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Expert Review of Respiratory Medicine

ISSN: 1747-6348 (Print) 1747-6356 (Online) Journal homepage: http://www.tandfonline.com/loi/ierx20

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To cite this article: Ali A. Rabaan (2017): Middle East respiratory syndrome coronavirus: five years later, Expert Review of Respiratory Medicine, DOI: 10.1080/17476348.2017.1367288

To link to this article: http://dx.doi.org/10.1080/17476348.2017.1367288

Published online: 21 Aug 2017.

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EXPERT REVIEW OF RESPIRATORY MEDICINE, 2017
https://doi.org/10.1080/17476348.2017.1367288

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REVIEW

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Middle East respiratory syndrome coronavirus: five years later

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ABSTRACT

Introduction: In the past five years, there have been 1,936 laboratory-confirmed cases of Middle East Respiratory Syndrome coronavirus (MERS-CoV) in 27 countries, with a mortality rate of 35.6%. Most cases have arisen in the Middle East, particularly the Kingdom of Saudi Arabia, however there was a large hospital-associated outbreak in the Republic of Korea in 2015. Exposure to dromedary camels has been recognized by the World Health Organization (WHO) as a risk factor in primary cases, but the exact mechanisms of transmission are not clear. Rigorous application of nationally defined infection prevention and control measures has reduced the levels of healthcare facility-associated outbreaks. There is currently no approved specific therapy or vaccine available.

Areas covered: This review presents an overview of MERS-CoV within the last five years, with a particular emphasis on the key areas of transmission, infection control and prevention, and therapies and vaccines.

Expert commentary: MERS-CoV remains a significant threat to public health as transmission mechanisms are still not completely understood. There is the potential for mutations that could increase viral transmission and/or virulence, and zoonotic host range. The high mortality rate highlights the need to expedite well-designed randomized clinical trials for direct, effective therapies and vaccines.

ARTICLE HISTORY
Received 26 May 2017
Accepted 10 August 2017

KEYWORDS
MERS-CoV; coronavirus; transmission; infection prevention and control; therapy; vaccine

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1. Middle East respiratory syndrome-CoV overview

We are now five years on from the first confirmed Middle East Respiratory Syndrome (MERS) case, which occurred in June 2012 in a previously healthy 60-year-old Saudi man [1]. He was hospitalized on 10 June 2012 in Bisha in the Kingdom of Saudi Arabia (KSA) with severe respiratory symptoms, and was subsequently transferred to a Jeddah hospital on 13 June 2012. His condition worsened, with the development of acute respiratory distress syndrome (ARDS) and acute renal failure, and he died on 24 June 2012 [1]. A novel beta-coronavirus, subsequently termed MERS-CoV, was isolated from the man. Since then, the World Health Organization (WHO) has been notified of 2040 laboratory-confirmed cases of human MERS-CoV infection, resulting in 712 deaths [2]. While cases have been mainly confined to the Middle East, in particular the KSA, cases have arisen in 27 countries; this includes a major outbreak in the Republic of Korea in 2015 [3–5].

MERS Coronavirus (MERS-CoV) is a betacoronavirus, which falls into clade c (lineage 3) [6]. As with other coronaviruses, bats are considered to potentially be a main MERS-CoV mammalian reservoir [7]. A closely related virus, NeoCoV, was isolated from a *Neoromicia zuluensis* bat in South Africa, while other close MERS-CoV relatives are the prototypic clade c betacoronaviruses, *Tylonycteris* bat virus HKU4, and *Pipistrellus* bat HKU5 virus [1,6,8–10]. Thus, MERS-CoV ancestors may be found in Old World bats of the *Vespertilionidae* family, of which the *Neoromicia* and *Pipistrellus* genera are members [9,10]. Phylogenetic analyses using NeoCoV as the root suggests that evolution of MERS-CoV occurred in camels prior to that in humans; genetic elements were exchanged among ancestral viruses either in bats, or in camels acting like a genetic 'mixing vessel, resulting in MERS-CoV emergence [9]. However, while bats are considered to host the evolutionary ancestor of Betacoronavirus clade C viruses, there may also have been a role for other mammalian taxa. Other animal hosts that may have played a role as MERS-CoV reservoirs include members of the *Eulipotyphla* taxon, which is the closest sister taxon to bats and includes hedgehogs [11]. A study on European hedgehog (*Erinaceus europaeus*) fecal samples in Germany revealed the presence of a novel CoV (EriCoV) in from 58.9% of samples [11]. Phylogenetic analyses showed that EriCoV had a sister relationship both to MERS-CoV and to the clade c bat CoVs [11].

Like its fellow betacoronavirus, the Severe Acute Respiratory Syndrome (SARS)-CoV, MERS-CoV is a positive-strand RNA virus which can cause acute respiratory illness in humans [2]. However, MERS-CoV differs from SARS-CoV in various important ways. Notably, MERS-CoV uses the human dipeptidyl peptidase 4 (DPP4; CD26) receptors for cell entry, rather than the angiotensin-converting enzyme 2 (ACE-2) receptors used by SARS [12–16]. MERS-CoV also has a wider cellular tropism than SARS-CoV [17–19]. Although it has not achieved the pandemic potential associated with SARS-CoV during the outbreak of 2003, its 34.9% mortality rate is substantially higher than the 14–15% estimated for SARS by WHO. Acute viral pneumonia is often associated with MERS-CoV infection, with gastrointestinal symptoms also occurring in some cases. Clinical severity can range from asymptomatic to death [2,20–23]. Death from MERS-CoV infection is usually due

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to development of ARDS. The role of asymptomatic subjects in viral transmission is one subject over which there are still questions. Various factors have been linked to poor outcomes and high mortality in MERS-CoV infection, including comorbidities, older age, and ICU admission [20–24]. High viral load and prolonged viral shedding has also been associated with severity of disease in studies from the Republic of Korea [12] and KSA [13].

The MERS-CoV genome is over 30,000 nucleotides in size, and contains seven predicted open reading frames (ORFs) and four structural genes encoding the spike (S), nucleocapsid (N), membrane (M) and envelope (E) proteins [14–16,25,26]. The S protein has been implicated in cross-species MERS-CoV transmission and host cell infection [26].

In 2015, Al-Tawfiq and Memish produced a review on 'MERS: 2 years later,' which documented the important MERS-related events that took place during the 2 years from its first identification in June 2012 [27]. The review described what was known about viral epidemiology, clinical features and diagnosis, genomic analysis, transmission, molecular mechanisms of cell entry and the available vaccines and therapies [27]. The authors pinpointed important unanswered questions and key issues needing clarification as part of a 5-year view. Routes and patterns of transmission, both human-to-human and zoonotic, were identified as one of the most important areas requiring clarification. Expanding such understanding would lead to better definitions of risk factors for animal-to-human transmission and for healthcare facility-related transmission, and hence better evidence-based infection prevention and control measures. Development of MERS-specific therapies and vaccines was also identified as a priority area, as well as repurposing of existing medications.

In this review, we propose to give an updated overview on MERS-CoV at five years, with particular emphasis on the key areas identified by Al-Tawiq and Memish [27], i.e. transmission, risk factors, infection control and prevention, and development of therapies and vaccines [27]. Some notable MERS-CoV-related events which have occurred since the publication of the Al-Tawiq and Memish review are shown in Figure 1.

2. Epidemiology update

WHO and the Centers of Disease Control and Prevention (CDC) have received reports of laboratory-confirmed cases of MERS from the national health authorities in the Middle Eastern countries of KSA, Bahrain, Iran, Jordan, Kuwait, Lebanon, Oman, Qatar, United Arab Emirates (UAE), and Yemen, as well countries outside the Arabian Peninsula including Algeria, Austria, China, Egypt, France, Germany, Greece, Italy, Malaysia, Netherlands, Philippines, Republic of Korea, Thailand, Tunisia, Turkey, the UK, and the USA. More than 85% of cases to date have occurred in KSA [2,8]. Cases occurring outside the Middle East have been associated with individuals with a travel history in the Middle East.

While most outbreaks since the period covered by Al-Tawiq and Memish have occurred in healthcare facilities in KSA, a major healthcare facility-associated outbreak occurred in the Republic of Korea in 2015 [3–5]. The outbreak, which ran between 20 May and 27 July 2015, involved 186 MERS-CoV cases, including 36 deaths [3,4,28]. As with other cases in countries outside the Middle East, the outbreak began with a

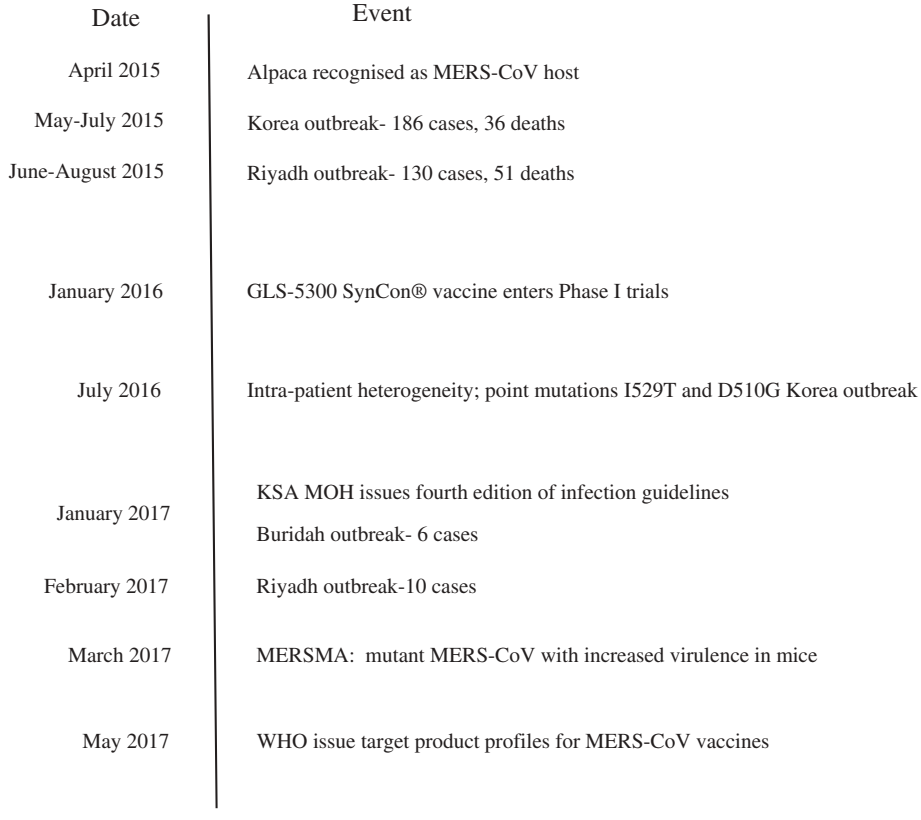

Figure 1. Timeline of notable MERS-CoV-related events.

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man with a preceding travel history to Middle Eastern countries, in this case Bahrain, UAE, KSA, and Qatar. Transmission dynamics analyses revealed that the outbreak was driven by 'superspreading' events connected with individuals who visited multiple healthcare facilities during the course of the outbreak and thus between them generated a disproportionately high number of secondary cases [3,4]. One of them was responsible for more than half of the tertiary cases that arose. Extensive contact tracing was carried out during this outbreak, and showed that infections arose in second- and third-generation contacts. The contact tracing proved effective in rapid diagnosis where MERS was suspected and subsequent isolation of infected individuals, and hence in rapid limitation of viral spread and reproduction numbers [3]. Prevention measures included quarantining of more than 3000 people and closing 700 schools [28]. Nevertheless, the first MERS case to arise in China was in a South Korean contact of confirmed MERS-CoV cases in the South Korean outbreak, who traveled to Guangdong Province in China [29,30].

Frequent small outbreaks have meanwhile occurred in KSA. Most recently, six cases were linked to a limited outbreak involving two hospitals in Buridah between 2 January 2017 and 10 February 2017, while an outbreak in a hemodialysis unit in a Riyadh hospital was linked to eight symptomatic and two asymptomatic cases between 23 February and 16 March 2017 [2]. Three clusters of cases occurred in three hospitals in the Riyadh area in June 2017, two of which were related, involving 49 individuals and 10 deaths [31]. A more major outbreak of 130 MERS cases occurred at King Abulaziz Medical City in Riyadh during late June–late August 2015, most of which were connected to the emergency department [32]. Cases included 43 healthcare workers. There were 51 deaths during the outbreak, but no deaths among the healthcare workers, who were younger than the patients (median age 37 and 66 years, respectively) and had fewer comorbidities. The outbreak resulted in initiation of the preexisting Infectious Disease Epidemic Plan (IDEP) in the hospital, which was based on WHO and CDC guidelines. The IDEP was ultimately raised to level III, which resulted in an almost complete shutdown of the hospital, including emergency department closure, cancelation of elective procedures, and suspension of outpatient appointments [32]. The last infected patient was recorded on 28 August 2015, and the end of the outbreak was officially declared on 28 September 2015. The measures that had to be taken to control this outbreak, which was a result of factors including overcrowding in the emergency department, lack of control of patient movement and high volumes of visitors, highlights the importance of rigorous adherence to stringent infection prevention and control measures in healthcare facilities.

A recent comparison of epidemiology of outbreaks in KSA compared to the outbreak in the Republic of Korea in 2015 revealed different patterns and risk factors [33]. The mean age of infected individuals was similar in both KSA and Republic of Korea (51 and 54 y, respectively) [33]. In both cases, older males (≥70 y) were at higher risk of infection or death from MERS-CoV, with males in general having a higher rate of comorbidity [30]. However, the transmission patterns were more complex in KSA compared to Republic of Korea. In KSA, there was evidence of zoonotic transmission, human-to-human transmission and unknown pathways, as well as nosocomial infection, while in Republic of Korea the pattern was almost exclusively nosocomial [33]. Of 1186 KSA cases identified in this study to have known risk factors, 13.3% were hospital-associated compared to 94.1% of the 175 Republic of Korea cases with known risk factors [33]. In KSA, unknown exposure risk was reported in 59.9% of cases.

3. Latest infection control and prevention recommendations

Extended outbreaks of MERS-CoV have centered on healthcare facilities. Transmission appears to require close human-to-human contact, for example unprotected care of an infected patient by a healthcare worker or family contacts. Current advice from WHO stresses the importance of infection prevention and control measures in limiting spread of MERS-CoV in healthcare facilities [2]. MERS-CoV outbreaks associated with clusters in healthcare facilities have been linked to defective or inadequate infection prevention and control measures [15,34–37]. Al-Tawfiq and Memish identified infection prevention and control as a key issue to be addressed [27]. Aggressive implementation of rigorous infection prevention and control measures has since been shown to limit viral spread and help in resolution of outbreaks [34,35]. WHO worked with public health authorities in KSA to produce detailed infection control and prevention guidelines designed to limit transmission from human to human in healthcare facilities [2]. In KSA, issues such as overcrowding in emergency department waiting rooms and inadequacies in the basic infection and prevention control measures such as handwashing were identified by WHO as contributory factors in MERS-CoV human-to-human transmission and hence outbreaks [2]. Technical meetings were also held in 2015 in Egypt and in Qatar in which control measures were discussed, along with evidence and risk factors associated with potential spread from camels to humans and the need for greater cooperation between the human and animal health sectors in the MERS-CoV context. WHO-led missions involving the Ministries of Health in both KSA and Korea resulted in updated guidelines in both of these countries in the areas of infection prevention and control [16,34,35]. These include the need for a comprehensive suite of basic measures such as hand hygiene, control of contacts, and use of personal protective equipment, as well as more advanced precautions for care of patients with acute respiratory infections, including use of effective triage, droplet and airborne precautions, safe patient transport and continuous training and education of healthcare workers. Cases have declined in both KSA and Korea as a result of following such guidelines. In January of 2017 the Ministry of Health in KSA issued the fourth edition of infection prevention and control guidelines for MERS-CoV in line with most up-to-date case definition and surveillance guidance [38].

4. Transmission of MERS-CoV

4.1. Transmission overview

In 2015, the mechanisms of zoonotic MERS-CoV transmission to humans was identified by Al-Tawiq and Memish as one of

the most important points to be clarified over a 5-year view [27]. This included the role of camels in transmission, possible intermediate hosts, and risk factors for transmission of MERS-CoV from animals to humans. Current thinking is that most MERS-CoV human infections arose from multiple zoonotic transfers with limited secondary human-to-human transmission in situations of close contact, particularly in family and healthcare settings, resulting in hospital-associated outbreaks in KSA, Korea, and UAE [25,36,37,39]. Now in 2017, the exact mechanism of transmission from animals to humans is still not entirely clear. The details of human to human transmission also remain incompletely understood, for example the role of asymptomatic patients in the transmission cycle. However, there has been progress in understanding the transmission of the virus and potential risk factors, even though the story is not yet complete.

4.2. Zoonotic transmission

Recent studies, including those based on serological evidence, support the role of dromedary camels as important zoonotic sources of human MERS-CoV infection. In a study on all MERS-CoV infection cases documented in KSA between May and November 2014, exposure to dromedary camels in the two weeks prior to illness onset was identified as a risk factor for MERS-CoV infection, along with comorbid illnesses including diabetes mellitus and coronary heart disease and lifestyle factors including smoking [40]. Exposure to infected dromedary camels or consumption of their raw milk is consistently cited as a contributing risk factor in many of the cases reported by WHO in their regular Disease Outbreak News bulletins [2]. Gossner et al. have suggested that changes in dromedary camel production and farming practices, including intensification and clustering close to cities, may contribute to a type of zoonotic 'spillover' effect [41]. While transmission of virus to humans by consumption of dromedary camel-derived food products such as milk, urine and meat is a potential risk, in reality the frequency of consumption of such products in Middle Eastern countries far exceeds the frequency of MERS-CoV infection [25]. Direct contact with dromedary camels rather than ingestion of food products is now considered to be the potentially most significant risk factor, with some genetic evidence available for direct contact transmission [25,42–44]. MERS-CoV antibodies have been identified in more than 90% of dromedary camels tested in the Middle East and in many African countries [25,45–49]. The recent identification of specific neutralizing antibodies in 39.5% of samples taken from 565 dromedaries in the Punjab in Pakistan suggests that there is a worrying expansion of enzootic MERS-CoV range in Asia [50]. The Punjab borders Rajasthan state in India, which is the location of India's largest dromedary camel population [50].

Studies on the transmission of MERS-CoV among dromedary camels in Africa suggest that there is circulation across broad areas [47]. However, despite the apparent zoonotic potential of camels in Africa, there has been a lack of reported MERS CoV infection in humans in Africa, which suggests that there may be unrecorded cases of human MERS-CoV, reminiscent of previous reports from KSA [51]. The reasons for this are not clearly understood. It is possible that deficiencies in public health systems or in the sensitivity of the detection tests used may lead to human cases going undetected in parts of Africa [41,51]. Other possibilities are circulation of less virulent strains in Africa, or exposure of different types of individuals, leading to more asymptomatic cases or resistance to infection [51]. It is important that suitable field epidemiology studies are established and that effective and extensive screening is carried out, to help uncover previously undetected cases, inform public health strategies and prevent the extent of transmission being underestimated, particularly outside the Middle East [25,40].

While MERS-CoV seropositivity has not been observed in other potential source animals including goats, cows, or water buffalo, alpacas have recently been shown to be another potential MERS-CoV zoonotic reservoir [52]. MERS-CoV antibodies were found in alpacas in Qatar, in an endemic region [52]. These animals were kept close proximity to a dromedary camel herd in which all but one animal tested positive for MERS-CoV antibodies. Nevertheless this cross-species transmission and apparent susceptibility of alpacas to infection suggests that they are a candidate for potential expansion in zoonotic host range if MERS-CoV were to be introduced in areas where there are large alpaca populations. This New World camelid is farmed in other parts of the world, including South America and the United States, thus this identification of viral antibodies represents a potential widening of MERS-CoV zoonotic range and geographic reach [52]. When the DPP4 receptor sequence of alpaca is aligned with that of other mammals, the 14 residues that interact with the MERS-CoV receptor-binding domain (RBD) are identical to those in the dromedary camel sequence. MERS-CoV antibodies were also identified recently in a sheep which had contact with seropositive camel herds in Egypt [45].

4.3. Human-human transmission

MERS-CoV is not currently considered to have pandemic potential, with human-to-human transmission apparently requiring close contact, and cases being mainly confined to the Middle East, or else occurring as a result of travel of infected persons from Middle Eastern countries. However, the outbreak in the Republic of Korea in 2015 highlighted the potential dangers of a combination of a primary source traveling from the Middle East, spread among secondary and tertiary contacts due to 'superspreading' events caused by movement of infected individuals between healthcare facilities, and deficiencies in infection prevention and control responses [2–5]. Since events covered by the Al-Tawfiq and Memish review, the outbreak in Republic of Korea is the only one outside the Middle East which featured further human-to-human transmission. Other cases reported to WHO, in the Philippines in February and July 2015, in Germany in February 2015, Thailand in June 2015 and August 2016, and Austria in September 2016, all involved single-infected individuals who were either resident in a Middle Eastern country and had traveled to the other country, or else were residents of the other country and had recently traveled in the Middle East [2]. In each case, contact tracing revealed no transmission of the virus in the non-Middle Eastern country. This is consistent with limited human-to-human transmission patterns [27].

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The role of asymptomatic patients in transmission of MERS-CoV is not yet fully understood. In a questionnaire-based study on expert opinion on MERS-CoV infection, all 14 experts surveyed considered that MERS-CoV-infected dromedaries and asymptomatic infected humans were most important in infection of humans, with the possible risk from bats and other species remaining undefined [53]. In a recent study of a case of asymptomatic MERS-CoV infection identified during the Korean outbreak, no evidence of transmission to 82 contacts was found [54].

4.4. Viral mutation and potential for enhanced transmission

As MERS-CoV has evolved different mutations, in particular in the S protein gene, have arisen in the various regions where outbreaks have occurred, and during the course of a single outbreak. The S protein plays a central role in MERS-CoV infection. Binding of MERS-CoV to cells via human dipeptidyl peptidase 4 (DPP4; CD26), a functional MERS CoV receptor, is mediated by the RBD in the S1 subunit, while viral fusion with the cell membrane is mediated by the S2 subunit [55,56]. Following fusion, the S protein is cleaved by host proteases at the S1/S2 border, thus dividing the N-terminal S1 subunit from the C-terminal S2 subunit [57]. The S2 subunit consists of a fusion peptide, two heptad repeat domains HR1 and HR2, and a transmembrane (TM) domain [57]. To facilitate fusion, S2 rearranges to form a six-helix bundle (6HB) fusion core, with HR1 and HR2 as important elements. The fusion peptide is exposed and inserted in the host cell membrane, allowing the proximity between host and viral cell membranes that permits fusion [26,57]. Host proteases which can carry out S protein cleavage include the serine protease TMPRSS2, the endosomal cathepsin L, and furin protease [26,58–61].

Since the events covered in the Al-Tawiq and Memish review [27], it has been established that introduction of two mutations, N762A and S746R, into the bat coronavirus HKU4S protein gene enables this virus to enter human cells [27,61]. These mutations within the S1/S2 junction are present in MERS-CoV, where they are part of human protease motifs, thus enabling cleavage of the S protein and infection of human cells. This suggests that these mutations were vital in transmission of MERS-CoV from bats to humans [61]. It also raises the possibility that other mutations in the S protein of MERS-CV could yet arise which would increase its animal-human or human-human transmissibility. However, mutations identified in 13 new viral genomes which arose during the outbreak in the Republic of Korea resulted in reduced viral affinity [62]. Two point mutations were identified in the RBD region of the S protein [62]. Both of these mutations, I529T (12 genomes), and D510G (one genome) resulted in reduction of S protein affinity for DPP4. Thus, pressure exerted by host immune responses may have driven viral adaptation in a direction that resulted in reduced viral affinity. The S protein is a major target of the host immune response against MERS-CoV, for example it is the main target for neutralizing antibodies during coronavirus infections [63]. Further genomic analyses of samples from the Republic of Korea outbreak supported the hypothesis that the host immune response was a significant driver of viral variations [64]. There was significant intrapatient heterogeneity, particularly in the individual responsible for transmission to the majority of the tertiary cases [64]. The combined frequency of the point mutations I529T and D510G was high, at approximately 87.7%, despite variation in inter-specimen frequency of their occurrence, suggesting that there was a strong host-driven selection pressure for the variants that reduced receptor affinity. Recently, two deletion mutants between ORF5 and the E protein were identified in samples from the Chinese MERS case, which may be defective in MERS CoV packaging [65]. It is currently unclear how these deletions occurred or what their effects are.

Genomic studies confirm that MERS-CoV, and the S protein gene in particular, has undergone multiple recombination events [26]. Nine positive selection sites were identified in the S gene, including six in the RBD. Notably, the ORF4b, S and N proteins have undergone more rapid substitution than the genome as a whole [26]. In the HR1 and HR2 regions of the S gene, for example, many positively selected sites have been identified such as R652 and V1060, which were associated with host range expansion [66]. Adaptive HR1 mutations at position 1020 (Q/R/H1020) in camels or a previous host have arisen recently in MERS-CoV evolution [66]. These resulted in small reductions in HR1 and HR2-mediated helical stability and bundle formation which increased infection efficiency in *in vitro* studies, and may be linked to viral spread to humans.

An ongoing issue in MERS-CoV studies is sourcing of a suitable small animal model. There is reliance on transgenic mice models as wild type mouse DPP4, in common with rat, hamster and ferret, does not support MERS-CoV infection [67,68]. Other models that have been developed include New Zealand white rabbit, rhesus macaque, and marmoset [67]. Thirteen DPP4 residues have been identified as being key to interfacing with the viral S protein RBD [69,70]. When phylogenetic comparisons are made, these residues are either conserved or differ by only one or two residues in DPP4 of other species that have shown to be permissive in either *in vitro* or *in vivo* studies, including camel, macaque, marmoset, goat, pig, civet, and horse [69], as well as alpaca [52]. However, DPP4 of non-permissive species including mouse, hamster, and ferret has multiple variations among these 13 residues. This underscores the importance of DPP4 recognition by MERS-CoV in viral transmission and the potential utility in targeting this interaction for therapeutic purposes. Structural differences, and/or posttranslational modification are also considered to be important. For example, removal of a glycosylation site in the mouse DPP4, which is not present in the human receptor but is found in the nonpermissive hamster receptor, conferred permissivity [71].

In a recent study aimed at generation of a mouse model of MERS, a mouse knockin model was generated by insertion of humanized exons 10–12 in the mouse *Dpp4* gene [72]. This model was permissive to MERS-CoV infection to the extent that viral replication occurred in the lungs, however the mice did not become ill. After 30 serial passages of virus through the lungs, a mutated virus termed MERSMA

emerged which harbored 13–22 mutations, many of which were in the S gene. The MERSMA virus was substantially more virulent in the knockin mouse than the MERS-CoV parent virus, and resulted in weight loss and fatal infection [72]. The adaptation of the virus to give MERSMA underscores the importance of furthering our understanding of the transmission mechanisms of MERS-CoV, as it illustrates the potential consequences of viral mutations that facilitate increased infectivity and/or virulence.

5. Therapy and vaccine candidates

5.1. Therapy overview

In 2015, the same year that Al-Tawiq and Memish published their review, Public Health England (PHE) and the World Health Organization-International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC-WHO) published a position paper on the evidence base for specific MERS-CoV therapies [73]. They suggested that benefit was likely to exceed risk for convalescent plasma, lopinavir/ritonavir, interferons and monoclonal/polyclonal antibodies [73]. For both ribavirin monotherapy and corticosteroids, risk would exceed benefit, while there was insufficient information available for interferon/ribavirin combination therapy, nitazoxanide and chloroquine [73].

Current and potential MERS-CoV therapies have recently been reviewed in some detail [74,75], as have MERS-CoV vaccines [76]. Al-Tawfiq and Memish concluded that while many agents have been tried both *in vitro* and *in vivo*, none been subject to well-organized, large clinical trials [74]. Instead, clinical studies have been confined to interferon in combination with ribavirin and/or lopinavir; the current first-line therapy is type 1 interferon with lopinavir/ritonavir [77]. However, these clinical trials to date consist of case reports or series rather than full-scale randomized clinical trials [74,78]. Thus, the need for prospective and randomized clinical trials for therapies specific to MERS-CoV remains a priority 5 years after the initial case, but the strategy is complicated by factors including the sporadic nature of cases beyond the large healthcare facility outbreaks [74].

5.2. Convalescent plasma

The PHE and ISARIC-WHO position paper suggested that convalescent plasma (or high neutralizing antibody titer products) could be considered for treatment of serious MERS-CoV infection [73]. Results of trials on SARS-CoV and H1N1 patients have suggested that administration early in the course of disease confers the greatest survival benefit, although quality of studies was not always high [79,80]. A recent survey of 327 physicians in KSA identified intensive care units (ICUs) in KSA as the likeliest clinical locations for performing clinical trials of MERS-CoV convalescent plasma therapy and suggested that most of them have prior experience of conducting RCTs [81]. However, only 21% of the respondents regularly took part in research networks. An ongoing clinical trial on convalescent plasma treatment safety and efficacy for critically ill MERS-CoV patients was initiated in May 2014 in KSA and is due to report in June 2017 [82; ClinicalTrials.gov Identifier: NCT02190799]. However, this trial has been hampered by logistical and technical problems, for example, insufficient donor availability and insufficient levels of viral-specific antibodies in samples [63,82]. While there are two case reports from 2014 in which IVIG was used in treatment of MERS-CoV, it is uncertain as to whether the IVIG contributed to patient recovery [83,84]. Thus, while convalescent plasma is theoretically a promising potential therapy for MERS-CoV, clinical evidence is so far very limited.

5.3. Monoclonal antibodies

Concerted research efforts have meanwhile focused on the other therapeutic option identified by PHE/ISARIC-WHO, namely antibody therapy. In particular, many mouse and human neutralizing mAbs directed against the S1 region of MERS-CoV have been trialed *in vitro* and/or in animal models, and have been recently extensively reviewed [75,85]. S1-targeted mAbs isolated and/or tested since the Al-Tawiq and Memish review [27] are summarized in Table 1 [86–96],

Table 1. Antibodies targeting S1 protein of MERS-CoV.

Antibody name	Source	Target region	Efficacy	Reference(s)
m336, m337, m338	Human from antibody library	RBD	*In vitro*; m336- *in vivo*- human DPP4 (hDPP4)-expressing transgenic mouse model; rabbits	[80–82]
MERS-4, MERS-27	Human from antibody library	RBD	*In vitro*	[83,84]
4C2	Humanized-mice	Partial RBD	*In vitro*; *In vivo*- Ad5-hDPP4 transgenic mouse model	[85]
hMS1	Humanized mice	RBD	*In vitro*; *In vivo*- human DPP4 (hDPP4)-expressing transgenic mouse model	[86]
LCA60	Human from B cells of MERS-CoV-infected volunteer	NTD and RBD (S1)	*In vitro*; *In vivo*- Ad5-hDPP4 transgenic mouse model and IFNAR-KO model	[87]
3B11-N	Human from antibody library	RBD	*In vitro*; *In vivo*- nonhuman primate (rhesus monkeys)	[88]
D12, F11, G2, G4	Mouse	RBD	*In vitro*; *In vivo*- human DPP4 (hDPP4)-expressing transgenic mouse model; NHP (Indian rhesus macaques)	[89]
REGN3048, REGN3051	Humanized mice	RBD	*In vitro*; *In vivo*- human DPP4 (hDPP4)-expressing transgenic mouse model	[90]

IFNAR: type I interferon receptor; KO: knock-out; NHP: non-human primate

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along with details of their sources and whether efficacy has been established *in vitro* or *in vivo*. Notably, human antibody LCA60, which was isolated from B cells of a MERS-CoV infected donor, targets both the N-terminal domain (NTD) and the RBD of the S1 region and was used to rapidly establish a stable CHO cell line which can be used as a convenient and reliable source of clinical grade antibody [93]. This is a promising development as the antibody has both potent prophylactic and therapeutic activities in Ad5/hDPP4 transgenic mice and type I interferon receptor (IFNAR)-KO mice [93]. Another notable candidate is the human 3B11-N antibody which has potent prophylactic effects *in vivo* in rhesus monkeys [94]. These antibodies offer potential sources for passive immunization strategies for MERS-CoV, but thus far they have not entered human clinical trials.

5.4. Active immunization

Active immunization strategies are dependent on successful delivery of MERS-CoV proteins; again S protein is the main focus of research efforts. Both viral and plasmid based delivery options have been tested *in vivo* [97–104]. Successful expression of S protein, and generation of neutralizing antibody and T cell immune responses in mice, have been achieved using replication-deficient vectors such as modified vaccinia virus Ankara (MVA), ad5 or ad41-type adenoviruses and measles viruses, all of which have good safety profiles in humans [97–100]. Recently, an S protein-expressing orthopoxvirus-based vaccine has been shown to induce mucosal immunity in MERS-CoV-infected dromedary camels [101]. This is potentially a very important step forward, as it opens up the possibility for veterinary use and hence interference with the cross-species transmission of virus from camels to humans, which is currently thought to lie behind many primary infection cases [101]. In the last few months, an S-protein expressing vaccine based on an inactivated rabies virus has been described, which fully protected hDPP4-expressing transgenic mice from MERS-CoV infection [102].

DNA plasmids are another possible vaccine delivery option. The S-protein expressing DNA-plasmid vaccine GLS-5300 is the first potential MERS-CoV vaccine to have entered human trials [103]. A 1-year phase I clinical trial in healthy volunteers began in 2016 to evaluate safety and immune response generation, following preclinical trials in mice, camels, and rhesus macaques [103,104]. Meanwhile, a novel vaccine consisting of chimeric virus-like particles (VLP) expressing the MERS-CoV RBD induced RBD-specific humoral and cellular immune responses in transgenic mice [105]. This vaccine, which assembles into chimeric, spherical VLP (sVLP), offers another promising prophylactic candidate in the event of a potential MERS-CoV outbreak situation [105]. However, despite the promise of these prophylactic vaccine candidates, barriers exist in terms of definition of the population who would be targeted in a vaccination program and/or randomized clinical trials, relatively low current human infection prevalence, and the difficulties in sourcing suitable small animal models [63,95,97]. In this context, WHO have just issued guidelines on the relevant considerations they will make on a case-by-case basis for proposed MERS-CoV vaccines [106]. They have distinguished between three vaccine types which would be aimed at three different defined target populations. These include dromedary camel vaccines aimed at prevention of camel–camel and camel–human transmission; protective human vaccines for those considered at long-term risk, for example healthcare workers and those working with animals that could potentially be infected; and finally human vaccines for use in an outbreak situation [106]. Both the preferred and minimally acceptable criteria for each of these vaccine types are defined.

5.5. Potential vaccine concerns

A note of caution is necessary when considering the potential effectiveness of proposed MERS-CoV vaccines as results from dromedary camels would suggest that preexisting monoclonal antibodies do not protect animals from reinfection [107]. Thus the recent demonstration of mucosal immunity and reduction of viral excretion conferred upon dromedary camels after use of an orthopoxvirus-based vaccine is particularly encouraging [101]. Candidates for prophylactic vaccines which can induce both cellular and humoral responses are also of potentially great importance in facilitating effective protection [97–100,105], while *in vivo* demonstration of protection from viral infection in small animal models or nonhuman primates is also promising [86–88,91–100].

Safety concerns have been raised around the use of full length S protein in vaccine. In a recent study, Th2-mediated hypersensitive-type lung pathology characterized by increased infiltration of eosinophils and raised IL-5 and IL-13 cytokines arose in mice vaccinated with whole inactivated virus (WIV), similar to previous studies on SARS-CoV [108]. In response to these types of safety concerns, immunogenicity of naked DNA vaccines expressing varying portions of MERS-CoV S protein was tested in mice within the last few months [109]. While both full-length (pS) and S1-subunit (pS1)-induced S1-specific antibodies were protective against MERS-CoV infection, different IgG isotype patterns were observed. Importantly, the pS1 vaccine induced a balanced Th1/Th2 response and higher IgG levels compared to the pS vaccine. Furthermore, only the pS1 vaccine induced a significant S1-specific cellular immune response [109]. The results of this study therefore are encouraging as they suggest that naked DNA vaccines based on the S1-subunit can give effective protection against MERS-CoV without the potential hypersensitivity concerns linked to the full-length S protein-based vaccines.

5.6. Antiviral peptides

Another potential therapeutic strategy that has developed is targeting of the HR regions of the S2 region of the S protein using antiviral synthetic peptides. *In vitro* effectiveness of the HR2P peptide in reducing viral replication and fusion had already been demonstrated in 2014 [110]. Importantly, an analog of this peptide, termed HR2P-M2, was subsequently also shown to have *in vivo* efficacy after intranasal

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administration to ad5-human DPP4-transgenic mice [111]. Coadministration of interferon-β enhanced this protection. The success of intranasal treatments *in vivo* also suggests the potential viability of a convenient nasal spray formulation as a delivery option.

5.7. Anti-proteases

Targeting of the protease-mediated S1/S2 cleavage event which is critical to MERS-CoV fusion with the host cell membrane is another strategy which has received recent research attention. The TMPRSS2 inhibitor camostat, which is already used clinically for chronic pancreatitis treatment, blocked spread and pathogenesis of SARS-CoV in a pathogenic mouse model and would be expected to similarly affect MERS-CoV [112]. Nafamostat, another TMPRSS2 inhibitor, had potent *in vitro* inhibitory effects against MERS-CoV in a split-protein-based cell-cell fusion assay [113]. Nafamostat has clinical approval for use in other conditions as an anticoagulant [113]. Thus, both camostat and nafamostat are potentially safe and effective candidate drugs for MERS-CoV treatment.

A recent high throughput screening of FDA-approved drugs led to identification of the cathepsin L inhibitor teicoplanin, a glycopeptide antibiotic, as an inhibitor of MERS-CoV, SARS-CoV and Ebola pseudoviruses entry into the cytoplasm [114]. Teicoplanin also has the advantage of being currently approved for clinical use in treatment of serious gram-positive bacterial infections, thus it is known to be safe for use in humans. Its derivatives, including dalbavancin, oritavancin, and telavancin, also block viral entry [114].

The viral protease PL^{pro} is also a potential therapeutic target. Its activity is inhibited *in vitro* by the SARS-CoV PL (pro) inhibitors, 6-mercaptopurine (6MP) and 6-thioguanine (6TG) [115]. Recently the commercial compound F2124–0890 (Life Chemicals), has been identified a MERS-CoV and SARS-CoV PLpro inhibitor [116–118]. However, the inhibitory mechanism differs between the two proteases due to differences in structural elements, with competitive inhibition of MERS-CoV PL(pro), but allosteric inhibition of SARS-CoV PL [pro) [116]. While potency of F2124–0890 may be compromised in physiological reducing environments, nevertheless this highlights the possibilities for PLpro targeting in MERS-CoV therapy [117]. Meanwhile, studies on a virulent murine coronavirus, mouse hepatitis virus (MHV), have recently identified matrix metalloproteinase and ADAM families as unexpected potential therapeutic targets for coronaviruses [118].

5.8. Other therapeutic candidates

Other recently emerging therapeutic candidates for MERS-CoV include Abl kinase inhibitors. In a screen of FDA-approved drugs, Abl kinase inhibitors, including the anticancer drug imatinib, emerged as candidate inhibitors of both SARS and MERS *in vitro* [119]. Abl-2, which is an imatinib target, has since been identified as essential for both MERS-CoV and SARS-CoV replication *in vitro* [120]. Thus, this opens up the possibility of repurposing of approved drugs such as imatinib, as well as camostat, nafamostat, and teicoplanin, in MERS-CoV treatment.

Meanwhile carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) has been identified as a novel cell surface binding target of MERS-CoV [121]. It appears to function as an attachment factor; it does not in itself render hamster cells permissive to MERS-CoV entry but does increase viral binding, and in permissive cells its overexpression increases MERS-CoV entry. Therefore, it augments MERS-CoV attachment and facilitates viral entry in conjunction with DPP4, presenting another potential therapeutic target.

6. Conclusions

Five years on from the first MERS-CoV case, we have advanced our understanding of viral transmission, identified as a priority by Al-Tawfiq and Memish in their 2-year review published in 2015 [27]. Exposure to dromedary camels is now recognized as a frequent risk factor in primary cases of human infection, and WHO have identified people who work with potentially infected animals as a target group for future prophylactic vaccination, along with healthcare workers [25,42–44,106]. However, our understanding of the exact viral transmission mechanisms remains incomplete, particularly in terms of human-human transmission and the role of asymptomatic individuals [53]. Emergence of alpacas as another potential MERS-CoV zoonotic reservoir is an indication that there may yet be zoonotic transmission routes of which we are as yet unaware [52]. Improvements in infection prevention and control strategies, another priority area identified by Al-Tawfiq and Memish[27], have contributed to an overall reduction in cases, and since 2015 there have been no major healthcare facility-associated outbreaks to match the outbreaks in the Republic of Korea or in Riyadh in KSA in that year [2–5,16,32–38]. Despite this, MERS-CoV remains a threat to public health as it exacts a high mortality rate, particularly among vulnerable individuals, and there is still no specific therapy available. Also, the virus has been shown to undergo frequent mutations; while this has not so far resulted in greater viral transmissibility, the potential for increased viral host range, virulence and transmission remains [61,62,64–66,72]. Thus, there is still an ongoing need, identified by Al-Tawfiq and Memish in 2015 and confirmed in a 2017 review by the same authors, to not only show potential efficacy of specific MERS – CoV therapies and vaccines *in vitro* and *in vivo*, but to move to well-designed RCTs for some of the candidates [27,74]. MERS-CoV patients currently depend on mainly supportive therapy extrapolated from SARS-CoV, with the indicated first-line therapy being type 1 interferon with lopinavir/ritonavir [77]. The plethora of S protein-specific monoclonal antibodies, the progress with possible active immunization strategies, the entry into phase I trials of the GLS-5300 vaccine, and the potential for repurposing some existing drugs offers hope that the aspiration for more targeted therapies, and a reliable vaccine, may be met [86–105,113–120].

7. Expert commentary and five year view

Over the next five years, it is important that we continue to narrow down the transmission mechanisms of MERS-CoV. Al-Tawfiq and Memish [27] identified this as a priority in their 2-year overview and, although progress has been made,

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neither zoonotic nor human-to-human transmission is still fully understood. For example, the role of asymptomatic individuals in spread is of particular interest and must be addressed. Improvements in test methodologies may be helpful in furthering our understanding of the role of asymptomatic and/or mildly affected patients and their role in transmission in the Middle East and elsewhere. Another area of particular interest is the identification of antibodies against MERS-CoV both in alpacas and sheep. Although this cross-infection probably arose due to close contact with infected camels, this cross-species transmission suggests that we must remain vigilant for potential expansions in zoonotic host range. The issue of possible unrecorded human cases in Africa should also be addressed by testing and validation of the serological testing methods being used and implementation of extensive and strategic sero-surveys. The need for well-validated human and animal sera panels has been identified and must be urgently addressed. Ongoing vigilance in implementation of infection prevention and control methods must be maintained, given its success in reducing the extent hospital-associated outbreaks.

In general, the clinical response to MERS-CoV has been too slow [122]. Thus, while there are many promising lines of research for specific therapies, there has been a lack of progression to well-organized RCTs. The ultimate goal is to make available therapies and vaccines which are specific to MERS-CoV, rather than relying on therapeutic agents extrapolated from SARS-CoV with uncertain efficacy in the specific context of MERS-CoV. There is an urgent human and clinical need for effective therapies. Cases of MERS-CoV are being reported every week in KSA and elsewhere, with a high mortality rate continuing to be observed. There is also the ongoing potential for a major outbreak outside the Middle East, such as that which occurred in the Republic of Korea in 2015. Thus, there is a pressing need to establish RCTs for prospective MERS-CoV vaccines, bearing in mind the three different target populations defined by WHO, i.e. dromedary camels, people who may be considered at long-term risk, for example healthcare workers and those working with animals that could potentially be infected; and people in an outbreak situation. Ensuring safety of proposed vaccines and targeting of both cellular and humoral responses are challenging areas, particularly given the difficulties inherent in preclinical studies, for example accessible small animal models. Alongside this, RCTs for some of the potential MERS-CoV-specific therapies that have shown promise *in vitro* and *in vivo* now need to be urgently progressed, including exploitation of the potential of re-purposing of existing drugs such as imatinib, camostat, nafamostat, and teicoplanin and its derivatives.

Key issues

- MERS-CoV remains a threat to public health. WHO has recorded 1,936 laboratory-confirmed cases in 27 countries, with a mortality rate of 35.6%.
- Contact with dromedary camels has been recognised as a risk factor in many primary cases. However, transmission mechanisms are still not fully defined.
- WHO have distinguished between vaccine types which would be aimed at three different defined target populations: dromedary camels; humans considered at long-term risk, for example healthcare workers and those working with animals that could potentially be infected; humans in an outbreak situation. No specific therapy or vaccine is yet available for use in humans or animals. A plasmid vaccine, GLS-5300, is the first potential MERS-CoV vaccine to have entered human trials.
- Monoclonal antibodies, anti-viral peptides and protease inhibitors are among the specific therapies that have shown promise *in vitro* and *in vivo*. There is also the potential to re-purpose existing drugs.
- Rigorous adherence to national guidelines on infection prevention and control appears to have reduced the extent of healthcare facility-associated MERS-CoV outbreaks.

Funding

This manuscript has not been funded.

Declaration of interest

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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