Parainfluenza Virus Type 3 Co-infection with Other Respiratory Pathogens Among Hospitalized Children with Acute Respiratory Infections in Wuhan, China

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Abstract

Background: Bacterial and viral co-infections are increasingly recognized as the cause of Acute Respiratory Infection (ARI). The role of co-infection in ARI patients with Parainfluenza Virus type 3 (PIV3) infection is unclear.

Objectives: This study aimed to determine the prevalence of PIV3 co-infections in hospitalized children and assess the co-infections' role in ARI patients with PIV3 infections.

Methods: Between January 2018 and December 2021, children were confirmed to have a PIV3 infection via throat swabs or nasopharyngeal aspirates. Some digital clinical data were analyzed, including demographic, epidemiological, diagnostic, and laboratory data.

Results: During the study period from 2018 to 2021, 2,539 patients were hospitalized with ARI caused by PIV3. Of them, 34.0% had co-infection with other pathogens, and 2.4% had co-infection with more than two pathogens. *Mycoplasma pneumoniae* was the most common co-infecting pathogen (71.3%), followed by other bacteria (13.3%) and viruses (8.2%). A significantly higher proportion of patients with *M. pneumoniae* co-infection was found in girls ($\chi^2 = 19.233$, P < 0.001). Co-infections with *M. pneumoniae* were observed principally in patients aged 1 – 2 years ($\chi^2 = 202.130$, P < 0.001). In contrast, viral (56.3%) and bacterial (66.1%) co-infections occurred mainly in children younger than one year. The diagnosis of PIV3 as a single infection included pneumonia (41.2%), bronchitis (39.9%), upper respiratory tract infections (15.0%), and laryngitis (3.9%), which were distinguished from those with bacterial co-infections ($\chi^2 = 16.424$, P = 0.001) and co-infections with more than two pathogens ($\chi^2 = 11.687$, P = 0.010). Co-infections of PIV3 with any pathogen were not associated with admissions to intensive care units or ventilator support. However, the mean hospitalization was significantly higher in *M. pneumoniae* co-infections ($t = 2.367$, P = 0.018), bacterial co-infections ($t = 2.402$, P = 0.016), and co-infections with more than two pathogens ($t = 2.827$, P = 0.006) than in single PIV3 infection.

Conclusions: Parainfluenza virus type 3 frequently occurs with other pathogens. The epidemiological and clinical characteristics of co-infections with different pathogens differed. *Mycoplasma pneumoniae* co-infections, bacterial co-infections, and co-infections with more than two pathogens lengthened the hospitalization. Bacterial co-infections and co-infections with more than two pathogens increased the severity of ARI and worsened the symptoms.

Keywords: Parainfluenza Virus Type 3, Co-infection, Acute Respiratory Infections

1. Background

Acute Respiratory Infections (ARIs) are the major cause of morbidity and mortality in children under the age of five, with developing countries having the highest number of deaths (1, 2). Various pathogens, including bacteria and viruses, can cause ARIs. The most common viruses that cause ARI are Respiratory Syncytial Virus (RSV), adenovirus (ADV), influenza virus A/B (FluA/B), and parainfluenza viruses (1, 3, 4). Parainfluenza Viruses (PIVs) can cause various respiratory infections, ranging from mild upper respiratory illnesses in healthy adults to croup, bronchiolitis, pneumonia in infants and children, and severe lower respiratory infections in elderly and immunocompromised people (3). In China, Parainfluenza Virus type 3 (PIV3) is the most virulent of the four PIV serotypes, accounting for 60 - 70% of primary PIV infections, and the PIV3 infection frequency is approximately 2.56 – 10.9% (1, 4-6).

Bacterial and viral co-infections are becoming more widely recognized as possible causes of ARIs (7, 8). The po-

tential pathogens are numerous, but improved detection methods have allowed for increased detection, resulting in greater epidemiological understanding. Also, PIVs were commonly co-infect with other respiratory pathogens in all PIV types, and PIV4 co-infection has been reported in previous research (6, 9). The role of PIV3 co-infection in the pathogenesis of ARIs is unknown; additionally, the possible impact of age, gender, and seasonality on the prevalence of co-infections is unknown.

2. Objectives

This study aimed to estimate the prevalence of PIV3 co-infections in hospitalized children and assess the co-infections' role in ARI patients with PIV3 infections.

3. Methods

3.1. Patient Selection

From January 2018 to December 2021, children hospitalized with ARIs in the Maternal and Child Health Hospital of Hubei Province were enrolled retrospectively in this study. Children with respiratory tract infection signs and symptoms (e.g., coughing, fever, nasal obstruction, and sneezing) or lower respiratory infection signs (e.g., tachypnea, dyspnea, or wheezing/rales upon auscultation) were classified as having ARI. Some digital clinical data were analyzed, including demographic, epidemiological, diagnostic, and laboratory data.

3.2. Laboratory Tests

3.2.1. Respiratory Virus Detection

We tested for PIV1–3 and four other common respiratory viruses, ADV, FluA/B, and RSV, using direct immunofluorescence assays (D3 Ultra DFA Respiratory Virus Screening and Identification Kit, Diagnostic Hybrids, Inc., USA). The kit comprised a DFA screening reagent that contained a mixture of Murine Monoclonal Antibodies (MAbs) directed against seven respiratory viruses. The DFA screening reagent is introduced to the specimens to assess the presence of viral antigens. The stained cells were rinsed with the diluted Wash Solution after incubation at 35°C to 37°C. A drop of the provided Mounting Fluid was poured, and the prepared cells were covered with a coverslip. The cells were inspected using a fluorescent microscope. When stained with the DFA screening reagent, virus-infected cells would show viral-specific apple-green fluorescence, whereas non-infected cells would show no fluorescence but would be stained red by the Evans Blue counter-stain.

3.2.2 Mycoplasma pneumoniae Detection

Passive particle agglutination kits (SERODIA®-MYCOII; Fujirebio Inc., Japan) were used to evaluate specific *Mycoplasma pneumoniae* antibodies. The kit detects a wide range of antibodies against *M. pneumoniae* but mostly detects IgM; therefore, its sensitivity is substantially higher than that of a single antibody test. The sera were serially diluted to 1:20, 1:40, 1:80, and 1:160, then co-incubated with sensitized gelatin particles in U-shaped 96-well microtiter plates to generate final dilutions of 1:40, 1:80, 1:160, and 1:320, mixed thoroughly, and stored at room temperature for three hours. An *M. pneumoniae* co-infection was defined as single titers of serum *M. pneumoniae* antibody $\geq$ 1:320 or seroconversion (increased antibody titers $\geq$ 4 folds) (10).

3.2.3. Microbial Culture and Identification

Evidence of bacterial co-infections was obtained from sterile or nonsterile sites, including bronchoalveolar lavage, sputum, and nasopharyngeal swabs. Microbial culture and identification were performed following routine diagnostic standard operating procedures in this hospital's clinical laboratory. Bronchoalveolar lavage and/or sputum were collected and cultured from patients with suspected ARI. A blood plate, a chocolate plate, and a MacConkey plate were used to inoculate the samples. Then, the blood plate and chocolate plate were incubated for 72 hours or until a positive result was observed in a carbon dioxide incubator with a concentration of 5%-10%, and the MacConkey plate for 24 - 48 hours in an incubator at 35 - 37°C. Bruker Matrix-assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry was used to identify positive cultures to the species level (Bruker Daltonik GmbH, Leipzig, Germany).

3.3. Exclusion Criteria

Exclusion criteria were as follows: (1) patients with chronic pulmonary disease that might affect the chest X-ray results, aspiration pneumonia, or interstitial lung disease, (2) patients with compromised immune systems or those taking immunosuppressive medications, (3) patients with suspected hospital-acquired infection or fungal infection, and (4) patients with insufficient clinical information.

3.4. Statistical Analyses

We used SPSS v21.0 software for statistical analysis (SPSS, Inc., Chicago, IL, USA). Chi-square or Fisher's exact tests were used to compare frequency distributions across groups. The independent sample *t*-test was used to analyze quantitative data with a normal distribution. The data are expressed as the mean ± standard deviation. A P-value $\leq$ 0.05 was considered statistically significant.
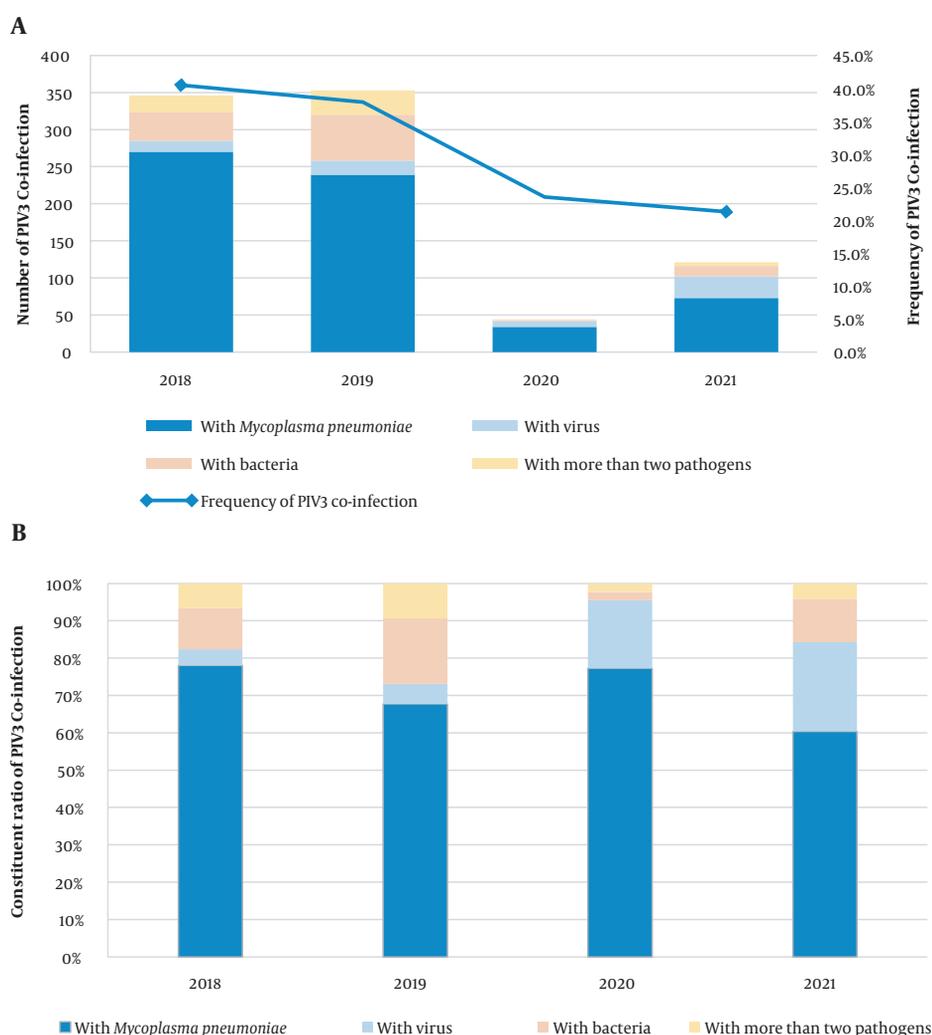
Figure 1. (A) Frequency of PIV3 co-infection; (B) Constituent ratio of PIV3 co-infection

4. Results

4.1. Frequency and Constituent Ratios for Parainfluenza Virus Type 3 Co-infections

During the study period from 2018 to 2021, 2,539 patients were hospitalized with ARI caused by PIV3. Of them, 34.0% (864/2539) had co-infections with other pathogens, and 2.4% (62/2539) were co-infected with more than two pathogens. Also, *M. pneumoniae* was the most common co-infecting pathogen (71.3%, 616/864), followed by other bacteria (13.3%, 115/864) and viruses (8.2%, 71/864). Figure 1 shows the co-infection rate for PIV3 and the constituent ratio of co-infecting pathogens for each year. The co-infection rate for viruses increased from 4.3% in 2018 to 5.4% in 2019, 18.2% in 2020, and 24.0% in 2021.

4.2. Respiratory Pathogens Co-infected with Parainfluenza Virus Type 3

In the viral co-infection cohort, the most frequently co-detected virus was RSV (60.6%), followed by PIV1 (25.4%), ADV (9.9%), FluB (2.8%), and PIV2 (1.4%). In the bacterial co-infection cohort, the most frequently co-detected bacteria were *Streptococcus pneumoniae* (51.3%), followed by *Haemophilus influenzae* (20.9%), *Staphylococcus aureus* (20.9%), *Klebsiella pneumoniae* (6.1%), and *Legionella pneumophila* (0.8%). The three most common co-infections with more than two pathogens were *M. pneumoniae* + *S. pneumoniae* (35.5%), *M. pneumoniae* + RSV (14.5%), and *M. pneumoniae* + *S. aureus* (11.3%) (Table 1).

Table 1. Parainfluenza Virus Type 3 Co-infection with Respiratory Pathogens

Variables	No. (%)
With *Mycoplasma pneumoniae* (n = 616)	
M. pneumoniae	616 (100)
With virus (n = 71)	
PIV1	18 (25.4)
PIV2	1 (1.4)
RSV	43 (60.6)
ADV	7 (9.9)
FluB	2 (2.8)
With bacteria (n = 115)	
Staphylococcus aureus	24 (20.9)
Haemophilus influenzae	24 (20.9)
Streptococcus pneumoniae	59 (51.3)
Klebsiella pneumoniae	7 (6.1)
Legionella pneumophila	1 (0.8)
With more than two pathogens (n = 62)	
M. pneumoniae + PIV1	2 (3.2)
M. pneumoniae + ADV	1 (1.6)
M. pneumoniae + FluA	4 (6.5)
M. pneumoniae + FluB	1 (1.6)
M. pneumoniae + RSV	9 (14.5)
M. pneumoniae + S. pneumoniae	22 (35.5)
M. pneumoniae + K. pneumoniae	1 (1.6)
M. pneumoniae + S. aureus	7 (11.3)
M. pneumoniae + H. influenzae	3 (4.8)
M. pneumoniae + RSV + S. pneumoniae	1 (1.6)
M. pneumoniae + PIV1 + S. pneumoniae	1 (1.6)
M. pneumoniae + RSV + ADV	2 (3.2)
M. pneumoniae + H. influenzae + K. pneumoniae	1 (1.6)
ADV + S. pneumoniae	1 (1.6)
ADV + S. aureus	1 (1.6)
RSV + H. influenzae	2 (3.2)
RSV + K. pneumoniae	2 (3.2)
H. influenzae + S. pneumoniae	1 (1.6)

4.3. Gender and Age Distribution of Parainfluenza Virus Type 3 Co-infections

As shown in Table 2, there were 1,061 boys and 614 girls in 1,675 cases of PIV3 single infections (male-to-female ratio = 1.73:1), and 328 boys and 288 girls in 616 cases of *M. pneumoniae* co-infections (male-to-female ratio = 1.14:1). A significantly higher proportion of girls with *M. pneumoniae* co-infection was observed than PIV3 mono-infection

patients (χ^2 = 19.233, P < 0.001). Compared with the PIV3 mono-infection group, *M. pneumoniae* co-infections were found mainly in patients 1 – 2 years of age (χ^2 = 202.130, P < 0.001). Co-infections with more than two pathogens also predominated in patients 1 – 2 years of age (χ^2 = 7.903, P = 0.056), although not statistically significant. As observed in the mono-infection group, viral (56.3%) and bacterial (66.1%) co-infections occurred mainly in children younger than one year.

4.4. Comparison of Clinical Characteristics of Patients with Parainfluenza Virus Type 3 Mono-infections and Co-infections

As shown in Table 3, PIV3 co-infections with any pathogen were not associated with ICU admissions and ventilator support. However, the mean length of stay of children with *M. pneumoniae* co-infections (t = 2.367, P = 0.018), bacterial co-infections (t = 2.402, P = 0.016), and co-infections with more than two pathogens (t = 2.827, P = 0.006) was significantly higher than that in children infected with a single PIV3 infection. Children with *M. pneumoniae* co-infections had a higher percentage of segmented neutrophils (t = 2.437, P = 0.015), band neutrophils (t = 5.009, P < 0.001), and monocytes (t = 2.090, P = 0.037), and a lower percentage of lymphocytes (t = 4.637, P < 0.001) than children with a single PIV3 infection. Similarly, bacterial co-infections and co-infections with more than two pathogens were presented with more segmented neutrophils and a lower percentage of lymphocytes. Bacterial co-infections also produced significantly higher white blood cell counts (t = 2.510, P = 0.012).

4.5. Comparison of Clinical Diagnosis of Patients with Parainfluenza Virus Type 3 Mono-infections and Co-infections

The clinical diagnoses for the six groups are shown in Table 4. The common diagnosis for PIV3 single infections were pneumonia (41.2%), bronchitis (39.9%), and upper respiratory tract infections (15.0%), which were distinguished from those with bacterial co-infections (χ^2 = 16.424, P = 0.001) and co-infections with more than two pathogens (χ^2 = 11.687, P = 0.010).

5. Discussion

The prevalence of PIV3 co-infections varies greatly across populations. Previously, PIV3 co-infections were observed in approximately 76% of children in Beijing, 43% in Changsha, 40.4% in Guangzhou, and 6.58% in Wenzhou (6, 9, 11, 12). Our findings revealed that among the 2539 ARI patients infected with PIV3, 34% had co-infections with other pathogens, and 2.4% had co-infections with more than two pathogens. The age group, comorbidities, disease severity

Table 2. Gender and Age Distribution of Parainfluenza Virus Type 3 Co-infections [a]

Variables	With *Mycoplasma pneumoniae*	With Virus	With Bacteria	With More Than Two Pathogens	Total	PIV3 Mono-infection
Gender						
Male	328 (53.2)	53 (74.6)	79 (68.7)	33 (53.2)	493 (57.1)	1061 (63.3)
Female	288 (46.8)	18 (25.4)	36 (31.3)	29 (46.8)	371 (42.9)	614 (36.7)
χ^2	19.233	3.797	1.333	2.625	9.477	
P-value	< 0.001	0.052	0.248	0.105	0.002	
Age, y						
< 1	133 (21.6)	40 (56.3)	76 (66.1)	25 (40.3)	274 (31.7)	917 (54.7)
1 - 2	378 (61.4)	25 (35.2)	33 (28.7)	31 (50.0)	467 (54.1)	591 (35.3)
3 - 5	96 (15.6)	5 (7.0)	6 (5.2)	5 (8.1)	112 (13.0)	160 (9.6)
≥ 6	9 (1.5)	1 (1.4)	0 (0.0)	1 (1.6)	11 (1.3)	7 (0.4)
χ^2	202.130	1.944	6.496	7.903	124.714	
P-value	< 0.001	0.561	0.098	0.056	< 0.001	

[a] Values are expressed as No. (%).

Table 4. Comparison of Clinical Diagnosis of Patients with Parainfluenza Virus Type 3 Mono-infection and Co-infection [a]

Clinical Diagnosis	With *Mycoplasma pneumoniae*	With Virus	With Bacteria	With More Than Two Pathogens	Total	PIV3 Mono-infection
Pneumonia	278 (45.1)	33 (46.5)	47 (40.9)	38 (61.3)	396 (45.8)	690 (41.2)
Laryngitis	32 (5.2)	2 (2.8)	1 (0.9)	1 (1.6)	36 (4.2)	66 (3.9)
Bronchitis	211 (34.3)	31 (43.7)	62 (53.9)	20 (32.3)	324 (37.5)	668 (39.9)
Upper respiratory tract infection	95 (15.4)	5 (7.0)	5 (4.3)	3 (4.8)	108 (12.5)	251 (15.0)
χ^2	7.082	3.854	16.424	11.687	6.257	
P-value	0.069	0.274	0.001	0.010	0.100	

[a] Values are expressed as No. (%).

of patients, antibiotic exposure, detection methods used, and spatiotemporal variations all contributed to the variability in the overall proportions of co-infections in the current study. It is worth noting that in 2018 and 2019, the co-infection rates of PIV3 were 40.5% and 37.9%, respectively, while in 2020 and 2021, after the outbreak of COVID-19 in Wuhan, the co-infection rate of PIV3 decreased to 23.5% and then to 21.3%.

Mycoplasma pneumoniae was found to be the most common co-infecting pathogen (71.3%), followed by other bacteria (13.3%) and viruses (8.2%). Infections with *M. pneumoniae* may be more common in China than in other countries (13-15). An investigation conducted in China found that the *M. pneumoniae* infection rate in 593 hospitalized children with segmental/lobar pattern pneumonia was 72.34%, while another study found that *M. pneumoniae* infections were responsible for 32.4% of community-acquired pneumonias (14, 15). Notably, the co-infection rate with viruses increased to 14.2% in 2020, while the co-infection rate with

bacteria decreased from 17.6% in 2019 to 2.3%. This could be due to a shift in the co-infection pattern caused by the COVID-19 epidemic in 2020, reduced personnel movement, and increased personal protection measures. These measures may have a greater impact on the spread of bacteria than viruses.

As known, RSV and PIV3 are the most prevalent viruses in patients with ARI in China (1, 4-6, 16). In the viral co-infection cohort, the most frequently co-detected virus was RSV (60.6%). Consistent with previous reports, in the bacterial co-infection cohort, we found that *S. pneumoniae* (51.3%) was the most frequent bacterial co-infection; however, both *S. aureus* (20.9%) and *H. influenzae* (20.9%) co-infections were also quite common (7, 17). The epidemiological characteristics of an illness can aid in determining the disease's etiological factors (13, 18, 19). Further research is required in the epidemiology of viral and bacterial co-infections, including information regarding gender, age, seasonality, appropriate diagnostic testing and method-

ologies, and biomarker utilization.

A significantly higher proportion of *M. pneumoniae* co-infections was observed in girls than in PIV3 mono-infection patients. As literature supports our findings, it is inferred that girls are more susceptible to *M. pneumoniae* infection, but more data and theoretical support are needed (13). It has been reported that PIV3 co-infection with a respiratory virus is most common in children under the age of one year (67.39%), which is consistent with our findings (9). Conversely, children with *M. pneumoniae* infections and co-infections with more than two pathogens occur mainly in children 1 – 3 years of age. This could be because *M. pneumoniae* infection is uncommon in children under one year (13). In addition, the co-infection rate of any pathogen in children over six years of age is less than 2%. The impact of co-infection on the severity of ARI remains questionable (11, 12, 17). Like earlier studies, the current investigation found no evidence that PIV3 co-infections with any pathogen are linked to ventilator support and ICU hospitalizations (11, 12). However, the mean length of stay for children with *M. pneumoniae*, bacterial co-infection, and co-infections with more than two pathogens was significantly higher than that for children infected with a single PIV3 infection.

We discovered that the lower respiratory tract infection rate in these cases is much higher than that for PIV3 infections alone, regardless of whether the cohort is a bacterial co-infection or a co-infection of more than two pathogens. Furthermore, among children with co-infections with more than two pathogens, the incidence of pneumonia was the highest in the five cohorts. Our findings suggest that bacterial co-infections and co-infections with more than two pathogens may impact the severity of ARI and result in worse symptoms. This contradicts previous reports' conclusion (12, 20). However, these reports do not explicitly cover cases of upper respiratory tract infections or specifically classify the pathogens involved in co-infection. Inconsistencies in the conclusions could be caused by variations in the cohort settings and case sources. These associations need to be confirmed by additional research.

In our study, children with *M. pneumoniae* co-infections had a higher percentage of monocytes, segmented and banded neutrophils, and a lower percentage of lymphocytes than children with PIV3 alone. Similarly, bacterial and multi-pathogen co-infections resulted in more segmented neutrophils and fewer lymphocytes. Bacterial co-infections also produced a significant increase in the white blood cell count. Common inflammatory markers may be useful in distinguishing infections caused by PIV3 alone from infections caused by other pathogens (21, 22). Our research has several limitations. One significant limita-

tion is that this was a retrospective study, and not all of the data was available, which may have influenced the outcome of the analysis. Second, pathogen detection methods and pathogen detection ranges are limited, and detection does not completely cover all respiratory pathogens. Finally, severe cases are more likely to be tested for PIV3 and other respiratory pathogen infections, while milder cases may be overlooked.

5.1. Conclusions

In conclusion, co-infection of PIV3 with other pathogens is common, and co-infection with different pathogens exhibits different epidemic and clinical characteristics. Our findings suggest that PIV3 co-infections may be associated with a longer hospital stay and support the link between PIV3 co-infections with bacterial or multiple pathogens and the severity of ARI with worse symptoms.

Footnotes

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Table 3. Comparison of Clinical Characteristics of Patients with Parainfluenza Virus Type 3 Mono-infection and Co-infection

	With Mycoplasma pneumoniae (n = 616)	χ^2/t	P-Value	With Virus (n = 71)	χ^2/t	P-Value	With Bacteria (n = 115)	χ^2/t	P-Value	With More Than Two Pathogens (n = 62)	χ^2/t	P-Value	Total (n = 864)	χ^2/t	P-Value	PIV3 Mono-infection (n = 1675)
PICU admission	4 (0.6%)	1.288	0.355	1 (1.4%)	0.026	1.000	2 (1.7%)	0.263	0.648	1 (1.6%)	0.088	1.000	8 (0.9%)	0.376	0.559	20 (1.2%)
Ventilator use	3 (0.5%)	0.364	0.587	1 (1.4%)	0.441	1.000	2 (1.7%)	1.450	0.226	1 (1.6%)	0.646	0.378	7 (0.8%)	0.067	0.795	12 (0.7%)
Hospitalization length of stay (d)		2.367	0.018		1.623	0.109		2.402	0.016		2.827	0.006		3.778	< 0.001	
Median (range)	7 (3 - 31)			6 (2 - 32)			7 (3 - 32)			7 (1 - 17)			7 (3 - 32)			6 (1 - 50)
Mean ± SD	7.07 ± 2.70			7.56 ± 4.23			7.45 ± 3.75			7.90 ± 3.19			7.22 ± 3.05			6.74 ± 3.02

Laboratory Data	With M. pneumoniae (n = 374)	t	P-Value	With Virus (n = 44)	t	P-Value	With Bacteria (n = 75)	t	P-Value	Multiple Co-infection (n = 45)	t	P-Value	Total (n = 538)	t	P-Value	PIV3 Mono-infection (n = 1003)
White blood cell counts (10^9/L)	8.56 ± 3.23	0.297	0.766	8.89 ± 2.99	0.534	0.593	9.64 ± 3.55	2.510	0.012	9.09 ± 3.76	0.922	0.357	8.78 ± 3.31	0.909	0.364	8.62 ± 3.39
Segmented neutrophils (%)	24.93 ± 13.53	2.437	0.015	25.70 ± 13.79	1.316	0.189	26.85 ± 11.66	2.408	0.016	28.40 ± 14.53	2.598	0.010	25.55 ± 13.40	3.626	< 0.001	22.89 ± 13.89
Banded neutrophils (%)	10.06 ± 7.22	5.009	< 0.001	7.45 ± 6.26	0.473	0.636	7.68 ± 6.73	0.331	0.740	9.47 ± 7.53	1.447	0.148	9.46 ± 7.16	4.073	< 0.001	7.95 ± 6.84
LyM. pneumoniaehocytes (%)	54.25 ± 17.19	4.637	< 0.001	58.70 ± 18.32	0.900	0.368	54.97 ± 15.01	2.012	0.044	52.31 ± 18.56	2.567	0.010	54.39 ± 17.10	5.121	< 0.001	59.11 ± 17.34
Monocytes (%)	6.91 ± 3.76	2.090	0.037	7.05 ± 3.02	1.074	0.283	6.97 ± 4.13	1.199	0.231	6.67 ± 4.15	0.402	0.688	6.91 ± 3.79	2.360	0.018	6.44 ± 3.69
C-reactive protein (mg/L)	4.02 ± 8.07	1.600	0.111	1.17 ± 3.00	2.97	0.004	4.54 ± 9.60	1.350	0.177	4.86 ± 12.59	1.29	0.197	3.97 ± 8.50	1.672	0.095	3.25 ± 7.93