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Middle East Respiratory Syndrome Coronavirus: Molecular Pathogenesis and Implications Towards Therapeutic Progressions

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Abstract

The recently emerged Middle East Respiratory Syndrome Coronavirus (MERS-CoV) causing severe respiratory tract infection in humans is now considered as a pandemic threat worldwide. It is a novel class of coronavirus group which uses a number of unidentified pathways for replication using nonconforming factors and pathogenesis in selective animal species. Currently, there is still dearth of information on foremost source of viral transmission along with exact pathogenic mechanism of action. The pathological effect is also diversified in different hosts. Besides this, the hospital outbreak of this super-spreading virus has made a greater concern about global health. The documented clinical studies accessible in this study represent the deadly outcome. The augmented rate of fatality of MERS-CoV induced disease makes it essential to develop safe and effective vaccines against this virus. Considering this issue, we reviewed on the factors responsible for the viral infection together with the promising mechanisms of pathogenesis initiated till date. In addition with the illustration of possible divergent targets of the virus, the evidences on pathological analysis developed through humans and other species could be momentum for therapeutic treatment strategies. This revelation may exert crucial guidance for the development of stable animal model in vivo trial as well as effective vaccines for the prevention of MERS-CoV spread.

Keywords: MERS-CoV, viral transmission, pathogenesis, therapeutics, vaccines

1. Introduction

A newly emerged highly pathogenic beta-coronavirus called Middle East Respiratory Syndrome Coronavirus (MERS-CoV) formerly known as HCoV-EMC (Human Coronavirus Erasmus Medical Center) was recognized as the causal agent of 50% lethality and fatal respiratory disease in humans during **2012** (Zaki *et al.* **2012**). As the first case was detected on June, **2012** in Saudi Arabia and the next was in Qatar where a 49 years old man was infected by the novel coronavirus (MERS-CoV) in September **2012** and there was a 99.5% sequence match between the two viruses separated from the patients (Bermingham *et al.* **2012**). The viral transmission from discriminating animal species to human has been evidenced and another study has also demonstrated that the pathogen has spread worldwide largely by human to human infection (Durai *et al.* **2015**). However the focus of infection has remained in countries on the Arabian neck of land (Saudi Ministry of Health **2014**). Jordan, Qatar, Saudi Arabia and the United Arab Emirates were reported as home cases of viral infection and additional cases included France, Germany, Italy, Tunisia and the United Kingdom

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whereas, this viral action is now well spread in South Korean state (WHO **2013**; Bermingham *et al.* **2012**). The largest cluster of cases to date occurred at a healthcare facility in 23 health care workers (Breban *et al.* **2013**) in Al-Hasa, Saudi Arabia (Assiri *et al*. **2013**; Perera *et al*. **2013**).

Another report documented that, in South Korea on 20 May **2015**, the first MERS-CoV case was found in a citizen travelling to Middle Eastern countries, while on 15 June **2015**, there was a spread in South Korea, with substantial deaths of 186 cases confirmed in laboratory testing. Besides this, as the first imported case in China, South Korean officer while visiting Guangdong Province was diagnosed with MERS-CoV (Roujian *et al*. **2015**). Globally, since September **2012**, WHO has been alerted about 1,595 laboratory-confirmed cases of infection with MERS-CoV, including at least 571 related decease.

Till August **2015**, 498 deaths were found among 1165 cases in the Saudi Arabian territory (ECDC **2015**). Current knowledge indicates that human MERS-CoVs emerged from animal ancestors and that various animal MERS-CoVs also passed along species to species. Several 2c betacoronaviruses are highly identical to MERS-CoV sequence were found among bats in Europe, Ghana and a little in Mexican countries (Annan *et al*. **2013**; Anthony *et al*. **2013**).On that basis, bats are one of the source of MERS-CoV virus. In vitro study demonstrates that MERS-CoV is of broad host range with dromedaries as in dromedary camels, antibodies against MERS-CoV have been acknowledged (Ali *et al*. **2015**; Muller *et al*. **2012**). The efficiency of genetic recombination and mutation of MERS-CoVs make them unusually adaptable to new hosts and (Woo *et al*. **2009**; Woo *et al*. **2006**; Lau *et al*. **2011**; Zeng *et al*. **2008;** Lai *et al*. **1997**; Herrewegh *et al*. **1998**). The mechanism(s) of pathogenesis of MERS-CoV is yet to be delineated as the virus utilizes a number of pathways to disseminate them as throughout the host cell with rapid fatality rate. We hereby demonstrated the overall pathways of MERS-CoV pathogenic mechanism(s) to well establish animal model for further discovery of drug or other prophylactic, vaccine and/or therapeutic intervention strategies to certify proper application in vivo which is a must. In addition, the therapeutic options hypothesized by previous studies are outlined here till date.

2. Animal reservoirs and transmission of MERS-CoV

Bats harbor high divergence of cognate infections and are associated to be the hoard of MERS-CoV (Ithete *et al*. **2013**; Cotten *et al*. **2013**; Annan *et al*. **2013**). Phylogenetic knowledge about MERS-CoV together with other correlated coronaviruses suggested the closeness of MERS-CoV with bat CoVs HKU4 and HKU5 of 2c class of Betacoronavirus cluster (Raj *et al*. **2014**a; Zaki *et al*. **2012**; van Boheemen *et al*. **2012**). Mers-Cov like firmly related CoVs were found in 24.9% of Nycteris bats and 14.7% of Pipistrellus bats from Ghana and neighbouring nations (Annan *et al*. **2013**), likewise in Africa, Asia, USA, and Eurasia (Raj *et al*. **2013**). Taphozous perforatus bat has 181 base pair of RNA dependent RNA polymerase enzyme which is hereditarily indistinguishable to MERS-CoV was accounted for to be found in a human MERS case (Memish *et al*. **2013**a). Another investigation says strikingly that, RNA-dependent RNA polymerase (RdRp) gene containing 190 nucleotide sequence was found to be 100% identical with a MERS-CoV isolate from the first patient in Saudi landmass; perceived once again from the Taphozous perforatus bat captured from close-by territory of the patient house (Mclntosh **2015**; Muller *et al*. **2014**). Modelling of the DPP4 (dipeptidyl peptidase-4) and MERS-CoV RBD collaboration anticipated the capacity of MERS-CoV to bind the DPP4s of camel, goat, dairy animals, and sheep. Expression of the DPP4s of these species on BHK cells bolstered MERS-CoV replication highly which recommends, together with the abundant DPP4 vicinity in the respiratory tract that these species may have the capacity to work as a MERS-CoV intermediate reservoir, (van Doremalen *et al*. **2014**). Eight MERS-CoV clusters have been documented, suggestive of transmission of the contamination over the persons from them (Zaki *et al*. **2012**). Studies have uncovered that dromedary camels are probably the moderate host and a prominent example was a 44 years old man had no comorbidities just came into contact with nasal swab of his own residential sick camels while offering prescription to them (Mclntosh **2015**; Muller *et al*. **2014**). It was suspected the transmission of infection could be through saliva, droplets in food while blending them up amid direct contact with infected camels or uncooked meat (Durai *et al*. **2015**). The WHO provided details in **2014** that, the air course of transmission of the infection was found in three air tests of a camel stable (Azhar *et al*. **2014**a) with droplet, contact and fomites (WHO **2014**a). Locally procured cases by MERS-CoV contagion was first found in ICU or medicinal services center where essential or secondary contact may bring about deadly release of infection. Then again, antibodies against MERS-CoV have been distinguished in dromedary camels with a critical numbers were recorded as among 203 serum samples,150had antibodies against MERS-CoV and the seropositivity was higher in grown-up camels (Meyer *et al*. **2014**; Reusken *et al*. **2014**a; Haggmans *et al*. **2014;** Reusken *et al*. **2013**). In past study, it was found that infection was transmitted all through diverse genomic variations of infected camels (Briese *et al*. **2014**) into human body

when ranch labourers, veterinarians dealt with caring the creature and around 6.5% of 76 camels demonstrated similitude with human viral sequences(Nowotny and Kolodziejek **2014**; Memish *et al*. **2014**a; WHO **2014b**). Yet again, the entire genomic arrangements of MERS-CoV from camels' nasal swab, also rectal swab (WHO **2014**b; Reusken *et al*. **2014**a) were investigated and stated to be nearly identical with human MERS-CoV sequences. A few analysts showed that, horse DPP4 can competently enhance viral infection through expression into various human cell lines studied in another section of this paper (Barlan *et al*. **2014**). So the investigation of seroepidemiology of potential animals cluster for MERS-CoV particular antibody is an appropriate way to deal with candidate species for further experimentation (Perera *et al*. **2013**). There were unambiguous evidence in Jeddah-Saudi Arabia 85.8 %, (Memish *et al*. **2014**a; Azhar *et al*. **2014**b), 8.1% in the United Arab Emirates, 1.7% in Jordan, and 1% in Qatar (MERS coronoa map. **2014**; Haggmans *et al*. **2014**), MERS-CoV was recognized from camel by polymerase chain reaction. It was generally considered that the transmissibility of MERS-CoV was not as much as SARS-CoV and the other related infections while it has not been cleared up yet, rather these days the high scattering rate of MERS-CoV is witnessed globally (Zumla *et al*. **2014**). Additional information illustrated a certain variation in genotypes from animal source and human source virus hence the transmission is either by zoonotic hosts or environmental sources which may spread this virus between camels and humans (Gardner and MacIntyre **2014**). In Arabian countries, consumption of camel milk has been found to be the source of infecting human seriously with MERS-CoV and (Durai *et al*. **2015**; Reuskin *et al*. **2014**a; WHO **2014a,** van Doremalen *et al*. **2013**) the spill over to human population was thus acquired though only a few cases were reported on this issue. In a survey apart, 87 camel shepherds and 140 slaughterhouse workers were tested in Saudi Arabia, of whom 7 were found seropositive. By studying all the cases overall, it has been suggested that the least number of viruses having common genotypes are responsible for causing infection in both animals and humans (Briese *et al*. **2014**). Hospitals are the primary location where human to human transmission of MERS-CoV has been observed (Memish *et al*. **2014**b; The WHO MERS-CoV Research Group, **2013**; Drosten *et al*. **2013**) although limited spread among family members has also been confirmed (HPA investigation team, **2013**).In flight transmission of MERS-CoV was estimated to be new infection site,both in a 5 hours flight in first class with one and 15 infections from a 'super-spreader' travelling 13 hrs in an economy class (Coburn *et al*. **2014**). In USA, among the travellers two people who travelled to Saudi Arabia were found to be infected by MERS when tested after return (Bialek *et al*. **2014**).

3. Epidemiologic outbreak and Clinical Manifestations of MERS-CoV Infection

The epidemiology of MERS-CoV was deliberate after outbreak in the hospital of Al-Hasa, Saudi Arabia and another rush in Al-Zarqa in Jordan in April **2012** (Assri *et al*. **2013**b; Hijawi *et al*. **2013**). Each observed feature of MERS-CoV epidemiology was summarized and found either as animal or premeditated release (MacIntyre **2014**). As seen on June **2014**, 688 people were apparently infected only and died 282, with707 laboratory-confirmed cases of MERS-CoV infection have been reported to the WHO including 252 (36%) fatal cases (WHO, **2014**). The WHO Regional Office for Africa reported two cases on 31st May **2014** in Algeria with travel history to Saudi Arabia, the MERS-CoV spreading peninsula, where they took part in international congregation Umrah (ECDC **2014**a), one of these cases was found to be dead. MERS-CoV had a low reproductive number and epidemic potential till **2014**, however, there was an outbreak in a number of countries **2015** (Cauchemez *et al*. **2013**a, Breban *et al*. **2013**) and the contagion has been persisted in human over a far more prolonged period which is still ongoing after four years. In 21st September **2015**, a 38 years old Saudi Arabian male developed symptoms and tested positive for MERS-CoV on 30th September. Five Jordan health workers (29-69yrs) were detected recently with MERS-CoV symptomatically in September **2015** (WHO **2015**a).

The outburst of this virus infected patients are long-lasting in Saudi, Republic of Korea, China, Thailand, Philippines, United Arab Emirates, Oman, Qatar, Iran, Germany (WHO **2015**a). The male patients were found to be dominant over female ones (male-to-female ratio 2.8:1) (Assiri *et al*. **2013**a) and severity resulted in due to the comorbid diseases like people with diabetes, renal failure, chronic lung disease and compromised immune system are considered to be at high risk of severe disease from MERS-CoV infection (WHO **2015**a, Al-Tawfiq *et al*. **2013**). Around 81 Healthcare workers with confirmed MERS-CoV are acknowledged to have had direct or indirect contact with patient in Korea (The Korean Society of Infectious Diseases **2015**).

In early days of MERS-CoV emergence the elderly people (56 to 60 years) were mostly infected by this virus. In support of this, reports have been published in **2014** that, 67 years old Iranian woman who was being treated for chronic obstructive pulmonary disease (COPD) in Algeria infected with MERS-CoV. Again, two travellers of Mecca diagnosed for MERS-CoV and

when they were back on 23rd May 2014 they appeared with dyspnoea and influenza like disease and latter one died on 10 June 2014 (WHO , 2014b; ECDC 2014a; Assiri *et al*. 2013a, Penttinen *et al*. 2013; The Who MERS-CoV Research Group, 2013). Whereas, some of the primary cases revealed childhood MERS-CoV and only two were found to be asymptomatic (WHO, 2014b). MERS-CoV has a more sporadic pattern so serological surveys, contact tracing and other surveillance in affected areas with animal model testing are needed to quantify with proper identification of exposures to non human sources of infection (Cauchemez *et al*. 2013b). Some camels were detected as seropositive for MERS-CoV in Kenya, Nigeria, Ethiopia, suggesting that there may be MERS-CoV cases unrecognized in Africa (Corman *et al*. 2014; Reusken *et al*. 2014b; Chu *et al*. 2014).Through vast research and investigational demonstrations, the incubation period of MERS-CoV has been developed as 5–14 days (Assiri *et al*. 2013b). It takes 3–4 days from symptom beginning of MERS-CoV patients to hospitalization thereafter ICU to death only 5 and 11.5 days, respectively (Assiri *et al*. 2013a; Assiri *et al*. 2013b). Common presenting symptoms include: fever, cough, dyspnea, chills, rigor, headache, myalgia, and malaise (Hui *et al*. 2010; Rainer *et al*. 2007; Fan *et al*. 2006; Liu *et al*. 2004; Christian *et al*. 2004; Leung *et al*. 2004; Lee *et al*., 2003; MMWR 2003). Among 3000 close contacts of patients screened with RT-PCR in Saudi Arabia by using nasopharyngeal swab, two were found asymptomatic and five were symptomatic (Mclntosh 2015). Leucopoenia, lymphopenia can be also caused due to MERS-CoV infection besides, lactate dehydrogenase of the patient gets high as mentioned in **Table 1**. Death rate of MERS-CoV disease was about 70% initially, but the frequency got to a lesser extent later in 2015 (Al-Tawfiq *et al*. 2014; Arabi *et al*. 2014; Penttinen *et al*. 2013; Assiri *et al*. 2013a). The clinical appearance of MERS ranges from asymptomatic to acute respiratory syndrome, septic shock, dysfunction of organs, tissue damage, multi-organ disorder, pneumonia and resultant death. An isolated experiment declared that about one-third of the tested patients had abdominal disorders (Durai *et al*. 2015). It was found that mild or asymptomatic infection resulted in due to intrafamilial transmissions (Health Protection Agency 2013; Euro surveill 2013 and Pro-med mail 2013). Hospital-to-hospital outbreak in (17 in numbers) Korea is an alarming situation these days (Al Abdallat *et al*. 2014). Immune compromised patients and people with persistent comorbidities show clinical severity in MERS-CoV infection (ECDC 2015a). A cluster of clinical features are given in **Table1**.

4. Genome organization

MERS-CoV is an enveloped ssRNA virus and contains few structural proteins of relatively long (around 30 kb) positive-stranded genome in lineage C of the genus of Betacoronavirus within the subfamily Coronavirinae (Zaki *et al*. 2012; van Boheemen *et al*. 2012). The 5' and 3' end of MERS-CoV contains untranslated regions of 278 and 300 nucleotides respectively (**Fig 1**). The genomic organization of MERS-CoV consists of the sub-genomic mRNA which translates the two large open reading frame called ORF1a and ORF 1b along with 11 functional ORFs (Zhang *et al*. 2014) and subsequently produce two main polyproteins as pp1a and pp1ab which are thereafter cleaved into 15/16 non structural proteins called nsps by the action of papain-like protease (PLpro) and 3C-like protease (3CLpro). These proteases are cleaved from polyprotein 1ab (pp1ab) along with other ORFs encoding nsps required to activate the viral RNA dependent RNA polymerase, helicase, exoribonuclease activity, endoribonuclease activity and methyltransferase activity identified as nsp12, nsp13, nsp14, nsp15 and nsp16 respectively. The nsp14 protein is indispensable in proofreading by analysing the mutation, as RNA virus gets changed ubiquitously (Durai *et al*. 2015; Smith *et al*. 2013; Gorbalenya *et al*. 2006; Snijder *et al*. 2003; Ziebuhr *et al*. 2000). The coronavirus membrane contains three or four viral proteins. The membrane (M) glycoprotein is the most abundant structural protein; it spans the membrane bilayer three times, leaving a short NH2-terminal domain outside the virus (or exposed luminally in intracellular membranes) and a long COOH terminus (cytoplasmic domain) inside the virion (Rottier 1995). Some major replicase proteins are coded in 5' terminal which are non-structural may be needed for the above polyproteins processing and the successful entry into the host cell for replication (Yang *et al*. 2013). At the downstream portion of ORF1 b it has some important protein coding genes similar to the other known CoVs (McBride and Fielding 2012) as spike(s) proteins are the type I membrane glycoprotein that constitute the peplomers which decorates the periphery of virion, systematized membrane (M) proteins, nucleocapsid (N) and the ion channel producing envelope (E) proteins. These are all structural proteins and are translated from sub-genomic mRNAs of which 5' leader sequence is similar to viral genomic 5' terminal but 3' quarter is different so that various ORFs can be produced. These ORFs are transcribed by transcription regulatory sequences (TRSs) found in 5' end as leader TRS and body TRSs in the proximal region of upstream of 3'

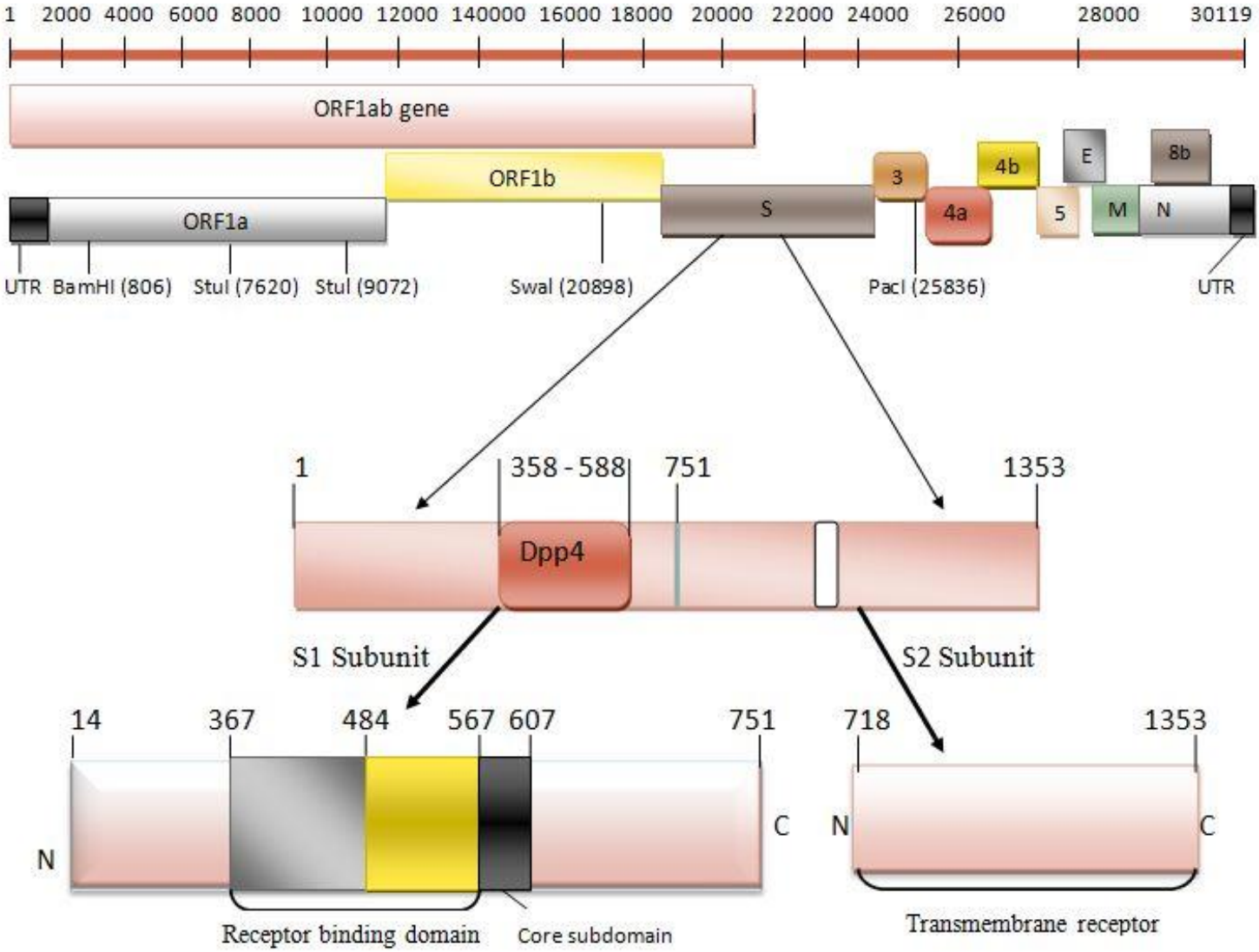

Fig 1. Genomic organization of MERS-CoV; Viral genes (ORF 1a, ORF 1b, S, 3, 4a, 4b, 5, E, M, 8b and N) are illustrated by boxes in this genome scheme with corresponding nucleotide sequences. Some relevant restriction sites used for the assembly of the infectious cDNA clone and their genomic positions (first nucleotide of the recognition sequence) are indicated. UTR, untranslated region, RBD and transmembrane receptor are indicated. Fig 1: MERS-Coronavirus genomic organization: S1353 amino acid, ORF3: 103 aa., ORF4a:109aa, ORF4b: 246aa, ORF5:224aa, E: 82aa, M: 219aa, N: 413aa.(Brand *et al.* 2015).

Table 1 Cluster of clinical features of MERS-CoV infected diseases

Country/patient age (year)	Clinical symptom/severity%	Total cases/ WHO reports Oct, 2015	Ref.
Qatar/49	pneumonia and kidney failure/fatal 99%	13	Bermingham *et al.* (2012)
Yemen/ 44	clinical symptoms NA/fatal 100%	1	Schweisfurth *et al.* (2014)
Kuwait/43	Symptoms NA/fatal 100%	3	Schweisfurth *et al.* (2014)
Jordan/25	Renal failure/Fatal	20	Pollack *et al.* (2013)
UK ex Qatar/49	Renal failure/Fatal	4	Bermingham *et al.* (2012)
Saudi peninsula/48	Respiratory symptoms(Cough, hemoptysis, chest pain, sore throat, runny nose, fever, chills)/ Fatal 70%	NA	Jaffar *et al.* (2013)
Saudi peninsula/65	Gastro-intestinal symptoms (abdominal pain, nausea, vomiting, diarrhea, myalgia, headache)/ fatal 22%	NA	Jaffar *et al.* (2013)
Saudi Arabia /Median 49-70	Influenza like symptoms, Fever and chills, Dry cough, Respiratory disorders affect mortality/fatal Renal failure/fatal Asymptomatic/acute febrile illnesses/ upper respiratory tract disease with 44% mortality	1166	Schweisfurth *et al.* (2014) Memish *et al.* (2013b); ECDC (2014) Alimuddin *et al.* (2015)
United Arab Emirates/(24-94)	acquired pneumonia, asymptomatic/ fatal 65%	76	Alimuddin *et al.* (2015)
France ex Saudi Arabia/64	Renal failure/fatal	2	Bermingham *et al.* (2012)
Germany ex Saudi Arabia/73	Renal failure/fatal	3	Drosten *et al.* (2013)
Zarqa, Jeddah /Median 49-50)	Fever (>38°C),Chills or rigors, Cough, Productive, Haemoptysis, Headache, Myalgia, Malaise, Shortness of breath, Nausea, Vomiting, Diarrhea, Sore throat, Rhinorrhoea/fatal 40-60% Influenza/fatal	2	Alimuddin *et al.* (2015) Schweisfurth *et al.* (2014)
Middle East(Al Hasa)/ 47 - 60[20,22]	Severe pulmonary consolidation acute hypoxic respiratory failure, hyperkalaemia, cardiac arrest, pericarditis and multi-organ failure elevated lymphopenia, lymphocytosis, thrombocytopenia, renal failure [20,21,30,32], with diabetes-2 and renal **co-morbidities** [40–47]/ fatal around 30%	1298	Brand *et al.* (2015)
South Korea /40-79	Symptomatic, fever and myalgia, pneumonia /around 21-40%	186	Moran ki (2015); The Korean Society of Infectious Diseases, (2015)
China/median	NA	1	Lu *et al.* (2015)
Greece/69	prolonged fever, diarrhea and pneumonia/fatal 100%	1	Kossyvakis *et al.* (2015)
Philippines/36	NA	3	WHO/MERS/RA/15.1 (2015b)
Thailand/median	Symptomatic	3	WHO/MERS/RA/15.1 (2015b)

NA: Not available

domain while sgRNAs are nested along with 3' end and are joined to a common leader. The total genome encapsidation is done by N proteins (Zumla *et al*. 2015). MERS-CoV genome codes five unique accessory proteins as 3, 4a, 4b, 5 and 8b coded by the five different amino acids like Ala291, Ile295, Arg336, Val341, and Ile346) (van Doremalen *et al*. 2014).

Among which 4a has been reported to inhibit the production of interferon in patient's body (Niemeyer *et al*. 2013). Furthermore, the virus is equipped with arsenals to elude innate immunity (Joshi 2013). The ORF1a encodes two of the protease domains as papain like (PL2pro) and 3C like (3CL pro). Seven mRNA with 67-nucleotide common leader sequence were found to be produced in MERS-CoV invaded cells.

5. Replication of MERS-CoV

Exact host for replication of MERS-CoV to a great extent is still an inconsistency. Researchers showed Syrian hamster could be a small animal model for MERS-CoV isolates (de Wit E *et al*. 2013). Middle East Corona viruses for their replication connect to specific receptor on the cellular surface. This process is the prerequisite for the nucleocapsid entrance into the host cell. MERS-CoV appears to replicate in

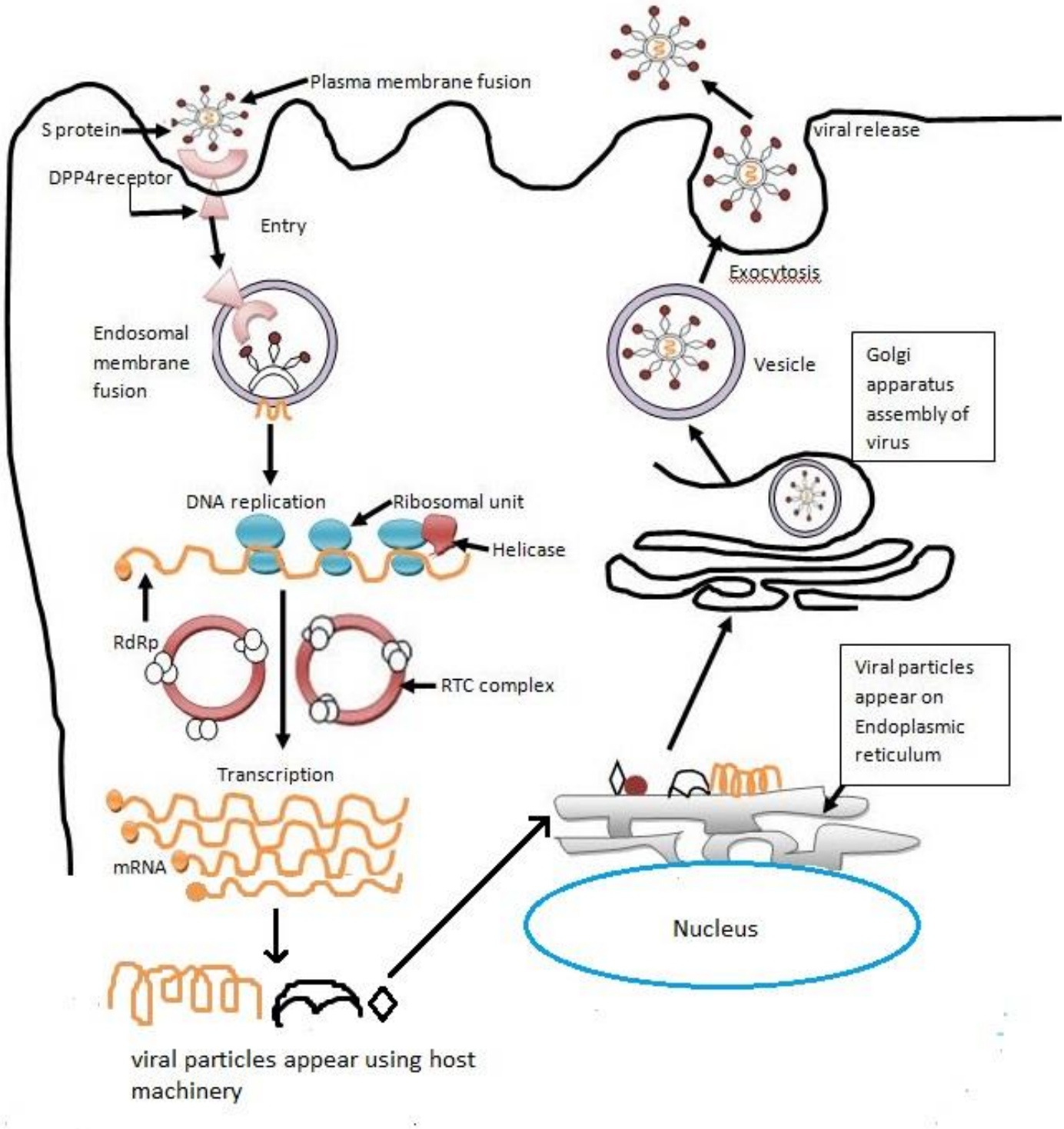

Fig 2. Replication mechanism of MER-CoV (Kilianski& Baker 2014). The most important finding was the cellular receptor-dipeptidyl peptidase 4 (DPP4). The DPP4 binds to a 231-residue region in the spike (S) protein of MERS-CoV for entry. The RNA genome is pumped in through a plasma or endosomal membrane fusion, into the target cell. The RNA immediately transcribes to proteins and RNA, which is packaged and released. (Kilianski & Baker 2014).

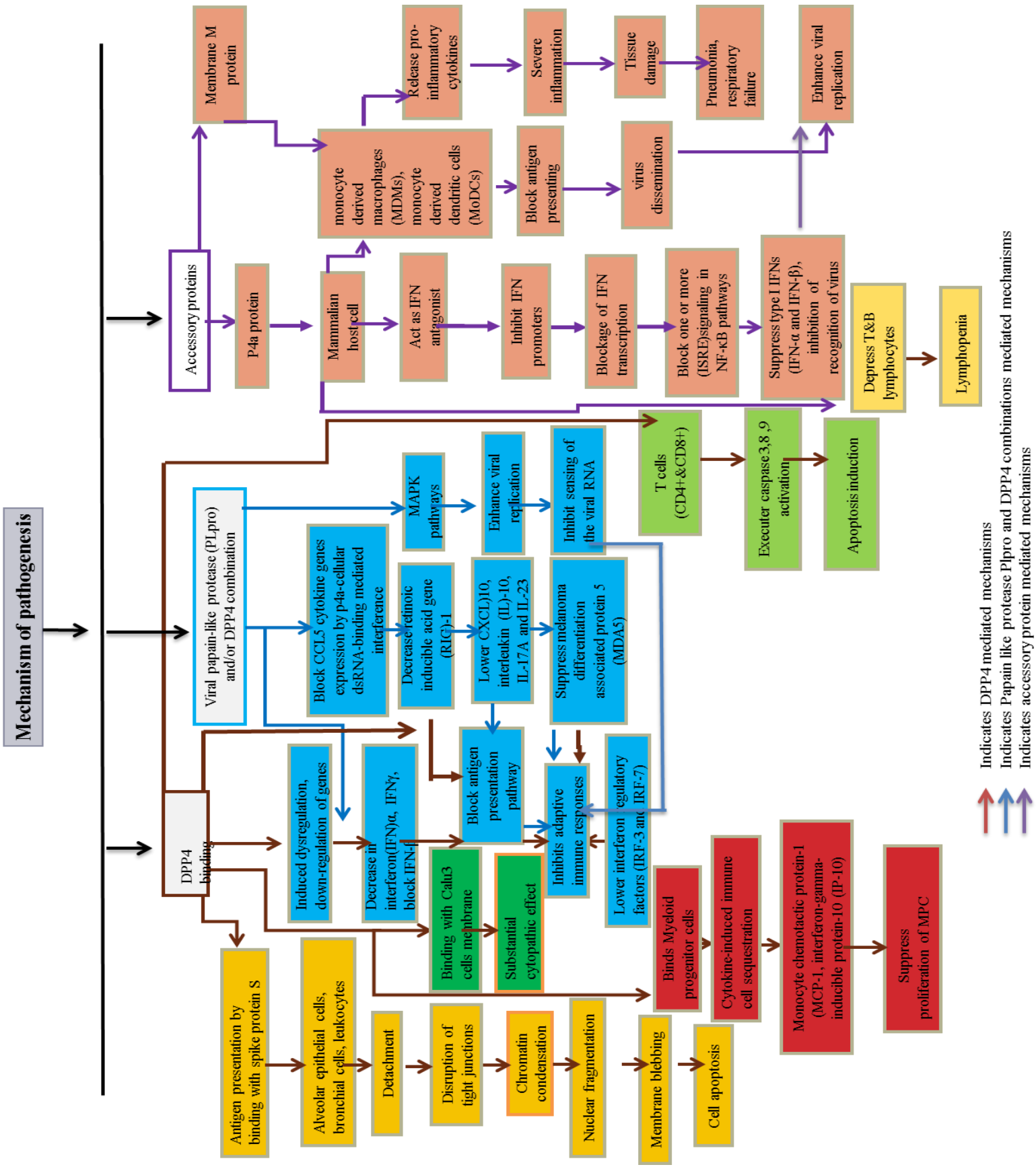
Mechanism of pathogenesis
Membrane M protein
Release pro-inflammatory cytokines
Severe inflammation
Tissue damage
Pneumonia, respiratory failure
Enhance viral replication
monocyte derived macrophages (MDMs), monocyte derived dendritic cells (MoDCs)
Block antigen presenting
virus dissemination
Accessory proteins
P4a protein
Mammalian host cell
Act as IFN antagonist
Inhibit IFN promoters
Blockage of IFN transcription
Block one or more (ISRE)signaling in NF-κB pathways
Suppress type I IFNs (IFN-α and IFN-β), inhibition of recognition of virus
Depress T&B lymphocytes
Lymphopenia
Viral papain-like protease (PLpro) and/or DPP4 combination
MAPK pathways
Enhance viral replication
Inhibit sensing of the viral RNA
T cells (CD4+&CD8+)
Executer caspase3,8 ,9 activation
Apoptosis induction
Block CCL5 cytokine genes expression by p4a-cellular dsRNA-binding mediated interference
Decrease retinoic inducible acid gene (RIG)-1
Lower CXCL)10, interleukin (IL)-10, IL-17A and IL-23
Suppress melanoma differentiation associated protein 5 (MDA5)
DPP4 binding
Induced dysregulation, down-regulation of genes
Decrease in interferon(IFN)α, IFNγ, block IFN-f
Block antigen presentation pathway
Inhibits adaptive immune responses
Lower interferon regulatory factors (IRF-3 and IRF-7)
Binding with Calu3 cells membrane
Substantial cytopathic effect
Binds Myeloid progenitor cells
Cytokine-induced immune cell sequestration
Monocyte chemotactic protein-1 (MCP-1, interferon-gamma-inducible protein-10 (IP-10)
Suppress proliferation of MPC
Antigen presentation by binding with spike protein S
Alveolar epithelial cells, bronchial cells, leukocytes
Detachment
Disruption of tight junctions
Chromatin condensation
Nuclear fragmentation
Membrane blebbing
Cell apoptosis
Indicates DPP4 mediated mechanisms
Indicates Papain like protease Plpro and DPP4 combinations mediated mechanisms
Indicates accessory protein mediated mechanisms

Table 2: Therapeutic strategies developed against MERS-CoV till date

Therapeutic target	Strategies	Ref.
Animal model expressing antibodies	reverse genetics engineering of a replication-competent, propagation-defective MERS-CoV develop attenuated viruses (lacking the structural E protein)as vaccine in mice. combination of DNA and protein immunogens Antibodies preparation as D12 and F11 protection in non-human primates	Wang *et al.* (**2015**)
S protein	Recombinant modified vaccinia virus Ankara (MVA) with S residues vaccination in mice Expression of the full-length S protein of MERS-CoV high levels of neutralizing antibodies leads to vaccine development Design of viral fusion peptide HR2P inhibitors against heptad repeat region HR2 of S. HR2P binds with the HR1 domain & form a stable six-helix bundle inhibit viral fusion core formation cell-cell fusion	Song *et al.* (**2013**); Zhang *et al.* (**2014**)
RBD	recombinant 212-amino acid RBD fragment with 377–588 residue of MERS-CoV S protein S-specific antibodies induction block the binding of MERS-CoV RBD to receptor DPP4 neutralization against MERS-CoV infection subunit candidate vaccine RBD protein fused with Fc of human IgG Intranasal immunization with subunit candidate vaccine strong anti-RBD- and anti-S1-specific neutralizing antibody responses develop effective MERS mucosal vaccines	Du *et al.* (**2013a**); Du *et al.* (**2013b**); Ma *et al.* (**2013**) Zhang *et al.* (**2014**)
SARS-CoV PLpro inhibitor	co-expression of MERS-CoV protease domain-cleavage activated luciferase identifying of profiles of protease activity limit efficacy of MERS-CoV PLpro	Kilianski *et al.* (**2013**)
3CLpro inhibitor of SARS-CoV	structurally similar to MERS-CoV effectively blocks the activity of MERS-CoV 3CLpro	Ren *et al.* (**2013**)
Protease activity	chloropyridine esters, CE-5, CE-10 benzotriazole esters locate active site of MERS-CoV 3CLpro needed for proteolysis covalently modify the catalytic cysteine residue and block protease activity act as suicide inhibitor	Verschueren *et al.* (**2008**) Ghosh *et al.* (**2008**) Doulkeridou S (**2013**)
TMPRSS2 activity	inhibitor of cathepsin L with camostat combination inhibit MERS-CoV syncytia formation inhibit entry into cells	Shirato *et al.* (**2013**)
Human lung epithelial cell	pegylated IFN-α, IFN-β, IFN-λ3 in pseudo-stratified HAE cultures reduction of the viral RNA levels Inhibition of MERS-CoV-induced CPE	de Wilde *et al.* (**2013**) Kindler *et al.* (**2013**) Zielecki *et al.* (**2013**)
(CD26/DPP4)	production of monoclonal antibodies against CD26 as m336,8MERS-4,93B11,10and Mersmab1,11 human mAb m336 of the IgG1 subclass very promising drug candidate Antibody isolation from memory B cells of infected patient LCA60, binds to novel site S protein and neutralizes infection with MERS-CoV by interfering with the binding to the cellular receptor CD26	Lu *et al.* (**2015b**)
Epitope	HR2P-M2 - m336mAb combination Strong neutralizing activity against authentic MERS-CoV multiple epitope, both within and outside the RBD potentially improve immunogenicity and reduce the likelihood of escape mutations. Preparation of Spike trimmer with native conformation after DNA immunization Cause diverse set of antibodies neutralize MERS-CoV by targeting the RBD, epitopes outside the RBD more capable than RBD-specific antibodies at preventing viral escape variants.	Wang *et al.* (**2015**); Ying *et al.* (**2014**)
Genomic expression	five siRNA and four miRNA effective aspirant against ORF1ab gene gene expression	Nur *et al.* (**2015**)
Amino acid residues at different places of genome	Site specific Mutation eliminate hydrogen bonding between amino acids significant reduction in binding of RBD to DPP4 hinder viral entry	Wang *et al.* (**2013**)
virion	modified HR2P peptide by introducing Glu (E)and Lys (K)residues suppress viral replication in epithelial cells	Lu *et al.* (**2015**)

	reduce the release of virions prevention of the spread	
MERS-CoV replication	Ribavirin known inhibitor at nanomolar levels MAPK inhibitor SB203580 Attack vero cells & hinder inhibit MERS-CoV replication	Falzarano *et al.* (2013); Coleman *et al.* (2013) Josset *et al.* (2013)
MERS-CoV titre	type 1 interferons (IFN-α and especially IFN-β), IFN-α2b-ribavirin readily inhibited Drug administration as ciclosporin and mycophenolic acid chloroquine, chlorpromazine, loperamide, and lopinavir)	Zielecki *et al.* (2013); Chan *et al.* (2013); de Wilde *et al.* (2013)
upE and ORF1a	highest sensitivities in detection followed by gene sequencing by PCR amplicons reliable diagnosis for drug treatment	Shirato *et al.* (2014)
MERS-CoV entry	HIV-1 gp41 HR2 region, C34 and T20 moderate inhibitory activity on MERS-CoV entry into NBL-7 cells	Zhao *et al.* (2013)
In vitro culture of MERS-CoV	convalescent plasma, hyper-immune globulin or human monoclonal antibodies that contain most strong in vitro activity Antagonize over mers-cov growth and dissemination	Sharif-Yakan *et al.* (2014)
MERS-CoV infection	Paracrine production by lymphoid cells relatively high ADA concentrations binds to the viral binding site locally block MERS-CoV infection provide clues to help develop other antagonists	Raj *et al.* (2014)

various human and other mammalian cell types in vitro (Chan et al. 2013a; Kindler *et al.* 2013; Zielecki *et al.* 2013; Muller *et al.* 2012) the only reported animal model for MERS-CoV is the rhesus macaque (Macacamulatta), in which it replicates and causes pneumonia and pulmonary infiltration (Munster *et al.* 2013). It exhibits an expanded host cell tropism, readily replicating in a variety of human lung cell types including fibroblasts, microvascular endothelial cells, and type II pneumocytes etc. (Scobey *et al.* 2013). MERS-CoV does not replicate in mice unless the animals are first transduced with adenovirus vectors encoding the receptor for entry, human dipeptidyl peptidase-4 (DPP4) (Zhao *et al.* 2014). First of all, the viral spike protein binds to the host receptor through the S1 subunit and then the fusion of host and viral membrane occur by S2 subunit with the subsequent release of fusion peptide (Zumla *et al.* 2015). To attain the fusion, there is a strategy needed to breakdown the S1-S2 region by the host proteases (Simmons *et al.* 2013; Belouzard *et al.* 2012; Heald-Sargent *et al.* 2012; Simmons *et al.* 2005; Simmons *et al.* 2004). Various host proteases are important for the target named as, furin, extracellular elastase, surface proteases angiotensin converting enzyme type 2 transmembrane serine protease, endosomal cathepsin L (Belouzard *et al.* 2012; Heald-Sargent *et al.* 2012). The mostly used protease for human cell entry is transmembrane serine protease (TMPRSS2) or low pH mediated cathepsin entry (Gierer *et al.* 2013; Qian *et al.* 2013; Shirato *et al.* 2013, Simmons *et al.* 2004). The interesting feature of MERS-CoV is that it can fuse with the host cell either at the interface of the receptor binding (S1) or fusion (S2) domains (S1/S2), in addition to a new location next to a fusion peptide within S2 (S2′) (Belouzard *et al.*, 2009; Yamada *et al.* 2009). For replication, spike protein of the virus attaches to the DPP4 receptor and release nucleocapsid to enter into the cell (Zelus *et al.* 2003, Matsuyama *et al.* 2002). After the entrance, positive-sense ssRNA genome' transcription occurs to form negative sense ssRNA. The replication of Coronavirus mRNA is made as a sub-genomic positive-sense RNA that contains a common 5′ primer leader sequence derived from the 5′ end of the genomic RNA, followed by the ORF of the viral gene (Pasternak *et al.* 2006). The transcription mediates the synthesis of sub-genomic mRNA (Enjuanes *et al.* 2005). Viral proteins pp1a and pp1ab are expressed by 5' ORF of the genomic mRNA along with the replicase E proteins are the result of ORF 5b expression (Jendrach *et al.* 1999). All of the required proteins need some co translational proteolysis for growing further. It leads to the localization of M and E proteins into the Golgi apparatus and these proteins have capability to form mature virus (Corse *et al.* 2003; Corse *et al.* 2000). The spike (S) glycoprotein, trimers of which form the virion peplomers, is another major structural protein. It is involved in binding of virions to the host cell and in virus-cell and cell-cell fusion. Intracellular membrane and the plasma membrane contain this most responsible spike protein which assembles with M protein and nucleocapsid (Haan *et al.* 1999). Then the new virions are moved towards the intracellular membrane and get them released (**Fig 2**).

6. Descriptions of the factors for the virus infection

An amino peptidase named as dipeptidyl peptidase-4 (DPP4, also known as CD26) is used by MERS-CoV (Raj *et al*. 2013b; Mou *et al*. 2013) as the crucial receptor to enter into the human cell (Raj *et al*. 2013b), predominantly found on nonciliated bronchial epithelial and alveolar cells in the lower parts of respiratory area (Muller 2014a). The profuse expression of DPP4 on T cells may cause to be the cells highly subject to MERS-CoV infection from the peripheral blood, spleen and tonsil in association with binding and fusion mechanism of Spike protein with the host cells (S1and S2 respectively). S1 region of the protein contains the domain of binding receptor to a 231-amino acid fragment which is about 358 to 588 residues (Mou *et al*. 2013) which degrades incretin to enhance glucose metabolism by T-cell activation, apoptosis and cell adhesion. DPP4 homologues are there in a range of cell lines together with the human Calu-3, Huh-7, HEK, His-1, HFL and Caco-2 cell lines (Muller *et al*. 2012; Chan *et al*. 2013b). All of these cells expressed cytopathic effects a few days later of MERS-CoV infection (Shirato *et al*. 2013). Researchers have found MERS-CoV as highly pathogenic virus in the lungs and the kidney which suggests for investigation on supplementary factors along with DPP4 are needed to elaborate the knowledge on viral tropism. To promote viral growth, viruses encode proteins antagonize cellular signaling which acted for host sustainment (Tortura *et al*. 2012). Among them, nsp3 is the multifunctional protein of about 1484-1802 amino acids has abundant domains, counting papain-like protease (PLpro) domain act as multifunctional cysteine protease. The (PLpro) domains of coronavirus are monomeric enzymes capable of multiple cellular functions to assist viral replication (Mielech *et al*. 2014). The essential role is recognizing and dealing out the viral replicase polyprotein at the boundaries of nsp1/2, nsp2/3 and nsp3/4 (Yang *et al*. 2014; Kilianski *et al*. 2013; Harcourt *et al*. 2004) that the hydrolyzation of peptide and isopeptide bonds occur in viral and cellular substrates, a prerequisite for coronavirus replication. Yang *et al*. (2014) demonstrated that MERS-CoV PLpro inhibits the signalling path that leads to the activation of IFN regulatory factors (IRF-3,IRF-4) which were key players to block viral attack, so this protein helps a lot to infect cells by MERS-CoV (Yahira *et al*. 2014). On the other hand, ORF 1a and ORF 1b polyproteins have the unique influence of infecting host cell by using the accessory proteins of the genome. In another review, they mentioned cellular proteases type II transmembrane serine protease (TMPRSS2 and cathepsin family) act as initiators of the major viral spike (S) glycoprotein activation which has significant role in binding the receptor DPP4 and finally viral entrance by formation of peplomeric structure on envelope of MERS-CoV (Gierer *et al*. 2013; Du *et al*. 2009) leads toward infection in Caco-2 cell lines (Gierer *et al*. 2013), giving support to the function of the proteases in viral entry as route. Whenever proteases are not available in cellular lipid bilayer surface area of the enveloped MERS-CoV it has been confirmed reportedly that they pierce cells by a cathepsin-mediated way. On the other hand, TMPRSS2 helps to infect cells through cell surface and/or via the endosomal pathway (Gierer *et al*. 2013). Consequently, TMPRSS2 provides role in case of lung as an initial location of virus contagion in Vero-TMPRSS2 cells and Calu-3 human bronchial epithelial cells by MERS-CoV as well in pseudotyped MERS-CoV colon-derived Caco-2 cells. Researchers have confirmed with a repeated result of mRNA levels of TMPRSS2, cathepsin L, and DPP4 the same in Calu-3 cells when determining susceptibility to MERS-CoV (Gierer *et al*. 2013). Interestingly, besides with cysteine, serine, threonine proteases and proteases from the extracellular environment may be exploited by MERS-CoV to enter into MRC-5 and WI-38 cells (Yang *et al*. 2014; Shirato *et al*. 2013; Muller *et al*. 2012a; Yoshikawa *et al*. 2010; Kawasw *et al*. 2009). Furin also mediates proteolytically activated MERS-CoV S1/S2 cleavage while it occurs during biosynthesis of S, and the S2′ cleavage occurs during virus entry confirmed with evidence (Millet and Whitaker 2014). The interaction between Trp535 of RBD and the DPP4 has essential influence on receptor binding and entry of MERS-CoV. These critical RBD residues considered to be occupied in viral entry (Yu *et al*. 2015). These amino acids' roles were validated through site-specific mutagenesis that points to the crucial affect in the propeller (blades 4 and 5) region of DPP4 for binding MERS-CoV (12–14) and DPP4-mediated entry of MERS-CoV (Raj *et al*. 2014a). So these can be the best targets for vaccination or prevention of viral entrance.

7. Unusual molecular mechanism of MERS-CoV pathogenicity

MERS-CoV has the ability to infect a number of cell lines of various species mostly observed in vitro, notably in human body they attack in different level of intensity. In (cell line) Calu-3, (fibroblast line) HFL, (lung adenocarcinoma cell line) A549, (embryonic kidney cell) HEK, Caco-2, liver cells like (hepatocellular carcinoma cell line) Huh-7 were detected through immunostaining where the viral nucleoproteins were identified (Chan *et al*. 2013b). Proteolytic activation unlocks the fusogenic potential of viral envelope glycoproteins and is often a critical step in the entry of enveloped viruses, the modulation of which can have a profound effect on cell tropism, host range, and pathogenicity. Non structural proteins as nsp1 has negative regulatory power on host gene

expression by blocking host mRNA translation and at times degradation of host mRNAs by endonucleolytic cleavage ability. The most unusual molecular tactic exerted by the virus upon cells is selective recognition of the mRNAs which are translationally proficient and thereby inhibit them from further expression. Unlike the SARS-CoV, nsp1 does not bind strongly with 40s ribosomal unit to attain accessibility to mRNAs to hinder them to translate depicts that they are distinct in targeting host machinery and they are distributed over the cytoplasm as well as nucleus. The most promising scenario here is that the nsp1 protein inhibits the host mechanism of protein expression through mRNA translation in cytoplasm but spared the viral particle (mRNA) to enter into host cell and being processed in cytoplasm which recapitulates the novel strategy of virus mRNA to abscond from the inhibitory action of nsp1 leads to their mechanism of atypical pathogenicity towards human cell lines (Chu *et al*. **2015**).

8. Pathology of MERS-CoV infection in humans

Human pathology was determined in case of MERS-CoV by using computer tomography where bilateral sub pleural, basilar airspace modification found with expansive ground-glass opaqueness over consolidation. The peribronchovascular tendency is analogous to pneumonia arrangement (Schweisfurth **2014**). Another detection through computational ways showed middle and lower lung field contagion by MERS-CoV (Banik *et al*. **2015**). Even though virus has been identified in urine and blood of some MERS patients, mainly the respiratory tract and kidneys are substantial in infection may result in pneumonia, acute renal failure, pericarditis, coagulopathy. The radiographic features of MERS-CoV disease are inconsistent due to the variability in the severity (Wiwanitkit **2015**). Plain radiographs have evidenced chest x-ray features in a case series of 55 patients (Das *et al*. **2015**) peripheral ground glass opacity (65%), consolidation (20%), pneumothoraces, pleural effusions and progressive involvement of all lungs zones are associated with higher mortality rate (Durai *et al*. **2015**; Ajlan *et al*. **2014;** Milne-Price *et al*. **2014**; Zhang *et al*. **2014;** Coleman *et al*. **2013**; de Groot *et al*. **2013**). The consequences of infection include inflammation of the pericardium, increase in leukocytes and neutrophils, proinflammatory cytokines, leading to severe inflammation and tissue damage, which may manifest clinically as severe pneumonia and respiratory failure (Raj *et al*. **2014**b) and lower numbers of lymphocytes, platelets and RBCs. Moreover, hyponatremia and low blood levels of albumin were detected during the case study (Durai *et al*. **2015;** The Who MERS-CoV research group **2013**). The most crucial cells of human innate immune system is the macrophages; works vitally to get rid of pathogens, to present epitopes to T cells containing CD3+ and CD8+ to enhance chemokines and cytokines production for keeping equilibrium and adjust strong immune response in organs(Murray *et al*. **2011**). MERS can create a dynamic infectivity in monocyte-derived macrophages (MDMs) along with macrophages. As because MERS-CoV receptor DPP4 is expressed in different human cells and tissues, so vascular endothelial cells of pulmonary leydig cells may also be infected by MERS-CoV (Zhou *et al*. **2014**) leads to an observation on severity of MERS-CoV. Furthermore, more fascinating thing is similarity in disease formation by SARS-CoV and MERS-CoV like lymphopenia noticed in most clinical patients (Al-Abdallat *et al*. **2014;** Assiri *et al*. **2013**b). This may be the outcome of cell sequestration induced by cytokine and chemokine through the release of monocyte chemotactic protein-1 (MCP-1) and interferon-gamma-inducible protein-10 (IP-10). These proteins considerably restrain the multiplication of human myeloid progenitor cells (Broxmeyer *et al*. **1993**) and thereby mediate infection. Gastrointestinal symptoms as well as fever, chill, vomiting, and abdominal pain are also infrequently observed (Raj *et al*. **2014**b).

9. Pathology of MERS-CoV infection in animals

Animal models mainly developed a transient lower respiratory tract infection through MERS-CoV virus. Infection of rhesus macaques with MERS-CoV caused for the fast expression of pneumonia in host body, so in that case rhesus macaque model will be instrumental in evolving vaccine and treatment options for this rising corona pathogen with pandemic potential. Clinical signs of MERS-CoV-infected macaques included cough and increased respiration rate, and lung samples showed lesions characteristic of mild to marked pneumonia with pulmonary infiltrates (Wang *et al*. **2015**; Munster *et al*. **2013**; de Wit *et al*. **2013**b),transient fever in infected monkeys and MERS-CoV specific antibody response in the macaques started at 7 days post-infection (Yao *et al*. **2014**). A potentially more sustainable transgenic lethal mouse model has been reported by using adenovirus vector mediated transduction of human DPP4 gene demonstrated productive, disseminated MERS-CoV infection, with most viral recovery in the lungs and brain of mice with a number of lacking in expression (Zhao *et al*. **2014**). In contrast, mice, ferrets, and guinea pigs do not appear to be susceptible to MERS-CoV infection (Yao *et al*. **2014**).

In hAd5-DPP4 mouse viral pathology occurs by causing weight loss and immune knockouts (Zhao *et al*.

2014). In marmoset, it has been found that infection with MERS-CoV results in lethal Pneumonia (Falzarano *et al*. 2014).

Besides these, the infectious MERS-CoV virus was found in the dromedary camels most remarkably in the larynx of respiratory tract, nasal passages, and olfactory membrane. The nasal passage infection infers that camel to human spread of viral infection may occur voluntarily due to getting in touch with them and droplet of saliva or probably the transmission through fomites. In the lower portion of trachea infection was detected and additionally in the lymph nodes of tracheobronchia, pharyngeal area, mild to acute submucosal membrane swelling which caused cell death of the tissue. Pseudostratified epithelial cells have been found to be damaged along with accelerating injure of mediastinum similar to the human cold normally seen. Moreover, destruction of epithelial cell and squamous transformation of tissue were experienced by farm camels. Histopathologic experimentation discovered that the URT, specifically the respiratory membrane in the nasal passage, is the principal location of MERS-CoV reproduction in camels. (Adney *et al*. 2014). A wide range of primates, bats, farm animals like sheep, goats, boars etc. have been ascertained to be infected with MERS-CoV (Eckerle *et al*. **2012**). Quite a number of mammalian DPP4 and viral receptor fusion spike(S) protein sequences were studied and their comparative research demonstrated higher percentage of resemblance in nucleotide sequences which are of crucial importance in some location for virus to bind and enter into the host. Particularly, this can be emphasized on human and horse DPP4 were found extremely compatible than human and dromedary DPP4 (Bosch *et al*. 2013). MERS-CoV is well capable of utilizing horse DPP4 which are expressed on non-susceptible cell lines (Barlan *et al*. 2014) and the viral multiplication level in horse are as good as in African bat cell (Meyer *et al*. 2015; Eckerle *et al*. 2012). Mice transduced with Ad5-hDPP4 drop some weight and incapable of expressing IFN (alpha/beta) receptor showed more extensive inflammation. They either remain asymptomatic or severe encephalitis like disease can be turned up (Zhao *et al*. 2015). Another confirmation supported that a variety of marmoset forms acute clinical syndrome (Falzarano *et al*. 2014; de Wit *et al*. 2013b).

10. Current treatment strategies for MERS-CoV infected disease

An assortment of in vitro approaches is available now-a-days as therapeutic initiatives against MERS-CoV infection. FDA approved drugs, like loperamide, chlorpromazine, lopinavir and chloroquine, were acknowledged to block MERS-CoV activity in host cell (Durai *et al*. 2015). Furthermore, interferon products have been found to have significantly trammelling ability like IFN-α and IFN-β whilst IFN-beta has 41-fold advanced performance than interferon gamma and 117-fold over interferon alfa-2a. Either singly or in association with Ribavirin, administering these products significantly lower the concentration of viral infection (Hart *et al*. 2014; Chan *et al*. 2013a; de Wilde *et al*. 2013). Likewise, a certain number of inhibitors were found during investigation, most outstandingly neurotransmitter inhibitors(Chlorpromazine), inhibitors against kinase signalling of virus(Imatinib, Dasatinib), antagonist of accessory protein- processing (Gemcitabine) which highly restrain viral DNA synthesis (Dyall *et al*. 2014). Correspondingly, Mycophenolic acid and cyclosporin A were experimented with success that they effectively inhibited MERS-CoV replication and spread (Durai *et al*. 2015). Among 27 compounds tested for antiviral activities K22, a small molecule and SSYA10-001 hindered membrane binding of MERS-CoV followed by replication, was identified by screening strain samples of MERS-CoV (Adedeji *et al*. 2014; Lundin *et al*. 2014). A recent study corroborated that structural and accessory proteins of MERS-CoV may function as candidate targets for developing MERS vaccines because of their importance in host interaction with the virus (Zhang *et al*. 2014).

Antibodies have been the most encouraging treatment strategies from the earlier days of the viral propagation. Antibodies like REGN3051as well as REGN3048 aimed at receptor binding domain which is essential for spike protein to bind with DPP4, have been used in vitro as the potential inhibitors of RBD-DPP4 interaction due to a high affinity binding with RBD than DPP4. In mouse model they showed promising expression against rec-hDPP4 (Kristen *et al*. 2015). A clear example of lately found MERS-27 antibody actively inhibited the mentioned interaction by attaching with RBD and prevented Asp539-Lys267 bridge formation crucial for the viral entry into host (Xiaojuan *et al*. 2015). We cannot disregard the traditional manipulation strategy of gene, which shows immense potential, as proof Spy Tag/Spy Catcher was recently developed and found to be highly proficient for site-specific protein conjugation. Synthetic vaccine technology inspired to prepare this tactic may be useful for easily arranging vaccine particles in an organized way (Zhida *et al*. 2015). Viral specific peptide fusion inhibitors may be used as novel approach in controlling further spread of MERS-CoV. The most promising strategies involved in therapeutic purposes till date is presented in **Table 2**.

11. Concluding remarks and future perspectives

The innate origins, variability in host susceptibility, all about factors, infectivity degree of MERS-CoV are unknown. Shortage of information about these aspects is hindering the drug discovery, biomarkers, and *in vivo* vaccines development. So, genomic studies needed for further acknowledgement on molecular basis about mutation rates with dissemination dynamics leading towards novel treatment tactics. The gathered facts provided here about currently available pathogenesis mechanisms is highly suggestive for a more rapid drug development and immediate implementation of proper infection control practices to prevent further spread. As from the very beginning of viral disease spread worldwide, vaccination is one of the most efficient strategies to prevent viral disease. It is essential against this infectious disease. Antibodies found in many dromedary animals can be extensively studied against antigen of MERS-CoV with evaluation *in vivo* animal models like marmoset which is less expensive to use in research purpose. Now-a-days, Rhesus macaque and transduced mice are being used by recombination technology but they are not well stable in preventing MERS-CoV consortium formation. That is the reason to well establish the *in vivo* model active against the virus. Finally, given the evidence that camels may play in transmission of the virus. Staying away from taking care of herd and consuming raw/unpasteurized milk could be the suggestion for controlling epidemic contagion.

12. Conflict of interest

There is no conflict in interest with authors during the work accomplishment.

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