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Etiology and characteristics of community-acquired pneumonia in an influenza epidemic period

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ABSTRACT

Purpose: The etiology of community-acquired pneumonia (CAP) in hospital patients is often ambiguous due to the limited pathogen detection. Lack of a microbiological diagnosis impairs precision treatment in CAP.
Methods: Specimens collected from the lower respiratory tract of 195 CAP patients, viruses were measured by the Single-plex real-time PCR assay and the conventional culture method was exploited for bacteria.
Results: Among the 195 patients, there were 46 (23.59%) pure bacterial infections, 20 (10.26%) *yeast* infections, 32 (16.41%) pure viral infections, 8 (4.10%) viral-*yeast* co-infections, and 17 (8.72%) viral-bacterial co-infections. The two most abundant bacteria were *Acinetobacter baumannii* and *klebsiella pneumoniae*, whereas the most common virus was influenza A.
Conclusions: Non-influenza respiratory microorganisms frequently co-circulated during the epidemic peaks of influenza, which easily being ignored in CAP therapy. In patients with bacterial and viral co-infections, identifying the etiologic agent is crucial for patient's therapy.

1. Introduction

Community-acquired pneumonia (CAP) is a common disease and a significant cause of morbidity and mortality worldwide [1]. The severity of the disease can be affected by various factors including age, immune status of the host, and the single or mixed infection. The etiology of CAP is essential because most respiratory illnesses lead to similar clinical presentations. Currently, the ambiguous of etiology and lack of a microbiological diagnosis in CAP impairs pathogen-directed antimicrobial therapy [2]. In the last decade, the long-term implications of respiratory viral infection among susceptible hosts have increasingly been recognized [3]. Although approximately 200 million cases of viral CAP occur every year, the incidence of viral pneumonia still has been underestimated. In hospital, bacteria remain the first consideration in pulmonary infections, and antibiotic therapy is still the cornerstone of the majority of CAP.

Influenza virus infection (IVI) causes annual epidemics that result in estimated 1 billion infections, including 3–5 million severe cases and 250,000–500,000 mortality cases worldwide each year [4]. IVI can cause primary viral pneumonia, which may progress to a potentially fatal outcome [5]. It is difficult to distinguish the cause of CAP induced by flu, other respiratory viruses or bacteria based solely on clinical symptoms, and the conventional lab biomarkers of viral and/or bacterial infections do not differ in influenza-positive compared with influenza-negative patients [6]. Influenza can temporarily suppress host immune defenses, leading to bacterial complications [7]. Secondary bacterial pneumonia is a frequent cause of excess mortality during influenza epidemics, but the epidemiology remains unclear [8]. Hospital admissions during the 2009 influenza epidemic season showed a moderate to strong association between influenza and bacterial pneumonia, whereas the interaction is modest or non-existent during non-epidemic periods [9]. Bacteria including streptococcus pneumoniae, hemophilia influenzae, and staphylococcus aureus, often interact with respiratory viruses (esp. the influenza virus), and shape the outcome of respiratory infection [10]. Studies have shown the evidence of bacterial invasion is more than 90% in influenza-infected cases, and the high rates of co-infections exist in these patients, indicating that viral infection may enhance both susceptibility and severity of subsequent bacterial infection [8,11,12]. In vitro studies have shown that influenza infection enhanced susceptibility to pneumococcal pneumonia by about

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100-fold in a week, but the interaction between influenza and bacteria is not limited in pneumococcal pneumonia [13]. In the hospital, viral testing among patients with respiratory symptoms is uncommon [14], and the determination of the microbiological etiology is severely hampered in CAP by the difficulty of obtaining specimens from the infected area (esp. from lower respiratory tract), samples could be easily contaminated by respiratory conditioned pathogens. At present, the epidemiological history of influenza-like illness (ILI) and the incidence and clinical presentation of CAP caused by viruses other than influenza during an influenza epidemic season were limited [15–17]. In this study, we report on the surveillance of respiratory microorganisms, and its laboratory biomarkers, in CAP patients admitted to a hospital during the January to August 2016. The specific microbiome patterns, their clinical significance, and the antibacterial/antiviral treatment were analyzed in these patients simultaneous.

2. Material and methods

2.1. Patients

We collected sputum specimens from CAP patients who were admitted to the First Affiliated Hospital of Shantou University Medical College, Shantou city, Guangdong province in China from January to August 2016. Pneumonia was diagnosed as an acute illness with fever, cough, or dyspnea / tachypnea, and at least one new focal chest sign, which was confirmed by finding lung shadowing on the chest radiographs that were likely to be new and without other obvious causes [18]. Patients with cystic fibrosis or HIV infection were excluded from our study.

2.2. Single-plex real-time PCR assay for respiratory viruses

The sputum specimens were collected and stored in a swab storage solution (COPAN, Italy) and stored at −80 °C. The nucleic acid was extracted using a beads viral DNA/RNA extraction kit (TIANLONG, China) and NP968 nucleic acid extractor (TIANLONG, China). Respiratory viruses were detected using an AgPath-ID™ One-Step RT-PCR kit (Applied Biosystems, USA) and using a 7500 Real-Time PCR System (Applied Biosystems, USA). Negativecontrols (DEPC-treated water) were included in each analytical procedure. Fourteen respiratory viruses including influenza A, influenza B, parainfluenza viruses 1–3 (PIV1-3), respiratory syncytial virus (RSV), coronaviruses 229E, OC43, HKU1, and NL63, human metapneumovirus (MPV), human rhinovirus (HRV), bocavirus (BOV), and adenovirus (ADV) were measured. All the above procedures were followed the manufacturer's protocol.

2.3. Bacteria and yeast detection

All sputum specimens were cultured according to China national standard protocols to detect respiratory bacteria and *Yeast*. Specimens were inoculated in blood agar, eosin methylene blue agar, and chocolate agar and incubated for 24–48 h at 37 °C. Colony identification was undertaken using a VITEK 2 Compact full automatic identification system (Biomérieux, France). Fourteen bacterium including *Acinetobacter baumannii(A. baumannii), Enterobacter aerogenes (E.aerogenes), Escherichia coli(E.coli), Haemophilus influenzae(H. influenzae), Branhamella catarrhalis(B. catarrhalis), Stenotrophomonas maltophilia(S. maltophilia), Enterobacter cloacae(E.cloacae), Serratia marcescens(S. marcescens), Klebsiella ornithinolytica(K. ornithinolytica), Klebsiella pneumoniae(K. pneumoniae), Pseudomonas aeruginosa(P. aeruginosa), Staphylococcus aureus(S.aureus), Staphylococcus haemolyticus(S. haemolyticus) and Streptococcus pneumoniae(S. pneumoniae)*.

2.4. Statistical analysis

Data were analyzed by SPSS 19.0. Categorical variables were summarized and compared by χ^2 test or Fisher's exact test, the comparisons between the two groups were made by using the Bonferroni test level adjustment method. Continuous variables with normal distributions were used mean ± standard deviation(SD) to describe and compare using multiple variance of multiple samples. Medians and interquartile ranges (IQR) were used to describe non-normal distribution of continuous variables, with comparisons based on the nonparametric Kruskal-Wallis H test for significance of the differences among more than two groups and the Mann-Whitney U test was used for significance of the differences between two groups.

Table 1
The number of cases and the percentage of patients the results of Clinical-laboratory testing.

	Cases	Percentage
Total	195	
Age		
0–14	15	7.69%
≥15	180	92.31%
Gender		
Male	130	66.67%
Female	65	33.33%
Pathogen infection		
Virus	32	16.41%
Bacterium	46	23.59%
Yeast	20	10.26%
Virus + bacterium	17	8.72%
Virus + *Yeast*	8	4.10%
Unidentified	72	36.92%
Associated lung disease		
COPD	6	3.08%
Tuberculosis	3	1.54%
Emphysema	4	2.05%
Pulmonary heart disease	3	1.54%
Asthma	2	1.03%
Bronchiectasis	1	0.51%
Bronchitis	1	0.51%
Systemic diseases		
Diabetes	24	12.31%
High blood pressure	54	27.69%
Heart disease	11	5.64%
Tumor	6	3.08%
Hepatitis	5	2.56%
Epilepsy	6	3.08%
Severity		
Mild - moderate	95	48.72%
Severity	100	51.28%
Treatment		
Antibiotic	113	57.95%
Antiviral	18	9.23%
Antiviral + antibiotic	39	20.00%
Clinical symptom		
Cough	99	50.77%
Pharyngeal discomfort	12	6.15%
Snivel	9	4.62%
Fever	124	63.59%
Shortness of breath or dyspnea	59	30.26%
Prognosis after healing		
Death	9	4.62%
Alive	186	95.38%

Abbreviations: COPD, chronic obstructive pulmonary disease.

3. Results

3.1. Clinical findings of patients with CAP

In Table 1, 195 patients included 130 males and 65 females. Fifteen cases were children with a mean age of 0–14 yrs, and 180 cases were adults with a mean age of 60 (15–91 yrs). Twenty patients (10.26%) previously had a pulmonary-associated disease, which included 6 cases of COPD, 3 cases of tuberculosis, 3 cases of pulmonary heart disease, and 4 cases of emphysema. A total of 106 patients (54.36%) had at least

1 underlying disease, including high blood pressure, diabetes, heart disease, epilepsy, tumors and hepatitis. After admission, broad-spectrum antibiotics were prescribed to 113 patients (57.95%) as an empiric or definitive therapy, and 18 patients were given antiviral treatment. 39 patients had been given antiviral plus antibiotic treatment. The most frequently used antibiotic were quinolones, cephalosporins and β-lactams, and the most frequently used antiviral agents were oseltamivir, ribavirin, recombinant human interferon α-1b. Unfortunately, 9 of the 195 patients died, all were adults (male: 6; female: 3), aged 55–91 yrs, with an average age of 69.89 yrs.

3.2. Pathogen detection in patients with CAP

A single-bacterial infection was found in 46 patients (23.59%), a single-*Yeast* infection was found in 20 patients (10.26%) and a single-viral infection was found in 32 patients (16.41%). Co-infections (virus + bacterium and virus + *Yeast*) was found in 25 patients (18.82%), and the pathogens could not be unidentified in 72 cases (36.92%) (Table 1).

Bacterial infection was identified in 63 (32.31%) of the 195 patients. The two frequently detected bacteria were *A. baumannii* and *K. pneumoniae*, which accounting for 49.21% of the bacterial infection. In addition, there were 7 cases infected by *P. aeruginosa* and *S. aureus*, respectively, and 6 cases were *E. coli* (Table 4). Three cases had more than one bacterial infection, which was *S. aureus* plus *K. ornithinolytica, A. baumannii* plus *P. aeruginosa* and *S. pneumoniae* plus *E. aerogenes,* respectively.

Viral infection was identified in 57/195 (29.23%) of the patients. Influenza A, influenza B, PIV3 and RSV were the most common viruses among all cases, followed by 229E, MPV, OC43, and PIV2 (Table 4). Specifically, 8 patients had more than one virus, accounting for 4.10% of the virus-positive group. Dual viral infection was observed in RSV co-infected with HRV, PIV3 and HKU1, respectively, PIV2 co-infected with BOV, HKU1 and 229E, respectively, and Influenza A co-infected with MPV and HRV, respectively.

25 patients had co-infections, including a single virus and a single bacterial/*Yeast* co-infections in 21 cases, two viruses and a single bacterium /*Yeast* co-infections in 4 cases. In these patients, bacterial/*Yeast* co-infections with non-influenza viruses were more frequent than bacterial /*Yeast* and influenza co-infections (Table 5).

3.3. Clinical presentation in relation to etiological findings

Fever, cough, and shortness of breath were the most common clinical signs observed in 50%, 30.43%, and 23.91% of the bacterial cases, in 75%, 78.13%, and 34.38% of the viral cases, in 60%, 35%, and 40% of the *Yeast* cases, and 64%, 68% and 44% of the co-infections cases, respectively. The bacterial group was significantly different from the viral group and the co-infected group in cough ($p < 0.0083$), at the same time, the viral group and the *Yeast* group in cough also has significant differences($p < 0.0083$) (Table 2).

3.4. Etiological type associated with disease severity and lab findings

4 patients with pure non-influenza infection and 4 patients with non-influenza and bacterium / *Yeast* co-infection were admitted to the ICU, and 3 patients died in the non-influenza co-infected with bacterium / *Yeast* group (Table 3). In these patients, pharyngeal discomfort was only found in the pure influenza group. Bacterium / *Yeast* co-infected with non-influenza viruses were more common than bacterium/ *Yeast* and influenza co-infections. WBC counts in the non-influenza groups were significantly higher than in the influenza groups, and the highest WBC counts were observed in the non-influenza and bacterium / *Yeast* co-infected group. The PLT count ($p < 0.01$) also had the similar trend.

3.5. The status of bacterial infections and viral infections in the influenza season and non-flu seasons

In the flu season, the cases of *A. baumannii* 's infection was greater than those in the non-flu season (15 cases vs. 1 case). It was noteworthy that bacteria such as *H. influenzae, B. catarrhalis, S. maltophilia, E. cloacae, K. ornithinolytica, S. marcescens, S. haemolyticus, S. pneumonia* were not detected in the non-flu season. The emergence of influenza viruses (Flu A and Flu B) was more common in the flu season, other virus like MPV and 229E were smaller than flu in the influenza season (Table 4).

4. Discussion

Pathogen-directed therapy avoids unnecessary antibiotic or antiviral use, facilitating more timely and hence more effective use of drugs, help prevent secondary spread of infection, all of which shorten hospital stays and have a major impact on patient management and disease prognosis [19–21]. Accurate and rapid etiologic diagnosis is crucial to pathogen-directed therapy. To date, the gold standard for CAP diagnosis is still based on the radiographic findings [22]. The radiological findings of multilobar infiltration or pleural effusion generally indicate greater severity [23]. Although etiological studies have been performed, current microbial diagnostic tests frequently do not allow clinicians to rule out bacterial infection with certainty [24]. Obtaining an qualified sample may be a challenge as specimens were easily contaminated by the bacteria located in upper airways [25]. For increasing the detection rate of pathogens, esp. for infection occurred in the lower respiratory tract, alveolar lavage fluid may be better in reflecting infectious status than the sputum. However, alveolar lavage fluids require semi-invasive surgery, which is certainly not routine in non-intubated patients, esp. to children [2]. Under this condition, taking sputum as the sample is more practical. Therefore, the relevant CAP guidelines recommend sputum examinations for patients with moderate to severe CAP. In this study, a high microbial yield can be achieved when real time PCR assays plus the conventional diagnostic methods, such as bacterial cultures [26,27]. The single-plex real time

Table 2
The clinical symptoms of bacterium group, virus group, *Yeast* group and co-infected group.

	Bacterium group	Virus group	*Yeast* group	Co-infected group	*p* value
Fever	23/46	24/32	12/20	16/25	0.166
Cough	14/46**	25/32$^{\#\#}$	7/20	17/25$^{\blacktriangle\blacktriangle}$	0.000
Shortness of breath or dyspnea	11/46	11/32	8/20	11/25	0.317
Pharyngeal discomfort	0/46	5/32	0/20	0/25	—
Snivel	1/46	3/32	0/20	0/25	—

p Value by R × C χ^2 test(Cell frequency was 0, not suitable for χ^2 test analysis).
Co-infected group : virus + bacterium and virus + *Yeast*.
$^{**}p < 0.0083$ when the bacterium group is compared with virus group, $^{\#\#}p < 0.0083$ when the virus group is compared with *Yeast* group and $^{\blacktriangle\blacktriangle}p < 0.0083$ when the bacterium group is compared with co-infected group (Comparisons between the two groups were made by using the Bonferroni test level adjustment method, inspection level = $0.05/ C_4^2 = 0.0083$).

Table 3
The clinical symptom and lab detection of Influenza group and non-Influenza group.

	Influ	Non-influ	Influ +bacterium /*Yeast*	Non-influ +bacterium /*Yeast*
Clinical symptoms				
Cough	14/16	11/16	3/5	14/20
Pharyngeal discomfort	5/16	0/16	0/5	0/20
Snivel	2/16	1/16	0/5	0/20
Fever	13/16	11/16	2/5	14/20
Shortness of breath	5/16	6/16	2/5	9/20
Lab detection				
WBC($\times 10^{\wedge}9$/L)	6.53 ± 3.02$^{\#\#**}$	11.46 ± 5.85	9.47 ± 6.37▲▲	14.56 ± 6.20
L($\times$10^9/L)	0.93 ± 0.41	1.64 ± 1.30	0.74 ± 0.31	1.56 ± 1.56
N(%)	72.53 ± 10.84	72.87 ± 24.35	74.31 ± 20.98	79.79 ± 14.88
L(%)	17.22 ± 9.24	18.81 ± 19.61	13.07 ± 12.76	12.46 ± 12.11
Hb(g/L)	120.68 ± 23.35	117.35 ± 35.24	119.14 ± 14.40	124.07 ± 29.29
PLT($\times 10^{\wedge}9$/L)	172.05 ± 76.96$^{\#\#}$	226.10 ± 82.73$^{\Delta\Delta}$	131.06 ± 63.80▲▲	210.03 ± 132.68
PH	7.45 ± .027	7.40 ± .027	7.43 ± .039	7.41 ± 0.15
PCO_2(mmHg)	28.86 ± 3.84	34.22 ± 7.09	39.76 ± 6.20	35.92 ± 10.25
SO_2(%)	92.00 ± 5.47	90.76 ± 7.48	94.49 ± 1.88	91.48 ± 14.29
Severity				
ICU	1	4	1	4
Death	0	0	0	3

Abbreviations: Influ, Influenza A+ Influenza B; Non-influ, other 13 viruses; WBC, white blood cell; L, lymphocyte; N, neutrophil; PLT, platelet; PH, power of hydrogen; PCO_2, carbon dioxide partial pressure of tension; SO_2, oxygen saturation; ICU, intensive care unit.
$^{\#\#}p < 0.01$ when the influ group is compared with non-influ group$^{**}p < 0.01$ when the influ group is compared with non-influ + bacterium/*Yeast* group; $^{\Delta}p < 0.01$ when the non-influ group is compared with influ + bacterium/ Yeast group; ▲▲$p < 0.01$ when the influ + bacterium/*Yeast* group is compared with non-influ + bacterium/ *Yeast* group.

Table 4
The number of bacterial infections and viral infections in the influenza seasons and non-flu seasons in 2016.

	Influenza seasons					Total	Non-influenza seasons			Total
Months	Jan.	Feb.	Mar.	Apr.	May		June	July	Aug.	
Yeast	1	0	5	3	3	12	3	1	12	16
A. baumannii	4	3	2	2	4	15	0	0	1	1
K. pneumoniae	0	1	1	4	2	8	0	3	4	7
P. aeruginosa	2	0	0	1	1	4	0	1	2	3
S. aureus	1	1	3	1	0	6	0	0	1	1
E. coli	1	2	0	0	0	3	3	0	0	3
H. influenzae	1	0	1	1	0	3	0	0	0	0
E. aerogenes	0	1	0	0	0	1	1	0	1	2
B. catarrhalis	0	2	0	1	0	3	0	0	0	0
S. maltophilia	0	1	0	0	0	1	0	0	0	0
E. cloacae	0	1	0	0	0	1	0	0	0	0
K. ornithinolytica	0	0	1	0	0	1	0	0	0	0
S. marcescens	0	0	1	0	0	1	0	0	0	0
S. haemolyticus	0	0	0	0	1	1	0	0	0	0
S. pneumoniae	0	1	0	0	0	1	0	0	0	0
Flu A	2	0	6	4	1	13	0	0	1	1
RSV	1	0	0	2	3	6	1	1	0	2
Flu B	0	1	4	0	1	6	0	0	1	1
PIV3	0	1	0	1	2	4	0	2	1	3
OC43	1	0	0	0	0	1	2	1	1	4
MPV	0	2	1	1	1	5	0	0	0	0
229E	1	1	2	1	0	5	0	0	0	0
PIV2	0	0	1	2	0	3	0	0	2	2
HKU1	1	0	0	1	0	2	1	0	0	1
BOV	0	0	0	0	0	0	0	0	3	3
HRV	0	0	0	1	0	1	0	0	1	1
NL63	0	0	0	0	0	0	0	0	1	1
PIV1	0	0	0	0	0	0	0	0	0	0
ADV	0	0	0	0	0	0	0	0	0	0

PCR assay were exploited for detecting 14 respiratory viruses, which could improve diagnostic efficacy, particularly in diagnosing respiratory viral infections [28]. Etiologic diagnosis in the CAP patients reached 63.08%, we still had 36.92% CAP patients without clearly microorganisms diagnose. For patients with comorbidities, the use of antibiotics prior to sampling may obscure a potential bacterial detection [29]. Previous studies revealed that etiology remains unknown in approximately one-half of the cases [30,31], indicating that establishment of a precise pathogen diagnosis for CAP patients is challenging. Better diagnostic methods are warranted to distinguish viral from

Table 5
Composition and quantity of co-infections of bacterium/*yeast* and virus.

	Flu A	Flu B	MPV	229E	RSV	PIV2	PIV3	OC43	BOV
Yeast	2	–	–		2*	–	1	1	2
A. baumannii	–	–	1	1(PIV2)	–	–	–	–	–
B. catarrhalis	1	–	–	1	–	–	–	–	–
S. aureus	–	1	–	1	1(HRV)	–	–	–	–
K. pneumoniae	1	–	–	–	–	2#	2	1	–
S. haemolyticus	–	–	–	–	1	–	–	–	–
E.aerogenes	–	–	–	–	–	–	–	2	–
P. aeruginosa	–	–	–	–	–	1	–	–	–

1(PIV2): *A. baumannii* and 229E, PIV2 three pathogens **co**-infections.
1(HRV): *S. aureus* and RSV, HRV three pathogens **co**-infections.
* indicates that one of the two cases are **co**-infected with three pathogens (*Yeast* and RSV, PIV3).
\# indicates that one of the two cases are **co**-infected with three pathogens (*K. pneumoniae* and PIV2, HKU1).

bacterial CAP, which will clarify if these viral infections cause CAP on their own or merely predispose the patient to bacterial co-infections [32].

Differences in epidemiology make the knowledge of local etiology crucial for the appropriate choice of empirical antimicrobial treatment [28]. Even there was a severe influenza outbreak during our study, other respiratory viral infections were more than influenza infection (63.16% vs. 36.84%). In all pathogens that cause CAP, bacteria occupied the majority of pathogens, and the most common bacterium was *A. baumannii*. Our results was not in keeping with the previous studies, in which the most frequently identified pathogens were *Burkholderia pseudomallei* (29%), *S. pneumoniae* (20%), *K. pneumoniae* (19%) and *H. influenza* (11%), respectively [33]. Although *A. baumannii* is an important pathogen of nosocomial infections, we often isolated it from infectious patients who is first admitted to the hospital, indicating that A. baumannii has a certain popularity in this area. Most community-acquired cases of *A. baumannii* pneumonia are from tropical or subtropical countries in the Asia-Pacific region. In Thailand, severe CAP is caused by *A. baumannii*, and its mortality is 10 times higher than hospital-acquired *A. baumannii* pneumonia [33,34]. In another aspect, most of our patients had underlying diseases, we could not exclude the possibility that patients had the bacterial infection in last hospital admission. The pathogen remains in the respiratory tract, causing clinical symptoms when the body's immunity is reduced. The outbreak of *A. baumannii* infection is also associated with multidrug resistance, and carbapenems have been considered to be a major factor in resistance to multidrug-resistant *A. baumannii* infection [34]. Moreover, the mortality of carbapenem-resistant *A. baumannii* pneumonia patients is higher than that of carbapenem-sensitive *A. baumannii* pneumonia patients [35,36].

S. pneumoniae and *H. influenza*, as co-pathogens, often seem to be part of a mixed infection (virus and bacterium) in adults with CAP [26], representing the most common combination with respiratory viruses [2]. The incidence of mixed infections was significant among patients admitted to the hospital with CAP [37]. In our study, 16.41% of CAP patients had single virus, and the co-infections (virus + bacterium and virus + *Yeast*) were found in 12.82% CAP patients. The incidence of co-infections was in agreement with a previous study [38]. The most common bacterium co-detected with a virus was *K. pneumoniae*, as well as the *Yeast* in our study, indicating that geographical variation exists in the prevalence of bacterium or *Yeast* strains expressing factors that enable efficient disease potentiation during viral epidemics, which should be considered one explanation for regional differences in severity [8]. The diversity in etiology revealed that understanding the local etiology of CAP is essential to inform clinical management decisions.

Respiratory viruses usually followed seasonal patterns of activity [39], and epidemic influenza was noted in our studied area, which was active throughout the study period, and there was a high detecting rate of influenza A/influenza B was from January to May. During an influenza epidemic season, a high percentage of samples from CAP patients contained at least one virus, and dual infections were very common [40]. The respiratory virus other than influenza accounted for 63.16% viral CAP in our study, revealing that respiratory viruses can be co-circulating even during the highest epidemic peaks of influenza that showed a seasonal distribution. Bacterial or fungal infections secondary to influenza viral infections were common [41], though we could not exclude that the increasing co-infections CAP was due to secondary viral infection. As the clinical signs and symptoms overlapped between influenza and non-influenza viruses, pathogen-directed therapy is difficult if only based on clinical symptoms. Studies have shown that delayed antiviral treatment is associated with high risk of progression to severe disease required ICU admission or prolonged hospital stay, and even causing death [42].

The microbiome's characterization and diversity are closely linked to the host status, and the host immune response may play a determining role in the infectious exacerbation of CAP. The intrinsic complexity of the host-microbiome relationship currently limits the clinical indications of microbiome analysis [25]. Diabetes and high blood pressure were the most common underlying disease, which were known to be associated with an increased risk of complications and death among critically ill patients [43]. Detection of respiratory secretions by PCR method has provided a rapid and definitive diagnosis for the ILIs in hospitalized adults [44]. Unfortunately, it is not routine test in most clinic labs. Appropriate empiric therapy is likely to be suboptimal without narrowing diagnostic possibilities to the most likely possibilities pending definitive pathogen identification [44]. Effective, targeted therapy for CAP requires an understanding of the heterogeneity of etiology and the individual host response to infection [45].

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Declaration

This study was approved by the Ethics Committee of Shantou University Medical College and informed consents were obtained from the patients.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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