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Original Article

Molecular detection of respiratory pathogens in community-acquired pneumonia involving adults

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Abstract *Background:* Community-acquired pneumonia (CAP) causes substantial morbidity and mortality in adults worldwide. The etiology of CAP often remains uncertain, and therapy is empirical. Thus, there is still room for improvement in the diagnosis of pneumonia.
Methods: Adults aged >20 years who presented at the outpatient or emergency departments of Linkou and Keelung Chang Gung Memorial Hospital with CAP were prospectively included between November 2016 and December 2018. We collected respiratory specimens for culture and molecular testing and calculated the incidence rates of CAP according to pathogens.
Results: Of 212 hospitalized adult patients with CAP, 69.3% were male, and the median age of the patients was 67.8 years. Bacterial pathogens were detected in 106 (50%) patients, viruses in 77 (36.3%), and fungal pathogens in 1 patient (0.5%). The overall detection rate (culture and molecular testing method) was 70.7% (n = 150). Traditional microbial culture yielded positive results in 36.7% (n = 78), molecular testing in 61.3% (n = 130). The most common pathogens were influenza (16.1%), followed by *Klebsiella pneumoniae* (14.1%), *Pseudomonas aeruginosa* (13.6%), human rhinovirus (11.8%), and *Streptococcus pneumoniae* (9.9%). Multiple pathogen

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co-infections accounted for 28.7% (n = 61), of which co-infection with *K. pneumoniae* and human rhinovirus comprised the largest proportion.
Conclusions: Molecular diagnostic testing could detect 23.6% more pathogens than traditional culture techniques. However, despite the current diagnostic tests, there is still the possibility that no pathogen was detected.

Introduction

Community-acquired pneumonia (CAP) causes substantial morbidity and mortality in adults in developed countries, leading to high rates of hospitalization, especially in the elderly.[1] The 2010 Global Burden of Disease Study reported that lower respiratory tract infections, including pneumonia, are the fourth-most common cause of death globally, exceeded only by ischemic heart disease, stroke, and chronic obstructive pulmonary disease (COPD), and represent the second-most frequent reason for years of life lost.[2] The annual incidence of pneumonia analyzed by the US Centers for Disease Control and Prevention was 24.8% per 10,000 adults, with the highest rates among adults aged 65—79 years (63 cases per 10,000 adults).[3] An etiologic agent is not identified in 30 %—65% of patients with pneumonia.[4] The etiology of CAP often remains uncertain, and therapy is empirical. Hence there is still room for improvement in the diagnosis of pneumonia.

It has been estimated that 17%—41% of CAP cases in the US are caused by *Streptococcus pneumoniae*.[5] CAP caused by *S. pneumoniae* is frequently associated with high mortality, risk of shock, and the need for mechanical ventilation.[6] In Taiwan, *S. pneumoniae* accounted for 23.5% of pathogens associated with CAP in 2005.[7] Pneumococcal pneumonia can be further subdivided into invasive (bacteremic) and noninvasive (non-bacteremic) cases, the latter being responsible for the majority of disease cases; nonetheless, a definitive pneumococcal diagnosis is often not established.[7] Culture methods are of limited value for the detection of noninvasive pneumococcal diseases. On the other hand, viral pneumonia, which is typically associated with childhood disease, is increasingly recognized as a disease-causing agent in adults. For example, > 80% of adenovirus-related infections, including pneumonia, occur in children, especially those <4 years old, whereas they account for 1%—7% of adult respiratory infections.[8] In contrast, respiratory syncytial virus (RSV) is a significant cause of pneumonia in adults. In a study of patients admitted to hospital for CAP, Dowell and colleagues reported that RSV was the third-most common cause of CAP in hospitalized patients following *S. pneumoniae* and influenza virus infection.[9]

The development of molecular methods with improved sensitivity and specificity has paved the way for the detection of novel viruses, for identification of pathogens that are difficult to culture, and for the detection of pathogens later in the disease process. In the past, relatively few studies have used new molecular methods to investigate pathogens causing pneumonia in Taiwan.[10] This study will apply conventional and molecular diagnostics to identify and survey viruses and bacteria associated with community-onset pneumonia in Taiwan.

Materials and methods

Patients

From November 2016 to December 2018, adults aged >20 years who presented at the outpatient or emergency departments of participating hospitals (inclusive of Linkou Chang Gung Memorial Hospital and Keelung Chang Gung Memorial Hospital) diagnosed with pneumonia were eligible for enrollment and screened for inclusion in this study. The definition of pneumonia is characterized by new pulmonary infiltrates on thoracic imaging and one or more of the following conditions: 1) novel or increased cough with or without sputum production and/or purulent respiratory secretions; 2) fever or hypothermia; 3) signs of systemic inflammation (leukocytosis >10,000 cells·cm^{-3}, bandemia >10%, leukopaenia <4000 cells·cm^{-3}), procalcitonin levels above the local upper limit of normal or increased C-reactive protein).[11]

Subjects were excluded if they had already been hospitalized for >48 h at other inpatient facilities (i.e., community hospitals), had hospital-acquired pneumonia (defined as pneumonia developing 48 h after hospital admission), had suspected tuberculosis or human immunodeficiency virus infection, had admitted to emergent department within 90 days, were nursing home residents, were unable to consent, were refused to join this study, or had been previously enrolled in this study within the past 30 days. Written informed consent was obtained from patients or their caregivers before enrollment. This study was approved by our institutional review board (104—2389B).

Bacterial study

Sputum samples displaying >25 leukocytes and <10 epithelial cells per 100 × power microscopic field after Gram staining were considered adequate samples. Blood, sputum, and, if available, pleural fluid, endotracheal aspirates, and bronchoalveolar lavage samples were sent for bacterial culture and processed according to standard techniques.[12] DNA was extracted from 200 μL of sputum using a DNA isolation kit (GeneMark, Georgia, USA) according to the manufacturer's instructions. Extracted DNA

was eluted in 100 μL of elution buffer and stored at −20 °C. To quantify the amount of bacterial DNA present in each sample, a standard curve was prepared using serially diluted DNA extracts from a known quantity (confirmed by plating) of *S. pneumoniae* ATCC 49619, *S. aureus* ATCC 29213, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Haemophilus influenzae* ATCC 33391, *Moraxella catarrhalis* ATCC 25238, *Acinetobacter baumannii* ATCC 19606, and *E. coli* ATCC 35218. These standard curves were used to calculate bacterial loads (DNA copies/mL). The amount of bacterial DNA present in each sample was obtained by direct extrapolation of PCR cycle threshold (C_T) values to the amount of DNA, as read from the concentration versus the C_T standard curve. Negative samples were defined as those with C_T values > 30. Real-time quantitative PCR (qPCR) targeting the fumarate

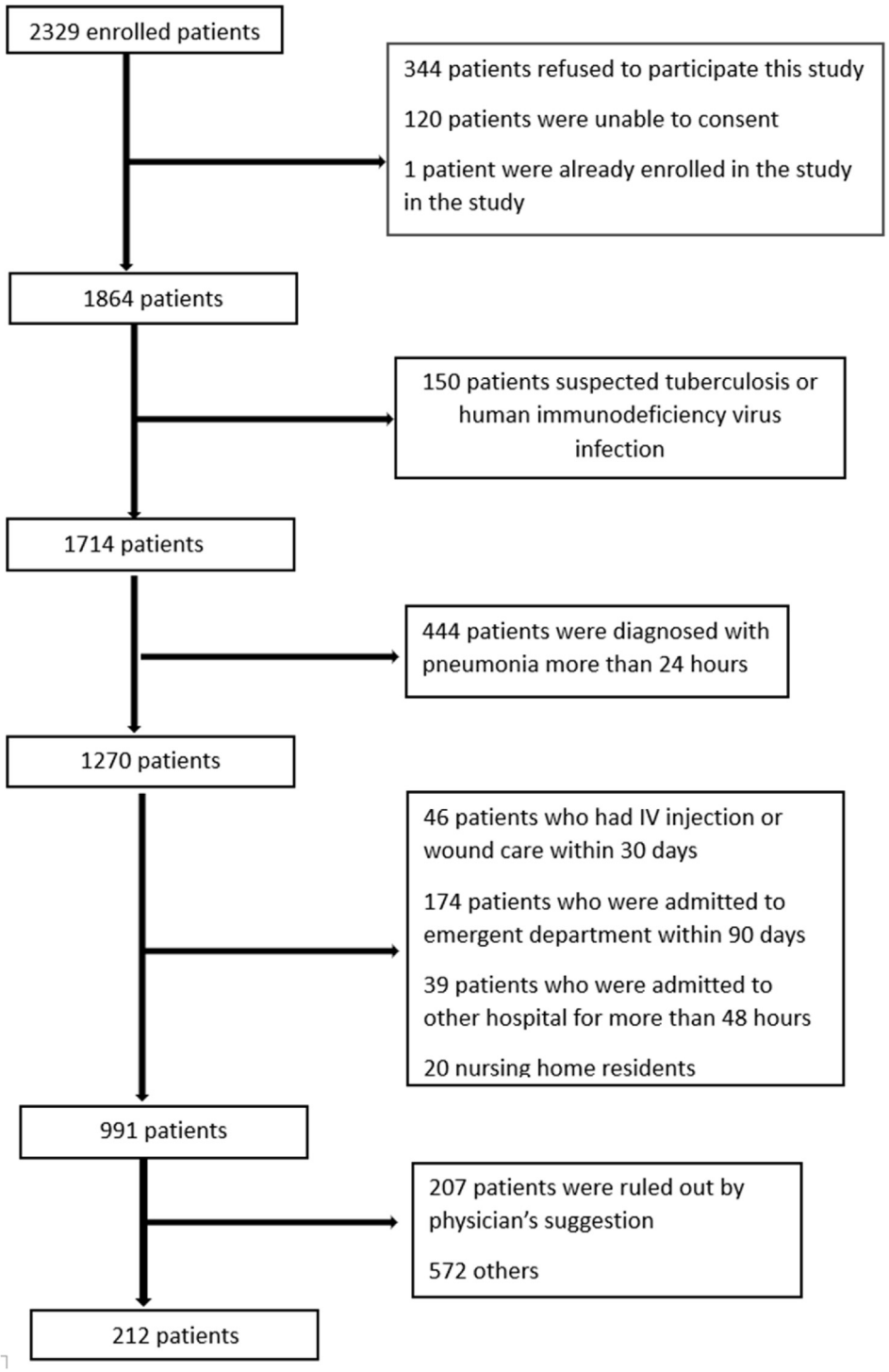

Figure 1. The flow chart of enrolled patients with exclusion criteria.

Table 1 Characteristics of included patients with community-acquired pneumonia.

Demographics	N (%)					
	Total (n = 212)	Only Bacteria (n = 71)	Only Virus (n = 42)	Virus and bacteria (n = 35)	Negative (n = 62)	P value
Male (%)	147 (69.3%)	55 (77.4%)	21 (50%)	28 (80%)	43 (69.3%)	0.015
Age, median year (interquartile range)	67.8 ± 16.8	67.1 ± 15.5	62.3 ± 18	64 ± 18.5	68 ± 16.4	0.298
Age≧65 y (%)	119 (56.1%)	37 (52.1%)	22 (52.3%)	18 (51.4%)	42 (67.7%)	0.414
Age≧75 y (%)	74 (34.9%)	26 (36.6%)	12 (28.5%)	13 (37.1%)	23 (37%)	0.86
Hospital admission	162 (76.4%)	61 (85.9%)	28 (66.6%)	25 (71.4%)	48 (77.4%)	0.06
Comorbidity						
Hypertension	63 (29.7%)	26 (36.6%)	12 (28.5%)	8 (22.8%)	17 (27.4%)	0.36
Diabetes mellitus	52 (24.5%)	21 (29.5%)	10 (23.8%)	6 (17.1%)	15 (24.2%)	0.49
Neoplastic disease	52 (24.5%)	17 (23.9%)	9 (21.4%)	9 (25.7%)	17 (27.4%)	0.97
Chronic renal insufficiency	26 (12%)	4 (5.6%)	7 (16.6%)	6 (17.1%)	9 (14.5%)	0.215
Coronary artery disease or congestive heart failure	22 (10.3%)	7 (9.8%)	5 (11.9%)	0 (0%)	6 (9.6%)	0.229
COPD	16 (7.5%)	7 (9.8%)	2 (4.7%)	2 (5.7%)	5 (8%)	0.746
ICU admission	18 (8.4%)	10 (14%)	2 (4.7%)	3 (8.5%)	3 (4.8%)	0.174
In-hospital mortality	13 (6%)	7 (9.8%)	2 (4.7%)	2 (5.7%)	2 (3.2%)	0.391
Mechanical Ventilator	21 (9.9%)	14 (19.7%)	2 (4.7%)	2 (5.7%)	3 (4.8%)	0.011
Septic shock	22 (10.3%)	13 (18.3%)	2 (4.7%)	2 (5.7%)	5 (8%)	0.059

Table 2 Bacterial Pathogen Detection in Patients with Community Acquired pneumonia.

Bacterial pathogen	No (%) of patients with positive finding (n = 212)	Only Sputum culture	Only Sputum PCR	Both Culture and PCR
K. pneumoniae	30 (14.1%)	4	20	6
P. aeruginosa	29 (13.6%)	3	14	12
S. pneumoniae	21 (9.9%)	2	18	1
H. influenzae	13 (6.1%)	8	4	1
S. aureus	13 (6.1%)	5	4	4
E. coli	9 (4.2%)	—	7	2
A. baumannii	9 (4.2%)	—	8	1
M. catarrhalis	7 (3.3%)	1	5	1
M. pneumoniae	2 (0.9%)	0	2	—
others	16 (7.5%)	13	0	3
total	106 (50%)	25	50	31

reductase (*frdB*) gene of *H. influenzae*, and the outer membrane protein (*copB*) gene of *M. catarrhalis* was performed as described by Kais et al.[13] Respiratory tract samples were also examined for *cpsA* for *S. pneumoniae*,[14] outer membrane lipoprotein gene (*oprL*) for *P. aeruginosa*,[14] *egc* for *S. aureus* outer membrane protein A (*OmpA*) for *A. baumannii*,[15] conserved protein (*yccT*) for *E. coli*,[16] and *Klebsiella* hemolysin gene (*khe*) for *K. pneumoniae* by means of qPCR.[17] Detection of *Mycoplasma pneumoniae* was performed by PCR on sputum as previously described.[18] The patients did not receive routine test for pneumococcal antigen in urine because the sensitivity is only 60–65% in several published studies. Therefore, the detection of pneumococcus was substituted by PCR on sputum.[19] Detection of Legionella species was performed by immunochromatographic tests on urinary antigen.

Viral study

Nasopharyngeal swabs were sent for viral detection using conventional viral culture techniques.[20] Specimens of cell suspensions were inoculated into MK2, MRC-5, and MDCK cells and incubated at 35 °C for 2 weeks. The cytopathic effect (CPE) of all culture tubes was checked every 2 days. For CPE-positive tubes, an immunofluorescence assay for respiratory virus (Chemicon Inc., Temecula, California, USA) was used for further examination of respiratory virus infection. Viral RNA was extracted from 200 μL of respiratory specimens using the QIAmp Viral RNA kit (Qiagen, Chatsworth, CA, USA), and reverse transcription reactions were performed for complementary DNA synthesis using the SuperScriptTM III One-Step RT-PCR kit (Invitrogen, Carlsbad, CA, USA). Real-time polymerase chain reaction (qPCR)

Table 3 Viral pathogen detection in patients with community-acquired pneumonia.

Viral pathogen	No (%) of patients with positive finding (n = 212)	Nasopharyngeal swab culture	Nasopharyngeal swab PCR	Both PCR and culture
Influenza A	26 (12.3%)	–	10	16
Influenza B	8 (3.8%)	–	8	0
Parainfluenza	2 (0.9%)	–	1	1
HCoV	6 (2.8%)	–	6	0
Rhinovirus-A	15 (7.1%)	–	12	3
Rhinovirus-B	2 (0.9%)	–	2	0
Rhinovirus-C	8 (3.8%)	–	8	0
Adenovirus	2 (0.9%)	–	1	1
Respiratory syncytial virus	4 (1.9%)	–	2	2
Human metapneumovirus	4 (1.9%)	–	4	0
HSV-1	1 (0.5%)	1	0	0
Total	77 (36.3%)	1	53	23

detection of the following viruses was performed: adenovirus, enterovirus, human bocavirus, human coronaviruses 229E, HKU-1, NL63, and OC43; human metapneumovirus; influenza viruses A and B; parainfluenza viruses 1–4; rhinovirus; and RSV (A and B).[21,22]

Statistical analysis

Chi-square or Fisher's exact tests were used to assess group differences in categorical variables. For continuous variables, one-way analysis of variance (ANOVA) was used. Statistical significance was set at $p < 0.05$. All probabilities were 2-tailed. All statistical analyses were performed using SPSS software (version 15.0, SPSS Inc., Chicago, IL, USA).

Results

A total of 2329 patients with pneumonia were eligible and screened for this study. After eliminating patients who met the exclusion criteria, a total of 212 hospitalized adult patients with CAP were included in the present study (Fig. 1); 69.8% were male and the median age was 67.8 years (Table 1). Patients who were infected with bacterial pneumonia were most commonly men ($p = 0.015$) and more likely to require hospital admission ($p = 0.06$). The most common comorbidities were hypertension (29.7%), diabetes mellitus (24.5%), and neoplastic diseases (24.5%). Among the 212 subjects, 18 patients (8.4%) experienced severe diseases requiring Intensive Care Unit (ICU) admission. A total of 21 (9.9%) patients have received mechanical ventilator and 22 patients (10.3%) had septic shock. Patients with bacterial infection only was more common in receiving mechanical ventilator during hospitalization ($p = 0.011$). Bacteria were detected in specimens from 106 (50%) patients, while viruses were detected in 77 (36.3%) cases. Combining both respiratory virus and bacterial results, the overall pathogen detection rate was 70.7% (n = 150). Molecular testing accounted for 61.3% (n = 130) and microbial culture 36.7% (n = 78). Multiple pathogen co-infections accounted for 28.7% (n = 61).

K. pneumoniae, *P. aeruginosa*, and *S. pneumoniae* were the most frequently identified bacteria in patients with CAP, detected in 30 (14.1%), 29 (13.6%), and 21 (9.9%) patients, respectively (Table 2). Bacterial PCR detected more *K. pneumoniae* (n = 16, 7.5%), *S. pneumoniae* (n = 16, 7.5%), *P. aeruginosa* (n = 11, 5.2%), *A. baumannii* (n = 8, 3.8%), and *E. coli* (n = 7, 3.3%) than standard microbial culture-based methods. Of the 56 positive bacterial culture specimens, 25 (44.5%) were also PCR-positive for the same species of bacteria; the 11 discrepant specimens were *K. pneumoniae* (n = 2), *P. aeruginosa* (n = 2), *S. aureus* (n = 4), *S. pneumoniae* (n = 2), and *M. catarrhalis* (n = 1), which were culture-positive but PCR-negative. The remaining 16 were β-*Streptococcus* (n = 8) and *M. chimaera-intracellulare* group (n = 2). *Mycobacterium tuberculosis* complex (n = 2), *Neisseria meningitides* (n = 1), *Serratia marcescens* (n = 1), *Stenotrophomonas maltophilia* (n = 1), and Group A *Streptococcus* (n = 1), which were not our target pathogens and were not included in the PCR assays. *M. pneumoniae* was also found in two patients, but they were detected via PCR rather than the culturing method. *Legionella pneumophila* was not detected in these patients. In terms of bacterial pathogens, molecular testing detected 38.6% (n = 82) of bacteria and culture identified 25.9% (n = 55). In total, bacterial PCR was able to detect bacteria in 50 (23.5%) microbial culture-negative specimens.

Viruses were detected in 77 (36.3%) patients (Table 3), and influenza A (12.3%, n = 26) and human rhinovirus (11.8%, n = 25) were the most commonly detected viruses. There were 35 viral-bacterial co-detection incidences. Viral PCR detected more influenza (n = 18, 8.5%), human rhinovirus (n = 22, 10.3%), coronavirus (n = 6, 2.8%), RSV (n = 2, 0.9%), and human metapneumovirus (hMPV) (n = 4, 1.9%) than standard culture-based methods. Of the 24 virus culture-positive specimens, 23 (95.8%) were also PCR-positive for the same virus; one specimen was herpes

simplex virus (HSV) (n = 1), which was not one of our target pathogens and was not included in PCR assays. Viral PCR was able to detect viruses in 54 (25.4%) culture-negative specimens.

35 specimens were simultaneously positive for both viruses and bacteria (Table 4). Influenza A virus (n = 16) and human rhinovirus (n = 14) were the most common viruses associated with bacterial infections. *K. pneumoniae* (n = 11), *S. pneumoniae* (n = 11), and *P. aeruginosa* (n = 10) were the most common bacteria associated with viral infections. One virus combined with one bacterium was the most common type, accounting for 26 (74.2%) patients, followed by single virus infection combined with multiple bacteria (n = 8, 22.8%) and single bacteria combined with multiple viruses (n = 1, 2.9%). Multiple pathogen co-infections accounted for 28.7% (n = 61), of which co-infection with *K. pneumoniae* and human rhinovirus comprised the largest proportion.

Only one fungal pathogen was detected as *Pneumocystis jirovecii* via PCR method and did not represent co-infection with other pathogens.

On clinical presentation, patients infected with *K. pneumoniae* was more prevalent in ventilated (p = 0.05) and in underlying disease of leukemia (p = 0.047). On the other hand, patients infected with *P. aeruginosa* had more common in ICU admission (p = 0.025) and in underlying disease of leukemia (p = 0.045). Besides, patients having *S. aureus* infection were more common in septic shock (p = 0.005) and required ventilator (p = 0.029). Patients infected with *S. aureus* was also more common in underlying disease of neoplastic disease (p = 0.019).

On the multivariate analysis, patients with community-acquired pneumonia having autoimmune disease (p = 0.012) and tumor with metastasis (p = 0.012) are the independent risk factors of in-hospital mortality (Table 5).

Table 4 Bacteria-virus coinfection in patients with community-acquired pneumonia.

	Influenza A	Influenza B	Parainfluenza	HCoV	Human Rhinovirus A	Human Rhinovirus B	Human Rhinovirus C	Adenovirus	Respiratory syncytial virus	Human metapneumovirus
K. pneumoniae	3	2	–	–	5	1	1	–	–	–
P. aeruginosa	2	2	–	–	3	–	1	–	2	–
S.pneumoniae	5	–	–	–	1	–	2	–	2	1
H. influenzae	–	–	–	2	1	–	–	–	–	–
A. baumnnii	–	–	–	–	2	–	–	–	–	–
E. coli	1	–	–	–	–	–	1	–	–	–
S. aureus	1	–	–	–	1	–	–	–	–	–
M. catarrhalis	1	–	–	–	–	–	–	1	1	–
others	3	1	–	–	1	–	1	–	–	–

Discussion

Among the 212 participants enrolled in the present study, pathogens were detected in 70.7%, with viruses detected in 36.3% and bacteria in 50%. Influenza (A and B), *K. pneumoniae*, *P. aeruginosa*, rhinovirus, and *S. pneumoniae* were the most frequently identified pathogens in patients with CAP. In general, PCR was able to detect bacteria in 50 (23.5%) culture-negative samples and 54 (25.4%) of viruses.

Although *K. pneumoniae* is not a common cause of CAP in Western countries such as North America and Europe,[23] it is an important pathogen involved in lower respiratory tract infections in Asian countries and South Africa.[24–29] In the current study, it was the most commonly detected bacterial pathogen. This result was similar to a study conducted in eight Asian countries, which showed that *K. pneumoniae* (15.4%) was only secondary to *S. pneumoniae* as the common pathogen in cases of CAP between 2002 and 2004.[26] The decrease in pneumococcal pneumonia in the current study, compared with previous studies, could be the consequence of the national immunization program of 13-valent pneumococcal vaccine (PCV13) among children since 2013 in Taiwan. According to Lu et al.,[30] the PCV13 national immunization program reduced the incidence of invasive pneumococcal disease (IPD) by 69% in children

aged 0–5 years, with a herd immunity effect by showing a 37% reduction in the incidence of IPD in the elderly. Taiwan is a *K. pneumoniae*-related endemic area for primary liver abscesses caused by hypermucoviscous serotypes K1 and K2.[31] Serotypes K1 and K2 account for half of the *K. pneumoniae* isolates causing pneumonia.[29] Although we did not study the serotype distribution of *K. pneumoniae* isolates in this study, we showed that community-onset *K. pneumoniae*-related pneumonia is still a major threat in Taiwan.

P. aeruginosa is a rare cause of CAP but, nevertheless, an important etiological factor in patients who lived in health care facility before admission to hospital.[11] In a multinational prevalence study of CAP, the overall rate of *P. aeruginosa* was 4.2%, but increased to 67% in patients with prior infection or colonization due to *P. aeruginosa*, tracheostomy, bronchiectasis, and/or very severe chronic obstructive pulmonary disease.[11] The rate of *P. aeruginosa* detection was 13.6% in patients with CAP in the study, which was slightly higher than that in a previous report that included patients with culture-positive bacteremic pneumonia, exhibiting a rate of 7.9% associated with *P. aeruginosa*.[32] Generally, Pseudomonas was indeed recognized as a nosocomial pathogen. However, in some previous reports, the high proportion of *P. aeruginosa* from community may be due to patients having pre-existing structural abnormality, such as malignancy, cystic fibrosis, aplastic anemia, chronic obstructive pulmonary disease, and bronchiectasis. In our study, 24.5% patients had neoplastic disease and 7.5% patients had chronic obstructive pulmonary disease. These may cause the high proportion of *P. aeruginosa* infection.[32] Besides, both *Klebsiella* and *Pseudomonas* species are highly virulent pathogens that cause CAP and result in poor prognoses.[31,32]

Viral pneumonia generally accounts for 20%–30% of adult patients with CAP.[33–36] Influenza and human rhinoviruses are the most common pathogens, accounting for 6 %-10% and 9–12% of cases, respectively.[35,36] There is no doubt that the influenza virus is capable of causing pneumonia since its discovery in 1933, leading to substantial morbidity and mortality every year. The frequency of other viruses is varied in different studies, such as parainfluenza virus at 1.6–2%, respiratory syncytial virus in 1.2–7.1%, adenovirus involving 1.6–4%, human metapneumovirus accounting for 0.9–4.2%, and coronavirus 2–13.1%.[33–36] Human rhinovirus infection can result in pneumonia and acute bronchiolitis in young children, and is strongly associated with CAP in adults.[34,36] It is not clear whether human rhinovirus is the primary cause of lower respiratory tract disease or a predisposition to bacterial pneumonia. Of note, 84% (21/25) of patients who were positive for human rhinovirus were simultaneously co-infected with a bacterial pathogen. Co-infections are a common finding in CAP because various viral mechanisms may decrease bacterial clearance, increase bacterial adherence, and suppress immunity during recovery.[37] Specific bacterial species also promote viral infections.[38,39] In this study, we found *K. pneumoniae*/influenza virus and human rhinovirus to be the most common bacterial or viral pathogen mixed with other pathogens in our co-infection cases.

The limitations of this study include the following: First, it lacks asymptomatic controls to understand the frequency of viral detection in individuals without symptoms. However, viral detection from upper respiratory samples was rare in asymptomatic adults compared with those with CAP in a previous study.[35] Second, the results came from our experience in a single medical center; they may not apply to every medical setting. Third, in our study, only 44.5% positive bacterial culture specimens were also PCR-positive for the same species. We assumed that culture has a specificity of 100%, and those samples detected by PCR were considered as false negatives, which is a limitation in this study. When considering the apparent lack of sensitivity for PCR, it was reported that PCR was performed with relative low colony growth, which is probably below the detection limit of PCR techniques.[40] Forth, we didn't perform viral culture or PCR for the sputum from the enrolled patients and this is the limitation for detection of lower respiratory tract specimens causing pneumonia.

In conclusion, molecular diagnostic testing could detect 23.6% more pathogens than traditional microbial culture techniques in patients with COP. However, despite the current diagnostic tests, there is still the possibility that no pathogen was detected. Co-infection with multiple pathogens accounted for approximately one-third of patients, of which one virus combined with one bacterium was the most common type. Moreover, co-infection with *K. pneumoniae* and human rhinovirus comprised the largest proportion.

Table 5 Risk factors of in-hospital mortality in adults with community-acquired pneumonia.

Risk factor	Univariate analysis	P value	Multivariate analysis	P value
	Odds ratio (95% CI)		Odds ratio (95% CI)	
Sex (male)	2.49 (0.54–11.6)	0.25	–	–
Coronary artery disease	4.99 (0.93–26.89)	0.062	5.66 (0.82–38.8)	0.078
Autoimmune disease	4.99 (0.93–26.89)	0.062	10.6 (1.68–67.4)	0.012*
Tumor with metastasis	9.8 (2.94–32.7)	<0.001	29.4 (2.13–406.1)	0.012*
Lymphoma	16.5 (0.97–280)	0.05	22.1 (0.36–1364.3)	0.142
Neoplastic disease	2.85 (0.91–8.9)	0.07	0.31 (0.02–4.47)	0.39
Age>75	1.18 (0.37–3.74)	0.782	–	–

Variables with $p < 0.1$ in the univariate analysis were included in the multivariate analysis. CI: confidence interval; -: no data in multivariate analysis.

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