Clinical Infectious Diseases

MAJOR ARTICLE

Environmental Contamination and Viral Shedding in MERS Patients During MERS-CoV Outbreak in South Korea

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Background. Although Middle East Respiratory Syndrome coronavirus (MERS-CoV) is characterized by a risk of nosocomial transmission, the detailed mode of transmission and period of virus shedding from infected patients are poorly understood. The aims of this study were to investigate the potential role of environmental contamination by MERS-CoV in healthcare settings and to define the period of viable virus shedding from MERS patients.

Methods. We investigated environmental contamination from 4 patients in MERS-CoV units of 2 hospitals. MERS-CoV was detected by reverse transcription polymerase chain reaction (PCR) and viable virus was isolated by cultures.

Results. Many environmental surfaces of MERS patient rooms, including points frequently touched by patients or healthcare workers, were contaminated by MERS-CoV. Viral RNA was detected up to five days from environmental surfaces following the last positive PCR from patients' respiratory specimens. MERS-CoV RNA was detected in samples from anterooms, medical devices, and air-ventilating equipment. In addition, MERS-CoV was isolated from environmental objects such as bed sheets, bedrails, IV fluid hangers, and X-ray devices. During the late clinical phase of MERS, viable virus could be isolated in 3 of the 4 enrolled patients on day 18 to day 25 after symptom onset.

Conclusions. Most of touchable surfaces in MERS units were contaminated by patients and health care workers and the viable virus could shed through respiratory secretion from clinically fully recovered patients. These results emphasize the need for strict environmental surface hygiene practices, and sufficient isolation period based on laboratory results rather than solely on clinical symptoms.

Keywords. MERS-CoV; South Korea; transmission mode; environmental contamination; prolonged viral shedding.

Middle East Respiratory Syndrome coronavirus (MERS-CoV) was first identified in an isolate from a patient who had died of severe pneumonia in Saudi Arabia in September 2012 [1]. MERS-CoV is a positive-sense, single-stranded RNA virus, a new member of the genus Betacoronavirus, lineage C, which is distinct from severe acute respiratory syndrome (SARS) coronavirus [2]. It can cause a wide range of clinical manifestations in humans, from asymptomatic infections to fatal diseases, with a 40% mortality rate as of 31 May 2015 [3]. Although MERS-CoV has caused limited disease outside the Arabian Peninsula, epidemiologic investigation revealed that sporadic infection has continued to be exported by travelers from the Middle East to countries in North America [4], Europe [5–8], and Thailand [9].

Although the mode of human-to-human transmission of MERS-CoV is not completely understood, the virus has frequently caused healthcare-associated outbreaks at hospitals in Saudi Arabia [10, 11]. Similarly, following the first MERS-CoV outbreak in South Korea on 20 May in 2015, widespread contamination of the hospital environment was suspected as a cause of the rapid transmission, although direct evidence is limited. During the first 30 days following the primary case, 166 laboratory-confirmed cases were diagnosed resulting in 24 deaths and comprising the largest outbreak of MERS-CoV infection outside the Arabian Peninsula [12]. Recently, the World Health Organization (WHO) and coinvestigators of the Ministry of Health of South Korea reported that the initial spread of MERS-CoV was expanded in several hospital clusters due to overcrowding in emergency rooms and medical wards, as well as poor infection control measures in hospitals during the early period of the outbreak. Furthermore, they pointed out unique cultural factors in South Korea that contributed to spread, including patients seeking medical opinions in multiple

Received 22 September 2015; accepted 4 December 2015; published online 17 December 2015.

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Clinical Infectious Diseases® 2016;62(6):755–60

DOI: 10.1093/cid/civ1020

healthcare facilities and inviting family members and relatives to the healthcare setting [13, 14]. Although MERS-CoV is assumed to be transmitted through large droplets, contact, and aerosols, the relative importance of aerosol transmission vs spread by large droplets or contact is unknown [15]. Therefore, there is an urgent need to investigate whether environmental contamination by droplets or other factors play a role in nosocomial transmission of the MERS-CoV.

The roles of fomites and environmental factors should not be overlooked in healthcare settings because many patients with underlying diseases share common spaces and environments. Hence, the aims of this study were to investigate the potential contribution of environmental contamination to MERS-CoV spread, as well as the length of time that viable virus is shed by patients. To this end, we sampled environmental surfaces in rooms used by laboratory-confirmed MERS patients, and measured virus by reverse transcription polymerase chain reaction (RT-PCR) as well as by culture. In addition, we monitored the viable virus shed from laboratory-confirmed MERS patients in late stage clinical disease.

MATERIALS AND METHODS

Patients and Rooms

Four laboratory-confirmed MERS patients, hospitalized in 2 hospitals (Chungbuk National University Hospital, Hallym University Kangnam Sacred Heart Hospital) from 8 June 2015 to 3 July 2015, were enrolled in this study. The rooms of those patients were selected for the environmental study. Respiratory specimens and environmental samples were collected and cultured from all patients during the later stages of clinical disease. After discharge of the MERS patients, we continued to collect environmental specimens for up to 24 or 120 hours. The respiratory specimens of the patients and the environmental specimens were tested for MERS by reverse transcription and PCR, and by virus culture [16]. The average temperature and humidity condition of the 4 rooms were 25.6°C and 52.2%. Three rooms of Chungbuk National University Hospital had 12 air-changes per hour and the average pressure gradient between the patient room and the anteroom was 9.1 hPa. One room of Hallym University Kangnam Sacred Heart Hospital had 24 air-changes per hour, and the average pressure gradient between the patient room and the anteroom was 2.5 hPa.

Respiratory Sample Collection

Patients were encouraged to expectorate sputum for more than 20 minutes with deep breathing. Expectorated sputum was collected in a specimen cup. The sputum in the specimen cup was soaked into the viral transport medium (200 U Penicillin, 200 μg Streptomycin, 68 μg Amphotericin B per mL phosphate-buffered saline [PBS]) in Hallym University Kangnam Sacred Heart Hospital for transportation to Biosafety Level 3 facilities (BSL3) at Chungbuk National University. Sputum samples from Chungbuk National University Hospital were delivered to the BLS3 immediately without mixing with VTM. If a patient could not expectorate sputum, tracheal aspirate was collected through nasotracheal suction.

Environmental Surface Sampling

Dacron swabs premoistened with viral transport medium were collected from environmental surfaces that were frequently touched by patients or healthcare workers. In patients' rooms, bed sheets, bedrails, bed tables, bed controllers, shelves, door buttons, bathroom door knobs, and floors were swabbed (Table 2). Patient care equipment such as patient monitor buttons, thermometers, IV fluid hangers, portable X-rays, and computed radiography cassettes were also swabbed. The anteroom floors, anteroom desks, and the inlets of air-ventilating equipment on the ceiling were also sampled. The swabbed specimens were immediately delivered to the BSL3.

Reverse Transcription-PCR (RT-PCR) and Sequencing

Viral RNA was extracted from all clinical or environmental specimens using the QIAampViral RNA Mini kit (QIAGEN, Valencia, California). RT-PCR conditions for quantifying MERS-CoV RNA and amplification parameters have been described previously [16–18]. Amplicons were purified using the GeneAll gel extraction kit (GeneAll, Korea) and sent to Cosmo GeneTech (Seoul, Korea) for commercial sequencing with an ABI 373 XL DNA sequencer (Perkin-Elmer, Foster City, California). The DNA sequences were compiled and edited using the Lasergene sequence analysis software package (DNASTAR, Madison, Wisconsin).

Cell Culture and Virus Isolation

Beside viral RNA detection from the environmental samples, all specimens collected in this study were also cultured to confirm virus viability. Vero E6 cells (ATCC, CRL 1586) were cultured in Eagle minimal essential medium (EMEM; Lonza) with 8% fetal calf serum (FCS) (Gibco) and antibiotics. Huh7 cells were grown in Dulbecco's modified Eagle's medium (DMEM; Lonza) containing 8% FCS, 2 mM L-glutamine (PAA), nonessential amino acids (PAA), and antibiotics. Infection of Vero E6 and Huh7 cells with each specimen was carried out in PBS containing 50 μg/mL DEAE-dextran and 2% FCS as previously described [19]. We monitored the cells daily for 12 days for cytopathic effects (CPE). Only cultures positive for MERS-CoV by both RT-PCR and sequencing were considered positive for virus isolation. All work with live MERS-CoV was performed in BioSafety Level 3 (BSL3) facility at Chungbuk National University.

Ethics Statement

This study was conducted in accordance with the study protocol approved by the Institutional Review Board of Chungbuk National University Hospital regarding specimen collection and permission obtained from patients for the use of their specimens (IRB no. 2015-07-022). Informed consent was waived

by the Institutional Review Board of Chungbuk National University Hospital to eliminate the possibility of spreading MERS-CoV via patient contact with the informed consent forms. The clinical specimen collection method involved minimal risk to the participants. Patient identifiers were removed from the records prior to research analysis. All of the experiments were conducted in a BSL3 facility in Chungbuk National University permitted by the Korea Centers for Disease Control and Prevention.

RESULTS

Demographic and Clinical Features of the MERS-CoV Infected Patients

The median age of the four enrolled patients was 68 years and all patients were female. Their underlying diseases and antiviral treatments are summarized in Table 1. All of the patients had pneumonia. Three patients recovered, but 1 patient died 23 days after symptom onset due to sudden aspiration. Patients 2 and 3 were hospitalized in the same room (room B) on the same day and shared the room for 17 days due to the lack of air-bone infection isolation rooms (AIIRs) in our hospital. However, patient 3 was moved to another room (room C) after the death of patient 2 and stayed there for 6 days until discharge. Patient 1 stayed in room A for 16 days, and patient 4 stayed in room D for 23 days.

Viral Shedding From the Patients (RT-PCR and Culture)

When we started to collect the respiratory samples for MERS RT-PCR and viral culture, all the patients had no clinical symptoms. In sum, 3 of the 4 enrolled patients showed positive results for virus isolation during this study. Although the duration of viral shedding through respiratory secretions varied among the patients, the latest point at which MERS-CoV could be detected from patient sputum was on the 25th day of disease onset (patient 3). The last day of virus isolation for the other cases was on the 22nd (patient 2 and patient 3) and 18th (patient 4) days after symptom onset (Table 2). Although we failed to isolate the virus, we could detect MERS-CoV RNA by RT-PCR from patient 4's respiratory specimen on day 23 post-disease onset.

Environmental Contamination of the Patient's Rooms

In room A, only the ceiling air inlet tested positive for viral RNA performed 1 day after the patient's discharge following full recovery (Tables 2 and 3). However, in room B the patient's room and medical equipment tested positive for viral RNA as late as 96 hours after the death of patient 3 from sudden aspiration (Table 2). It is noteworthy that medical equipment in the patient's room and anteroom began to test positive for viral RNA 1 day after the entrance of the MERS patient into room C. In room D, the patient's room and medical equipment was positive for the virus up to 120 hours after the patient's last positive PCR (Table 2). The RT-PCR results with the environmental specimens revealed that bed controllers (5 of 15 specimens, 33.3%), IV fluid hangers (5 of 14 specimens, 35.7%), anteroom desks (3 of 7 specimens, 42.8%), and bedrails (4 of 15 specimens, 26.7%) were frequently positive (Table 3). The detailed numbers of positive samples from all tested environmental specimens are summarized in Table 3. The duration of viral RNA detection from environmental specimens since the patient's last positive PCR ranged from 2 to 5 days. The environmental sampling was continued in rooms A, B, and C for the same time period due to the disinfection schedule for the new patients. Of note, viral RNAs were also detected in samples from the anteroom floors and anteroom desks of room C and room D, areas the patients couldn't reach by themselves. Furthermore, we also tested the environmental specimens from X-ray machines and thermometers, which were used for routine patient check-ups. RT-PCR and sequencing results revealed that 1 of 6 and 1 of 5 specimens from X-ray devices and thermometers, respectively, were positive for viral RNA suggesting that medical instruments could also be a source of virus spread. Infectious virus was also found in some environmental samples including those from bed sheets (room C), IV fluid hanger (room C), bedrail (room D), anteroom table (room D), and X-ray devices (room D) (Tables 2 and 3). It is noteworthy that the virus was recovered from the specimen taken from the IV fluid hanger in room C even on the day when the patient was negative for viral RNA as assessed by RT- PCR (Table 2).

DISCUSSION

In the current study, we provide evidence of the potential for MERS-CoV nosocomial transmission through contamination of surface of materials in the rooms of MERS patients, including many points frequently touched by patients and healthcare workers (bed sheets, bed controllers, and bedrails). We could also detect MERS-CoV RNA on medical devices and air-ventilation equipment in isolation rooms. During the late clinical phase of MERS-CoV infection, viable virus could be isolated from 3 of

Table 1. Patient Characteristics

Patient Number	Age	Sex	Underlying Diseases	Antiviral Treatment	Outcome
1	60	F	DM, Hypertension	peg-IFN + ribavirin + lopinavir/ritonavir (9 d)	Recovered
2	78	F	DM, Cerebral infarct	None	Died on day 22
3	76	F	DM, Hypertension, Left femur fracture	peg-IFN + ribavirin + lopinavir/ritonavir (12 d)	Recovered
4	25	F	None	peg-IFN + ribavirin + lopinavir/ritonavir (10 d)	Recovered

Abbreviations: DM, diabetes mellitus; peg-IFN, pegylated interferon.

Table 2. Polymerase Chain Reaction and Culture Results for Respiratory and Environmental Specimens

Room	Patient's Status; Sampling Location; Method of MERS CoV Detection	Sample Collection Day After the Patient's Symptom Onset												
		18 d	19 d	20 d	21 d	22 d	23 d	24 d	25 d	26 d	27 d	28 d	29 d	30 d
A	Patient's status (patient 1)		Discharged											
	Patient's respiratory specimen; PCR	(–)	(–)											
	Patient's respiratory specimen; viral culture	(–)	(–)											
	Patient's room; PCR		(–)	(–)										
	Patient's room; viral culture		(–)	(–)										
	Medical equipment; PCR		(–)	(–)										
	Medical equipment; viral culture		(–)	(–)										
	Anteroom/ventilation system; PCR		(–)	(+)[1]										
	Anteroom/ventilation system; viral culture		(–)	(–)										
B	Patient's status (patient 2 and 3)						P2 died, P3 moved to Room C							
	Patient's respiratory specimen; PCR					(+)*								
	Patient's respiratory specimen; viral culture					(+)*								
	Patient's room; PCR							(+)[2]	(–)	(+)[3]	(+)[4]			
	Patient's room; viral culture							(–)	(–)	(–)	(–)			
	Medical equipment; PCR							(–)	(–)	(+)[5]	(+)[5]			
	Medical equipment; viral culture							(–)	(–)	(–)	(+)[5]			
	Anteroom/ventilation system; PCR							(–)	(–)	(–)	(–)			
	Anteroom/ventilation system; viral culture							(–)	(–)	(–)	(–)			
C	Patient's status (patient 3)						P3 moved to Room C					Discharged		
	Patient's respiratory specimen; PCR					(+)			(+)		(–)	(–)		
	Patient's respiratory specimen; viral culture					(+)			(+)		(–)	(–)		
	Patient's room; PCR							(+)[2]	(+)[6]	(+)[2]	(+)[7]			
	Patient's room; viral culture							(+)[2]	(–)	(–)	(–)			
	Medical equipment; PCR							(–)	(–)	(–)	(+)[5]			
	Medical equipment; viral culture							(–)	(–)	(–)	(+)[5]			
	Anteroom/ventilation system; PCR							(–)	(+)[8]	(+)[8]	(–)			
	Anteroom/ventilation system; viral culture							(–)	(–)	(–)	(–)			
D	Patient's status (patient 4)								Discharged					
	Patient's respiratory specimen; PCR	(+)			(+)		(+)		(–)					
	Patient's respiratory specimen; viral culture	(+)			(–)		(–)		(–)					
	Patient's room; PCR	(–)			(+)[9]		(+)[10]		(–)	(+)[11]	(+)[4]	(+)[3]		(–)
	Patient's room; viral culture	(+)[11]			(–)		(–)		(–)	(–)	(–)	(–)		(–)
	Medical equipment; PCR	(+)[12]			(+)[13]		(–)		(–)	(+)[5]	(+)[5]	(+)[14]		(–)
	Medical equipment; viral culture	(+)[12]			(–)		(–)		(–)	(–)	(–)	(–)		(–)
	Anteroom/ventilation system; PCR	(+)[15]			(+)[15]		(+)[15]		(–)	(–)	(–)	(–)		(–)
	Anteroom/ventilation system; viral culture	(+)[15]			(–)		(–)		(–)	(–)	(–)	(–)		(–)

The dotted line box for each room indicates that patient was in the room.

Abbreviations: MERS CoV, Middle East Respiratory Syndrome coronavirus; P1, Patient 1; P2, Patient 2; P3, Patient 3; P4, Patient 4; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction.

* Both P2 and P3 were positive for RT-PCR and virus isolations.

[1]The inlet of air-ventilating equipment on the ceiling, [2]bed sheet, [3]bed controller, [4]bedrail, bed controller, [5]IV fluid hanger, [6]bed controller, bathroom door knob, [7]door button, [8]anteroom floor, [9]bed table, [10]bedrail, bed table, [11]bed rail, [12]computed radiography cassette, [13]portable X-ray device, [14]thermometer, [15]anteroom table.

the 4 enrolled patients between days 18 and 25 post-symptom onset. In addition, we could isolate viable MERS-CoV from some environmental surfaces, suggesting that MERS-CoV can be transmitted through contaminated environments and fomites.

Our results clearly demonstrate that the unwitting actions by both patients and healthcare workers potentially induce viable virus contamination of the surface of various environments and medical devices (X-ray machines, thermometers) around MERS patients. In rooms B and C, viral RNA of MERS-CoV was detected on environmental points where the patients could not reach due to their limited activity. This finding suggests that the contamination was caused by healthcare workers potentially exposed to high levels of infectious virus unwittingly contaminating the environment during patient care. These results might explain the unusual, rapid spread of MERS-CoV to visitors and people who did not share the same room during the MERS outbreak in South Korea. The massive contamination around the patients including beds, IV fluid stands, and X-ray

Table 3. Frequency of Environmental Sample Positivity for Middle East Respiratory Syndrome Coronavirus in Reverse Transcription Polymerase Chain Reaction or Viral Culture

Swab Site	PCR Results (Positivity Percent, %)	Culture Results (Positivity Percent, %)
Bed sheet	3/15 (20.0)	1/15 (6.7)
Bedrails	4/15 (26.7)	1/15 (6.7)
Bed tables	2/5 (40.0)	0/5 (0.0)
Bed controllers	5/15 (33.3)	0/15 (0.0)
Shelves	0/14 (0.0)	0/14 (0.0)
Door buttons	1/10 (10.0)	0/10 (0.0)
Bathroom door knobs	1/10 (10.0)	0/10 (0.0)
Patient room floor	0/7 (0.0)	0/7 (0.0)
Patient monitor buttons	0/5 (0.0)	0/5 (0.0)
Thermometers	1/5 (20.0)	0/5 (0.0)
IV fluid hangers	5/14 (35.7)	2/14 (14.3)
Portable X-rays	1/5 (20.0)	0/5 (0.0)
Computed radiography cassette	1/1 (100.0)	1/1 (100.0)
Anteroom floors	2/14 (14.3)	0/14 (0.0)
Anteroom tables	3/7 (42.8)	1/7 (14.3)
Entrances of air-ventilating equipment	1/6 (16.7)	0/6 (0.0)

Abbreviation: PCR, polymerase chain reaction.

machines prior to recognition of the first patient (20 May 2015) might have been a critical factor responsible for the amplified spread of MERS-CoV infection to visitors and people who did not share the same room, even in the emergency room of each hospital. Similar findings were made during the severe acute respiratory syndrome (SARS) outbreak in Canada, in which PCR-positive swab samples were recovered from frequently touched surfaces in patient rooms, on patient care equipment, and ventilation-system components [20].

Although several reports have shown that MERS-CoV viral RNA can be detected in respiratory secretions for 2 weeks [21–23], we found that the virus could be isolated from the sputum up to the 25th day after disease onset. This finding suggests that infected patients can shed MERS-CoV in respiratory secretions for more than 20 days, which is longer than previously thought [21–23]. This prolonged viral shedding may result in the persistent contamination of the patient's environment even when clinical symptoms and laboratory abnormalities have resolved. Thus, careful infection control and prevention strategies should be applied to isolated MERS patients not only during the acute phase but also during recovery to prevent viral spread and further nosocomial infections. Our results also suggest that isolation and quarantine policy during a MERS outbreak should be considered on laboratory results (PCR or viral culture) rather than solely on improvements in patient symptoms.

Furthermore, MERS-CoV was isolated from medical equipment in patient rooms even after respiratory specimens were PCR negative. In 1 study, van Doremalen and colleagues reported that MERS-CoV could be recovered from samples after storage for 48 hours at 20°C in 40% relative humidity. Moreover, they found that MERS-CoV was more stable than influenza A under various environmental settings [24]. This characteristic of MERS-CoV increases the likelihood of contact and fomite transmission, and therefore, when possible medical devices such as thermometers, X-ray devices, or IV fluid hangers should be retained in the patient room to avoid cross contamination. Furthermore, all medical devices should be cleaned thoroughly before leaving the patient's room.

Anterooms are necessary to maintain the pressure gradient of airborne infection isolation rooms and reduce the migration of infectious particles from the isolation room into the corridor [25]. In addition, an anteroom provides space for healthcare workers to put on and take off Personal Protective Equipment (PPE). Usually, medical devices used in patient rooms are disinfected in anterooms; thus, the anteroom is commonly considered to be a buffer zone. In our study, MERS-CoV was detected on anteroom floors and desks. This suggests that unless the anteroom is frequently disinfected, viable virus can be spread to the corridor by medical personnel or medical devices leaving patient rooms.

Based on the results of our study it can be concluded that the environment around MERS patients is widely contaminated; in order to protect healthcare workers, more thorough infection control guidelines are necessary, including emphasizing methods to prevent contact transmission of MERS-CoV. Current guidelines suggest gloves, gowns, respirators, and eye protectors as PPE for MERS patient care [26]. In addition, during large MERS outbreaks, where hastily assembled anterooms may not be equipped with sinks for proper hand washing, double gloving may help to avoid spread by contact. Furthermore, because PPE may be contaminated by MERS-CoV during patient care, there is a need for more careful and comprehensive procedures for putting on and removing PPE and respirators, including double gloving, in order to improve infection control. To this end, mirrors placed in anterooms can assist healthcare workers in checking their PPE status before entering patient rooms and during removal of PPE to avoid contamination. Moreover, it is reasonable to assume that the surface of sample bottles and specimen cups may be contaminated by MERS and should be disinfected before transport to the hospital laboratory.

Aerosol transmission of a highly virulent agent could extensively infect personnel quickly causing a large epidemic; however, prior to the current study evidence for MERS-CoV transmission was limited to family and hospital-associated clusters, implying viral spread through close contact and droplets rather than airborne-virus particles [12, 27]. Although we failed to isolate viable virus by cell culture (Table 3), it is noteworthy that viral RNA was detected on the entrance to air-ventilating equipment in 1 room, suggesting the potential existence of airborne-viral particles. Because our study included small number of patients (4 patients) in their late clinical phase, to confirm the airborne transmission of MERS-CoV, more case studies and additional well-designed experimental studies will be needed.

Taken together, our data demonstrate that environmental contamination by patients and healthcare workers commonly occurs in airborne infection isolation rooms of MERS patients and viable virus can be shed through respiratory sputum for as long as 25 days after disease onset. Frequent and thorough environmental cleaning and disinfection are therefore critical to reduce spread of this highly contagious virus, especially in the hospital setting. In addition, detailed, pre-existing guidelines should be in place to disinfect the environment, protect healthcare workers from contaminated hospital surfaces, and isolate patients for a sufficient time period in order to limit virus spread.

Notes

Author contributions. S. Y. B., J. Y .H., M.-S. S., J. L., H. W. J., and Y. K. C. designed research; S. Y. B., J. Y. H., M.-S. S., J. L., E.-H. K., S.-J. P., H.-i. K., S. m. K., Y.-i. K., Y.-J. S., I.-W. L., W.-S. C., Y. H. B., J. M., H. W. J., and Y. K. C. performed experiments; S. Y. B., J. Y. H., M.-S. S., J. L., E.-H. K., S.-J. P., H.-i. K., S. m. K., Y.-i. K., Y.-J. S., I.-W. L., W.-S. C., H. W. J., and Y. K. C. analyzed data; S. Y. B., J. Y. H., M..-S. S., J. L., H. W. J., and Y. K. C. wrote the article.

Financial support. This work was partly supported by a grant of the Korea Healthcare technology R&D project, Ministry of Health and Welfare, Republic of Korea (grant no. A103001) and by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by Ministry of Science (NRF-2007-0054930).

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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