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Detection of multiple viral sequences in the respiratory tract samples of suspected Middle East respiratory syndrome coronavirus patients in Jakarta, Indonesia 2015–2016

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ABSTRACT

Objectives: The identification and analysis of viral etiological agents from suspected Middle East respiratory syndrome coronavirus (MERS-CoV) cases admitted to Prof. Dr. Sulianti Saroso Infectious Disease Hospital (IDH) using molecular assays.
Methods: Biological samples were collected from 13 hospitalized patients suspected of MERS-CoV infection in Prof. Dr. Sulianti Saroso IDH from July 2015 to December 2016. The majority of patients presented with pneumonia, with symptoms including fever (≥37.5 °C), labored breathing, and cough, and with a history of travel to the Middle East. Viral RNA was isolated and converted to cDNA, which was used as a template for the detection of 12 viral panels using conventional PCR and sequencing.
Results: Viral etiological agents detected in the patients were enterovirus D68, dengue virus type 3, rhinovirus C, human coronavirus 229E, herpes simplex virus type 1, influenza virus H1N1, influenza virus H3N2, human metapneumovirus, and rhinovirus A60.
Conclusions: The sequences of nine viral agents under different taxa were detected in suspected MERS-CoV patients, including influenza virus, paramyxovirus, coronavirus, enterovirus, human metapneumovirus, and herpesvirus.

Introduction

Since 2012, Middle East respiratory syndrome coronavirus (MERS-CoV) has been reported worldwide, with a total of 2206 confirmed cases and 787 deaths in 27 countries (WHO, 2019). The spectrum of MERS-CoV infection ranges from a mild viral respiratory illness to a rapidly fatal pneumonia complicated by acute respiratory distress syndrome and acute renal failure (Chong et al., 2015), with a mortality rate of around 35–50% (Noorwali et al., 2015; Yang et al., 2017; Hui et al., 2018). No specific licensed antiviral treatment is currently available for MERS-CoV infection (Rabaan et al., 2017).

The most common clinical symptoms of MERS-CoV are fever, cough, and dyspnea. The positive detection rate is significantly higher in hospitalized patients with pneumonia compared to those with upper respiratory tract infections. These symptoms usually occur with a mean incubation period of 2–13 days. Comorbidities such as diabetes mellitus, cirrhosis, and others affecting the respiratory, renal, and cardiac systems have been shown to increase the severity of the illness (Yang et al., 2017). However, there is lack of specific clinical symptoms to distinguish MERS-CoV from other respiratory pathogens (Butler, 2015), making it imperative that early detection and laboratory identification of coronavirus infection is performed in suspected patients.

Three neighboring countries in Southeast Asia have reported MERS-CoV infections associated with a history of travel to the Middle East. In April 2014, a man in his mid-50s presented to the emergency ward of a public hospital in Malaysia. He had returned from a pilgrimage to Saudi Arabia 13 days earlier and was confirmed as the first MERS-CoV-infected case in the country (Premila Devi et al., 2014). One year later, in February 2015, a 31-year-old woman in the Philippines was also diagnosed with MERS-CoV; she had a history of being resident in Saudi Arabia as a health worker (Racelis et al., 2015). Four months later, MERS-CoV

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infection was also detected in an Omani citizen who had visited Thailand (Plipat et al., 2017). MERS-CoV has never been reported in Indonesia, although thousands of pilgrims have visited the Middle East every year. Despite extensive efforts to screen for MERS-CoV in 28 197 returning pilgrims in Indonesia in 2015, the study did not identify any positive cases (Amin et al., 2018).

Prof. Dr. Sulianti Saroso Infectious Disease Hospital (IDH) in Indonesia is one of the referral hospitals for the evaluation of MERS-CoV in suspected patients. Up until 2018, none of the suspected patients returning from the Middle East were positive for MERS-CoV, although they were screened with relevant assays. In addition, none of the specimens were further evaluated to determine other etiologies that could tailor and improve patient management and reduce the inappropriate use of antibiotics.

The aim of this study was to identify emerging viral etiologies in suspected MERS-CoV patients admitted to IDH by screening bio-archived respiratory samples accumulated in 2015–2016.

Methods

Ethical approval

The testing of archived specimens was approved by Prof. Dr. Sulianti Saroso IDH (ethical clearance number 54/VII.10/IX/2017) and the Eijkman Institute Research Ethics Commission (number 66, January 2017).

Patients, clinical data, and specimens

Clinical specimens were collected from a total of 13 hospitalized patients with suspected MERS-CoV infection in Prof. Dr. Sulianti Saroso IDH from July 2015 to December 2016. The majority of patients presented with pneumonia and symptoms including fever ($\geq$37.5 °C), dyspnea, and cough. All patients had a history of travel to the Middle East <14 days prior to biological sampling. All biological specimens collected were sent to the bio-repository of Prof. Dr. Sulianti Saroso IDH for future research. The specimens (when available) including sputum, oropharyngeal swab, nasal swab, nasopharyngeal swab, and serum had tested negative for MERS-CoV by viral-specific PCR performed by the Indonesian Ministry of Health. The primers used in the PCR were designed to amplify the protein E, ORF1b, and ORF1a genes of MERS-CoV, as mentioned in the guidelines for MERS-CoV specimen sampling and laboratory examination of the Indonesian Ministry of Health. The specimens that had tested negative for MERS-CoV were further tested for additional respiratory viruses at the Eijkman Institute for Molecular Biology, Jakarta. Demographic data, risk factors, and clinical data were also analyzed.

Nucleic acid extraction and reverse transcription

Total viral RNA was isolated from 140 μl of specimen (serum/respiratory swab) using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Of a total of 60 μl of viral RNA, 4 μl was added to 1 μl random primer for complementary DNA (cDNA) synthesis using the GoScript Reverse Transcription System (Promega, Madison, WI, USA) to develop 20 μl of cDNA. The cDNA was then used as template for the detection of 12 viral families based on conventional PCR.

PCR assays for emerging viruses

Panels of a singleplex PCR assay were used for the detection of 12 taxa of viruses in respiratory specimens, including coronavirus, paramyxovirus, influenza virus, enterovirus, arenavirus, astrovirus, bocavirus, Nipah virus, adenovirus, hantavirus, herpesvirus, and respiratory syncytial virus. In addition, testing of serum for two families of arbovirus – flavivirus and alphavirus – was performed because of their endemicity in Indonesia.

All of the primers and positive controls used in the amplification reaction are based on the protocols from PREDICT USAID (Anthony et al., 2013). The singleplex PCR reaction was performed in a thermal cycler ProFlex PCR system. A recombinant plasmid containing all sequences of PCR target or viral genetic materials from vaccines and isolates was used as a positive control in each PCR.

Gel visualization

Electrophoresis was done with 1% agarose gel in an electrophoresis chamber at 100 V for 30 min. Five microliters of PCR products were added to each agarose well, and a 100-bp DNA ladder (Invitrogen, Carlsbad, CA, USA) was used as a DNA size marker. Visualization of positive bands was performed using the Gel Imaging BioRad Gel Doc XR System and Quantity One 1-D Analysis Software.

Sequencing

Samples with positive viral amplicons were characterized by Sanger sequencing method after purification. Purification of PCR products was performed using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, followed by PCR sequencing and precipitation reaction before loading into an ABI 3130 sequencer. Sequencing results were analyzed using Geneious Software R8 version 8.1 and were compared with sequences in the GenBank database.

Results

During admission, 30 clinical specimens (seven oropharyngeal swabs, 11 nasal swabs, and 12 serum samples) were collected from 13 patients with pneumonia and risk factors associated with MERS-CoV infection, including history of travel to the Middle East <14 days before illness. There was no history of contact with animals or sick people during their visit. Demographic and clinical characteristics of the patients are presented in Table 1. The numbers of male and female patients were similar (male-to-female ratio, 7:6), and the median age of all patients was 61 years.

All patients were diagnosed with pneumonia, confirmed by chest X-ray and categorized in the low-severity pneumonia risk group based on the British Thoracic Society guidelines (NICE, 2019). The duration of hospital stay varied between 2 and 10 days, with an average duration of 5.8 days. The patients were placed in isolation rooms dedicated for airborne infectious diseases. All patients recovered fully.

A total of 18 respiratory clinical specimens that tested negative for MERS-CoV (by the Indonesian Ministry of Health) were retrospectively tested for 12 respiratory panels. In addition, 12 serum samples were tested for alphavirus and flavivirus to exclude the possibility of endemic viral infection. The coronavirus panel did not detect any MERS-CoV in any of the respiratory specimens, confirming the Indonesian Ministry of Health results. In this study, sequences of nine viral pathogens were detected in seven patients: four with single infections and three with multiple infections (Table 2). In patient ID MER004 072015, a co-infection of enterovirus and flavivirus was detected from nasal swab and serum, respectively. Nucleotide comparison of 357 nucleotides of the polyprotein gene using BLAST showed 99% similarity with enterovirus D68; for the flavivirus, 274 bases of the NS5 gene showed 98% similarity with dengue virus type 3. In patient ID MER002 092016, both herpesvirus and coronavirus were detected

Table 1
Demographic characteristics and clinical presentation of suspected MERS-CoV patients in Prof. Dr. Sulianti Saroso Infectious Disease Hospital.

Demographics	Patient ID													Prevalence (Average)
	MER004 072015	MER012 042016	MER001 092016	MER011 122016	MER002 092016	MER009 102015	MER003 042016	MER013 112015	MER006 102015	MER005 102015	MER007 102015	MER008 102015	MER010 092016	
Age, years	32	66	56	60	34	75	65	37	71	59	61	71	61	57.5 Median: 61 (32–75)
Sex	F	M	M	M	F	M	F	F	M	F	F	M	M	M: 7 (53.8%)
Travel history to Middle East[a]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	1 (100%)
Purpose of travel	Umrah	Umrah	Umrah	Umrah	Hajj	Hajj	Umrah	Hajj	Hajj	Hajj	Hajj	Hajj	Hajj	
Date of travel	May 2015	Apr 2016	Sept 2016	Dec 2016	Sept 2016	Nov 2015	Apr 2016	Nov 2015	Sept 2015	Oct 2015	Oct 2015	Oct 2015	Sept 2016	
Days of Hospitalization	6	NA	NA	5	5	8	7	3	5	8	5	10	2	5.8 days
Diagnosis	Pneumonia	Pneumonia	Pneumonia	Pneumonia	Pneumonia	Pneumonia	Pneumonia	Pneumonia	Pneumonia	Pneumonia	Pneumonia	Pneumonia	Pneumonia	
Curb-65 severity score	1	0	0	0	1	1	1	0	1	0	0	1	1	
Outcome	Recovered	Recovered	Recovered	Recovered	Recovered	Recovered	Recovered	Recovered	Recovered	Recovered	Recovered	Recovered	Recovered	
Clinical manifestations and laboratory investigations														
Fever (>37.5 °C)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	13 (100%)
Cough	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	13 (100%)
Chest pain	No	No	No	No	Yes	No	Yes	No	No	Yes	Yes	No	No	4 (30.8%)
Difficulty breathing	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	12 (92.3%)
Ronchi	No	No	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	9 (69.2%)
Wheezing	No	No	No	No	No	No	No	No	No	No	Yes	Yes	No	2 (15.4%)
Respiratory rate (/min)	24	25	24	28	29	28	28	24	24	22	22	27	30	25.8
Chest radiography	Infiltration	Infiltration	Infiltration	Infiltration	Infiltration	Infiltration	Infiltration	Infiltration	Infiltration	Infiltration	Infiltration	Infiltration	Infiltration	
Leukocytes 1st (/l)	4.4	4.9	26.5	25.2	31.2	8.5	20.9	6.2	8.1	NA	7.7	32.8	11.9	15.6917
Leukocytes 2nd (/l)	3.0	NA	NA	12.0	20.7	9.3	20.4	8.3	NA	NA	9.3	32.9	NA	14.4875
Leukocytes 3rd (/μl)	5.1	NA	NA	NA	17.0	7.3	12.3	10.4	NA	NA	19.2	19.1	NA	12.9143
Lymphocytes 1st (%)	41	23	13	13	4	3	13	17	30	NA	13	5	12	15.6
Lymphocytes 2nd (%)	NA	NA	NA	24	14	5	14	NA	NA	NA	19	4	NA	13.3
Antibiotics[b]	Cef, Levo	Cef	Mero	Mero	Mero, Levo	Mero	Cefix	Mero	Azi	Cef	Cef, Levo	Mero, Levo	Levo	

MERS-CoV, Middle East respiratory syndrome coronavirus; F, female; M, male; NA, not available.
[a] History of travel to Middle East <14 days before admission to the hospital.
[b] Cef, ceftriaxone, Levo, levofloxacin, Mero, meropenem, Azi, azithromycin, Cefix, cefixime.

Table 2
Conventional PCR test results for viral agents from suspected MERS-CoV patients.

Patient ID	Specimen	Virus panel												Sequencing results
		Corona virus	Influenza virus	Enterovirus	Para myxovirus	Herpes virus	Henipavirus	Hanta virus	Arena virus	Adenovirus	Astrovirus	RSV	Flavivirus	Alpha virus	
MER004 072015	Oropharyngeal swab	–	–	–	–	–	–	–	–	–	–	–	NA	NA	
	Nasal swab	–	–	+	–	–	–	–	–	–	–	–	NA	NA	Enterovirus D68
	Serum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	–	Dengue 3 virus
MER012 042016	Oropharyngeal swab	–	–	–	–	–	–	–	–	–	–	–	NA	NA	
	Nasal swab	–	–	–	–	–	–	–	–	–	–	–	NA	NA	
	Serum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	
MER001 092016	Nasal swab	–	–	+	–	–	–	–	–	–	–	–	NA	NA	Rhinovirus C
	Serum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	
MER011 122016	Oropharyngeal swab	–	–	–	–	–	–	–	–	–	–	–	NA	NA	
	Nasal swab	–	–	–	–	–	–	–	–	–	–	–	NA	NA	
	Serum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	
MER002 092016	Oropharyngeal swab-1	–	–	–	–	+	–	–	–	–	–	–	NA	NA	Herpes simplex 1 virus
	Oropharyngeal swab-2	+	–	–	–	+	–	–	–	–	–	–	NA	NA	Coronavirus 229E, herpes simplex 1 virus
	Serum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	
MER009 102015	Nasal swab	–	+	+	–	–	–	–	–	–	–	–	–	–	Influenza virus H1N1, rhinovirus A60
MER003 042016	Serum	–	–	–	–	–	–	–	–	–	–	–	–	–	
MER013 112015	Serum	–	–	–	–	–	–	–	–	–	–	–	–	–	
MER006 102015	Nasal swab-1	–	–	–	–	–	–	–	–	–	–	+	NA	NA	Human metapneumovirus
	Nasal swab-2	–	–	–	–	–	–	–	–	–	–	+	NA	NA	Human metapneumovirus
	Serum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	
MER005 102015	Nasal swab	–	–	–	–	–	–	–	–	–	–	–	–	–	
	Serum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	
MER007 102015	Nasal swab	–	+	–	–	–	–	–	–	–	–	–	–	–	Influenza virus H1N1
	Serum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	
MER008 102015	Oropharyngeal swab	–	–	–	–	–	–	–	–	–	–	–	NA	NA	
	Nasal swab	–	–	–	–	–	–	–	–	–	–	–	NA	NA	
	Serum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	
MER010 092016	Oropharyngeal swab	–	+	–	–	–	–	–	–	–	–	–	NA	NA	Influenza virus H3N2
	Nasal swab	–	+	–	–	–	–	–	–	–	–	–	NA	NA	Influenza virus H3N2
	Serum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	

NA, not available.

in the patient's oropharyngeal swab. Herpes simplex virus type 1 (HSV-1) was identified based on sequence matching of 400 bp through the online database (NCBI). Meanwhile, nucleotide comparison of 387 bases of the RNA-dependent RNA polymerase (RdRp) gene obtained from coronavirus showed 100% similarity with human coronavirus 229E (HCoV-229E). For patient ID MER009 102015, amplification of 400 bp untranslated region (UTR) gene of the enterovirus family and 243 bp M-gene region of influenza virus in a nasal swab revealed a rhinovirus A60 and influenza virus H1N1 (similarity 99% and 100%, respectively) co-infection. Single infections with rhinovirus C, influenza virus H3N2, human metapneumovirus (HMPV), and influenza virus H1N1 were identified in the respiratory swabs of patients with ID MER001 092016, MER007 102015, MER006 102015, and MER010 092016, respectively.

Discussion

Although patients with MERS-CoV usually present with an influenza-like illness, asymptomatic infection or cases with mild respiratory symptoms have also been reported (Mackay and Arden, 2017; Ahmed, 2017a). In late infection, most of the MERS-CoV patients have been reported to develop pneumonia. Age, pneumonia, and a history of travel to the Middle East are important markers of MERS-CoV (Mackay and Arden, 2015). Other factors increasing the risk of infection are male sex, contact with a camel or sick patient, diabetes mellitus, severe illness, low white blood cell count, low alanine aminotransferase, and high aspartate aminotransferase (Ahmed, 2017b). The coronavirus family panel used in this study has been validated to detect MERS-CoV (Anthony et al., 2017). However, no MERS-CoV was detected, which could be due to the small sample size. Although the severity of pneumonia of all suspected MERS-CoV patients was categorized as low risk based on the British Thoracic Society guidelines (Mackay and Arden, 2015), they would be categorized as severe pneumonia cases under the Korean guidelines, due to lung infiltrations on chest X-ray and the requirement for oxygen therapy (Park et al., 2018).

A co-infection of enterovirus D68 and dengue virus type 3 was identified in patient ID MER004 072015. She was 32 years old and presented with fever, cough, wheezing, and difficulty breathing. In adults, enterovirus D68 is rarely reported as a cause of severe illness. However, the virus has attracted international attention since it was associated with community-acquired pneumonia in children in China and Italy (Esposito et al., 2015; Bosis and Esposito, 2017; Zhang et al., 2015; Esposito et al., 2016). It is possible that the pneumonia in this case was due to the enterovirus D68 infection; further phylogenetic analysis is needed to compare this strain with other enterovirus D68. In 2014, a large outbreak of enterovirus D68 was confirmed in 49 states in the USA involving at least 1153 cases and 14 deaths. Recent reports have shown a possible association between enterovirus D68 and acute flaccid paralysis (Messacar et al., 2016; Dyda et al., 2018). However, no sign of neurological disorder was observed in this patient.

Patient ID MER002 092016 suffered from pneumonia, sepsis, hypoalbuminemia, and leukocytosis (26.5×10^9/l). This patient was co-infected with HCoV-229E and HSV-1. With the exception of varicella zoster and human herpesvirus 6, human herpesviruses are rarely associated with pneumonitis (Duke and Sagar, 2018; Kuwahara-Ota et al., 2018). However, one case study reported a lower respiratory tract infection caused by HSV-1 in a 72-year-old man with underlying disease (Luginbuehl et al., 2017). HSV-1 is very infectious and highly prevalent in the world, with more than 60% of the population exposed to this virus in 2012 (Looker et al., 2015). It usually causes orolabial ulcers that can lead to neurotrophic complications with lifelong potential for symptomatic or asymptomatic viral shedding episodes (Looker et al., 2015; Kaufman et al., 2005). HCoV-229E is one type of coronavirus commonly infecting humans. An epidemiological study conducted in adults with community-acquired pneumonia confirmed that four human coronaviruses (HCoV-229E, HCoV-NL-63, HCoV-HKU1, and HCoV-OC43) accounted for 0.6–2.5% of pneumonia cases (Yin and Wunderink, 2018). In this case, HCoV-229E was the most likely cause of pneumonia.

In patient MER001 092016, rhinovirus C genetic material was detected from a nasal swab. Rhinoviruses have often been associated with pneumonia in adults. Rhinovirus C was recently discovered; this is not as well characterized as rhinoviruses A and B (Choi et al., 2015). There is controversy regarding the clinical severity of rhinovirus C infection (Choi et al., 2015; Arden et al., 2010). The virus can induce diverse clinical outcomes, with factors including species and type-specific differences as determinants of severity (Yin and Wunderink, 2018; Choi et al., 2015; Arden et al., 2010; Annamalay et al., 2016). Globally, rhinovirus C has been linked to lower respiratory tract infections such as bronchitis, bronchiolitis, and pneumonia, especially when there is co-infection with other viruses, such as bocavirus and influenza virus (Langelier et al., 2017). In Indonesia, data on rhinovirus C are limited, with only one report related to myocarditis (Wiyatno et al., 2018).

Influenza virus H1N1 was detected in patients MER009 102015, MER007 102015, and MER010 092016. In addition, patient MER009 102015 was co-infected with rhinovirus A60. Influenza virus H1N1 is known to cause a serious infection that can lead to the development of pneumonia and death if it progresses to acute respiratory distress syndrome. In the present study, HMPV was detected from a nasal swab of a 71-year-old patient with pneumonia. HMPV is one of the most significant causes of upper and lower respiratory tract infections in children and the elderly, especially the immunocompromised and those with underlying respiratory conditions (Oong et al., 2018; Taniguchi et al., 2019; Jallow et al., 2019). Two genotypes of HMPV have been identified, which can be classified into five lineages, A1, A2a, A2b, B1, and B2. However, their association with disease severity remains unclear (Kumar and Srivastava, 2018). There is only a single report on HMPV from Indonesia, documenting the association of HMPV lineage A1 and A2 with asthma exacerbations and pneumonia, indicating that HMPV should be screened in those with chronic and severe respiratory infections (Prasetyo et al., 2015).

This study, together with that of Amin et al., strengthens the conclusion that Indonesia remained MERS-CoV-free, at least up until 2015–2016. There are some limitations to this study, such as the weakness of statistical power due to the lack of information on the total number of subjects screened and the small number of patients involved in this study. Secondly, although the specimens were mostly obtained during the acute febrile phase, beneficial for molecular screening, serology was not performed for a more comprehensive investigative approach. Thirdly, the detection of viral pathogens in swabs did not imply causality, as additional viral and bacterial panels need to be evaluated.

In conclusion, the sequences of nine viral pathogens were detected in samples from seven suspected MERS-CoV patients hospitalized in Prof. Dr. Sulianti Saroso IDH, Indonesia. Etiological agents detected in the patient samples were enterovirus D68, dengue virus type 3, rhinovirus C, HCoV-229E, HSV-1, influenza virus H1N1, influenza virus H3N2, HMPV, and rhinovirus A60. This study also confirmed that there was no MERS-CoV genetic material detected from these 13 patients. Along with results reported by Amin et al., we confirmed that Indonesia remained MERS-CoV-free in 2015–2016. In conclusion, the early detection and identification of the etiological agent is crucial for the containment of MERS-CoV

in those with a history of travel to the Middle East, and if results are negative, testing should be performed for additional pathogens to improve patient management.

Conflict of interest

No conflict of interest to declare.

Author contributions

TYS, TSH, KSM, VL, and DS were responsible for the initial design of the study. EH and R collected samples from eligible patients and collected clinical data. TYS, AW, and EH, conducted the experiment and data analysis. TSH, R, and AKD performed the data analysis. TYS, AW, and DS wrote the first draft of the paper. All of the authors contributed to the interpretation of the data and reviewed the manuscript for important intellectual content. All authors read and approved the final manuscript.

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References

Ahmed AE. The predictors of 3- and 30-day mortality in 660 MERS-CoV patients. BMC Infect Dis 2017a;17(September) [Cited 23 May 2018]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5594447/.

Ahmed AE. The predictors of 3- and 30-day mortality in 660 MERS-CoV patients. BMC Infect Dis 2017b;17(September) [Cited 23 May 2018]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5594447/.

Amin M, Bakhtiar A, Subarjo M, Aksono E, Widiyanti P, Shimizu K, et al. Screening for Middle East respiratory syndrome coronavirus among febrile Indonesian Hajj pilgrims: A study on 28,197 returning pilgrims. J Infect Prev 2018;19(September (5)):236–9.

Annamalay AA, Jroundi I, Bizzintino J, Khoo S-K, Zhang G, Lehmann D, et al. Rhinovirus C is associated with wheezing and rhinovirus A is associated with pneumonia in hospitalized children in Morocco. J Med Virol 2016;(September).

Anthony SJ, Epstein JH, Murray KA, Navarrete-Macias I, Zambrana-Torrelio CM, Solovyov A, et al. A strategy to estimate unknown viral diversity in mammals. mBio 2013;4(5) [Internet] Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3760253/.

Anthony SJ, Johnson CK, Greig DJ, Kramer S, Che X, Wells H, et al. Global patterns in coronavirus diversity. Virus Evol 2017;3(June (1)) Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5467638/.

Arden KE, Faux CE, O'Neill NT, McErlean P, Nitsche A, Lambert SB, et al. Molecular characterization and distinguishing features of a novel human rhinovirus (HRV) C, HRVC-QCE, detected in children with fever, cough and wheeze during 2003. J Clin Virol 2010;47(March (3)):219–23.

Bosis S, Esposito S. Enterovirus D68-associated community-acquired pneumonia in the pediatric age group. Curr Infect Dis Rep 2017;19(March (3)):12.

Butler D. South Korean MERS outbreak is not a global threat. Nat News 2015;, doi: http://dx.doi.org/10.1038/nature.2015.17709 [Cited 21 April 2017]. Available from: http://www.nature.com/news/south-korean-mers-outbreak-is-not-a-global-threat-1.17709.

Choi S-H, Hong S-B, Kim T, Kim S-H, Huh JW, Do K-H, et al. Clinical and molecular characterization of rhinoviruses A, B, and C in adult patients with pneumonia. J Clin Virol 2015;63(February):70–5.

Chong YP, Song JY, Seo YB, Choi J-P, Shin H-S. Antiviral treatment guidelines for Middle East respiratory syndrome. Infect Chemother 2015;47(September (3)):212–22.

Duke JD, Sagar AS. Varicella pneumonia. QJM Mon J Assoc Phys 2018;111(November (11)):827.

Dyda A, Stelzer-Braid S, Adam D, Chughtai AA, MacIntyre CR. The association between acute flaccid myelitis (AFM) and enterovirus D68 (EV-D68)—what is the evidence for causation?. Eurosurveillance 2018;23(January (3)) Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5792700/.

Esposito S, Zampiero A, Ruggiero L, Madini B, Niesters H, Principi N. Enterovirus D68-associated community-acquired pneumonia in children living in Milan, Italy. J Clin Virol 2015;68(July):94–6.

Esposito S, Zampiero A, Bianchini S, Mori A, Scala A, Tagliabue C, et al. Epidemiology and clinical characteristics of respiratory infections due to adenovirus in children living in Milan, Italy, during 2013 and 2014. PLoS One 2016;11(4) e0152375.

Hui DS, Azhar EI, Kim Y-J, Memish ZA, Oh M-D, Zumla A. Middle East respiratory syndrome coronavirus: risk factors and determinants of primary, household, and nosocomial transmission. Lancet Infect Dis 2018;(April).

Jallow MM, Fall A, Kiori D, Sy S, Goudiaby D, Barry MA, et al. Epidemiological, clinical and genotypic features of human metapneumovirus in patients with influenza-like illness in Senegal, 2012 to 2016. BMC Infect Dis 2019;19(May) Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6532257/.

Kaufman HE, Azcuy AM, Varnell ED, Sloop GD, Thompson HW, Hill JM. HSV-1 DNA in tears and saliva of normal adults. Invest Ophthalmol Vis Sci 2005;46(January (1)):241–7.

Kumar P, Srivastava M. Prophylactic and therapeutic approaches for human metapneumovirus. Virusdisease 2018;29(December (4)):434–44.

Kuwahara-Ota S, Chinen Y, Mizuno Y, Takimoto-Shimomura T, Matsumura-Kimoto Y, Tanba K, et al. Human herpesvirus-6 pneumonitis in a patient with follicular lymphoma following immunochemotherapy with rituximab. Infect Drug Resist 2018;11:701–5.

Langelier C, White CV, Sontag MK, Mourani PM, DeRisi JL. Complete genome sequence of a divergent human rhinovirus C isolate from an infant with severe community-acquired pneumonia in Colorado, USA. Genome Announc 2017;5 (November (48)) [Cited 17 May 2018]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5722057/.

Looker KJ, Magaret AS, May MT, Turner KME, Vickerman P, Gottlieb SL, et al. Global and regional estimates of prevalent and incident herpes simplex virus type 1 infections in 2012. PLoS One 2015;10(10)e0140765.

Luginbuehl M, Imhof A, Klarer A. Herpes simplex type 1 pneumonitis and acute respiratory distress syndrome in a patient with chronic lymphatic leukemia: a case report. J Med Case Rep 2017;11(November) Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5700675/.

Mackay IM, Arden KE. MERS coronavirus: diagnostics, epidemiology and transmission. Virol J 2015;12(December (1)):222.

Mackay IM, Arden KE. An opportunistic pathogen afforded ample opportunities: Middle East respiratory syndrome coronavirus. Viruses 2017;9(December (12)) [Cited 20 April 2018]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5744144/.

Messacar K, Abzug MJ, Dominguez SR. 2014 outbreak of enterovirus D68 in North America. J Med Virol 2016;88(May (5)):739–45.

NICE. Pneumonia overview–NICE pathways [Cited 2019 May 27]. Available from: https://pathways.nice.org.uk/pathways/pneumonia. 2019.

Noorwali AA, Turkistani AM, Asiri SI, Trabulsi FA, Alwafi OM, Alzahrani SH, et al. Descriptive epidemiology and characteristics of confirmed cases of Middle East respiratory syndrome coronavirus infection in the Makkah Region of Saudi Arabia, March to June 2014. Ann Saudi Med 2015;35(June (3)):203–9.

Oong XY, Chook JB, Ng KT, Chow WZ, Chan KG, Hanafi NS, et al. The role of human metapneumovirus genetic diversity and nasopharyngeal viral load on symptom severity in adults. Virol J 2018;15(1):91.

Park WB, Jun KI, Kim G, Choi J-P, Rhee J-Y, Cheon S, et al. Correlation between pneumonia severity and pulmonary complications in Middle East respiratory syndrome. J Korean Med Sci 2018;33(June (24)):e169.

Plipat T, Buathong R, Wacharapluesadee S, Siriarayapon P, Pittayawonganon C, Sangsajja C, et al. Imported case of Middle East respiratory syndrome coronavirus (MERS-CoV) infection from Oman to Thailand, June 2015. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 2017;22(August (33)).

Prasetyo AA, Desyardi MN, Tanamas J, Suradi, Reviono, Harsini, et al. Respiratory viruses and torque teno virus in adults with acute respiratory infections. Intervirology 2015;58(1):57–68.

Premila Devi J, Noraini W, Norhayati R, Chee Kheong C, Badrul AS, Zainah S, et al. Laboratory-confirmed case of Middle East respiratory syndrome coronavirus (MERS-CoV) infection in Malaysia: preparedness and response, April 2014. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 2014;19(May (18)).

Rabaan AA, Alahmed SH, Bazzi AM, Alhani HM. A review of candidate therapies for Middle East respiratory syndrome from a molecular perspective. J Med Microbiol 2017;66(September (9)):1261–74.

Racelis S, de los Reyes VC, Sucaldito MN, Deveraturda I, Roca JB, Tayag E. Contact tracing the first Middle East respiratory syndrome case in the Philippines, February 2015. West Pac Surveill Response J WPSAR 2015;6(September (3)):3–7.

Taniguchi A, Kawada J-I, Go K, Fujishiro N, Hosokawa Y, Maki Y, et al. Comparison of clinical characteristics of human metapneumovirus and respiratory syncytial virus infection in hospitalized young children. Jpn J Infect Dis 2019;(February).

WHO. Middle East respiratory syndrome coronavirus (MERS-CoV). WHO; 2019 [Cited 23 May 2018]. Available from: http://www.who.int/emergencies/mers-cov/en/.

Wiyatno A, Febrianti EZ, Dewantari AK, Myint KS, Safari D, Idris NS. Characterization of rhinovirus C from a 4-year-old boy with acute onset dilated cardiomyopathy in Jakarta, Indonesia. JMM Case Rep 2018;(January).

Yang Y-M, Hsu C-Y, Lai C-C, Yen M-F, Wikramaratna PS, Chen H-H, et al. Impact of comorbidity on fatality rate of patients with middle east respiratory syndrome. Sci Rep 2017;7(September) [Cited 23 May 2018]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5596001/.

Yin Y, Wunderink RG. MERS, SARS and other coronaviruses as causes of pneumonia. Respirology 2018;23(2):130–7.

Zhang T, Ren L, Luo M, Li A, Gong C, Chen M, et al. Enterovirus D68–associated severe pneumonia, China, 2014. Emerg Infect Dis J 2015;21(May (5)) [Cited 17 May 2018]. Available from: https://wwwnc.cdc.gov/eid/article/21/5/15-0036_article.