

Brief communication

Definitive diagnosis in suspected Middle East Respiratory Syndrome Coronavirus cases

Elisa Rubio, Pharm. D^{1,2}, Miguel J. Martínez, PhD^{1,2}, Verónica Gonzalo, RN^{1,2}, Josep Barrachina, RN^{1,2}, Núria Torner, PhD³, Ana I. Martínez, PhD³, Mireia Jané, PhD³, Anna Vilella, PhD^{2,4}, Ana del Rio, PhD⁵, Natalia Rodriguez-Valero, MD^{2,6}, Maria Jesús Pinazo, PhD^{2,6}, José Muñoz, PhD^{2,6}, Alex Soriano, PhD⁵, Antoni Trilla, PhD^{2,4}, Jordi Vila, PhD^{1,2}, and Ma Ángeles Marcos, PhD^{1,2*}

¹Department of Clinical Microbiology, Hospital Clínic, University of Barcelona, Barcelona, Spain, ²ISGlobal Barcelona Institute for Global Health, Barcelona, Spain, ³Agencia de Salud Pública de Cataluña, Ciber de Epidemiología y Salud Pública CIBERESP, Spain, ⁴Public Health Department, Hospital Clínic, University of Barcelona, Barcelona, Spain, ⁵Department of Infectious Diseases, Hospital Clínic, University of Barcelona, IDIBAPS, Barcelona, Spain and ⁶Centre for Research in International Health (CRESIB), Hospital Clínic, University of Barcelona, Barcelona, Spain

*To whom correspondence should be addressed. Email: mmarcos@clinic.cat

Submitted 3 August 2017; Revised 7 November 2017; Editorial decision 12 November 2017; Accepted 16 November 2017

Abstract

We evaluated the microbiological diagnosis in 14 patients with epidemiological and clinical suspicion of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) attended in a non-endemic area between June 2015 and January 2017. While no MERS-CoV was detected, other respiratory viruses were identified in 12 cases and *Mycoplasma pneumoniae* in 1 case.

Key words: MERS-CoV, diagnosis, respiratory agents, multiplex PCR, FilmArray

Background

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a novel coronavirus first described in the Arabian Peninsula in 2012.¹ Humans may become infected through contact with contaminated respiratory secretions from infected patients or animals (mainly dromedaries). Although human-to-human transmission of the virus is low, MERS-CoV has been known to spread from ill people to others through close contact such as caring for or living with an infected person. Thus, transmission of MERS-CoV is more likely to occur in healthcare facilities,² with most of the cases reported having been associated with nosocomial outbreaks.³ As of July 2017, 2040 laboratory-confirmed cases of MERS-CoV have been reported to the World Health Organization. To date, all cases of MERS have been linked to travel or residence in countries in and near the Arabian Peninsula. Moreover, exported cases³ and outbreaks² outside the Arabian Peninsula have also been shown. The clinical syndrome

ranges from asymptomatic and mild respiratory symptoms to severe acute respiratory disease and death, with a case fatality rate of ~35%.^{4,5} To achieve early detection and avoid the transmission of the virus, suspicion of MERS-CoV should be raised whenever there are compatible clinical symptoms (acute febrile respiratory illness) and an epidemiological link (person resides or has travelled within a 14-day period before symptom initiation to the Middle East or any of the countries where MERS-CoV is known to be circulating in dromedary camels or where human infections have recently occurred).⁶ In addition, adequate measures of infection prevention and control should be taken until MERS-CoV infection is ruled out. Initial symptoms of MERS-CoV are non-specific and may resemble those of other respiratory agents. Having an accurate and early microbiological diagnosis without delaying MERS-CoV testing seems reasonable to improve patient management. In this study we evaluated the clinical and epidemiological aspects of the patients with suspicion of MERS-CoV

attended in our centre and the definitive respiratory aetiology identified through a comprehensive panel test.

Methods

We prospectively analysed all consecutive patients attending the Hospital Clinic of Barcelona with clinical and epidemiological suspicion of MERS-CoV according to the Interim patient under investigation guidance from the CDC specifications⁷ and as established by the MERS-CoV suspected case management guidelines⁸ between June 2015 and January 2017. These guidances specify that any patient with an epidemiologic risk and severe or milder respiratory illness should be considered as a patient under investigation for MERS-CoV. According to the protocol established by the Public Health Agency of Catalonia any clinical suspicion of MERS-CoV should be analysed in our hospital, which is the reference centre for MERS-CoV diagnosis in the region of Catalonia (North Eastern Spain) holding a laboratory with biosafety level II and providing rapid results 24 h a day, 7 days a week.

These patients remained in an isolation type III room until laboratory results became available. Respiratory samples (nasopharyngeal swabs and whenever possible a sputum) were collected and sent to the Microbiology laboratory. For the detection of MERS-CoV, RNA extraction of inactivated samples was performed using an automated system (QiaSymphony; Quiagen, Hilden, Germany) followed by real-time reverse-transcription polymerase chain reaction (RT-PCR) targeting upstream of the *npE* gene, encoding protein E, as described previously.^{9,10} Synthetic RNA standards were

used as the positive control. Additionally, all nasopharyngeal swabs samples were tested with a multiplex PCR system (FilmArray Respiratory Panel, BioMérieux, Marcy l'Etoile, France). This is an integrated and automated system which allows the detection of 17 viral agents (influenza viruses A and B, parainfluenza viruses 1–4, respiratory syncytial virus, adenovirus, bocavirus, coronaviruses HKU1, NL63, 229E and OC43, metapneumovirus, human rhinovirus/enterovirus) and 3 bacterial agents (*Bordetella pertussis*, *Chlamydomphila pneumoniae* and *Mycoplasma pneumoniae*) in 1 h. All tests performed in the patients in this article were part of the routine clinical assessment at Hospital Clínic.

Results

A total of 14 patients fulfilled the definition of MERS-CoV suspected case during the study period. The median age of the patients was 30 years (interquartile range: 24–65), seven (50%) were males and seven (50%) were females. All the patients resided in the Middle East except for patient 13 who had been in Singapore when there was an alert for prevention of transmission of MERS-CoV due to the outbreak in the Republic of Korea.¹¹ All patients presented respiratory signs or symptoms. Patients 1–11 presented milder respiratory illness (cough, odynophagia or dyspnoea) and patients 12–14 presented pneumonia based on radiological evidence (Table 1).

Real-time RT-PCR for the detection of MERS-CoV was performed in nasopharyngeal swab samples of all the patients and additionally from a sputum sample in patients 10, 12 and 14.

Table 1. Clinical and epidemiological characteristics of the patients included in the study and the identification of microorganisms using FilmArray Respiratory Panel

Patient	Age	Sex	City or country of origin	Clinical symptoms	Chest X-ray	Identification by FilmArray RP ^a
1	24	M	Dammam (Saudi Arabia)	Chills, headache, myalgia, odynophagia and fever	NAD ^b	Parainfluenza virus 2
2	24	F	Bahrain	Odynophagia, dyspnoea, wheezing and fever	NAD ^b	Rhinovirus
3	20	F	Bahrain	Odynophagia and cough with purulent sputum	Not performed	Rhinovirus and Parainfluenza virus 4
4	65	F	Bahrain	Cough and odynophagia	Not performed	Rhinovirus and Parainfluenza Virus 4
5	68	F	Mecca (Saudi Arabia)	Cough without purulent sputum and fever	Not performed	Rhinovirus Influenza A H3
6	26	M	Al Bahah (Saudi Arabia)	Cough and dysthermia	Not performed	Rhinovirus
7	24	M	Riyadh (Saudi Arabia)	Cough, fever and odynophagia	NAD ^b	Rhinovirus
8	29	F	Jeddah (Saudi Arabia)	Cough and fever	Not performed	Influenza A H3
9	36	F	Riyadh (Saudi Arabia)	Cough and fever	Not performed	Influenza B
10	53	F	Iraq	Cough, fever and myalgia	Not performed	Negative
11	21	F	Kuwait	Cough and fever	Not performed	Influenza B
12	30	M	Kuwait	Non-productive cough, fever and vomiting	Infiltrate in the left lower lobe	<i>Mycoplasma pneumoniae</i>
13	75	M	Singapore	General malaise and disorientation	Infiltrate in the upper right lobe	Respiratory syncytial virus
14	76	M	Arabian countries	Cough, fever	Consolidation in the left lower lobe	Coronavirus NL63

^aRP, respiratory panel.

^bNAD, no abnormality detected.

All the samples tested negative for the detection of MERS-CoV. However, in 13 cases a respiratory pathogen was identified performing FilmArray multiplex PCR of the nasopharyngeal swabs. A respiratory virus was identified as the causative agent in 12 cases and *M. pneumoniae* was identified as the causative agent in 1 case (Table 1).

Discussion

Although MERS-CoV does not seem to have an efficient human-to-human transmission, the high mortality rate of the virus and the fact that most of the cases have been related to healthcare-associated outbreaks reported in the Middle East and also in non-endemic areas from imported cases, makes necessary that potential new imported cases of MERS-CoV are early detected and isolated as soon as possible. Since the symptoms of MERS-CoV are non-specific and can be confused with infections due to other respiratory pathogens, the presence of other respiratory agents should also be investigated in patients with clinical suspicion of MERS-CoV. All the patients in the present study were negative for MERS-CoV, but the additional rapid testing for respiratory pathogens revealed the aetiological agent in most of the cases. This information allowed better patient management with rapid implementation of treatment and prevention and control measures.

Previous studies¹² have shown that lower respiratory tract specimens contain higher viral loads and are more sensitive to virus detection, although upper respiratory tract specimens remain important for diagnosis and are more frequently available.¹³ The fact that all suspected cases were analysed in the reference laboratory underscores the importance of good quality sampling for respiratory tract infections with unusual location for a more sensitive approach.

Our results are in agreement with previous studies evaluating respiratory pathogens among pilgrims returning from an endemic area.^{14,15} However, our group of patients is heterogeneous not restricted to pilgrims and with patients returning from different endemic areas. Our study supports a comprehensive evaluation of respiratory agents in addition to MERS-CoV in patients with epidemiological and clinical suspicion of MERS-CoV infection coming to non-endemic areas.

Conclusions

Patients with respiratory symptoms returning from endemic MERS-CoV areas should be urgently screened for the possible presence of MERS-CoV. Additionally, it is of critical importance to early rule out other possible respiratory agents to avoid their transmission, prevent potential outbreaks and provide adequate patient management.

Sources of funding

None.

Acknowledgments

We thank Biomerieux for providing the kits used in this study.

Conflicts of interest: The authors have declared no conflicts of interest.

Author contributions

Elisa Rubio: Study design, collection of microbiological and clinical data from the included patients and data analysis. Article writing. Miguel J. Martínez, Jordi Vila, Ma Ángeles Marcos: Control and validation of the microbiological procedures. Study design, article supervision and correction. Verónica Gonzalo, Josep Barrachina: Performance of the microbiological procedures. Núria Torner, Ana I Martínez, Mireia Jané, Anna Vilella, Antoni Trilla: Control and communication of the suspected cases. Article supervision and correction. Ana del Rio, Natalia Rodriguez-Valero, Maria Jesús Pinazo, José Muñoz, Alex Soriano: Patient management. Article supervision and correction.

References

- Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* 2012; **367**: 1814–20.
- Cho SY, Kang JM, Ha YE *et al.* MERS-CoV outbreak following a single patient exposure in an emergency room in South Korea: an epidemiological outbreak study. *Lancet* 2016; **388**: 994–1001.
- World Health Organization. *WHO MERS-CoV Global Summary and Risk Assessment*. 2016; WHO/MERS/RA/16.1.
- Al-Gethamu M, Corman V, Hussain R, Al-Tawfiq JA, Drosten C, Memish ZA. A case of long-term excretion and subclinical infection with Middle East respiratory syndrome coronavirus in a healthcare worker. *Clin Infect Dis* 2015; **60**: 973–4.
- Arabi YM, Balkhy HH, Hayden FG *et al.* Special report: Middle East respiratory syndrome. *N Engl J Med* 2017; **376**: 584–94.
- World Health Organization. Surveillance for human infection with Middle East respiratory syndrome coronavirus (MERS-CoV). *Interim Guidance*. 2015. WHO/MERS/SUR/15.1.
- Centers for disease control and prevention. *Interim Patient Under Investigation (PUI) Guidance and Case Definitions* [Internet]. <https://www.cdc.gov/coronavirus/mers/case-def.html#pui> (17 April 2017, date last accessed).
- de Catalunya AdSP. *Síndrome Respiratòria de l'Orient Mitjà Nou Coronavirus (MERS-CoV): Procediment d'actuació davant casos sospitosos produïts pel nou coronavirus*. 2016;1–18. http://canalsalut.gencat.cat/web/content/home_canal_salut/professionals/temes_de_salut/coronavirus/documents/procediment_mers_cov.pdf.
- Corman VM, Eckerle I, Bleicker T *et al.* Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. *Euro Surveill* 2012; **17**: 20285.
- Martinez M, Marcos MA, Gonzalo V *et al.* Lack of detection of Middle East respiratory syndrome coronavirus in mild and severe respiratory infections in Catalonia, northeastern Spain. *New Microbes Hum* 2014; **2**: 27–8.
- Zhang EX, Oh OSH, See W *et al.* Assessment of the risk posed to Singapore by the 2015 Middle East respiratory syndrome outbreak in the Republic of Korea. *West Pac Surveill Response J* 2016; **7**: 17–25.
- Memish ZA, Al-taw JA, Makhdoom HQ *et al.* Respiratory tract samples, viral load, and genome fraction yield in patients with Middle East respiratory syndrome. *J Infect Dis* 2014; **210**: 1590–4.
- Memish ZA, Assiri A, Almasri M *et al.* Prevalence of MERS-CoV nasal carriage and compliance with the Saudi Health Recommendations among pilgrims attending the 2013 Hajj. *J Infect Dis* 2014; **210**: 1067–72.
- Abdulhaq AA, Basode VK, Hashem AM *et al.* Patterns of human respiratory viruses and lack of MERS-coronavirus in patients with acute upper respiratory tract infections in Southwestern province of Saudi Arabia. *Adv Virol* 2017; **2017**: 1–7.
- Gautret P, Charrel R, Belhouchat K *et al.* Lack of nasal carriage of novel corona virus (HCoV-EMC) in French Hajj pilgrims returning from the Hajj 2012, despite a high rate of respiratory symptoms. *Clin Microbiol Infect* 2013; **19**: E315–7. <http://dx.doi.org/10.1111/1469-0691.12174>.