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Original article

Pathogen profiles and molecular epidemiology of respiratory viruses in Japanese inpatients with community-acquired pneumonia

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ABSTRACT

Background: The etiological profile of viruses among adult patients with community-acquired pneumonia (CAP) has not been characterized yet. The aim of this study was twofold: first, investigate the pathogen profiles and the molecular epidemiology of respiratory viruses among Japanese CAP patients; and second, explore the clinical significance of viral infections.
Methods: A cross-sectional observational study was conducted at Kyorin University Hospital. To identify respiratory pathogens, hospitalized CAP patients were enrolled, and reverse transcriptase–polymerase chain reaction technology was applied alongside conventional microbiological methods. Phylogenetic and pairwise distance analyses of 10 viruses were performed. CAP patients were divided into four etiological groups (virus alone, bacteria alone, co-detection of virus and bacteria, and not detected) and the clinical findings were compared.
Results: Seventy-six patients were enrolled. Bacteria alone were detected in 39.5% ($n=30$) of CAP patients. Virus alone or co-detection were found in 10.5% ($n=8$) and 11.8% ($n=9$) of cases, respectively. *Streptococcus pneumoniae* and human metapneumovirus were the most frequently detected bacterium and virus, respectively. Phylogenetic analyses of human metapneumovirus, human rhinovirus, and human respiratory syncytial virus showed that different subgroups and genotypes might be associated with CAP. Respiratory failure was more common when a virus was detected (both virus alone and co-detection groups; $n=17$, 100%, $p<0.05$) than when a bacteria alone was detected ($n=17$, 56.7%).
Conclusion: Prevalence of respiratory virus infection in CAP inpatients was 22.3%. The detected viruses display high genetic divergence and correlate with increased respiratory failure.

1. Introduction

Community-acquired pneumonia (CAP) is a life-threatening respiratory disease of worldwide importance [1]. According to several studies from developed countries, the annual incidence of CAP in adults is in the range of 0.5–1.1%, and the mortality rate of hospitalized CAP patients is 4–14% [2]. Previous reports have suggested that various pathogens including bacteria, fungi, and viruses are associated with CAP [3]. Among these, *Streptococcus pneumoniae* (pneumococcus) is a major cause of CAP in adult patients, particularly in those with severe diseases [2–5]. Bacteria such as *Haemophilus influenzae*, methicillin-sensitive *Staphylococcus aureus* (MSSA), *Legionella pneumophila*, and *Moraxella catarrhalis* are associated with CAP in adult inpatients [2–5], while other types such as *Mycoplasma pneumoniae* are detected in relatively mild CAP in outpatients [6].

Until recently, respiratory viruses such as human respiratory syncytial virus (RSV), human rhinovirus (HRV), human parainfluenza virus (HPIV), and human metapneumovirus (HMPV) have been mainly associated with lower respiratory tract infections (LRTI) including bronchitis, bronchiolitis, and pneumonia in children [7–9].

Respiratory viruses such as RSV, HRV, HMPV, and adenovirus are present in two-thirds of children with CAP [10].

Apart from causing LRTI, these viruses can exacerbate asthma and chronic obstructive pulmonary disease (COPD) [11–14]. For example, Dowell et al. showed that RSV was associated with 4.4% of adult cases of LRTI during winter [15]. Recent molecular epidemiological studies suggest that these viruses can be classified into numerous phylogenetic subtypes and genotypes. Specifically, there are over 150 genotypes of HRV species A to C (HRV-A to -C). Similarly, RSV and HPIV species can be subclassified into several genotypes. Recent studies have demonstrated the presence of these respiratory viruses and/or bacteria in adult patients with CAP [16,17]. However, the molecular epidemiology in adult CAP Japanese patients is poorly understood.

We conducted pathogen profiling and phylogenetic analyses of various respiratory viruses detected in hospitalized CAP patients in Japan and characterized these patients in terms of infectious viral and/or bacterial pathogens.

2. Patients and methods

2.1. Patients and study design

In this cross-sectional observational study, we recruited consecutive patients admitted to Kyorin University Hospital (Tokyo, Japan) between August 2012 and August 2014 with a diagnosis of CAP. Pneumonia was defined as the presence of new infiltrates on chest X-rays along with other suggestive signs and symptoms: cough, sputa, fever, chills, dyspnea, pleuritic chest pain, disturbance of consciousness, and crackles. Exclusion criteria included the following: (a) residence in a long-term nursing home or healthcare home; (b) hospitalization within the preceding 90 days; (c) elderly persons or physically disabled persons who needed healthcare; (d) continuous endovascular therapy (i.e., hemodialysis, anti-cancer, or immunosuppressive drugs); (e) onset of pneumonia 48 h after admission; and (f) active tuberculosis.

2.2. Clinical data collection

The following data were recorded on admission: age, sex, comorbid illnesses (chronic heart diseases, COPD, asthma, other lung diseases, diabetes mellitus, or active cancer), immunodeficiency status (i.e., use of immunosuppressive drugs or prednisolone dose of ≥ 5 mg/day, and HIV-positive patients), use of anti-microbial drugs (anti-bacterial or anti-influenza) before admission, and clinical or laboratory findings. Respiratory failure was defined as PaO_2 <60 mmHg or SpO_2 <90% in room air. In patients who underwent home oxygen therapy, respiratory failure was diagnosed at the point when further oxygen supply was needed to maintain the patient's previous condition.

The severity of pneumonia was assessed using the pneumonia severity index (PSI), a prediction rule with points assigned based on age, coexisting diseases, and abnormal physical findings. PSI stratifies CAP patients into five classes (I–V) to predict their risk of mortality [18]. Severe pneumonia is defined as PSI class IV or V. Follow-up variables included the need for mechanical ventilation (invasive and non-invasive) within 5 days of admission, and mortality within 30 days.

2.3. Samples

Samples collected on admission included sputum, nasopharyngeal swab (NPS), bronchoalveolar lavage fluid (BALF), blood, and urine. Serological tests for *Mycoplasma pneumoniae* were performed on admission and after several weeks, when possible. Invasive diagnostic methods were conducted according to clinical judgment. Respiratory samples for PCR-based detection of respiratory viruses, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae* were collected separately from those

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intended for bacterial culture and were stored at −80 °C until use.

2.4. Ethical approval

Samples were collected after written informed consent was obtained from the subjects or their legal representatives. The study protocol was approved by the Ethics Committee on Human Research of Kyorin University Hospital (H24-021) on July 31, 2012. The protocols were carried out in accordance with approved guidelines.

2.5. Bacteriological examination

Acceptable sputum and BALF samples were cultured on 5% blood agar, chocolate agar, and modified Conradi-Drigalski agar for the isolation and identification of bacterial pathogens. Blood cultures were incubated under aerobic and anaerobic conditions using the BacT/Alert system (bioMérieux, Marcy, l'Etoile, France). Urine samples were used for the detection of pneumococcus and *L. pneumophila* antigens using BinaxNOW® (Alere Medical Co., Ltd., Shinjuku, Japan).

2.6. RNA extraction, PCR, and gene sequencing of the pathogens

Samples were centrifuged at 3000*g* at 4 °C for 30 min. Viral RNA and DNA were extracted from supernatants using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA). Reverse transcription was performed using PrimeScript™ RT reagent Kit (Takara Bio, Otsu, Japan), according to the manufacturer's instructions. Using PCR, we aimed to detect various respiratory viruses such as HMPV, HRV, enterovirus, RSV, influenza viruses A, B, and C (InfV-A, B, and C), HPIV, human coronavirus, adenovirus, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, cytomegalovirus (CMV), human parvovirus B19, varicella zoster virus, and human bocavirus as described previously [19–26]. PCR products were purified using MonoFas DNA Purification Kit I (GL Sciences Inc., Shinjuku, Tokyo, Japan). The purified products were sequenced with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using the above primers [19–26]. Sequence analysis was performed on an ABI 3130 Genetic Analyzer (Applied Biosystems). The nucleotide sequences thus obtained were given GenBank accession numbers from LC020476 to LC020488.

2.7. Diagnostic criteria of causative pathogens

We considered that infection was caused by a specific pathogen if one or more of the following criteria were met. (1) Positive bacterial culture was obtained from acceptable sputum samples with a predominant species and compatible results from Gram staining. Samples were considered acceptable when >25 polymorphonuclear cells were observed under low-power magnification [27]. (2) The pathogen was cultured from blood samples and, except for the lungs, no other organs were known to be involved. (3) A urinary antigen test was positive for pneumococcus or *L. pneumophila*. (4) A fourfold increase in complement fixation titers for *Mycoplasma pneumoniae* was detected from paired sera. Finally, (5) respiratory viruses *Mycoplasma pneumoniae* or *Chlamydophila pneumoniae* were detected in respiratory samples by PCR [28]. CAP patients were classified into 4 etiological groups: virus alone, bacteria alone, co-detection of virus and bacteria, and not detected. Clinical characteristics including respiratory failure were compared between the groups.

2.8. Phylogenetic analyses by the neighbor-joining (NJ) method and genotyping of HMPV, RSV, and HRV

We performed phylogenetic analyses using Molecular Evolutionary Genetics Analysis (MEGA) software, version 5.0. Phylogenetic analysis of HMPV, RSV, and HRV were based on parts of the *F* gene (317 bp), *G* gene (240–312 bp on RSV-A, 234–294 bp on RSV-B), and *VP4/VP2* coding region (390 bp), respectively. Evolutionary distances were estimated using Kimura two-parameter method, and phylogenetic trees were constructed using the NJ method. Reliability of the trees was estimated using 1000 bootstrap replications.

2.9. Calculation of pairwise distances of detected respiratory viruses

We calculated pairwise distances (*p*-distance) of the HMPV, RSV, and HRV species detected in this study using MEGA software, version 5.0. Calculations were based on the nucleotide sequence of each virus, as described in Section 2.8.

2.10. Statistical analysis

Data was analyzed using StatView 5.0 (SAS Institute, Cary, NC, USA). Differences in proportions between the groups were assessed using Fisher's exact test (2 groups) and the chi-squared test (4 groups). Other comparisons between groups were done using Mann–Whitney *U* (2 groups) and Kruskal–Wallis (4 groups) tests. Statistical significance was defined by a two-sided α-level of 0.05.

3. Results

3.1. Data obtained from patients

Seventy-six patients were enrolled in this study. Forty-eight respiratory samples (sputum $n=46$, BALF $n=2$) were acceptable for bacterial culture and identification. Respiratory samples (NPS $n=59$, BALF $n=2$, sputum $n=15$) were used to detect viral and atypical pathogens by PCR-based methods. Urine antigen tests and blood cultures were examined in 66 and 72 patients, respectively.

3.2. Pathogen profiles of inpatients with CAP

In this study, 76 CAP inpatients were enrolled between August 2012 and August 2014, as detailed in Tables 1 and 2. Bacteria alone were detected in 30 patients (39.5%). Pneumococcus was the most frequently detected bacterium ($n=11$, 14.5%) followed by *Mycoplasma pneumoniae* ($n=4$), MSSA ($n=4$), *H. influenzae* ($n=2$), *M. catarrhalis* ($n=2$), and *Streptococcus anginosus* ($n=2$). Neither

Table 1 – Summary of patient characteristics.

	All patients	Virus alone	Bacteria alone	Co-detection	Not detected	*p* value
Number of patients	76 (100)	8 (10.5)	30 (39.5)	9 (11.8)	29 (38.2)	
Age*	71.5 (58.3–78.0)	76.5 (69.5–78.0)	67.5 (48.8–74.8)	68.0 (64.0–77.0)	73.0 (52.0–78.0)	NS
Male	51 (67.1)	4 (50.0)	22 (73.3)	5 (55.6)	20 (69.0)	NS
Severe pneumonia (PSI class IV or V)	39 (51.3)	4 (50.0)	15 (50.0)	6 (66.7)	14 (48.3)	NS
Wheezing	20 (26.3)	2 (25.0)	8 (26.7)	5 (55.6)	5 (17.2)	NS
Respiratory failure	51 (67.1)	8 (100) [a, b]	17 (56.7) [a, c]	9 (100) [c, d]	17 (58.6) [b, d]	<0.05
Mechanical ventilation	7 (9.2)	1 (12.5)	2 (6.7)	2 (22.2)	2 (6.9)	NS
(Invasive mechanical ventilation)	5 (6.6)	0 (0)	1 (3.3)	2 (22.2)	2 (6.9)	NS
Mortality within 30 days#	3/64 (4.7)	0/8 (0)	1/22 (4.5)	1/8 (12.5)	1/26 (3.8)	NS
Duration of respiratory failure (days)	5.0 (0–10.0)	7.0 (5.0–11.3)	3.0 (0–8.8)	7.0 (4.0–16.0)	3.0 (0–10.0)	NS
Laboratory findings						
White blood cell count, 10^6 cells/mL*	11.1 (7.9–15.4)	7.7 (6.8–9.7)	13.1 (9.0–15.3)	9.4 (5.8–15.3)	11.1 (8.6–15.6)	NS
C-reactive protein, mg/dL*	11.4 (4.1–23.1)	4.1 (2.8–11.3)	13.0 (6.8–22.0)	6.6 (2.7–20.2)	17.1 (2.7–25.9)	NS
Procalcitonin, ng/mL*	0.39 (0.09–2.46)	0.13 (0.06–0.60)	0.80 (0.11–2.84)	0.29 (0.09–4.89)	0.41 (0.11–0.94)	NS
Comorbidity						
Asthma	8 (10.5)	1 (12.5)	3 (10.0)	3 (33.3)	1 (3.4)	NS
COPD	20 (26.3)	2 (25.0)	9 (30.0)	2 (22.2)	7 (24.1)	NS
Chronic heart disease	18 (23.7)	3 (37.5)	5 (16.7)	1 (11.1)	9 (31.0)	NS
Immunodeficiency	10 (13.2)	0 (0)	2 (6.7)	2 (22.2)	6 (20.7)	NS
Diabetes mellitus	13 (17.1)	2 (25.0)	6 (20.0)	1 (11.1)	4 (13.8)	NS
Malignancy	4 (5.3)	0 (0)	3 (10.0)	0 (0)	1 (3.4)	NS
Other lung disease	8 (10.5)	1 (12.5)	3 (10.0)	0 (0)	4 (13.8)	NS
Antimicrobials						
Anti-bacterial drug	22 (28.9)	5 (62.5) [e, f]	4 (13.3) [e, g]	0 (0) [f, h]	13 (44.8) [g, h]	<0.05
Anti-influenza drug	4 (5.3)	1 (12.5)	1 (3.3)	0 (0)	1 (3.4)	NS

Data are expressed as number (percentage) unless otherwise stated.
* Data are expressed as median (IQR).
# Data are expressed as number of deaths/*n* (percentage) and missing date was excluded. NS: not significant.
[a] $p=0.035$: virus alone vs bacteria alone.
[b] $p=0.036$: virus alone vs not detected.
[c] $p=0.018$: co-detection vs bacteria alone.
[d] $p=0.036$: co-detection vs not detected.
[e] $p=0.010$: virus alone vs bacteria alone.
[f] $p=0.010$: virus alone vs co-detection.
[g] $p=0.016$: bacteria alone vs not detected.
[h] $p=0.010$: co-detection vs not detected.

Mycoplasma pneumoniae nor *Chlamydophila pneumoniae* were detected by PCR. All patients infected with *Mycoplasma pneumoniae* were diagnosed using paired sera.

In 8 of the 76 patients (10.5%), viral infections alone were detected; they included HMPV ($n=3$), CMV ($n=2$), RSV ($n=1$), InfV-A ($n=1$), and HPIV ($n=1$). Furthermore, bacterial and viral co-detection was found in 9 (11.8%) patients, and in all those with HRV, where the main pathogenic pattern was HRV+pneumococcus ($n=2$). No pathogens were detected in 29 (38.2%) patients. Three patients (MSSA/*Pseudomonas aeruginosa*, $n=1$; unknown, $n=1$; HRV/pneumococcus, $n=1$) died within 30 days of admission. The first two patients died of respiratory failure on days 3 and 17, while the third died on day 2 from severe sepsis.

3.3. *Proportion of patients with severe pneumonia among the groups*

No significant differences were found between the etiological groups (virus alone, bacteria alone, co-detection, or not detected) and the proportions of patients with severe pneumonia (PSI IV or V) in each of them (Table 1).

However, significant differences between the groups were found in relation to rates of respiratory failure and antibiotics use.

Table 2 – Distribution of detected pathogens.

Virus alone	8
HMPV	3
CMV	2
RSV	1
InfV-A	1
HPIV	1
Bacteria alone	30
Pneumococcus	11
M. pneumoniae	4
MSSA	4
M. catarrhalis	2
H. influenzae	2
S. anginosus	2
P. aeruginosa	1
E. coli	1
S. marcescens	1
Pneumococcus+*H. influenzae*	1
P. aeruginosa+MSSA	1
Virus+bacteria	9
HRV	
Pneumococcus	2
Group G *Streptococcus*	1
Pneumococcus+*H. influenzae*	1
HMPV	
Pneumococcus	1
MSSA	1
Pneumococcus+*H. influenzae* +MSSA	1
RSV	
Pneumococcus	1
CMV+pneumococcus	1

HMPV: human metapneumovirus; CMV: cytomegalovirus; RSV: respiratory syncytial virus; InfV-A: influenza virus A; HPIV: human parainfluenza virus; HRV: human rhinovirus; Pneumococcus: *Streptococcus pneumoniae*; MSSA: methicillin sensitive *Staphylococcus aureus*.

3.4. *Virus-positive (virus alone and co-detection groups) vs bacteria alone groups*

Among CAP patients, the frequency of respiratory failure was more common when a virus was detected (both virus only and co-detection groups, $n=17$, 100%, $p<0.05$) than in bacteria alone cases ($n=17$, 56.7%; Table 1).

Respiratory failure in the virus-positive groups (virus alone, median 7, interquartile range (IQR) 5–11.3 days; co-detection, median 7, IQR 4–16 days) lasted longer than in the bacteria alone group (median 3, IQR 0–8.8 days).

3.5. *Co-detection group vs bacteria alone group*

The co-detection group (100%) was more susceptible to respiratory failure than the bacteria alone group (56.7%, $p<0.05$). The combinations of pathogens found in the co-detection group are shown in Table 2. The need for invasive mechanical ventilation was higher in the co-detection group ($n=2$, 22.2%) than in the bacteria alone (*Mycoplasma pneumoniae*) group ($n=1$, 6.7%), but this result was not statistically significant ($p=0.06$). The combinations of causative pathogens in the co-detection group were HRV+pneumococcus ($n=1$), and HRV+pneumococcus+*H. influenzae* ($n=1$).

3.6. *Genetic and phylogenetic properties of respiratory viruses detected in CAP inpatients*

We constructed phylogenetic trees based on the nucleotide sequences of genes from the various viruses. Phylogenetic trees constructed by the NJ method are shown in Fig. 1a–c. The most commonly detected virus was HMPV (HMPV alone, 3 patients; HMPV+bacteria, 3 patients). Phylogenetic analysis confirmed the HMPV subgroups to be A2 (2 strains), B1 (1 strain), and B2 (3 strains), which may be prevalent in Japan. Three RSV strains (RSV alone, 1 patient; RSV+bacteria, 2 patients) were detected among inpatients, and their genotypes were confirmed as ON1 (1 strain) and BA9 (2 strains). In addition, 4 HRV strains (HRV+bacteria) were detected, all of which belonged to HRV-A genotypes (HRV-A1, HRV-A29, HRV-A103, and HRV-A71).

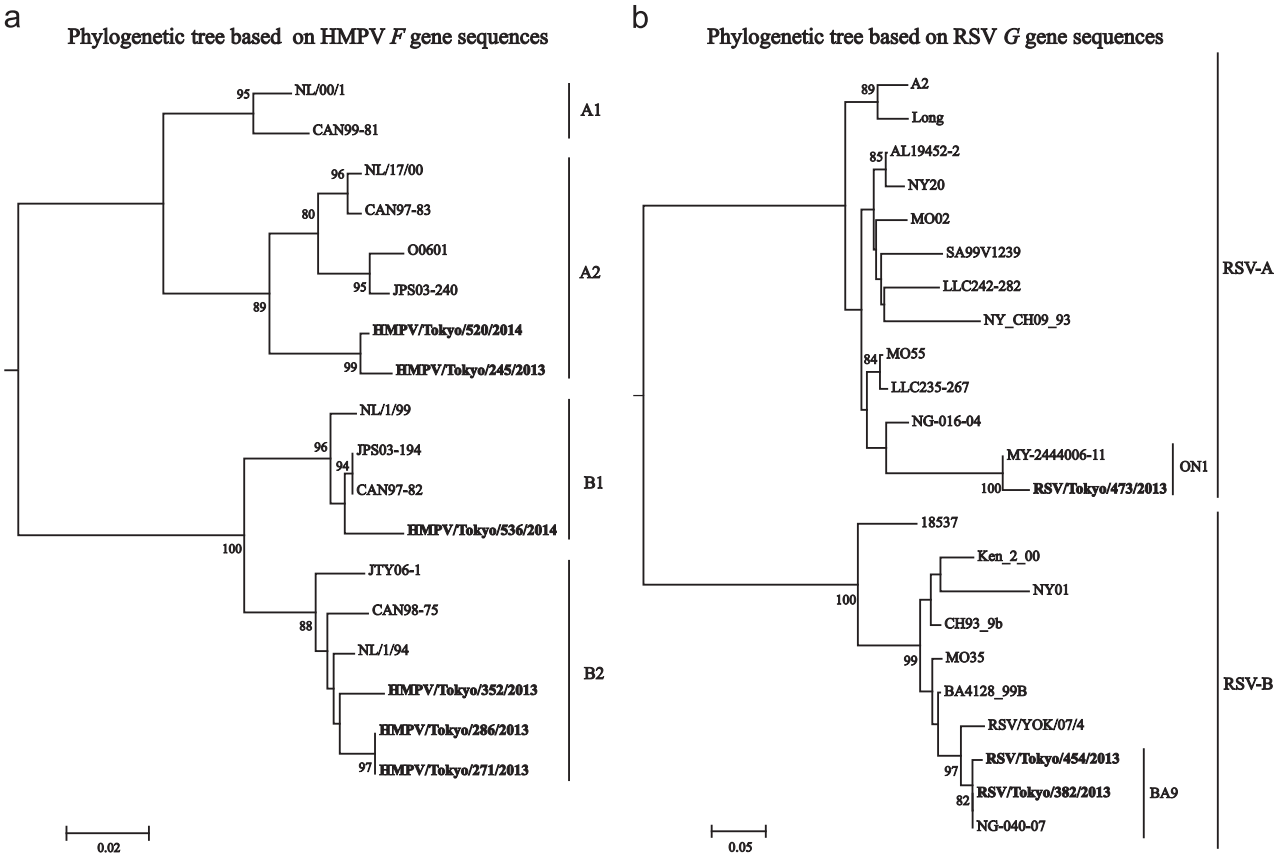
The *p*-distance values between the detected HMPV, RSV, and HRV strains were 0.096 ± 0.061, 0.47 ± 0.40, and 0.22 ± 0.031, respectively. These results indicate that the viruses belonged to various subgroups and genotypes, and the strains displayed relatively high genetic diversity.

4. Discussion

We conducted pathogen profiling of Japanese inpatients (76 cases) with CAP and performed genetic analyses of the various respiratory viruses detected such as HMPV, RSV, and HRV. Bacteria alone were detected in 39.5% patients (30 cases), while viruses alone were detected in 10.5% patients (8 cases). Co-detection was noted in 11.8% patients (9 cases). Pneumococcus and HMPV were the most commonly detected bacterium and virus, respectively. In addition, phylogenetic analyses of HMPV, RSV, and HRV indicate that different subgroups and genotypes of the viruses may be associated with CAP inpatients in Japan.

Many previous reports have suggested that pneumococcus is a major causative pathogen in CAP [2–5,28]. For example, Lim et al. showed that pneumococcus was the predominant causative bacterium in hospitalized CAP patients in the United Kingdom, followed by *Chlamydophila pneumoniae* and *H. influenzae* [29]. Ishida et al. reported that the bacterial pathogens in Japanese CAP patients were pneumococcus, *H. influenzae*, and *Mycoplasma pneumoniae*, in descending order of prevalence [30]. The detection frequencies of the isolated bacteria were similar to those in the present study.

Few studies have been reported on pathogen profiles, including respiratory viruses, in adult Japanese CAP patients [31]. In addition, the molecular epidemiology of CAP-associated viruses is poorly understood; although, some CAP-associated respiratory viruses such as HMPV and HRV-C have recently been confirmed [32–34]. Our results show that HMPV, RSV, HRV, and HPIV are associated with CAP in adult inpatients. The viruses were classified into subgroups and genotypes based on genetic divergence in the phylogenetic trees (Fig. 1a–c). This indicates that viruses such as HMPV, RSV, and HPIV could have been associated with over 10% of


a
Phylogenetic tree based on HMPV F gene sequences
NL/00/1
CAN99-81
NL/17/00
CAN97-83
O0601
JPS03-240
HMPV/Tokyo/520/2014
HMPV/Tokyo/245/2013
NL/1/99
JPS03-194
CAN97-82
HMPV/Tokyo/536/2014
JTY06-1
CAN98-75
NL/1/94
HMPV/Tokyo/352/2013
HMPV/Tokyo/286/2013
HMPV/Tokyo/271/2013
A1
A2
B1
B2
0.02
b
Phylogenetic tree based on RSV G gene sequences
A2
Long
AL19452-2
NY20
MO02
SA99V1239
LLC242-282
NY_CH09_93
MO55
LLC235-267
NG-016-04
MY-2444006-11
RSV/Tokyo/473/2013
ON1
RSV-A
18537
Ken_2_00
NY01
CH93_9b
MO35
BA4128_99B
RSV/YOK/07/4
RSV/Tokyo/454/2013
RSV/Tokyo/382/2013
NG-040-07
BA9
RSV-B
0.05
c
Phylogenetic tree based on HRV VP4/VP2 coding region
HRV_A1
HRV/Tokyo/419/2013
HRV_A55
HRV_A90
HRV_A49
HRV_A31
HRV_A29
HRV/Tokyo/305/2013
HRV_A101
HRV_A103
HRV/Tokyo/529/2014
HRV_A51
HRV_A71
HRV/Tokyo/185/2013
HRV-A
HRV_B4
HRV-B
HRV_QPM
HRV-C
0.1

CAP inpatients. Although we found no correlation between the phylogenies of these viruses and disease severity, the estimate was inferred from the seasonal prevalence of these strains [35]. Regarding CMV detection, it is not possible to distinguish between active respiratory infection and asymptomatic/latent infection, especially in clinically ill patients [36,37].

The present study confirmed two previous observations. First, bacteria (alone) were the most frequently detected pathogens among CAP patients (39.5%), and there was equal prevalence of virus only (10.5%) and co-detection (11.8%), as reported before [28,38,39]. Thus, viral infections should be considered major pathogens of CAP, even in adult patients.

Second, respiratory failure on admission was significantly more common ($p<0.05$) and lasted longer in the virus or co-detection groups than in the bacteria alone group. Similarly, Johansson et al. reported that co-infected CAP patients suffered from tachypnea and required oxygen therapy over a longer period compared with subjects infected with bacteria alone [40].

Bacterial and viral co-infections may increase the severity of pneumonia. Several mechanisms have been proposed such as alteration of the host immune response, deterioration of the respiratory status following co-infection with pneumococcus and influenza virus in mice [41], and increased susceptibility to bacterial (pneumococcus) and/or viral infection (HMPV) caused by previous viral (HMPV/influenza virus) infections in mice and *in vitro* [42,43].

Here, we show that 2 of the 4 patients in the co-detection group with HRV and bacteria (pneumococcus or pneumococcus+*H. influenzae*) required mechanical ventilation. Previous studies reported that HRV caused secondary bacterial infections in up to 60% of COPD patients due to cleavage of antimicrobial peptides, secretory leukoprotease inhibitor or elafin, by virus-induced neutrophil elastase, supporting a hypothesis that co-detection (HRV+bacteria) itself is vulnerable to more severe respiratory status. [44–46]. Here, bacteria were co-detected in all 4 HRV patients, which might explain the high frequency of invasive mechanical ventilation. Comorbidity may have an impact on the etiology of CAP; some studies have shown that patients with cardiovascular disease are vulnerable to viral CAP [47,48]. However, we found that the proportions of comorbid illnesses (i.e., COPD, asthma, and chronic heart disease) did not differ between groups (virus alone, bacteria alone, co-detection) (Table 1).

Thus, we conclude that viral and bacterial co-infection could lead to deterioration of respiratory status in adult CAP patients.

The rate of detection of influenza virus in CAP patients (1.3%) was lower than previously reported (4.4–9.5%) [14,28,39]. This might reflect the high frequency of neuraminidase inhibitors (NAIs) administered to patients with influenza in Japan [49].

Our study had the following limitations. First, it was conducted at a single tertiary center in the west side of Tokyo; hence, results may be affected by both anti-bacterial and anti-influenza drugs prescribed prior to admission by doctors in that area. Second, seasonal influenza virus was detected in only one case. This may be a result of the characteristic facilities of a university hospital. Hence, there may have been some bias in the selection of the present subjects.

Finally, upper respiratory samples, such as NPS, were used for viral detection in this study. It has been suggested that some respiratory viruses such as HRV and HMPV can be detected in these samples in up to 2.1% of healthy adults [17,50]; however, we did not examine healthy adult controls. Self et al. reported that any respiratory virus could be associated with adult CAP; therefore, the viruses detected in this study were possible pathogens.

To the best of our knowledge, this is the first genetic and phylogenetic investigation of viral infections in Japanese adult CAP patients.

5. Conclusion

This study demonstrates that respiratory viruses (i.e., HMPV, RSV, or HRV) displaying high genetic divergence are present in approximately 22.3% of adult CAP patients in whom viral infections can accelerate respiratory failure.

Fig. 1 – (a) Phylogenetic tree of HMPV nucleotide sequences based on *F* gene (317 bp). The phylogenetic tree was constructed by the neighbor-joining method using MEGA software 5.0. Distances were calculated according to Kimura's two-parameter method. Bootstrap values ≥80% are shown at the branch nodes. The tree includes the present strains ($n=6$) and reference strains ($n=12$). The following references strains were used: NL/00/1 (AF371337), CAN99-81 (M18759), NL/17/00 (AY304360), CAN97-83 (AY145296), O0601 (EF589610), JPS03-240 (AY530095), NL/1/99 (AY304361), JPS03-194 (AY530094), CAN97-82 (EU814623), JTY06-1 (EU127917), CAN98-75 (M18761), and NL/1/94 (AY304362). (b) Phylogenetic tree of RSV nucleotide sequences based on the *G* gene (240–312 bp). The phylogenetic tree was constructed by the neighbor-joining method using MEGA software 5.0. Distances were calculated according to Kimura's two-parameter method. Bootstrap values ≥80% are shown at the branch nodes. The tree includes the present strains ($n=3$) and reference strains ($n=20$). The following references strains were used: A2 (M11486), Long (AY911262), AL19452-2 (AF233901), NY20 (AF233918), MO02 (AF233910), SA99V1239 (AF348808), LLC242-282 (AY114150), NY_CH09_93 (AF065254), MO55 (AF233915), LLC235-267 (AY114149), NG-016-04 (AB470478), MY-2444006-11 (JX256871), 18537 (M17213), Ken_2_00 (AY524575), NY01 (AF233931), CH93_9b (AF065251), MO35 (AF233929), BA4128_99B (AY333364), RSV/YOK/07/4 (AB551076), and NG-040-07 (AB470478). (c) Phylogenetic tree for HRV-A nucleotide sequences based on *VP4/VP2* coding region (390 bp). The phylogenetic tree was constructed by the neighbor-joining method using MEGA software 5.0. Distances were calculated according to Kimura's two-parameter method. Bootstrap values ≥80% are shown at the branch nodes. The tree includes the present strains ($n=4$) and reference strains ($n=12$). The sequences of the reference strains were obtained from picornaviridae.com (URL: http://www.picornaviridae.com/).

Conflict of interest

The authors have no conflicts of interest.

Authors' contributions

DK, TS, HI, HTs, AR, KO, HT, and HK designed the research; DK, YS, TS, HTs, KK, and TI performed the research; YS, HTs, MK, and KO contributed analytic tools; DK, YS, TS, and HK analyzed data; DK, YS, TS, HT, and HK wrote the paper.

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