVR) that varies in length among strains. Comparison of sequences of various isolates of the JHM strains as well as one isolate of the A59 strain shows “in-frame” deletions of up to 450 nucleotides. The carboxyterminal S2 subunit, which is conserved among all coronavirus spikes and is believed to form a stalk-like structure anchored in the membrane, contains two (or perhaps three heptad repeat (HR) domains as well as the putative fusion peptide A cysteine-rich domain that bridges the putative fusion junction of the anchor and the cytoplasmic tail is necessary for fusion, as is the transmembrane domain [11].

Receptor Interaction, Fusion and Entry: Coronaviruses attach to specific cellular receptors via the spike protein (Table 1). The first identified coronavirus receptor was CEACAM 1, utilized by MHV (141). Viral attachment triggers a conformational change in the spike protein that promotes the fusion of viral and cellular membranes (212, 369). While there are no crystal structures available for any coronavirus spike, it is believed that it may undergo changes similar to those of other type I fusion proteins, such as influenza virus hemagglutinin and human immunodeficiency virus gp120, in order to mediate fusion of viral and cellular membranes. The coronavirus spike protein plays vital roles in viral entry, cell-to-cell spread and determining tissue tropism. Coronavirus entry is, in general, not pH dependent and thus it has been believed to occur directly at the plasma membrane and not via an endosomal route. Furthermore, this inhibition may be overcome by protease treatment of virus that has attached to the cell. This, along with the observation that infection is blocked by inhibitors of the pH-sensitive endosomal protease cathepsin [12].

Clinical Features: MERS-CoV is known to infect cell lines of the intestinal tract. MERS-CoV has no specific symptoms, it generally shows all the symptoms which are occurred in pneumonia and other respiratory diseases. So it’s difficult to understand the disease with respect to the symptoms [1]. The typical symptoms of MERS are fever, cough and shortness of breath and it is often accompanied by pneumonia [13].

In general, the clinical appearances of MERS-CoV infection characterize a wide-ranging spectrum fluctuating from asymptomatic presentation and mild to severe acute respiratory illness to death. A distinctive presentation of MERS-CoV features are, fever with chills or rigors, generalized myalgia, malaise, drowsy, confused, dyspnea, cough, shortness of breath and radiological pulmonary presentation of pneumonia. The extra-pulmonary features include abdominal disorders, nausea, vomiting, diarrhea and acute renal failure [7].

The disease appears with severity in old age and debilitated immune systems, mainly in people with chronic diseases such as diabetes mellitus and pulmonary and renal diseases. The other clinical findings are pericardium inflammation, consumptive coagulopathy, an increase in White Blood Cells count, mainly neutrophils and decrease lymphocytes, platelets and red blood cells [14].

Identification and Viral Characterization
Molecular Detection of MERS-COV: Laboratory detection of MERS-CoV is based on molecular techniques and the most commonly used approach is real-time reverse-transcription polymerase chain reaction (rRT-PCR), which is an essential and indispensable method for laboratory molecular diagnostics [15]. Hydrolysis probe-based real-time PCRs targeting upstream of E gene (UpE) and open reading frame (ORF) 1a are used as recommended by the World Health Organization (WHO). Each specimen with broad-range reverse transcription PCR (RTPCR) conserved across the coronavirus family to detect other Coronaviruses [16].

MERS-CoV, a lineage C Betacoronavirus (MERS-CoV), has a positive-sense single-stranded RNA (ssRNA) genome about 30-kb in size. Phylogenetic analysis of MERS-CoV has been done on 182 full-length genomes or multiple concatenated genome fragments, including 94 humans and 88 from dromedary camels [17]. The MERS-CoV genomes share more than 99% sequence identity, indicating a low mutation rate and low variance among the genomes [4].

MERS-CoV genomes are roughly divided into two clades: clade A, which contains only a few strains and clade B, to which most strains belong. As with other CoV genomes, the first 5’ two-thirds of the MERS-CoV genome consist of the replicase complex (ORF1a and ORF1b).
The remaining 3' one-third encodes the structural proteins spike (S), envelope (E), membrane (M) and nucleocapsid (N), as well as five accessory proteins (ORF3, ORF4a, ORF4b, ORF5 and ORF8b) that are not required for genome replication, but are likely involved in pathogenesis. The flanking regions of the genome contain the 5' and 3' untranslated regions (UTR). Typical of the *Coronaviruses*, the MERS-CoV accessory proteins do not share homology with any known host or virus protein, other than those of its closely related lineage C βCoVs [20].

### Nucleic Acid Extraction

The collected samples which are either in RNAlater RNA stabilization Reagent (QIAGEN, Hilden, Germany, 1ml/vial) or transport media (COPAN, 1ml/vial) are thoroughly mixed and centrifuged at 2, 000 rpm for 5 min and then the supernatants are used for RNA extraction from 200ul of each swab sample in a fully automated MagNA pure 96 DNA and viral NA [nucleic acid] small volume kit (Roache Diagnostics, Germany), according to the manufacturer’s instructions. The concentration and purity of eluted NA is assessed using a nano-spectrophotometer. A ratio of approximately 2.0 is accepted as pure RNA yield and considered suitable for RT-PCR processing.

- **Up E-Fwd** (5'-GCAACGCCTATTAGTT-3') and **Up E-reverse** (5'-GGCTTACCACGGGACCATA-3')
- primers, 0.5 µM upE
- **Probe** (5' 6-FAM/CTCTTACATAATCGCCCCGAGCTCG/3'6-TAMSp) and 2 µL cDNA in 1 × reaction buffer (Takara, Kyoto, Japan). The reaction was carried out in Viia 7 real-time PCR system (Life Technologies) with 40 cycles of amplification. For the ORF1a quantitative PCR, the reaction was set up as above except with forward primer
  - **Orf1a-Fwd** (5'-CCACTACTCCATTGTTCGAG-3')
  - **Orf1a-reverse** (5'-CAGTATGGTAGCAGCAGATATAAGCA-3')
  - **And Orf1a-probe** (5' 6-FAM/TTGCAAAATTGGCTGCCCGACC/3'6-TAMSp) targeting the ORF1a gene. Cycle threshold was generated by the Viia7 system automatically with default settings [19].

### Real Time Reverse Transcription –Polymerase Chain Reaction

Recently, the World Health Organization (WHO) published an updated interim guidance for laboratory testing of MERS-CoV. Three assays for diagnosing MERS-CoV infections have been developed, which were based on the detection of specific regions of viral RNA by rRT-PCR. The tests include an assay targeting upstream of the envelope gene (upE), which is highly sensitive and recommended for laboratory screening, followed by confirmation with assays targeting either the open reading frame 1b (ORF 1b) or 1a (ORF 1a) [20].

Real-time quantitative RTPCR is the primary method for laboratory diagnosis of MERS-CoV infection and it requires at least two different genomic targets for a positive diagnosis according to the case definition announced by WHO as of July 26, 2017 [21].

The two RT-qPCR assays developed shortly after the first report of the disease were designated as recommended MERS-CoV molecular diagnostics by WHO. Both assays proved to be highly sensitive and were successfully used for the diagnosis of the majority of the MERS-CoV cases [3].

Criteria for detection of MERS-CoV RNA: a positive result for at least one of the following

- A positive result for at least two *MERS-CoV*-specific gene targets (*upE*, *ORF1a*, *ORF1b*, *N*) by real-time RT-PCR (recommended).
- A positive RT-PCR result for at least one *MERS-CoV*-specific gene target [ORF1b (*RdRp*), *N*], plus confirmation by base sequence analysis of the PCR product [22].

### Control and Prevention

Understanding the route of transmission of MERS-CoV and its pattern of transmission is important for the proper implementation of control and prevention of the disease. The pattern of the disease transmission will dictate the methods for prevention [5]. The WHO advises people at risk of MERS-CoV infection to avoid contact with camels, practice good personal hygiene and avoid drinking raw milk or eating contaminated food unless it is properly washed, peeled, or cooked [23].

Currently, there is no specific drug or vaccine available for the treatment of infection caused by MERS-CoV. Therefore,
CONCLUSIONS AND RECOMMENDATION

MERS-CoV is a zoonotic emerging disease with bats and dromedary camels as the important animals mainly involved as source for its emergence, outbreak and epidemiological pattern. Urgent epidemiological studies and molecular detection like of viral RNA by real-time reverse-transcriptase polymerase chain reaction of MERS-CoV are mandatory to better understand the transmission patterns of MERS-CoV. Both in human and camel samples, in camel rearing African countries like Ethiopia where camel dependent poor pastoral communities are found, even though current human cases are not reported. In Ethiopia, the study also implies the probability of circulation of MERS-CoV in human being in the study areas.

Based on this review, gap and future direction will be recommended:

- Avoid close contact with sick people, especially with those suffering from acute respiratory infections, avoid close contact with camels.
- Adhere to good food-safety practices, avoid undercooked meat and do not consume unpasteurized camel milk.
- In Ethiopia: for people especially live in the region of the sero-prevalancey is high like Oromia, Amhara. Somalia, Afar and Tigray, the government should be attentive to them since there is evidence of transmission.

REFERENCES


