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American Journal of Infection Control 47 (2019) 290–293

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major Article

Middle East respiratory syndrome coronavirus intermittent positive cases: Implications for infection control

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Key Words:
Middle East respiratory syndrome coronavirus
MERS-CoV
Outbreak Saudi Arabia

Background: Middle East respiratory syndrome coronavirus (MERS-CoV) continues to be reported from the Kingdom of Saudi Arabia. Data on the phenomenon of intermittent positive results for MERS-CoV on reverse-transcription polymerase chain reaction (RT-PCR) with negative results in between are lacking. Here we describe cases with intermittent positive MERS-CoV test results and highlight the required number of tests to rule out or rule in MERS-CoV infection based on a large retrospective cohort of patients with confirmed MERS-CoV.

Methods: This analysis included cases admitted between January 2014 and December 2017. The included patients had a minimum of 3 nasopharyngeal MERS-CoV RT-PCR tests for confirmation and needed 2 negative samples for MERS-CoV evaluated 48 hours apart with clinical improvement or stabilization apart to ensure clearance.

Results: A total of 408 patients with positive MERS-CoV test results were treated at the referring hospital. We excluded 72 patients who had only 1 swab result available in the system and were treated in the initial years of the disease. Of the remaining 336 patients, 300 (89%) had a positive result after 1 swab, 324 (96.5%) had a positive result after 2 consecutive swabs, and 328 (97.6%) had a positive result after 3 consecutive swabs. Of the total cases, 46 (13.7%) had a positive MERS-CoV test then a negative test, followed by positive test results.

Conclusions: Our data indicate that 2 to 3 nasopharyngeal samples are needed to produce the highest yield of positive results for MERS-CoV. In addition, 2 negative results 48 hours apart with clinical improvement or stabilization are needed to clear patients from MERS-CoV. Evaluation of the yield of sputum samples is needed to assess the effectiveness against nasopharyngeal swabs.

Since the emergence of Middle East respiratory syndrome coronavirus (MERS-CoV) in the Kingdom of Saudi Arabia (KSA) in June 2012, a total of 2,229 cases have been reported to the World Health Organization (WHO) from 27 countries, with an overall case fatality rate of 35.6%.[1] Most reported cases of MERS-CoV have been from the Arabian Peninsula, with the majority from the KSA.[2] Outside of the Arabian Peninsula, South Korea had a large MERS-CoV outbreak, with a total of 186 cases and 36 deaths, stemming from an index patient who had returned from a business trip to the KSA, Bahrain, United Arab Emirates, and Qatar.[3-5] Much has been learned about the virus and the disease over the last 5 years, but some knowledge gaps remain in disease pathogenesis, transmission, diagnostics, and the best infection control measures to prevent disease acquisition and transmission.

In diagnostics, real-time reverse-transcription polymerase chain reaction (RT-PCR) is considered the gold standard test for diagnosing MERS-CoV. Viral cultures are not recommended for routine testing, because cultures require a biosafety level 3 facility, and final results are not available for 2-3 days. Published data show that lower respiratory tract sampling gives the highest yield, followed by sputum and

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Conflicts of interest: None to report.

0196-6553/
https://doi.org/10.1016/j.ajic.2018.08.020

nasopharyngeal swabs.[6-9] In addition, lower respiratory samples have highest MERS-CoV viral loads.[6,10-14] The number of samples required for confirmation of diagnosis or clearance from positivity has not been clearly defined, and the pattern of positivity of repeat sampling has not been looked at systematically.

In an attempt to learn from the large cohort of patients cared for at Prince Mohammed Bin Abdulaziz Hospital (PMAH), a corona reference center for the central region of the KSA based in Riyadh we performed a critical review of the yield of PCR results in diagnosis and clearance, and here we report the results.

METHODS

We included all patients who tested positive and who underwent more than 1 MERS-CoV test of respiratory tract samples. PMAH policy calls for a minimum of 2 samples obtained 48 hours apart for suspected MERS-CoV cases; more samples can be obtained in the event of very high suspicion, at the treating physician's discretion. However, the attending physician can override this policy if he or she deems that more testing is needed. Clearing a negative MERS-CoV case requires a minimum of 2 negative samples obtained 48 hours apart with clinical improvement or stabilization. Some patients underwent repeat testing at the discretion of the treating clinicians, and the testing was nonsystematic. MERS-CoV tests were done on either Cobon-flocked nasopharyngeal swabs or sputum samples.

The respiratory samples were tested using RT-PCR amplification targeting the upstream E protein gene (*upE*) and *ORF1a* for confirmation, as described previously.[8,15] A probable case was defined as a patient testing positive for 1 of the genes who underwent no further testing but had a history of potential exposure and consistent clinical signs and symptoms. A confirmed case was defined as a patient testing positive for the 2 genes. Early in the course of MERS-CoV, all samples were analyzed at the Riyadh regional laboratory, but starting in 2015, after a period of validation, samples were tested at the PMAH laboratory to expedite the reporting process. Obtaining the results takes 6-8 hours; usually all samples are run first thing in the morning, but samples can be run any time during the day or night depending on urgency. We included patients who had intermittently positive MERS-CoV test results after an initial negative test. We considered a case negative if 2 or more consecutive samples were negative by RT-PCR.

RESULTS

During the study period from January 2014 to December 2017, a total of 408 patients positive for MERS-CoV were treated at PMAH. We excluded 72 patients who had only 1 swab result available, because these patients were treated during the initial years of the disease. Of the remaining 336 patients, 300 (89%) had a positive result after 1 swab, 324 (96.5%) had a positive result after 2 consecutive swabs, and 328 (97.6%) had a positive result after 3 consecutive swabs (Fig 1). The majority of samples (70%) were nasopharyngeal samples, but in critically ill and intubated patients, most samples were tracheal aspirates, with only a few sputum samples collected. A total of 1,745 tests were done for all the patients, of which 967 (55.4%) were positive, 662 (38%) were negative, and 116 (6.64%) were probable. Of the total patients, 46 (13.7%) had a positive MERS-CoV test results, then a negative test result, followed by positive test results (Fig 2). Of those patients, 8 (19%) were health care workers, 17 (40.5%) were primary cases, and 10 (23%) died. All patients were symptomatic, and 72% had evidence of pneumonia on chest radiography. Seventeen patients (40.5%) did not receive steroid therapy, and 10 patients (23%) received ribavirin/interferon. Thus, it was not possible to correlate the effect of any medications with the intermittent positive samples.

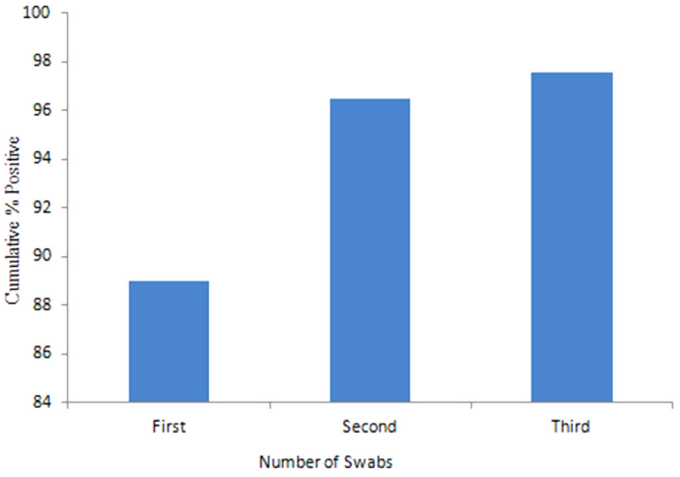

Fig 1. Bar graph showing the cumulative increase in the positivity rate of nasopharyngeal swabs with an increasing number of swabs (first, second, and third swabs).

DISCUSSION

RT-PCR became the standard test for diagnosing MERS-CoV immediately after the emergence of MERS-CoV in the KSA in September 2012, with the WHO recommendation of a standardized test that can be used worldwide. Despite the great value of molecular testing, several concerns were raised early in its application. The poor reliability of upper respiratory tract samples (ie, nasopharyngeal and oropharyngeal) necessitates deep sampling from the lower respiratory tract (sputum and tracheal aspirates).[6,8] Lower respiratory tract specimens, such as tracheal aspirates and sputum, have been found to be more reliable for detecting MERS-CoV including viral loads, and throat swabs are considered a useful alternative.[10]

Repeat sampling is needed to confirm the diagnosis in patients with high suspicion of MERS-CoV in the face of negative initial test results. The significance of positive RT-PCR (viral shedding) as it relates to infectivity is unclear, because most positive patients have positive results for up to 6 weeks. The required number of negative results to clear a positive patient is not known, given that RT-PCR can alternate between positive and negative results before it becomes negative.

We attempted to evaluate 2 of the questions: 1) The number of samples required for confirmation of diagnosis or 2) number of samples required for clearance from positivity, by reviewing our database of all patients with MERS-CoV managed at our institution, which serves as a reference center for MERS-CoV for the central region of the KSA. Specially assigned staff have been trained in nasopharyngeal sampling for MERS-CoV, and these are the only staff allowed to sample patients for MERS-CoV. Our results demonstrate the need for a minimum of 2 samples to confirm MERS-CoV, and that a third sample will increase the yield by only 1% (from 96.5% to 97.6%). Concerns about the infectivity of RT-PCR–positive patients[16,17] have been confirmed by a recent report of a positive MERS-CoV culture from the upper respiratory tract of an asymptomatic positive case from KSA obtained at 15 days after illness onset.[18] There is an urgent need to verify how many negative results are needed to confirm negativity. In our series, only 30% of patients had negative-positive-negative results necessitating confirmation of negativity. The KSA Ministry of Health recommends that "two negative lower respiratory samples 24 hours apart are required for ventilated patients and one negative respiratory sample in other patients including home-isolated individuals."[19] We concur with the recent WHO guideline recommending 2 MERS-CoV –negative samples obtained 1 week apart to ensure clearance.[20] This is particularly important because most MERS-CoV cases are linked to hospital transmission.[2]

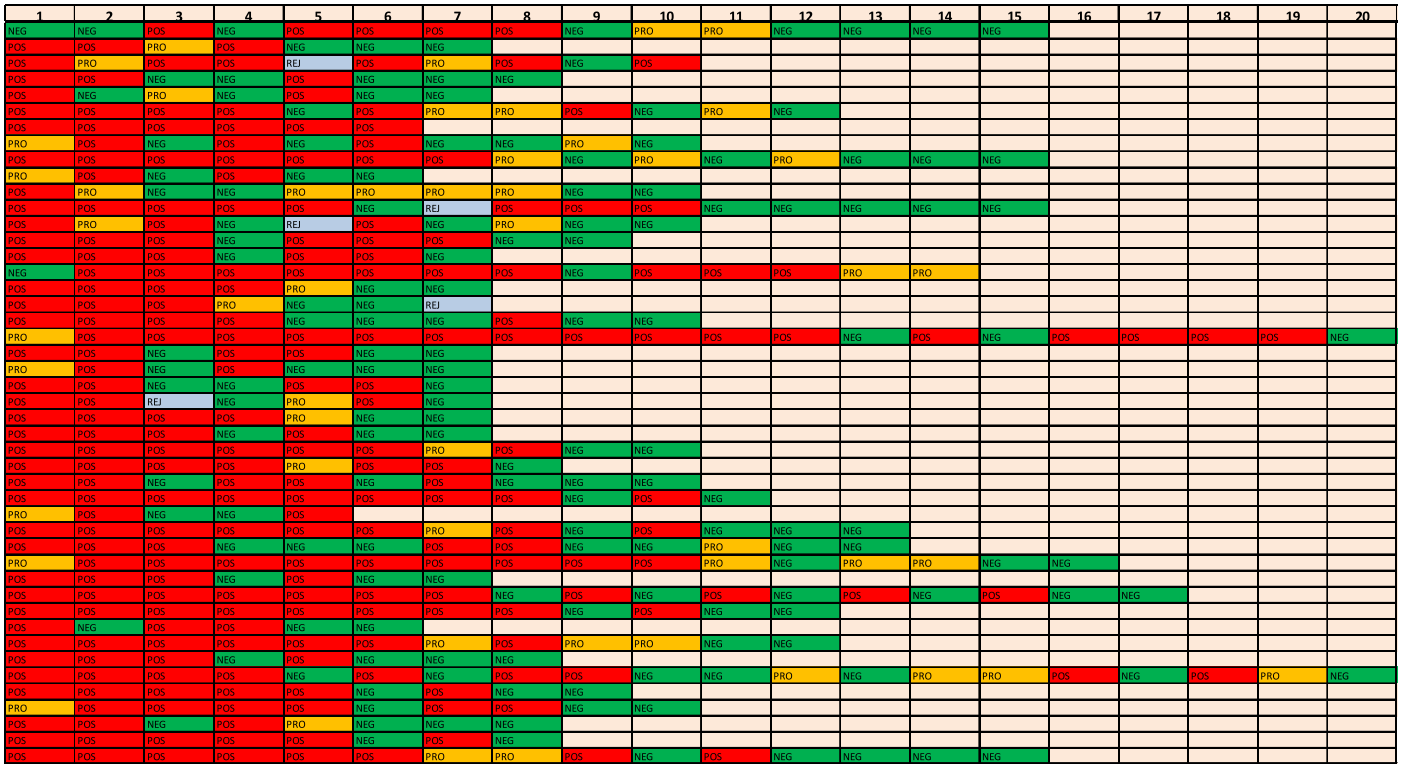

Fig 2. Graph showing intermittent positive samples (red) and the occurrence of probable tests (yellow), REJ (light blue) refers to rejected samples by the lab and the negative tests (green). Individual patients are shown on the vertical axis, and the number of swabs is shown on the horizontal axis. The abbreviations in the graph refer to the result of the RT-PCR test for MERS-CoV, as follows: POS, positive; PRO, probable; NEG, negative.

It is unfortunate that some alternative MERS-CoV testing methodologies, such as serology, have proved to be less reliable, whereas others, such as rapid point-of-care testing, have not yet been thoroughly investigated. Rapid, sensitive, and specific point-of-care tests have been reported but have yet to be validated in large samples in KSA.[21-23] A significant limitation of our study is the lack of comparative data on the value of lower respiratory tract vs upper respiratory tract sampling to confirm what other investigators have shown.[6,7]

CONCLUSIONS

In conclusion, our data indicate that 2 or 3 nasopharyngeal samples are required to ensure the highest yield of positive results for MERS-CoV. In addition, 2 negative results 48 hours apart with clinical improvement or stabilization are needed to clear patients of MERS-CoV. Evaluation of the yield of sputum samples is needed to assess the effectiveness of this approach compared with using nasopharyngeal swabs.

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