Extensive viable Middle East respiratory syndrome (MERS) coronavirus contamination in air and surrounding environment in MERS outbreak units

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40-word summary

The presence of MERS-CoV was confirmed by RT-PCR from viral cultures of 4 out of 7 air samples and 15 out of 68 surface swab samples from three MERS patients’ rooms, calling for epidemiologic investigation for contact and airborne transmission.
Abstract

**Background:** The largest outbreak of Middle East respiratory syndrome (MERS) outside the Middle East occurred in South Korea in 2015 and resulted in 186 laboratory-confirmed infections, including 36 (19%) deaths. Some hospitals were considered epicenters of infection and voluntarily shut down most of their operations after nearly half of all transmissions occurred in hospital settings. However, the ways that MERS-coronavirus (MERS-CoV) is transmitted in healthcare settings are not well defined.

**Methods:** We explored the possible contribution of contaminated hospital air and surfaces to MERS transmission by collecting air and swabbing environmental surfaces in two hospitals treating MERS-CoV patients. The samples were tested by viral culture with reverse-transcriptase polymerase chain reaction (RT-PCR) and immunofluorescence assay (IFA) using MERS-CoV Spike antibody, and electron microscopy (EM).

**Results:** The presence of MERS-CoV was confirmed by RT-PCR of viral cultures of four out of seven air samples from two patients’ rooms, one patient’s restroom, and one common corridor. In addition, MERS-CoV was detected in 15 of 68 surface swabs by viral cultures. IFA on the cultures of the air and swab samples revealed the presence of MERS-CoV. EM images also revealed intact particles of MERS-CoV in viral cultures of
the air and swab samples.

Conclusions: These data provide experimental evidence for extensive viable MERS-CoV contamination of the air and surrounding materials in MERS outbreak units. Thus our findings call for epidemiologic investigation of the possible scenarios for contact and airborne transmission, and raise concern regarding the adequacy of current infection control procedures.
Introduction

Many factors are thought to have contributed to the large outbreak of Middle East Respiratory Syndrome (MERS) in South Korea in 2015: the unfamiliarity of physicians with MERS-CoV, sub-optimal infection control measures in some hospitals, overcrowding in emergency rooms, patients occupying rooms with many beds, the habit of seeking medical advice from multiple health care facilities, and visits to hospitalized patients by friends and family members [1]. Added to these was the initial failure to trace contacts. Although the transmission routes of MERS are not completely understood [2], Centers for Diseases Control and Prevention (CDC) guidelines define close contact as being within 6 feet of an infected patient or within the room or care area of such a patient for a long time [3]. The Korea Centers for Diseases Control and Prevention (KCDC) initially quarantined and followed-up, by personal interview or close-circuit television review, only those who had been in close contact (6 feet) with the index patient or had shared the same room. However, many patients and guardians became infected and that were later recognized to have been more than 6 feet away from the index patient, though in the same ward [4]. Eventually, the 4 hospital outbreak clusters (91, 36, 14, 11 cases, respectively) accounted for 82% of all the cases that occurred [5, 6].
Therefore, identifying the possible transmission routes for the distant contacts of index patients is important for our ability to reduce the spread of MERS. The distant transmission could be explained by two scenarios; (i) contaminated environmental surfaces including fomites and/or (ii) airborne transmission. A previous study revealed that most of the accessible surfaces in MERS units were contaminated by MERS-CoV, as by reverse transcriptase-polymerase chain reaction (RT-PCR) and culture [7]. Although viral RNA was detected on inaccessible surfaces such as the entrance of air-ventilating equipment [7], the authors did not test air samples. Therefore, we have investigated whether contamination of the air or of accessible/inaccessible surfaces in three MERS-CoV-infected patient rooms could explain the transmission of MERS-CoV.

Methods

1. Study sites and patients data

On 1 July 2015, during the 2015 MERS outbreaks in South Korea, air and environmental samples were collected from two MERS-CoV-infected patients in Hospital A and one in Hospital B. The MERS-designated wards in Hospital A had been newly constructed in 2012 and were specially designed for highly pathogenic respiratory viral pathogens. Each negative-pressure room had an anteroom (Figure 1A).
In Hospital A, one room was occupied by Patient 1, a 69-year-old male with pneumonia who received mechanical ventilation and extracorporeal membrane oxygenation on day 22 from the onset of symptoms (Figure 1B) and whose respiratory specimens (tracheal aspirates) persistently tested positive for MERS-CoV by RT-PCR up to the time of environmental sampling. The other room in Hospital A was occupied by Patient 2, a 54-year-old male with pneumonia who received mechanical ventilation on day 16 from the onset of symptoms (Figure 1C); his respiratory specimens (tracheal aspirates) also persistently tested positive for MERS-CoV by RT-PCR up to the time of environmental sampling. The MERS-designated wards in Hospital B were switched to isolation wards during MERS outbreaks. The rooms lacked anterooms, had portable negative-pressure devices (Cleanroom H13, IQAir, Goldach, Switzerland), and shared a common corridor (Figure 2A). One room was occupied by Patient 3, a 74-year-old male with pneumonia who was not using any mechanical ventilator on day 19 from the onset of symptoms, his respiratory specimens (sputum samples) tested positive for MERS-CoV by RT-PCR 6 days before the time of environmental sampling. At the time of environmental sampling, on day 19, his respiratory symptoms persisted but further respiratory samples were not taken because the attending physician thought that continued positive results from his respiratory specimens would not alter any clinical...
decisions about management and isolation in this resource-limited hospital. The patient was bed-ridden and had not used the restroom (Figure 2B). Environmental sampling of the rooms occupied by Patient 1 and 2 was performed 6 to 7 hours after the daily routine cleaning, and environmental sampling of Patient 3 was performed 3 to 4 hours after the daily routine cleaning.

2. Sample collection

Air was sampled using an MD8 airscan sampling device (Sartorius, Goettingen, Germany) and sterile gelatin filters (80-mm diameter and 3-μm pores, Sartorius, Goettingen, Germany). Air was sampled twice at a speed of 50 liters/min for 20 min in the negative-pressure room and its associated restrooms. The filters were dissolved aseptically in 30 mL viral transport medium (sterile phosphate buffer with 10% fetal calf serum, 10,000 U/mL penicillin, 10 mg streptomycin, 25 μg amphotericin-B) and stored at -80°C until analyzed.

Dacron swabs premoistened with viral transport medium were used to swab surfaces aseptically. The following types of surface were swabbed: (1) fixed structures in the elevators (i.e., buttons, guardrails, and doors), (2) fomites (i.e., stethoscopes, ambu bags, blood pressure cuffs, nasal prongs, pillows, and keyboards), (3) fixed structures
in the rooms and its associated restrooms (i.e., door knobs, bed guardrails, toilet seats, and hand soap dispensers), and (4) the ventilation exits on the restroom ceiling and the ventilation exits on the ceilings and walls of the negative-pressure rooms. All the environmental samples were collected after daily cleaning and disinfection of the rooms. Surface swabbing was focused especially on surfaces such as ventilator exits and the tops of television sets, which are easily missed by daily cleaning.

3. Laboratory procedures

The MERS-CoV-Korea- isolate MERS-CoV/KOR/KNIH/002_05_2015 (accession number: KT917527) for use as a positive control was kindly provide by Dr. Sung Soon Kim, Division of Respiratory Viruses, Korea National Institute of Health. Vero cells (ATCC CCL-81) were grown in T-75 flasks, inoculated with MERS-CoV, and incubated at 37°C in a CO₂ incubator. Three days after inoculation, the MERS-CoV infected Vero cells were harvested.

The detailed procedure for RT-PCR and sequencing of environmental samples are described in the online Supplemental Material. Air and surface swab samples were filtered through 0.1-µm pore syringe filter units (Pall, New York, USA) to minimize bacterial contamination. Vero E6 (ATCC, CRL-1586) cells were incubated with the
filtered samples in Dulbecco’s minimal essential medium (Welgen, Korea) supplemented with 100 IU/mL penicillin and 100 μg/mL streptomycin at 37°C in CO₂ incubator, and checked daily for cytopathic changes. Fourteen days after inoculation, culture supernatants and lysates of Vero E6 cells were harvested and used for detecting MERS-CoV by RT-PCR. The harvested cells were centrifuged for 3 min at 1,000 rpm to remove cellular debris. The pellets were resuspended in washing buffer (0.1 M phosphate buffer) and centrifuged for 5 min at 3,000 rpm. After thoroughly removing washing buffer, the cells were fixed with 2.5% glutaraldehyde at 4°C overnight and photographed with a transmission electron microscope (JEOL model GEM-1400, Tokyo, Japan). The same culture supernatants were used to infect Vero cells for immunofluorescence analysis. Immunofluorescence antibody test was conducted at the tissue culture cells on 2 dpi for MERS-CoV/KOR/KNIH/002_05_2015 and 7 dpi for environmental samples, respectively. Anti-MERS-CoV Spike antibody was purchased from Sino Biological Inc. (Beijing, China). All images were acquired using the Operetta High-Content Imaging System (Perkin Elmer, USA) at 20 × magnification.

All experiments were done at the Institut Pasteur Korea in compliance with the guidelines of the Korea National Institute of Health using enhanced BSL-3 containment procedures in laboratories approved for use by the Korea Centers for Disease Control and
Results

1. RT-PCR procedure

The RT-PCR procedure was optimized using the control MERS-CoV-Korea-isolate (accession number: KT917527). Primers specific for the spike gene (nt 22300-22628) were used for RT-PCR (Supplemental Figure 1A, black arrow). Using the optimized RT-PCR procedure, a single DNA band of the expected size (328 bp) was detected from Vero cells infected with the MERS-CoV-Korea-isolate (Supplemental Figure 1B). Sequencing of the amplification product confirmed the expected sequence.

2. Air samples

A summary of the patient case status and environmental test results in the two MERS-designated hospitals is given in Table 1. To examine the possibility of airborne transmission of MERS-CoV, air samples collected at Hospitals A and B were subjected to RT-PCR. All were positive for MERS-CoV (Table 1 and Supplemental Table 1).

Next, the presence of viable MERS-CoV was tested by viral culture. MERS-CoV was cultured in Vero E6 cells from four of the seven air samples: from two of the patients’
rooms, one patient’s restroom, and one common corridor (Figures 1B, 1C, 2A, 2B, Supplemental Figure 2B, Table 1, and Supplemental Table 1). RT-PCR results of viral cultures and subsequent sequencing confirmed RT-PCR amplification of the MERS-CoV spike gene in 14-day culture material from all four air samples (Table 1, Supplemental Table 1, and Supplemental Figure 2). In addition, high-resolution EM images of the cultured virus revealed intact virus particles compatible with MERS-CoV (Supplemental Figure 3A) and immunofluorescence assay using MERS-CoV Spike antibody on the tissue cultures revealed green granular intracytoplasmic reaction (Supplemental Figure 3B).

3. Surface swab samples

Of the 68 swab samples collected at Hospitals A and B, 42 samples tested positive for MERS-CoV by RT-PCR and sequencing (Table 1 and Supplemental Table 1). In particular, 13 of 16 fomite swabs and 29 of 52 fixed structure swabs were positive. Furthermore, MERS-CoV was cultured from 15 swabs comprising seven of 16 swabs from fomites including a stethoscope, and eight of 52 swabs obtained from fixed structures including doorknobs and bed guardrails (Figures 1B, 1C, 2B, Table 1, and Supplemental Table 1). Interestingly, one of five swabs obtained from the air exhaust
damper and one of ten swabs obtained from elevators were positive for MERS-CoV, as
determined by viral culture (Table 1, Supplemental Table 1, and Supplemental Figure
2). In addition, EM images of the cultured virus from swab samples revealed intact
virus particles compatible with MERS-CoV (data are not shown) and
immunofluorescence assay using MERS-CoV Spike antibody on the tissue cultures
revealed green granular intracytoplasmic reaction (Supplemental Figure 3B).

Discussion

Our data demonstrate the presence of MERS-CoV in the hospital environment including
air, fomites, and environmental surfaces, although they do not provide direct insight into
the routes of transmission. However, the presence of MERS-CoV on the environmental
surfaces and in the air of a MERS-CoV-infected patient’s room that was routinely
disinfecteda by standard procedures suggests that MERS-CoV can be transmitted via
contact and aerosols. Therefore, our findings provide important evidence for the possible
routes for distant transmission of MERS-CoV. In addition, our findings call for
epidemiologic investigation of the possible scenarios for remote transmission, and raised
concern regarding the adequacy of current infection control procedures.

As mentioned above, transmission beyond 6 feet from the index patient could be
explained by two scenarios; (i) contaminated formites or environmental structures and/or (ii) airborne transmission. It has been proposed that the SARS outbreak in the Hong Kong M Hotel was due to contaminated environmental surfaces [8], and that those in Amoy Garden in Hong Kong, in airplanes, and in the Prince of Wales Hospital in Hong Kong were due to airborne transmission [9-11]. Roy and Milton have suggested that SARS-CoV is not transmitted by either droplet transmission or airborne transmission by a process lying somewhere between two [12]. They proposed that the transmission mode of SARS-CoV would lie somewhere between droplet transmission and airborne transmission [12]. Given that we found that air and surrounding surfaces including accessible and inaccessible areas were all contaminated by MERS-CoV, we agree that the transmission mode of MERS-CoV differs from droplet transmission. Further studies are needed to clarify this question.

There is evidence supporting aerosol transmission of MERS. Experimental aerosolization of MERS-CoV did not decrease its stability at 20°C and 40% relative humidity [13] and MERS-CoV RNA was detected in an air sample from the barn of an infected camel that transmitted MERS-CoV to a patient who died [14]. In addition, a recent study demonstrated that the human lung parenchyma has abundant dipeptidyl peptidase 4 (DPP4) receptors for MERS-CoV while the human nasal mucosa has few
DPP4 receptors for MERS-CoV [15]. Hence, we assume that aerosol transmission of
MERS-CoV is possible, and that an aerosol with a high concentration of infectious
particles, and contamination of the surrounding environment, might mimic that expected
of large-droplet sprays and surface contact [12]. We therefore cautiously recommend that
contact tracing and infection-control precautions equivalent to those involved in cases of
airborne transmission are needed in hospitals where patients who are severely ill with
MERS stay. It is worth noting that many environmental swab samples contained MERS-
CoV despite daily cleaning and disinfection of the patients’ rooms. These findings are
consistent with previous studies that demonstrated survival of MERS-CoV for 2 days on
plastic and steel surfaces [13], survival of SARS-CoV for 3 days on various surfaces [16],
and survival of hCoV for 6 days in air [17]. Therefore, the extensive environmental
contaminations and prolonged environmental presence of MERS-CoV may partially
explain why MERS is easily spread in health-care setting. In addition, our data emphasize
the importance of strict adherence to infection-control precautions, including hand
hygiene.

An independent research group in South Korea has isolated viruses from swab samples
in the environment of MERS-CoV-infected patients and demonstrated that most
accessible surfaces in MERS units were contaminated by MERS-CoV, confirmed by RT-
PCR and viral culture [7]. On the other hand, a group in China conducted environmental sampling of the room of a MERS-infected patient, on days 13 and 15 post-symptom onset, and the swabs were negative results for viral RNA [18]. Our study results partially overlap with those of the study of Bin et al. [7], who performed environmental sampling of the 4 rooms of 4 MERS-infected patients between days 18 and 30 post-symptom onset [7]. However, the positive rates of RT-PCR and viral culture in the previous study [9] were 20% (30/148) and 4% (6/148), respectively, whereas those in the present study were 65% (49/75) and 25% (19/75), respectively. Caution is needed on comparing the results of our study to those of the previous study [7], because ours was more focused on air-borne transmission. Actually, Bin et al. showed that MERS-CoV was detected by RT-PCR but not by viral culture on the entrance to air-ventilating equipment in one patient’s room, and they suggested the existence of air-borne-virus particles. However, they did not perform air sampling. We tested air samples and swabbed surfaces such as the ventilator exit and the top of televisions, which were inaccessible, and areas remote from the patients as well as areas easily missed by daily cleaning. So, it is possible that MERS-CoV particles could have been concentrated in exhaust air grilles and the corners of rooms that were not routinely disinfected.
This study has a few limitations. First, it was performed late in the Korean MERS outbreak. The three patients included in the study were at similar stages of disease progression, between 16 and 22 days after symptom onset, and patients at a stage, less than 1 week after symptom onset, were not included. In addition, it would be interesting to examine the environmental contamination surrounding less severely ill patients.

Second, some may question the isolation of viable virus from the surroundings of Patient 3 in whom the last positive RT-PCR for MERS-CoV was in a respiratory specimen taken 6 days prior to the date of the environmental sampling. Actually, since a respiratory sample was not taken at the time of the environmental sampling, we cannot know whether the results of RT-PCR of a respiratory specimen would have been positive or not. However, in the previous study [7], the patient’s room and medical equipment were positive for virus up to 5 days after the patient’s last positive PCR for a respiratory specimen. We thus assume that virus can still be detected several days after negative PCR conversion of respiratory specimens. Hence, the absence of results for a respiratory specimen from Patient 3 at the time of environmental sampling does not significantly affect the interpretation of our results. Finally, the experimental data indicating extensive surface and air contamination only provide some insight into the possible routes of transmission; they do not fully identify the route(s) of transmission. Further
epidemiologic and experimental studies are urgently needed in this area.

In conclusion, these data provide experimental confirmation for extensive viral contamination of the air and materials surrounding patients with MERS, pointing to the possibility of airborne and contact transmission during 2015 MERS outbreaks in Korea. Our demonstration that MERS-CoV can be shed into the air and surface environment will no doubt guide the response to future MERS outbreaks.

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Potential conflict of interest:

There are no potential conflicts of interest for any authors.
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Figure 1. Floor plan of the well-equipped MERS-designated hospital where each negative-pressure room had an anteroom and postroom (A). The nimrod (x) and slash (/) indicates the air supply and air exhaust, respectively. Patient 1 was a 69-year-old male with pneumonia who received mechanical ventilation and ECMO on day 22 from the onset of symptoms. The results of viral culture of air and swabs from Patient 1’s room are depicted (B). Patient 2 was a 54-year-old male with pneumonia who received mechanical ventilation on day 16 from the onset of symptoms. The results of viral cultures of air and swabs from Patient 2’s room are depicted (C). The solid blue lines radiating from the large blue ovals indicate the angles of observation used for drawing the cartoons of the patient’s room.

Figure 2. Floor plan of the MERS-designated hospital that was switched to isolation wards in the MERS outbreaks where each room had a portable negative-pressure device and no anteroom and shared a common corridor (A). The nimrod (x) and slash (/) indicates air supply and air exhaust, respectively. Patient 3 was a 54-year-old male with pneumonia who received mechanical ventilation on day 19 from the onset of symptoms. The results of viral culture of air and swabs from Patient 3’s room are depicted (B). The solid blue lines radiating from the large blue oval indicate the angle of observation used to draw the cartoon of the patient’s room.
Table 1. Summary of patient case status and environmental test results in two MERS-designated hospitals

<table>
<thead>
<tr>
<th>Hospital</th>
<th>No</th>
<th>Case status</th>
<th>Time of sampling for PCR (days post-symptom onset)</th>
<th>MERS-CoV PCR results</th>
<th>Environmental data</th>
<th>RT-PCR from samples</th>
<th>RT-PCR from viral culture</th>
</tr>
</thead>
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<tr>
<td></td>
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<td></td>
<td></td>
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<td>Environmental</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sampling</td>
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</tr>
<tr>
<td>A</td>
<td>1</td>
<td>Pneumonia on mechanical</td>
<td>22</td>
<td>(+) at the time of</td>
<td>Air samplingb</td>
<td>2/2</td>
<td>1/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ventilation and ECMO</td>
<td></td>
<td>sampling</td>
<td>Fomites swab</td>
<td>4/6</td>
<td>2/6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fixed structure swab</td>
<td>7/13</td>
<td>2/13</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Pneumonia on mechanical</td>
<td>16</td>
<td>(+) at the time of</td>
<td>Air samplingb</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ventilation</td>
<td></td>
<td>sampling</td>
<td>Fomites swab</td>
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<td>3/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elevator</td>
<td></td>
<td></td>
<td>Fixed structure swab</td>
<td>12/12</td>
<td>5/12</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>Pneumonia and bedridden</td>
<td>19</td>
<td>(-) at the time of</td>
<td>Air samplingd</td>
<td>3/3c</td>
<td>1/3</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sampling</td>
<td>Fomites swab</td>
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<td>2/6</td>
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<td></td>
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<td>Fixed structure swab</td>
<td>8/17</td>
<td>0/17</td>
</tr>
</tbody>
</table>

Note: Data are number of samples with a positive test result/number of samples tested, unless otherwise indicated.
Abbreviations: ECMO, extracorporeal membrane oxygenation; (+), positive result; (-), negative result

*aHospital A was a well-equipped MERS-designated hospital specially designed for highly pathogenic respiratory virus pathogens.*
Air samples were obtained from each patient’s room and its affiliated restroom.

Hospital B was a general hospital with wards that were switched to isolation wards in the MERS outbreaks.

Three air samples were obtained from the patient’s room, its affiliated restroom, and the corridor as a common anteroom.
Positive from viral culture
Negative from viral culture

Fomites not depicted in the Figure

- Restrain band
- Ambu bag, blood pressure cuff, stethoscope, hand washer tip, fire extinguisher
- Fixed structure not depicted in the Figure
- Upper part of television
- Door knob in the restroom, call button, the patient’s opposite corner of the floor
Fornites not depicted in the Figure
- Stethoscope, ambu bag, call button, keyboard
- Blood pressure cuff

Fixed structure not depicted in the Figure
- The patient’s opposite corner of the floor, the floor in the restroom
- Monitor display above the patient
Positive from viral culture
Negative from viral culture

Patient #3

Nasal prong, telephone button
Blood pressure cuff, moving cart, hand washer tip
Door knob in the room, door knob in the restroom, the patient’s opposite corner of the floor, call button & handrail, floor, exit door knob in common corridor