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Detection of Common Respiratory Viruses and *Mycoplasma pneumoniae* in Patient-Occupied Rooms in Pediatric Wards

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Abstract: Few studies have assessed viral contamination in the rooms of hospital wards. This cross-sectional study evaluated the air and objects in patient-occupied rooms in pediatric wards for the presence of common respiratory viruses and *Mycoplasma pneumoniae*.

Air samplers were placed at a short (60−80 cm) and long (320 cm) distance from the head of the beds of 58 pediatric patients, who were subsequently confirmed to be infected with enterovirus (n = 17), respiratory syncytial virus (RSV) (n = 13), influenza A virus (n = 13), adenovirus (n = 9), or *M pneumoniae* (n = 6). Swab samples were collected from the surfaces of 5 different types of objects in the patients' rooms. All air and swab samples were analyzed via real-time quantitative polymerase chain reaction assay for the presence of the above pathogens.

All pathogens except enterovirus were detected in the air, on the objects, or in both locations in the patients' rooms. The detection rates of influenza A virus, adenovirus, and *M pneumoniae* for the long distance air sampling were 15%, 67%, and 17%, respectively. Both adenovirus and *M pneumoniae* were detected at very high rates, with high concentrations, on all sampled objects.

The respiratory pathogens RSV, influenza A virus, adenovirus, and *M pneumoniae* were detected in the air and/or on the objects in the pediatric ward rooms. Appropriate infection control measures should be strictly implemented when caring for such patients.

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Abbreviations: CAP = community-acquired pneumonia, PBS = phosphate-buffered saline, PCR = polymerase chain reaction, RSV = respiratory syncytial virus.

INTRODUCTION

Typical hospital air quality indices are temperature, relative humidity,[1] and levels of carbon dioxide, particulate matter,[2,3] toxic metals,[3] volatile organic compounds,[1] bacteria,[2,4–6] fungi,[5,7,8] and viruses.[9] Previous studies of air quality primarily focused on intensive care units,[4,7,10,11] operating rooms,[12,13] negative-pressure patient isolation rooms,[6,9] and public areas in hospitals.[3,14–19] However, few studies assessed viral contamination in the patients' rooms in a given hospital ward.

Airborne microorganisms in a hospital can infect susceptible patients.[20] Aerosolized droplets are generally 4 to 8 μm in diameter,[21] while most viruses are 25 to 400 nm in length.[22] In nature, airborne viruses associate with larger particles and aggregate.[23–25] However, the size distribution of airborne viral particles is rarely determined. Two mechanisms underlie the person-to-person transmission of viral infections of the respiratory system: exposure to large-droplet infectious nuclei that remain suspended in air for short periods, and exposure to small-particle infectious nuclei that can remain suspended in air for long periods.[26] Generally, large-particle aerosols are believed to account for viral transmission.

Viral infections of the respiratory system are very common. In Taiwan, the predominant viruses isolated from patients with respiratory infections are enterovirus, respiratory syncytial virus (RSV), influenza A and B viruses, adenovirus, cytomegalovirus, herpes simplex virus-1, and parainfluenza virus.[27] Enterovirus causes herpangina, hand-foot-and-mouth disease, myocarditis, encephalitis, and death. RSV is the most common pathogen of the lower respiratory tract in infants[28] and a common cause of nosocomial infections in pediatric wards.[29] Influenza A and B viruses cause seasonal epidemics in Taiwan, especially in winter.[30] Adenovirus causes acute respiratory tract infections in children younger than 5 years of age and circulates throughout the year.[31] Further, *Mycoplasma pneumoniae*, a small bacterium, is a common pathogenic agent of community-acquired pneumonia (CAP) in children and young adults.[32,33]

RSV has been detected in air samples collected at distances of 30 to 700 cm from the head of patients' beds,[34] and RNA analysis of such samples at distance of 700 cm from the bedside may be useful for identifying small RSV-containing particles in hospital wards. Moreover, ubiquitous objects (e.g., telephones, door knobs, tables, air filters, ventilators) in hospitals have been shown to harbor adenovirus,[35,36] varicella-zoster virus,[37,38] *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.[39]

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Few studies have investigated the concentrations of respiratory viruses and *M pneumoniae* aerosols in the rooms of pediatric wards. To provide more detailed information, we measured the concentrations of 4 respiratory viruses common in children (enterovirus, influenza A virus, RSV, and adenovirus) and *M pneumoniae* in air samples collected at 2 locations (relative to the head of the bed) in patient-occupied rooms in the pediatric wards of a university-affiliated hospital in northern Taiwan. We also inspected the objects in the pediatric ward rooms to determine whether they were contaminated.

METHODS

Patient Enrollment and Sampling Locations

Chang Gung Children's Hospital is a part of Chang Gung Memorial Hospital, which is a 3700-bed university-affiliated teaching hospital in northern Taiwan that provides primary to tertiary care. The general pediatric wards of the Children's Hospital currently contain 220 beds distributed among 6 floors, 2 of which house most of the patients with infectious diseases. In each pediatric room, there are 1 to 3 beds and, hanging on the wall near the door, 1 bottle of alcohol-based hand-sanitizing solution. There are also 3 one-bed and 2 two-bed negative-pressure isolation rooms. Patients with pulmonary tuberculosis, measles, or varicella are always placed in the negative-pressure isolation rooms.

In general practice, patients with clinically suspected enteroviral infections, such as herpangina or hand, foot, and mouth disease, are segregated in a given set of rooms. The care providers who stay in these rooms (e.g., parents, grandparents) receive specific instructions regarding infection control procedures, such as wearing a mask, maintaining hand hygiene, and using disinfectants. The caregivers of patients with documented influenza (a positive rapid test) also follow these guidelines as much as possible. There are no specific guidelines for the caregivers of patients with adenovirus, RSV, or *M pneumoniae* infection, although hygiene is emphasized. The air in the ward rooms was air-conditioned but not heated during the study period.

The study period was from October 2009 to September 2010. For this study, 75 hospitalized children who we suspected were infected with enterovirus, influenza A virus, RSV, adenovirus, or *M pneumoniae* were recruited after obtaining written informed consent from their parents or guardians. Ultimately, it included 58 patients with infections subsequently confirmed via virus culture or polymerase chain reaction (PCR) (enterovirus, 17 of 24 patients; influenza A virus, 13 of 16 patients; RSV, 13 of 15 patients; adenovirus, 9 of 13 patients; and *M pneumoniae*, 6 of 7 patients). Air and object surface samples were obtained from the rooms of these patients. The study protocol was approved by the institutional review board of the hospital (97-1394B).

Air Sampling

Air sampling of the patients' rooms was conducted within 2 days after the patients had moved in. Air samplers were placed 60 to 180 cm (defined as ''short distance'' sampling) and 320 cm (defined as ''long distance'' sampling) from the head of the each patient's bed and collected 2 and 24 h, respectively, after placement. Air sampling was performed 1.2 to 1.5 m above the floor (i.e., in the breathing zone). Indoor air was filtered through a closed-face, 3-piece disposable plastic cassette with a 0.2 μm polytetrafluoroethylene filter (Whatman, Sigma-Aldrich, St. Louis, MO) at airflow rates of 4 and 12 L/min for bioaerosol sampling at the short distance and long distance, respectively. After air sampling, the filters were stored immediately at −80°C until analysis. Ten percent of the blank samples were examined to detect the RNA or DNA of the viruses and *M pneumoniae*.[40] For quantitative analysis of the viruses and *M pneumoniae*, 116 air samples were collected.

Object Surface Sampling

Object surface sampling was performed within 2 days after the patients had moved into their rooms. The surfaces of the following items were swabbed in each patient's room: nursing call button, bed handrail, television remote control buttons, light switch, bathroom door knobs (2 samples from both the inner and outer door knobs), and inner ward room door knob. After surface sampling, the swabs were immediately immersed in 2 mL phosphate-buffered saline (PBS) (Sigma-Aldrich) and stored at −80°C until analysis. Again, 10% of the blank samples were examined to detect the RNA or DNA of the viruses and *M pneumoniae*. A total of 406 swab samples were collected.

Preparation of Filter Sample Extracts

Filters were placed in 60 mm petri dishes, and 1.5 mL PBS was added to each dish. The filters were shaken on an orbital tabletop shaker at 150 rpm for 60 min at room temperature. All samples were stored at −80°C until RNA and DNA were extracted.

RNA and DNA Extraction

RNA and DNA were isolated by using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) and the Qiagen DNA Mini Kit (Qiagen Instruments AG, Hombrechtikon, Switzerland), respectively, according to the manufacturer's directions. Briefly, 500 μL of each sample was extracted, and the nucleic acid was eluted at a final volume of 30 μL. Isolates were stored at −80°C until analysis. For negative controls, sterile distilled water was used in place of the sample.

Real-Time Quantitative PCR Assay for Enterovirus, RSV, Influenza A Virus, Adenovirus, and *M pneumoniae*

Table 1 shows the sequences of the primers and probes for enterovirus, RSV, influenza A virus, adenovirus, and *M pneumoniae*. For enterovirus detection, the PCR reaction mixtures contained 5 μL RNA, 20 μL 2× TaqMan universal master mix (Applied Biosystems, Waltham, MA), 10 μM of each primer, and 5 μM probe. The PCR thermal profile was 30 min at 48°C, 10 min at 95°C, and 45 cycles of 15 s at 95°C and 1 min at 58°C. The PCR products were amplified and detected by using an ABI Prism 7900 sequence detection system (Applied Biosystems). For influenza A virus and RSV detection, the PCR reaction mixtures contained 5 μL RNA, 20 μL reverse transcription-PCR buffer (OneStep RT-PCR kit; Qiagen), 10 mM dNTPs, 25 mM $MgCl_2$, 2000 U RNAsin (Invitrogen, Paisley, Scotland), 50 μM of each primer, and 10 μM probe. The PCR thermal profile was 30 min at 50°C, 10 min at 95°C, and 50 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C. The PCR products were amplified and detected by using the CFX96 real-time detection system (Bio-Rad, Veenendaal, The Netherlands). The concentrations of adenovirus and *M pneumoniae* in the filter samples were quantitatively measured as described previously.[41] In this study, the detection limits in the real-time PCR assay were 1 and 100 copies/mL for RSV and enterovirus, respectively, and 10 copies/mL for the other viruses and *M pneumoniae*.

TABLE 1. Sequences of Primers and Probes of Different Viruses and *Mycoplasma pneumoniae*

Oligo Names	Sequences (5′→3′)	Genes	References
RNA viruses			
Enterovirus		VP1	Wells[26]
EV-s	CCCCTGAATGCGGCTAATC		
EV-as	GATTGTCACCATAAGCAGC		
EV-probe	FAM-CGGAACCGACTACTTTGGGTGTCCGT-TEMRA		
Respiratory syncytial virus		L	Lin[27]
RSV-s	TTTCCACAATATYTAAGTGTCAA		
RSV-as	TCATCWCCATACTTTTCTGTTA		
RSV-probe	HEX-GCGAGCCCATGTGAATTCCCTGCATCAATGCTCGC-BHQ-1		
Influenza A virus		NS	Lin[27]
FluA-s	AAAGCGAATTTCAGTGTGAT		
FluA-as	GAAGGCAATGGTGAGATTT		
FluA-probe	FAM-GCTGCCAGGGCTTTCACCGAAGAGGGGGCAGC-Dabeyl		
DNA viruses			
Adenovirus		Hex	
Ad-s	GCCCCAGTGGGCTTACATGCACATC		
Ad-as	ATTGAAGTAGGTGTCTGTGGCGCGGG		
Ad-probe	FAM-AACTGCACCAGACCCGGGCTCAGGTACTCC-TAMRA		
Bacterium			
M pneumoniae		P1	Goldmann[29]
MP-TM1	CCAACCAAACAACAACGTTCA		
MP-TM2	ACCTTGACTGGAGGCCGTTA		
MP-probe	VIC-ATCCGAATAACGGTGACTT-MGB		

EV = enterovirus, MP = *Mycoplasma pneumoniae*, RSV = respiratory syncytial virus.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 19.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. The level of significance was 0.05. The figures were constructed by using GraphPad Prism 5.0 software. The chi-squared test was used to identify group differences in detection rates. The Kruskal–Wallis test and Mann–Whitney *U* test for non-normally distributed data were used to identify group differences in virus and *M pneumoniae* concentrations.

RESULTS

Enterovirus and influenza A virus were not detected in the air samples collected at the short distance (60–180 cm from the head of the patient's bed; Table 2). RSV, adenovirus, and *M pneumoniae* were observed in 7.69%, 11.11%, and 16.67% of these samples, respectively. Enterovirus and RSV were not detected in the air samples collected at the long distance (320 cm from the head of the patient's bed). Adenovirus was found in 67% of the long distance samples, whereas influenza A virus and *M pneumoniae* were found in only 15% and 17% of these samples, respectively. There were no statistically significant differences in the detection rates of any of these viruses or of *M pneumoniae* between the short and long distance samplings.

RSV was detected on all objects tested excepting the television remote control buttons (Figure 1A), with the bed rails having the highest detection rate (15.38%). Influenza A virus was found on the call buttons (15.38%) and the door knobs of the bathrooms and ward rooms (10.26%; Figure 1B). Adenovirus was detected on all 5 object types at very high rates (88.9–100%; Figure 1C). The detection rates for *M pneumoniae*

TABLE 2. Distribution of Airborne Viruses and *Mycoplasma pneumoniae* in the Specific Pathogen-Infected Patient-Occupied Isolation Rooms

	Detection Rate, n (%)			Concentration, Copies/m^3*	
Viruses and Bacterium	Short Distance From Patient	Long Distance From Patient	*P* Value	Short Distance From Patient	Long Distance From Patient
Enterovirus	0/17 (0.00)	0/17 (0.00)	–	ND	ND
Respiratory syncytial virus	1/13 (7.69)	0/13 (0.00)	1.000	73.5	ND
Influenza A virus	0/13 (0.00)	2/13 (15.38)	0.480	ND	108.8 (59.0–158.5)
Adenovirus	2/9 (11.11)	6/9 (66.67)	0.153	173.3	66.1 (23.8–78.4)
Mycoplasma pneumoniae	1/6 (16.67)	1/6 (16.67)	1.000	74,902	1781

*Median (range).ND = nondetectable value.

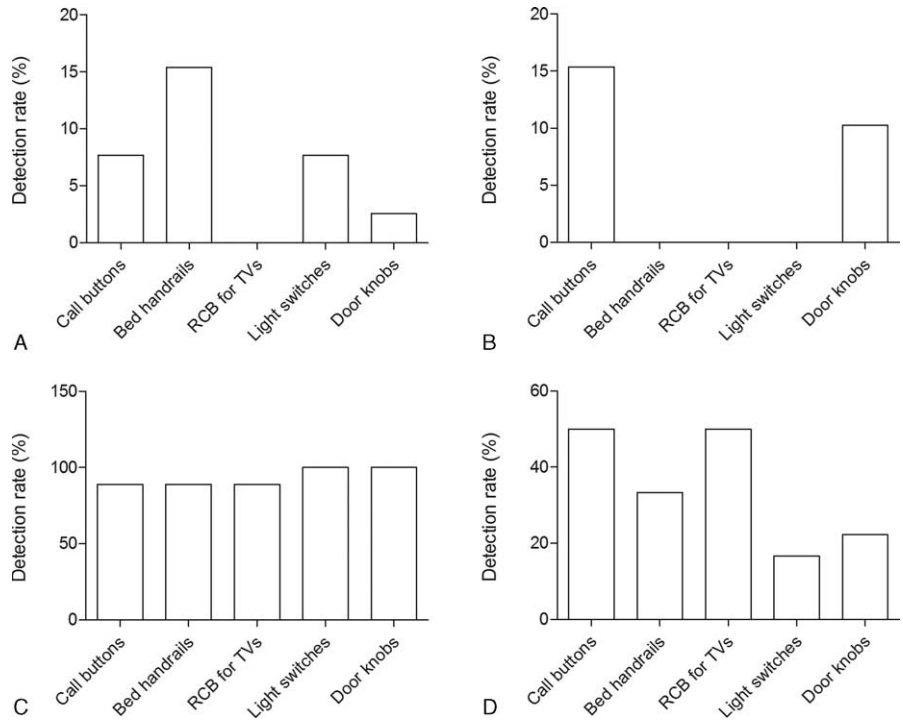

FIGURE 1. Detection rates of viruses and *Mycoplasma pneumoniae* in swab samples of the objects in pediatric isolation rooms. (A) Respiratory syncytial virus. (B) Influenza A virus. (C) Adenovirus. (D) *M pneumoniae*. RCB for TVs = remote control buttons for televisions.

were also high; the call buttons and television remote control buttons had the highest rates (both 50%), and the light switches had the lowest rate (16.67%; Figure 1D).

In the swab samples, the median concentrations of influenza A virus (930.15 copies/unit area, $P < 0.01$) and adenovirus (1893.05 copies/unit area, $P < 0.01$) were significantly higher than that of RSV (12.33 copies/unit area; Figure 2). The concentrations of *M pneumoniae* in the swab samples ranged from 317.58 to 96,894.9 copies/unit area, and the median concentration of *M pneumoniae* (3669.52 copies/unit area) was significantly higher than that of RSV ($P < 0.01$). There were no statistically significant differences between the concentrations of influenza A virus, adenovirus, and *M pneumoniae* in the swab samples.

Seventeen patients were excluded from the final analysis because viral or bacterial infection could not be confirmed in their clinical specimens. In these patients, 2 long distance air samples were positive for adenovirus, and all 5 sampled object types were positive for a microorganism in 3 of the 4 patients with suspected adenoviral infections. One door knob sample was positive for *M pneumoniae*.

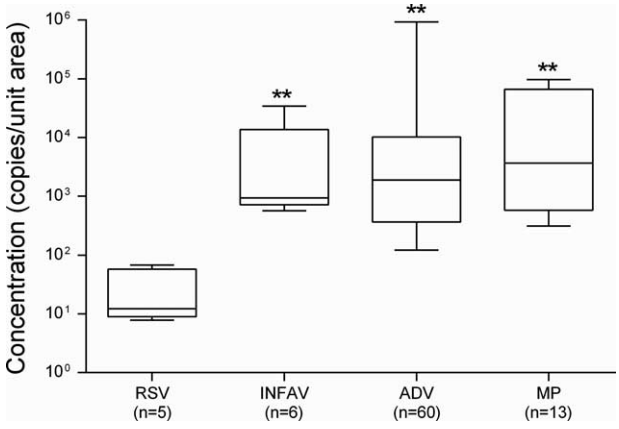

FIGURE 2. Concentrations of viruses and *Mycoplasma pneumoniae* in swab samples of the objects in pediatric isolation rooms. ADV = adenovirus, INFAV = influenza A virus, MP = *Mycoplasma pneumoniae*, RSV = respiratory syncytial virus. $^{**}P < 0.01$ compared with RSV.

DISCUSSION

To our knowledge, this study is the first to examine the distributions of aerosol particles of *M pneumoniae* and the viruses common in children in patient-occupied rooms in pediatric wards. Adenovirus and *M pneumoniae* were detected in the air samples obtained at both the short and long distance relative to the head of the patient's bed. RSV was only detected at the short distance, and influenza A virus was only detected at the long distance. Surprisingly, there was no enterovirus RNA in the air samples or object surface swabs. The absence of enterovirus from the air samples indicates that the likelihood of air and/or droplet transmission of this virus was extremely low. Its absence from the objects in the rooms housing the children with enteroviral infections is most likely explained by our periodic disinfection of these rooms with a 0.5% sodium hypochlorite solution. Our detection of RSV RNA in air samples collected only at the short distance (60–180 cm) is at odds with a previous study showing RSV in air samples collected 30 to 700 cm from the head of the patients' beds.[34] Additionally, influenza A virus RNA was detected in 15% of the

air samples obtained at the long distance but in no air samples obtained at the short distance. It is likely that differences in sampling flow rates, sampling durations at different distances, and the activities of the patients in the rooms affect the detection rates of these pathogens.

To reduce the exposure risk of patients, their families, and healthcare workers, the ventilation system in pediatric wards should be maintained and inspected routinely. In our previous study, adenovirus and *M pneumoniae* were the most prevalent airborne pathogens in the pediatric outpatient department and emergency rooms.[41] In this study, aerosol particles containing adenovirus and *M pneumoniae* were also found in the ward rooms at the 2 locations tested. Collectively, these findings indicate that there is a risk of airborne or droplet transmission of both adenovirus and *M pneumoniae* in pediatric ward rooms.

Previous studies indicated that adenovirus was frequently found on the surfaces of the furnishings in hospitals.[35,36] In this study, all microorganisms examined excepting enterovirus were detected on the surfaces of all tested objects in the pediatric ward rooms. Adenovirus and *M pneumoniae* were more often detected on these objects, and at higher concentrations, than were RSV and influenza A virus. More frequent disinfection of the objects' surfaces may be necessary to reduce the level of contamination. Also, the effectiveness of different disinfectants at different concentrations in terms of killing or inactivating these pathogens needs to be evaluated.

Similar to *M pneumoniae*, *Chlamydia pneumoniae* also commonly causes CAP in school-age children and young people in the United States.[42] Chlamydial infections can be transmitted via respiratory secretions and can result in pneumonia, bronchitis,[43–45] cough, and low-grade fever. They have also been associated with atherosclerosis[46] and sudden death in athletes.[47,48] To date, only a few studies have assessed the distribution of *Chlamydia pneumoniae* in the air and on the surfaces in patients' rooms, and investigation of this important issue is warranted.

This study has 3 limitations. First, during collection of the air samples, the patients did not necessarily remain in bed, but sometimes walked about. Consequently, the short-distance samplings did not represent the potential ''droplet'' transmission mode, which was initially presumed, and the long-distance samplings did not represent the potential ''airborne'' transmission mode. Second, because the surfaces of the designated objects were sampled only once, rather before and after disinfection, we could not evaluate the effects of disinfection. Third, the number of patients enrolled and the number of pathogens examined were relatively small, and further studies should be conducted to allow definite conclusions.

In conclusion, the common respiratory pathogens RSV, influenza A virus, adenovirus, and *M pneumoniae* were detected in the air and/or on the surfaces of the objects in the rooms occupied by pediatric patients infected with the corresponding pathogen. It cannot be overemphasized that appropriate infection control measures should be strictly implemented when caring for such patients.

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