HAYATI Journal of Biosciences 24 (2017) 53–56

Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences

Review paper

Emerging Developments on Pathogenicity, Molecular Virulence, Epidemiology and Clinical Symptoms of Current Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

Kannan Subbaram,[1*] Hemalatha Kannan,[2] Mansour Khalil Gatasheh[3]

[1] Department of Preparatory (Biology), Al-Ghad International Colleges for Applied Medical Sciences, Riyadh, Saudi Arabia.
[2] Department of Laboratory Sciences & Pathology, Jimma University, Ethiopia.
[3] Department of Clinical Laboratory Sciences, Al-Ghad International Colleges for Applied Medical Sciences, Riyadh, Saudi Arabia.

ARTICLE INFO

Article history:
Received 26 March 2017
Received in revised form
28 July 2017
Accepted 22 August 2017
Available online 14 September 2017

KEYWORDS:
genetic rearrangement,
MERS-CoV,
RT-PCR,
+ssRNA,
virulence

ABSTRACT

Middle East respiratory syndrome coronavirus (MERS-CoV) is a recently reported virus that is associated with severe, life threatening and rapidly spreading primarily respiratory illness called the Middle East respiratory syndrome. MERS-CoV possesses a unique positive-sense single-stranded RNA and can undergo rapid mutation in the viral genome. This results in antigenic switching and genetic variation, finally leading to the emergence of novel and new MERS-CoV subtypes which are uncontrollable by vaccines. Researchers are also finding difficulties to sort out therapeutic intervention strategies for MERS-CoV. This virus can spread from human to human, but transmission from dromedary camels to humans plays a crucial epidemiological significance. Dromedary camel acts as "gene mixing vessels" for MERS-CoV and these virus particles undergo rapid change in them. Viral receptors called dipeptidyl peptidase-4 are important receptors for attachment and spread of MERS-CoV in humans. The current method of laboratory confirmation is through real-time polymerase chain reaction on bronchoalveolar lavage, sputum and tracheal aspirates. Unfortunately, till today there are no definite anti-viral drugs available for MERS-CoV.

1. Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV) is a unique positive-sense single-stranded RNA (+ssRNA) virus belonging to the genus Betacoronavirus (World Health Organization, 2014). Previously, it was called novel coronavirus 2012 because it was isolated in 2012 by genomic sequencing from sputum of a patient, during the 2012 outbreak of new flu (Zaki *et al.* 2012).

Until August 2015, MERS-CoV outbreaks were reported in more than 21 nations like Saudi Arabia, Jordan, Egypt, Qatar, UAE, Turkey, Kuwait, Oman, Bangladesh, Indonesia, Algeria, South Korea, Philippines, Thailand, USA, UK and Austria (Abdullah *et al.* 2016). According to the European Center for Disease Prevention and Control, it was estimated that out of a total of 1082 MERS-CoV cases, there were 439 deaths thereby mortality rate leading to whooping 41% (Haagmans *et al.* 2015). As per the World Health Organization estimates, the fatality rate surged to 37%. MERS-CoV patients have acquired this deadly infection through various sources, namely, infected humans, camels, bats, other domesticated animals and pets (World Health Organization, 2015). The very nature and special feature of coronavirus is its ability to undergo rapid genetic rearrangement and profound molecular variation in its ssRNA. This rearrangement and variation of the viral genome resulting in new antigenic subtypes, leads to difficulties in developing vaccines and therapeutic interventions (Chu *et al.* 2016). Hence, MERS-CoV poses serious threat not only to the Middle East region, but also throughout the world, as a whole.

Genomes of MERS-CoV are phylogenetically grouped into clade A and clade B. Initially, reported MERS-CoV cases belonged to clade A (Jordan-N3/2012), recently, the newly reported cases were shown to be genetically different, as such, they were named as clade B. Based on molecular, genetic and antigenic studies, it was established that MERS-CoV is unique and different from severe acute

* Corresponding author.
E-mail addresses: subbaramkannan@gmail.com (K. Subbaram), hema_paramesh@yahoo.com (H. Kannan), mansour_gatasheh@yahoo.com (M. Khalil Gatasheh).

Peer review under responsibility of Institut Pertanian Bogor.

http://dx.doi.org/10.1016/j.hjb.2017.08.001

respiratory syndrome (SARS) coronavirus and common cold coronaviruses. Because of this fact, earlier MERS-CoV was called "Saudi SARS" (Azhar *et al.* 2016). This notorious virus was first identified and isolated in Saudi Arabia by Dr Ali Mohamed Zaki from a patient suffering from pneumonia-like illness, and these isolates exhibited cytopathic effects like syncytia formation. Through molecular clock analysis, zoonotic transmission was established from dromedary camels (*Camelus dromedaries*), particularly prevailing in Middle East countries.

2. Virology of MERS-CoV

2.1. Molecular Virulence

Both MERS-CoV and SARS coronavirus have similar +ssRNA in their genome.

This ssRNA in MERS-CoV encodes structural proteins (SPs), membrane protein (M), envelope (E), nucleocapsid (N), two non-structural replicase polyproteins (ORF1a and ORF1b) and spike (S) (Reusken *et al.* 2016). The two non-structural proteins (NSPs) initiate genomic replication and RNA synthesis. The huge replicase gene surrounds 5′ proximal side of RNA genome. Translation of ORF1a gives rise to polyprotein 1a (pp1a) and ribosomal frame shifting makes translation of ORF1b to give pp1ab. MERS-CoV uses one or two papain-like protease to release NSPs. It is established that MERS-CoV possesses 16 NSPs (Firth and Brierley, 2015).

Viral components like NSPs, SPs, M, E, N, S and other proteins, glycoproteins and enzymes play a crucial role in virulence in establishing and exaggerating the disease (Figure 1).

MERS-CoV's E protein is responsible for the attachment of virus particles to host cell receptors. S protein is in charge of fusion and entry in to the respiratory epithelia (Kilianski *et al.* 2016).

Virus-induced proteases help viral particles to spread to lower parts of the lungs, thus enhancing the severity of the infection. It results in inflammation and suppression of anti-viral interferons (IFN). At this point of the infection, there is excessive anti-viral immune responses like interleukins (IL-6, IL-8 and TNF-α), humoral IgG and IgM are inefficient to control the viral spread and replication in the lungs. The very nature of MERS-CoV virus ability to overcome and suppress host immune challenge is due to its +ssRNA, viral SPs and NSPs (Mielech *et al.* 2016).

2.2. Pathogenesis and symptomatology

MERS-CoV exhibits its degree of pathogenicity based on the host. It elicits maximum pathogenic potential, especially in humans. This is due to the fact that MERS-CoV shows a strong tropism for bronchial non-ciliated epithelia. During this time, it is proved that virus also arrests host bronchial IFN synthesis (Coleman *et al.* 2015; Mielech *et al.* 2016). At this juncture, it should be noted that most of other viruses causing respiratory diseases attack and damage epithelial cilia, including influenza type A. Studies revealed that cellular receptors for MERS-CoV is exopeptidase (angiotensin converting enzyme 2) (Coleman *et al.* 2015). Moreover, it was found that neutralization of angiotensin converting enzyme 2 by specific antibodies did not arrest the spread of infection into bronchus and lung alveolus. Extensive studies showed that another functional cellular receptors called dipeptidyl peptidase-4 were also involved in the severity of disease spread in

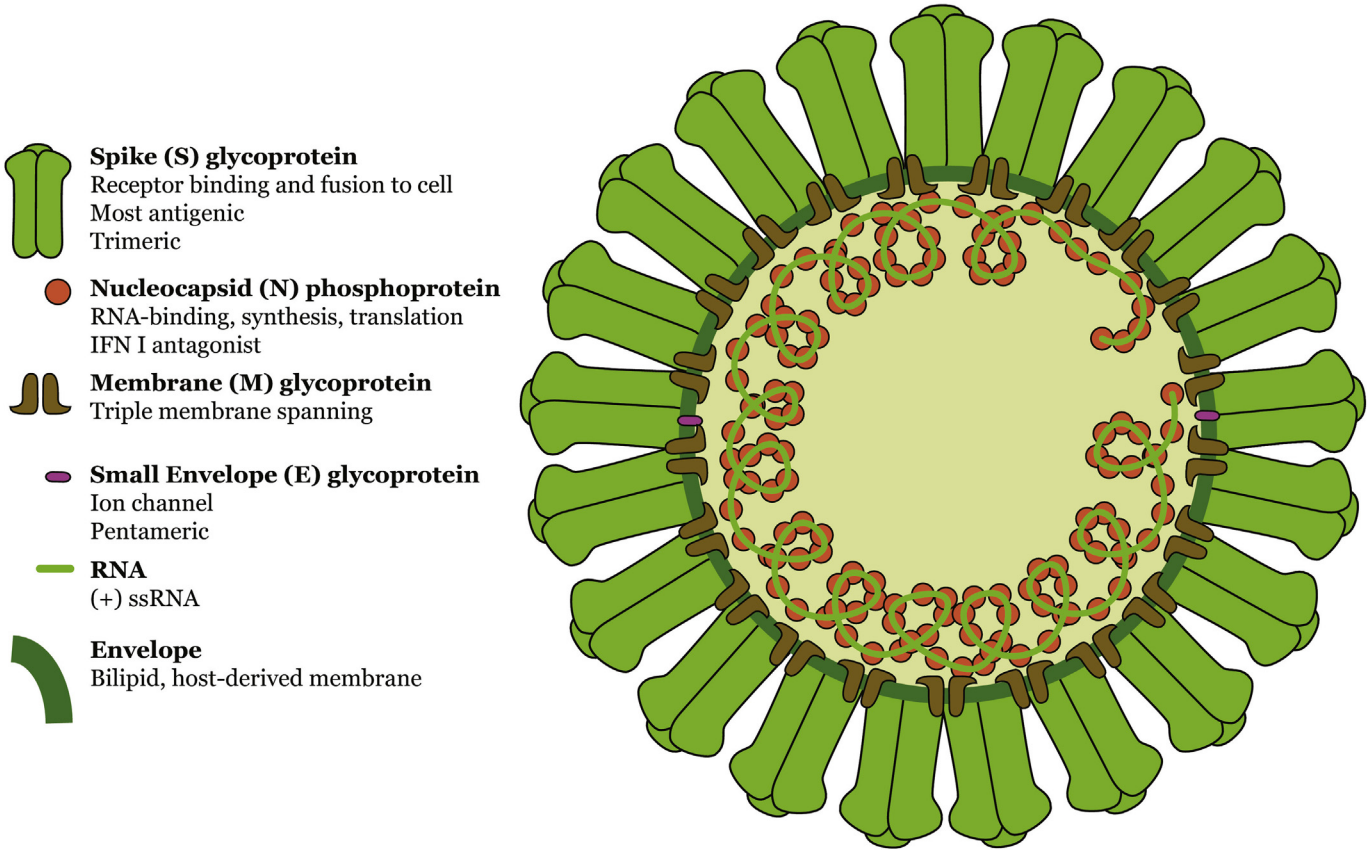

Figure 1. Molecular structure of MERS-CoV. MERS-CoV = Middle East respiratory syndrome coronavirus.
(Photo courtesy Dr Ian M Mackay, PhD)

the lungs due to MERS-CoV (de Wit *et al.* 2015). Moreover, receptors for dipeptidyl peptidase-4 are also located in nephrons of kidneys and heart. During the acute stage of the MERS-CoV infection, there is severe viremia, leading to the spread of MERS-CoV virus particles in blood stream. At this point, MERS-CoV exhibits the hall mark of infection leading to not only to the damage of lungs but also kidneys and heart, thereby resulting in respiratory, renal and cardiac failure, ultimately leading to coma and death (vanBoheemen *et al.* 2016). The severity is worsened by concurrent secondary bacterial infections. Recent research showed that bacterial infections due to *Staphylococcus aureus*, Group A *Streptococcus*, *Streptococcus pneumoniae* and *Haemophilus influenzae* type b augment the pathogenic potential of MERS-CoV, particularly in humans. These bacteria particularly dwell in the oral cavities, tonsils and pharynx of humans (Lau *et al.* 2016). Another interesting fact is that the reservoir host dromedary camels and other animals do not exhibit the presence of dipeptidyl peptidase-4 receptors in their kidneys and heart. In humans, after the entry of MERS-CoV viral particles in to lung alveoles, alveolar macrophages fail to contain the spread of infection. The strong host cellular immune response and cytokine release lead to inflammation and fluid accumulation in lungs. This will result in the characteristic symptoms of MERS-CoV; high fever, chills, rigors, severe cough, dyspnoea and hypovolemic shock (Lin *et al.* 2016). Many patients exhibited common symptoms of pneumonia, whereas a significant number of patients expressed symptoms of chronic obstructive pulmonary syndrome like disease. Another large number of patients also revealed laryngotracheobronchitis, which is called “croup”. The patterns of disease due to MERS-CoV also differed among different age groups. Clinical cases, morbidity and mortality rates due to MERS-CoV infections are more common and high (80%) in the age group of 35–45 years. Astonishingly, the severity of MERS-CoV is lower (40%) in the higher age groups, particularly above 55 years (Lei *et al.* 2015).

2.3. Epidemiology

Epidemiology and mode of spread of MERS-CoV play a crucial role in morbidity and fatality due to these life-threatening viruses. Different ways of transmission of MERS-CoV have been documented (Corman *et al.* 2015). They include dromedary camel to human mode, bats to camels, among camels, cattle to man, dogs to humans, cats to man, bats to humans and finally man to man transmission. Human to human spread is very effective and common in close contacts and crowded surroundings. Nosocomial, hospital-borne outbreaks have also been reported in many instances. There have been reported incidences of spread from patients to health care workers (Song *et al.* 2015). In MERS-CoV epidemiology, dromedary camels play an important role because these animals act as reservoir host. Dromedary camels also act as “gene mixing vessels”. When two different strains of MERS-CoV, from two different sources, infect dromedary camels, these two genetically different MERS-CoV exchange their +ssRNA resulting in the development (mutation) of new subtypes of MERS-CoV. The stunning features of these new subtypes of MERS-CoV have new antigens and novel virulence genes. In Saudi Arabia, there were possible cases of human transmission after drinking camel milk. But, there are no cases documented in humans through ingestion of camel meat (de Wit *et al.* 2016).

2.4. Recent laboratory testing techniques

Patients suffering from MERS exhibited low white blood cell count in particular of low lymphocytes. Confirmation and rapid molecular testing can be performed using real-time polymerase chain reaction (RT-PCR) test. RT-PCR testing can be performed on bronchoalveolar lavage, sputum and tracheal aspirates. Serological test like immunofluorescence technique (IFT) is also useful. But, the main issue with IFT is many other serologically related viruses may cross-react with MERS-CoV antibodies. So, IFT is unsuitable for confirmation. World Health Organization recommends RT-PCR for the confirmation of MERS-CoV in clinical samples (Báez-Santos *et al.* 2014).

2.5. Prevention and treatment

Because the main transmission is through camels, people should be advised to handle camels with precautions, especially camels with severe nasal discharge. Other general health care measures should be taken to arrest nosocomial hospital-borne MERS-CoV outbreaks. For the treatment of MERS-CoV cases, administration of ribavirin and IFN-α2b was found to be promising, but not very effective. Researchers are recently investigating many ways to overcome the outbreak of MERS-CoV. Many drugs are under evaluation (IFN, chloroquine, chlorpromazine, loperamide, camostat, mycophenolic acid and lopinavir (de Wit *et al.* 2016).

3. Conclusion

MERS-CoV causes severe, life threatening and rapidly spreading Middle East respiratory syndrome not only in Middle East region, but throughout the world. Dromedary camels and bats play an important role in the spread of MERS-CoV. Several nosocomial infections due to MERS-CoV have also been reported worldwide. Several viral SPs and NSPs are critical for the molecular virulence of MERS-CoV. Because of the +ssRNA, MERS-CoV can undergo molecular rearrangement leading to antigenic change, genetic variation and mutation. This attributes to the emergence of new and novel subtypes of MERS-CoV in the human populations. These unique subtypes are difficult to control by vaccinations and therapeutic intervention strategies. Another important issue faced by scientists and molecular virologists is related to these new MERS-CoV viral particles which possess enhanced molecular virulence, thus leading to increased pathogenicity and a more rapid spread than their parental strains. Studies revealed that dromedary camels act as “gene mixing vessels” for MERS-CoV. Prevention is mainly based on avoiding contact with dromedary camels and focussing on nosocomial infection outbreaks. Researchers are constantly trying to develop anti-viral drugs to combat MERS-CoV, but till today there is no breakthrough. The perspective of what can we expect in the future of this field is developing effective vaccine, conducting detailed studies on molecular research on mechanism of mutation and genetic recombination occurring in MERS-CoV.

Conflict of interest

There is no conflict of interest.

Funding support

We would like to acknowledge University Grants Commission (UGC), New Delhi for the financial grant supporting this work through their grant number 112050678/2016.

References

Abdullah MA, Holly MB, Glen RA, Xiaoyan L, Abdulaziz BS, Osman A, Mutaz M, Hail MA, Homoud SA, Rafaat FA, Malak MA, Ali AA, Randa N, Dean DE, Susan IG, John TW. 2016. Increase in MERS-CoV cases in Saudi Arabia linked to hospital outbreak with continued circulation of recombinant virus, July 1–August 31, 2016. *Infect Dis.* http://dx.doi.org/10.1093/ofid/ofw165.

Azhar EI, El-Kafrawy SA, Farraj SA, Hassan AM, Al-Saeed MS, Hashem AM, Madani TA. 2016. Evidence for camel-to-human transmission of MERS coronavirus. *N Engl J Med* 370:2499–505.

Báez-Santos YM, Mielech AM, Deng X, Baker S, Mesecar AD. 2014. Catalytic function and substrate specificity of the PLpro domain of nsp3 from the Middle East respiratory syndrome coronavirus (MERS-CoV). *J Virol* 88:12511–27.

Chu H, Zhou J, Ho-Yin Wong B, Li C, Cheng Z-S, Lin X, Kwok-Man Poon V, Sun T, Choi-Yi Lau C, Fuk-Woo Chan J, Kai-Wang To K, Chan K-H, Lu L, Zheng B-J, Yuen KY. 2016. Productive replication of Middle East respiratory syndrome coronavirus in monocyte-derived dendritic cells modulates innate immune response. *Virology* 454:197–205.

Coleman CM, Matthews KL, Goicochea L, Frieman MB. 2015. Wild-type and innate immune-deficient mice are not susceptible to the Middle East respiratory syndrome coronavirus. *J Gen Virol* 95:408–12.

Corman VM, Kallies R, Philipps H, Göpner G, Müller MA, Eckerle I, Brünink S, Drosten C, Drexler JF. 2015. Characterization of a novel betacoronavirus related to Middle East respiratory syndrome coronavirus in European hedgehogs. *J Virol* 88:717–24.

de Wit E, Rasmussen AL, Falzarano D, Bushmaker T, Feldmann F, Brining DL, Fischer ER, Martellaro C, Okumura A, Chang J, Scott D, Benecke AG, Katze MG, Feldmann H, Munster VJ. 2015. Middle East respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques. *Proc Natl Acad Sci* 110:16598–603.

de Wit E, Prescott J, Baseler L, Bushmaker T, Thomas T, Lackemeyer MG, Martellaro C, Milne-Price S, Haddock E, Haagmans BL, Feldmann H, Munster VJ. 2016. The Middle East respiratory syndrome coronavirus (MERS-CoV) does not replicate in Syrian hamsters. *PLoS One* 8, e69127.

Firth AE, Brierley I. 2015. Non-canonical translation in RNA viruses. *J GenVirol* 93: 1385–409.

Haagmans BL, Al Dhahiry SH, Reusken CB, Raj VS, Galiano M, Myers R, Godeke GJ, Jonges M, Farag E, Diab A, Ghobashy H, Alhajri F, Al-Thani M, Al-Marri SA, AlRomaihi HE, Al Khal A, Bermingham A, Osterhaus AD, Alhajri MM, Koopmans MP. 2015. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. *Lancet Infect Dis* 14:140–5.

Kilianski A, Mielech AM, Deng X, Baker SC. 2016. Assessing activity and inhibition of Middle East respiratory syndrome coronavirus papain-like and 3C-like proteases using luciferase-based biosensors. *J Virol* 87:11955–62.

Lau SK, Lau CC, Chan KH, Li CP, Chen H, Jin DY, Chan JF, Woo PC, Yuen KY. 2016. Delayed induction of proinflammatory cytokines and suppression of innate antiviral response by the novel Middle East respiratory syndrome coronavirus: implications for pathogenesis and treatment. *J GenVirol* 94:2679–90.

Lei J, Mesters JR, Drosten C, Anemüller S, Ma Q, Hilgenfeld R. 2015. Crystal structure of the papain-like protease of MERS coronavirus reveals unusual, potentially druggable active-site features. *Antiviral Res* 109:72–82.

Lin MH, Chuang SJ, Chen CC, Cheng SC, Cheng KW, Lin CH, Sun CY, Chou CY. 2016. Structural and functional characterization of MERS coronavirus papain-like protease. *J Biomed Sci* 21:54.

Mielech AM, Kilianski A, Baez-Santos YM, Mesecar AD, Baker SC. 2016. MERS-CoV papain-like protease has deISGylating and deubiquitinating activities. *Virology* 450:64–70.

Reusken CB, Farag EA, Jonges M, Godeke GJ, El-Sayed AM, Pas SD, Raj VS, Mohran KJ, Moussa HA, Ghobashy H, Alhajri F, Ibrahim AK, Bosch BJ, Pasha SK, Al-Romaihi HE, Al-Thani M, Al-Marri SA, AlHajri MM, Haagmans BL, Koopman MP. 2016. Middle East respiratory syndrome coronavirus (MERS-CoV) RNA and neutralizing antibodies in milk collected according to local customs from dromedary camels, Qatar. *Euro Surveill* 19:20829.

Song F, Fux R, Provacia LB, Volz A, Eickmann M, Becker S, Osterhaus AD, Haagmans BL, Sutter G. 2015. Middle East respiratory syndrome coronavirus spike protein delivered by modified vaccinia virus Ankara efficiently induces virus-neutralizing antibodies. *J Virol* 87:11950–4.

vanBoheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, Osterhaus AD, Haagmans BL, Gorbalenya AE, Snijder EJ, Fouchier RA. 2016. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. *mBio* 3, e00473–12. http://dx.doi.org/10.1128/mBio.00473-12.

World Health Organization. 2014. Middle East respiratory syndrome coronavirus (MERS-CoV): update (United Arab Emirates). Geneva: World Health Organization, Geneva.

World Health Organization. 2015. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. Geneva: World Health Organization, Geneva.

Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier AM. 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* 367:1814–20.