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Short Communication

Conservation of nucleotide sequences for molecular diagnosis of Middle East respiratory syndrome coronavirus, 2015

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SUMMARY

Infection due to the Middle East respiratory syndrome coronavirus (MERS-CoV) is widespread. The present study was performed to assess the protocols used for the molecular diagnosis of MERS-CoV by analyzing the nucleotide sequences of viruses detected between 2012 and 2015, including sequences from the large outbreak in eastern Asia in 2015. Although the diagnostic protocols were established only 2 years ago, mismatches between the sequences of primers/probes and viruses were found for several of the assays. Such mismatches could lead to a lower sensitivity of the assay, thereby leading to false-negative diagnosis. A slight modification in the primer design is suggested. Protocols for the molecular diagnosis of viral infections should be reviewed regularly after they are established, particularly for viruses that pose a great threat to public health such as MERS-CoV.

1. Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV) is an enveloped virus with a positive-sense RNA genome. Infection with the virus causes severe respiratory symptoms in humans, with a case fatality rate as high as 37%.[1] Camels may be a source of infection to humans.[2] Human-to-human transmission is also possible, but this requires close contact, such as health care-related contact without proper measures for infection control and prevention.[3] The earliest case of MERS was reported in Jordan, and MERS-CoV was subsequently isolated from cases in Saudi Arabia only a short time later.[4] Since then, infections have been endemic mainly in the Middle East. However, MERS-CoV has spread sporadically to other areas, including Europe, North America, Africa, and Southeast and East Asia, by travelers from the Middle East.[5]

The laboratory diagnosis of MERS-CoV infection is mainly performed using real-time reverse transcription PCR (RT-PCR) to detect viral RNA in specimens. Interim recommendations from the World Health Organization (WHO) in 2015 for the laboratory testing of MERS-CoV included protocols for RT-PCR that were developed by the University Hospital Bonn and the US Centers for Disease Control and Prevention.[6–9] This document included seven assays: (1) the UpE assay, which is considered highly sensitive and is recommended for screening,[7] (2) the ORF1a assay, which is considered equally as sensitive as the UpE assay,[6] (3) the ORF1b assay, which is considered less sensitive than the ORF1a assay,[6,7] and the (4) N2 and (5) N3 assays, which can complement UpE and ORF1a assays for screening and confirmation.[8,9] To date, these assays have shown no cross-reactivity with other human coronaviruses.[6–8] Sequencing protocols for further confirmation, namely the (6) RdRpSeq and (7) NSeq assays, were also developed.[6]

Because MERS-CoV is an RNA virus that can evolve rapidly, there remains concern that these protocols may not be suitable for the detection of current MERS-CoV because of a mismatch among sequences in the primer/probe regions. This study was performed to analyze recent viral genomic nucleic acid sequences and to discuss the efficacy of the RT-PCR protocols for the molecular diagnosis of MERS-CoV infections.

2. Methods

As of July 29, 2015, sequence data for 386 MERS-CoV were available at 'Virus Variation' (http://www.ncbi.nlm.nih.gov/genome/viruses/variation; GenBank accession numbers of the sequence data analyzed are available upon request). The data

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Table 1
Conservation of the primer and probe region sequences of the WHO-recommended assays for the molecular diagnosis of MERS-CoV

Assay	Regions	Sequence (5′ to 3′)[a]	Conservation[b]
UpE	Forward primer	G 'C' AACGCGCGATTCAGTT	99% (115/116)
	Reverse primer	GCCTCTACACGGGACCCATA	100% (115/115)
	Probe	C 'T' CTTCACATAATCGCCCCGAGCTCG	99% (115/116)
ORF1a	Forward primer	CCACTACTCCCATTTCGTCAG	100% (119/119)
	Reverse primer	CAGTATGTGTAGTGCGCATATAAGCA	100% (119/119)
	Probe	TTGCAAATTGGCTTGCCCCCACT	100% (119/119)
ORF1b	Forward primer	TTCGATGTTGAGGGTGCTCAT	100% (116/116)
	Reverse primer	TCACACCAGTTGAAAATCCTAATTG	100% (115/115)
	Probe	CCCGTAATGCATGTGGCACCAATGT	100% (116/116)
N2	Forward primer	GGCACTGAGGACCCACGTT	100% (127/127)
	Reverse primer	TTG 'C' GACATACCCATAAAAGCA	99% (126/127)
	Probe	CCCCAAATTGCTGAGCTTGCTCCTACA	100% (126/126)
N3	Forward primer	GGGTGTACCTCTTAAT 'G' CCAATTC	95% (125/131)
	Reverse primer	TCT 'G' TCCTGTCTCCGCCAAT	99% (130/131)
	Probe	ACCCC 'T' GCGCAAAATGCTGGG	92% (120/131)
RdRpSeq	Forward primer	TGCTATWAGTGCTAAGAATAGRGC	100% (119/119)
	Reverse primer	GCATWGCNCW 'G' TCACACTTAGG	0% (0/110)
	'Corrected reverse primer'	GCATWGCNCWATCACACTTAGG	100% (120/120)
	Reverse-nested primer	CACTTAGGRTARTCCCAWCCCA	100% (120/120)
NSeq	Forward primer	CCTTCGGTACAGTGGAGCCA	100% (127/127)
	Reverse primer	GATGGG 'G' TT 'G' CCAAACACAAAC	49% (53/108)
	'Modified reverse primer'	GATGGGRTTRCCAAACACAAAC	99% (107/108)
	Forward-nested primer	TGACCCAAAGAATCCCAACTAC	100% (128/128)

WHO, World Health Organization; MERS-CoV, Middle East respiratory syndrome coronavirus.
[a] The position of the mismatched nucleotide is indicated with quotation marks.
[b] In parenthesis: number of sequences perfectly matched/number of sequences available for the region.

included viruses detected between 2012 and 2015, including viral sequences from the big outbreak in eastern Asia in 2015.[1] Data for these 386 sequences, including complete as well as partial genome sequences, were obtained and analyzed. Sequence data were aligned with ClustalW to assess genetic changes in the nucleotide sequences of the primer and probe regions of the assays described above. The numbers of viral sequences that matched the primer/probe sequences perfectly were counted.

3. Results and discussion

As mentioned in the Introduction above, the UpE, ORF1a, N2, and N3 assays can be used for screening because of their high sensitivity.[6–9] Among these, only the primer and probe designs of the ORF1a assay showed 100% conservation of all sequence data available today (Table 1). Minor mismatches were found for the UpE assay (one nucleotide substitution in two sequences) and N2 assay (one nucleotide substitution in one sequence), and significant mismatches were found for the N3 assay. The primer/probe regions were found to be well conserved, except for the N3 assay. In addition, mismatches were not found in the 3′ end region of primers for the UpE and N2 assays (Table 1). The sensitivity of the assays may not be greatly affected. No mismatches were found for the ORF1b assay.

With regard to the sequencing assays, no sequence data that matched the sequence of the reverse primer for the RdRpSeq assay was found. However, a single common mismatch in all sequence data was found. When the mismatched nucleotide was corrected, the RdRpSeq assay matched all the sequence data perfectly ('corrected reverse primer', Table 1). In addition, viral sequences of the reverse primer region for the NSeq assay were not highly conserved; the sequence matched only 49% of strains. Based on these results, the use of a modified reverse primer for the assay is suggested, in order to reduce the possibility of a mismatch ('modified reverse primer', Table 1).

Several mismatches among viral sequences in the primer/probe regions for molecular diagnosis were identified in this study. Such mismatches could lead to a lower sensitivity of the assay, thereby leading to false-negative diagnosis. The mismatched sequence data could have been generated by errors in PCR or sequencing during viral nucleotide sequence analysis because of the incorporation of the wrong nucleotide.[10] However, it is more likely that the RNA virus has evolved and that this has accidentally resulted in the induction of mutation/s in the region targeted by the primer/probe for RT-PCR, only 2 years after the establishment of the protocols. Fortunately, no or few mismatches were found for most of the MERS-CoV screening assays. Nevertheless, protocols for the molecular diagnosis of viral infections should be reviewed regularly after they are established, particularly for viruses that pose a great threat to public health such as MERS-CoV.

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