Original Article
Epidemiological study on the respiratory pathogens in hospitalized patients with lower respiratory tract infection in Fujian Province

Daxuan Wang[1,2], Yusheng Chen[1,2], Hongru Li[1], Wei Zhang[2], Wensen Huang[2], Xiaohong Lin[2], Mengqing Lin[2], Nengluan Xu[1], Ming Lin[1], Baosong Xie[1], Xiaona Shen[3], Jianfeng Xie[3], Xinlan Hu[4]

[1]Department of Respiratory and Critical Care Medicine, Fujian Provincial Hospital, Fujian Provincial Medical College, Fujian Medical University, Fuzhou, Fujian Province, China; [2]Provincial Clinical Medical College of Fujian Medical University, Fuzhou, Fujian Province, China; [3]Department of Viral Diseases, Fujian Center for Disease Control and Prevention, Fuzhou, Fujian Province, China; [4]Clinical Microbiology Laboratory, Fujian Provincial Hospital, Fuzhou, Fujian Province, China

Abstract: Objective: To detect the probable pathogens in patients with lower respiratory tract infection (LRTI) using a bead-based suspension array, and analyze the epidemiological and clinical characteristics of these patients in Fujian Province. Methods: Sputum and/or bronchoalveolar lavage fluid was collected from 819 hospitalized patients diagnosed with LRTI from the Department of Respiratory Medicine of the four tertiary hospitals in Fujian Province from March 2012 to March 2016. The pathogens were detected using a bead-based suspension array and the data were statistically analyzed in combination with the clinical information. Results: The most common bacteria in LRTI were Pseudomonas aeruginosa (P. aeruginosa), Streptococcus pneumoniae (S. pneumoniae) and Klebsiella pneumoniae (K. pneumoniae); the viruses ranking the top three were influenza a virus (Flu-A), human cytomegalovirus (HCMV), and adenovirus. The analysis of the top three viruses with the highest morbidity revealed that the three most common bacteria in patients with community-acquired pneumonia, acute exacerbation of chronic obstructive pulmonary disease, and bronchiectasis were P. aeruginosa, K. pneumoniae, and S. pneumoniae, while viruses with the largest numbers were Flu-A and HCMV. Due to mixed infection of bacteria and viruses, the patients with LRTI were more likely to suffer from pleural effusion and were prone to have a large patchy shadow. Moreover, leukocyte levels, neutrophil/granulocyte ratio, C-reactive protein, and prognosis including invalid, died rates were all higher compared with the other three groups (P<0.05). Conclusion: The results showed that the study of epidemiological and clinical characteristics in Fujian Province could be used to guide clinical drug use.

Keywords: Bacteria, bead-based suspension array, lower respiratory tract infection, virus

Introduction

Lower respiratory tract infection (LRTI) is a common respiratory disease, which has high morbidity and mortality [1]. The pathogens of LRTI are numerous, and their clinical symptoms are highly similar, so they are difficult to diagnose and distinguish on the basis of clinical manifestations and pulmonary signs [2]. However, most of the current laboratories only aim at analyzing, and detecting traditional bacterial strains in culture, which are poorly adapted to the detection of fastidious bacteria and viruses [3, 4]. Therefore, a high-sensitivity, high-specificity, and high-throughput method for detecting respiratory viruses is essential to prevent the abuse of antibiotics and clear up LRTI.

The bead-based suspension array has the advantages of simple operation, high sensitivity, strong specificity, and rapid identification of several kinds of pathogens of LRTI [5]. It not only simplifies the experimental process, saves labor, and reduces the amount of test specimens, but also reduces the detection time of samples. It can provide diagnostic results timely for clinical treatment, and control the use of antibiotics for LRTI effectively [6, 7].

Specimens of hospitalized adults with LRTI were collected from the four tertiary hospitals (Fujian Provincial Hospital in Fuzhou, the Affiliated Hospital (Group) of Putian University, San Ming Hospital, and WuYiShan City Hospital) in this study and then an in-depth analysis was performed for the results of pathogen detection clinical data and data on regional conditions. This study aims to explore the species of respiratory pathogens and prognosis in patients with LRTI for guiding the clinical therapy.

Materials and methods

Subjects

A total of 819 hospitalized patients with LRTI in Fujian Provincial Hospital from March 2012 to March 2016 were enrolled in this study. The inclusion criteria were as follows: (1) patients who suffered from cough, sputum viscosity, and pulmonary rales, as well as one of the following conditions: (a) fever; (b) increase in the total number of leukocytes and (or) the neutrophil ratio; (c) invasive lesions of the lung revealed by chest x-ray; (2) patients with chronic airway disease at the stable stage (chronic bronchitis with or without obstructive emphysema, asthma, or bronchiectasis) who suffered from secondary acute infection, combined with pathogenic changes, or showed a significant change on a chest x-ray compared with that at admission or appearance of a new lesion.

The exclusion criteria were as follows: (1) patients who died during hospitalization or were discharged within 24 h after hospitalization; (2) patients who were clearly or highly suspected with pulmonary tuberculosis, pulmonary fungal disease, and pulmonary malignancies; (3) patients who had acquired immunodeficiency syndrome (acute respiratory distress syndrome) or human immunodeficiency viral infection, and other autoimmune diseases.

Data collection

The basic information of the enrolled subjects was collected including demographics ((age, gender, smoking history, comorbidities (chronic obstructive pulmonary disease, COPD), bronchiectasis, asthma, heart function insufficiency, cerebrovascular disease, diabetes, renal insufficiency, non-lung tumors)), use of antibiotics before admission, clinical symptoms, related signs, laboratory examination and imaging data.

Specimen collection and detection

Qualified sputum specimens were collected at hospitalization before receiving antiviral drugs or antibiotics (microscopic examination was performed for all the sputum specimens after homogenization, if the number of leukocytes under each low-power field was greater than 25, while the number of squamous cells under low-power field was less than ten).

Pathogens were detected using bead-based suspension array based on previous reports. Briefly, the extraction of the pathogen DNA of qualified sputum specimens was performed (Aidlab Biotechnologies Co. Ltd), besides the extraction of specimen pathogen RNA (the kits were purchased from Takara Company, Japan). The extracted RNA was subjected to reverse transcription into cDNA (the kits were purchased from Aidlab Biotechnologies Co. Ltd). Amplification was performed based on the established four groups of multiplex polymerase chain reaction (PCR) amplification system: the first group: six pairs of virus primers (human cytomegalovirus (HCMV)-F, HCMV-R, PIV1-F, PIV1-R, PIV2-F, PIV2-R, PIV3-F, PIV3-R, adenovirus (AD)-F, AD-R, RSV-F, and RSV-R); the second group: six pairs of virus primers (HMPV-F, HMPV-R, HBOV-F, HBOV-R, SARS-F, SARS-R, N1-F, N1-R, H5-F, H5-R, influenza A virus (Flu-A)-F, and Flu-A-R); the third group: three pairs of virus primers (SP-F, SP-R, SM-F, SM-R, MP-F, and MP-R); the fourth group: five pairs of virus primers (SA-F, SA-R, AB-F, AB-R, KP-F, KP-R, CP-F, CP-R, PA-F, and PA-R). PCR products and fluorescence-coded microspheres (Shanghai Jing Jing Life Polytron Technologies Inc, Shanghai, China) were hybridized, and Luminex 100 (Luminex Company, USA) detection was performed.

Meanwhile, the distribution of bacteria and viruses in different disease subtypes of LRTI, the relationship of the prognosis of patients and bacterial infection, the relationship of the incidence of mixed infection or the infection with a variety of bacteria and the prognosis were observed.

Data analysis

Statistical analysis was performed using SPSS 17 statistical software (SPSS, IL, USA). Continuous data were represented as Mean ±
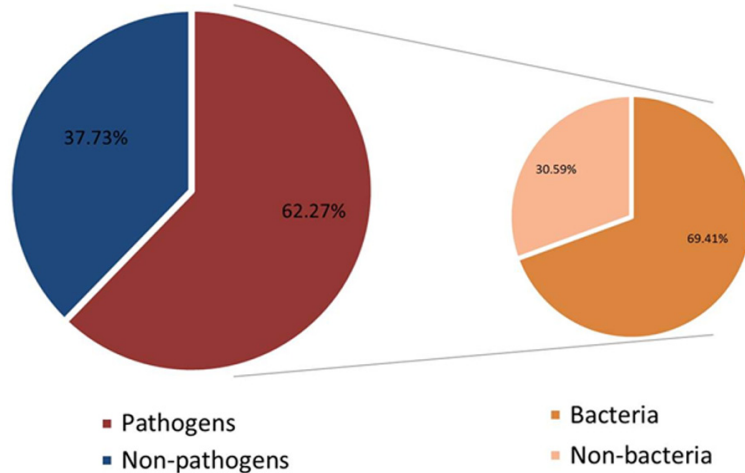
Figure 1. Composition of the positive detection rate of bacterial pathogens.
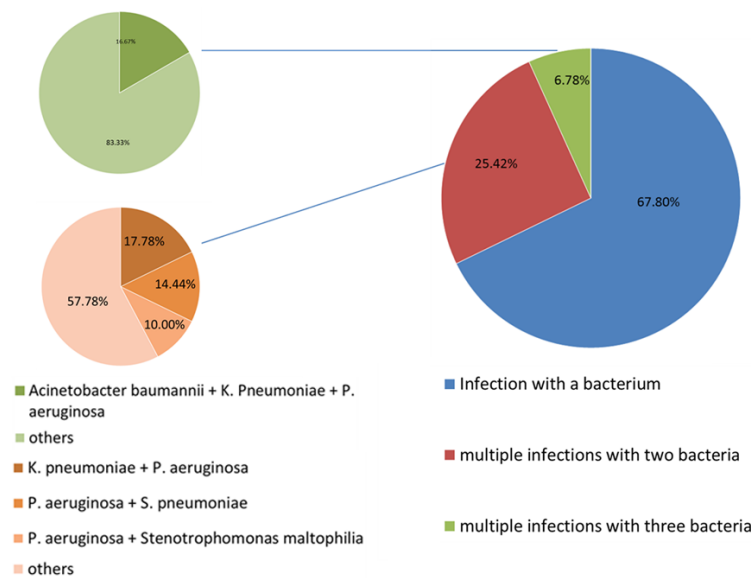

Figure 2. Composition of the 354 detected bacterial strains.

Standard deviation (SD), and the t test or one-way analysis of variance was used. The X^2 test was used for categorical data. A *P* value less than 0.05 was considered to be statistically significant.

Results

General information about patient demographics

A total of 819 effective cases (560 males (68.38%) and 259 females (31.62%)) aged 15-96 years old (average age of 63.96 years old) were reported, patients with complications accounted for 55.56% (455/ 819), including 396 cases with structural lung diseases (87.03%) (204 cases of CO-PD, 113 cases with bronchiectasis, and 79 cases of asthma), 141 cases with cardiac insufficiency (17.22%), 38 cases with cerebral vascular diseases (4.64%), 89 cases with diabetes (10.87%), 26 cases with renal insufficiency (3.17%), and 68 cases with non-lung tumors (8.30%).

Bacterial and viral infections in patients with LRTI

In these patients with LRTI, the positive rate of respiratory pathogens was 62.27% (510/ 819), and the rate of bacterial pathogens was 69.41% (354/ 510) in these respiratory pathogens (**Figure 1**).

There were 240 cases (240/ 354, 67.80%) with a bacterial infection, and 114 cases (114/ 354, 32.20%) with multiple infections. Among that, there were 90 cases with two kinds of bacterial infections, in which the top three comprised 16 cases with K. pneumoniae + P. aeruginosa (16/90, 17.78%), 13 cases with P. aeruginosa + S. pneumoniae (13/90, 14.44%), and 9 cases with P. aeruginosa + Stenotrophomonas maltophilia (9/90, 10%); while there were 24 cases with three bacterial infections, where most of the multiple infections with three kinds of pathogenic bacteria comprised 4 cases with Acinetobacter baumannii + K. pneumoniae + P. aeruginosa (4/24, 16.67%) (**Figure 2**).

The positive detection rate of viruses was 53.33% (272/510) in respiratory pathogens, and in these, 227 cases (227/272, 83.46%) had a viral infection, 43 cases (43/272, 15.81%) had multiple infections with two kinds of viruses (23 cases of Flu-A + HCMV (53.48%), four cases of HCMV + AD (9.30%), and 3 cases of Flu-A + PIV1 (6.98%)) (**Figure 3**).
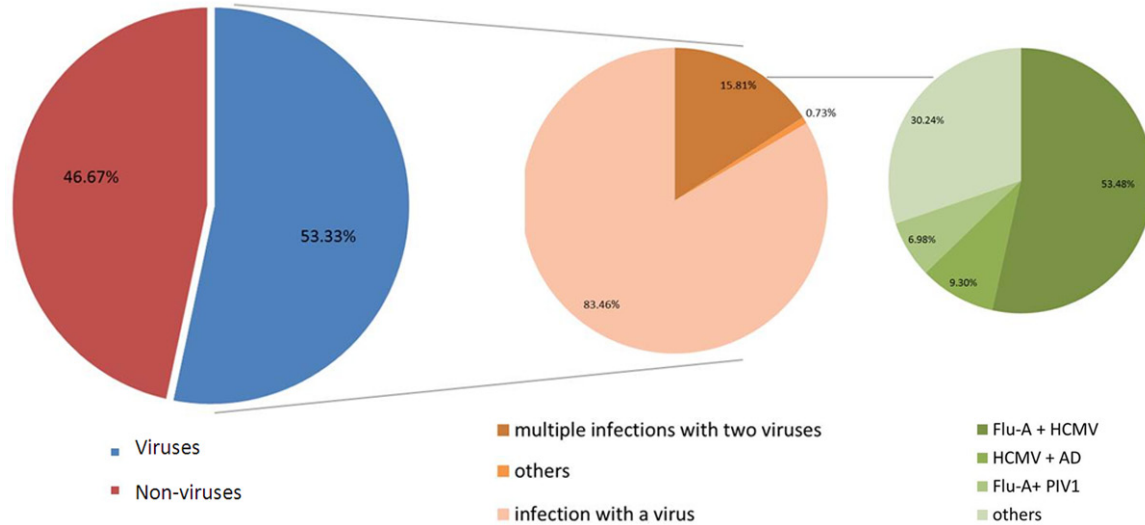
Figure 3. Composition of the 272 detected viral strains.

Table 1. Pathogens spectrum in the disease

Pathogenic bacteria	CAP (n=502)	AE-COPD (n=204)	AEB (n=113)
Pseudomonas aeruginosa	71 (14.14%)	25 (12.25%)	34 (30.09%)
Streptococcus pneumoniae	66 (13.14%)	33 (16.18%)	9 (7.96%)
Klebsiella pneumoniae	58 (11.55%)	23 (11.27%)	12 (10.62%)
Acinetobacter Bauman	33 (6.57%)	9 (4.41%)	6 (5.31%)
Staphylococcus aureus	24 (4.78%)	12 (5.88%)	6 (5.31%)
Stenotrophomonas maltophilia	26 (5.18%)	15 (7.35%)	4 (3.54%)
FLUA	86 (17.13%)	36 (17.65%)	24 (21.24%)
HCMV	65 (12.95%)	32 (15.69%)	16 (14.16%)
H5	6 (1.20%)	1 (0.49%)	0 (0.00%)
PIV1	4 (0.80%)	1 (0.49%)	1 (0.88%)
AD	3 (0.60%)	2 (0.98%)	1 (0.88%)
PIV2	4 (0.80%)	2 (0.98%)	0 (0.00%)
PIV3	10 (1.99%)	2 (0.98%)	0 (0.00%)
N1	2 (0.40%)	1 (0.49%)	0 (0.00%)
HBOV	4 (0.80%)	0 (0.00%)	0 (0.00%)
HMPV	1 (0.20%)	1 (0.49%)	1 (0.88%)
RSV	8 (1.59%)	1 (0.49%)	0 (0.00%)

Note: AEB, Bronchial dilation; AE-COPD, chronic obstructive pulmonary disease in acute phase; CAP, community-acquired pneumonia. Pseudomonas aeruginosa, Streptococcus pneumoniae and Klebsiella pneumoniae.

The analysis of the composition of disease spectrum in these 819 cases with LRTI showed 502 cases with community-acquired pneumonia (CAP), 204 cases with acute exacerbation of COPD, and 113 cases with bronchial dilation. The composition of the pathogenic bacteria of the three kinds is shown in **Table 1**.

Comparison of clinical features of mixed viral-bacterial infection with simple bacteria and virus infection in the lower respiratory tract

In the clinical data, 113 cases with mixed viral-bacterial infection of the lower respiratory tract, 241 cases with simple bacterial infection, 159 cases with simple viral infection, and 306 cases with no infection were compared. No significant difference in gender distribution, underlying disease, existence of wheezing, and extrapulmonary complications was found (P>0.05). The course of fever in the mixed infection group was long. Therefore, this group was more prone to pleural effusion, which was statistically compared with the other three groups (P<0.05). Regarding imaging manifestations, the mixed infection group was more prone to large patchy shadows, and pleural effusion frequently occurred in patients with high-density large patches, as shown by lung imaging. However, the data of simple bacterial infection and virus infection groups mainly showed high-density small patchy shadows, with significant differ-

Table 2. Comparison of the indexes between the mixed viral-bacterial infection and the non-infection groups

Items	Mixed infection (113 cases)	Simple bacteria (241 cases)	Simple virus (159 cases)	Cases without detected virus or bacteria strains (306 cases)	Statistics	P value
Gender (M/F)	82/31	161/80	101/58	216/90	3.168	0.306
Age	63.8 ± 16.24	61.3 ± 17.25	59.7 ± 18.31	64.5 ± 15.66	0.380	0.702
Underlying diseases	23 (20.35%)	58 (24.06%)	34 (21.38%)	70 (22.87%)	0.770	0.857
Pleural effusion	30 (26.54%)	62 (25.72%)	27 (16.98%)	24 (7.84%)	37.565	0.000
Wheezing	35 (30.97%)	83 (34.44%)	46 (28.93%)	87 (28.43%)	2.564	0.464
Chest x-ray						
Patchy shadows	65 (57.52%)	134 (55.60%)	62 (38.99%)	32 (10.46%)	150.572	0.000
Atelectasis	23 (20.35%)	61 (25.31%)	37 (23. 27%)	65 (21.24%)	1.691	0.639
Lung consolidation	8 (7.07%)	17 (7.05%)	10 (6.28%)	18 (5.88%)	0.388	0.943
Extrapulmonary complications	35 (30.97%)	57 (23.65%)	33 (20.75%)	31 (10.13%)	29.815	0.000
Experimental data						
Peripheral blood WBC	14.27 ± 4.99	12.07 ± 3.06	9.23 ± 2.98	8.05 ± 2.81	12.293	0.000
Proportion of neutrophils in peripheral blood	0.79 ± 0.21	0.73 ± 0.32	0.31 ± 0.23	0.29 ± 0.16	4.43	0.007
CRP	73.78 ± 4.71	66.67 ± 4.16	32.16 ± 2.17	26.81 ± 2.06	29.56	0.000
Prognosis						
Cured	30	120	78	201	34.33	0.000
Improved	31	105	69	98	14.13	0.003
Invalid	26	13	10	6	36.55	0.000
Died	16	3	2	1	32.81	0.000

ences among the four groups (P<0.05) (**Table 1**). During the course of disease (onset period) in patients with LRTI, mixed viral-bacterial infection, leukocyte level, neutrophil ratio, average of C-reactive protein (CRP) and prognosis including invalid, died rates were all higher than those in the other three groups with statistical significance (P<0.05). The results are shown in **Table 2**.

Discussion

In this study, the positive detection rate of bacterial pathogens was 43.22%, which was consistent with other studies [8-11]. Most of bacterial strains detected were gram-negative bacteria. The top three were P. seudomonas, S. pneumoniae, and K. pneumoniae, the detection rates of P. aeruginosa and K. pneumoniae were consistent with those reported in other published studies from Asia [12, 13]. The high detection rate of P. aeruginosa was related to the fact that most of the patients were elderly in this study, structural lung disease accounted for the majority. Therefore, P. aeruginosa should be taken into consideration for patients with LRTI due to structural lung disease. However, the high detection rate of K. pneumoniae was associated with colonization, or might be relat-

ed to the excessive use of antibiotics resulting in resistant strains of K. pneumoniae such as strains with Klebsiella pneumonia carbapenemase enzyme [14]. Furthermore, the detection methods were not in line with the higher detection rate of S. pneumoniae compared with other published studies [15]. S. pneumoniae belongs to fastidious bacteria, and the impacts of many factors result in the failure of the sputum culture for pathogens, however, the molecular biology detection method is superior to the traditional bacterial culture with a high detection rate, which is also related to the epidemic pathogens and the inclusion criteria of cases.

The top three of the detected viruses were (Flu-A) in 146 strains, HCMV in 113 strains, and PIV3 in 12 strains, while Flu-A had the highest detection rate, which was in keeping with other studies [16-18]. HCMV infection is widely popular in China. Generally, HCMV lurks in many normal people, but most immunocompetent individuals show symptomless infection, However, obvious symptoms might occur in immunosuppressed individuals, fetuses, and infants if they are infected. The patients in this study were older, and most of them also had underlying diseases. Therefore, their immunity was low, leading to the replication of HCMV in the body,

which had a higher detection rate [19, 20]. It was worth mentioning that 43 cases had co-infection of two kinds of viruses in this study. However, Flu-A + HCMV accounted for the majority in the co-infection of two kinds of viruses, which was inconsistent with the findings of Cebey-Lopez M. It was considered to be associated with the replication of HCMV due to low immunity during the course of Flu-A infection [21]. In fact, few reports are available about the mechanism of the co-infection of viruses, therefore, it needs further investigation [22].

The top three pathogenic bacteria were P. aeruginosa, S. pneumoniae, and K. pneumonia in CAP, which was basically in accordance with the multicenter survey of CAP pathogen detection by Zhao et al. [23]. This was because most of the enrolled patients with CAP were elderly, and the majority of them also had other underlying diseases, and low immunity. Other, COPD is a common and frequently occurring chronic respiratory disease, with a high mortality rate [24]. Moreover, 50%-80% AE-COPD is caused by respiratory tract infection [25, 26]. In AE-COPD, the top three were P. aeruginosa, S. pneumoniae, and K. pneumoniae. Polverino et al. studied the composition of the 281 strains of pathogenic bacteria in the sputum specimen separated and identified from patients with AE-COPD, and its composition was highly consistent with that reported in the present study [27]. A study reported that more than half of the patients with respiratory tract disease had potential colonization of pathogenic bacteria in the respiratory tract when the disease was stable, and the most common pathogen was P. seudomonas aeruginosa [28]. We demonstrated 71 strains bacteria were detected in the 113 cases of bronchial dilation in which P. aeruginosa accounted for the majority, while the other bacteria were fewer in number, which was attributed to the small sample size.

However, the detection rates of Flu-A and HCMV were high in the virological examination of the aforementioned three kinds of disease spectrum. Moreover, these patients previously were treated with broad-spectrum antimicrobial agents for multiple times. Also, some of them previously received invasive diagnosis and treatment such as mechanical ventilation and sputum suction. Therefore, the replication

and infection of HCMV were caused by a variety of factors. In addition, the mixed viral-bacterial infection includes secondary bacterial infection after viral infection, co-infection, and secondary viral infection after bacterial infection. At present, the first situation has been recognized by most scholars [22], while the other two have been found only in an animal model [29, 30].

Some studies of pathogens causing CAP suggested that mixed bacterial-viral infection could result in more severe inflammation and clinical symptoms in patients with LRTI [31]. Other studies have shown that patients with mixed infection have a higher proportion of cough, chest pain, and expectoration besides the general manifestation of infection, and they are more prone to complications such as pleural effusion and pulmonary edema [32]. However, the mixed infection group was more likely to have a pleural effusion. The mixed viral-bacterial infection group was more likely to have large patchy shadows in imaging manifestations. However, a significant difference in the infection index of inflammation was observed between the mixed viral-bacterial infection and the other groups. Clinically, patients with LRTI who also have increased leukocyte level, CRP content, and proportion of neutrophils in peripheral blood should be suspected to have bacterial and viral totally infections, and they might have the worse prognosis. So, future investigations should include more regions and hospitals to be representative of the entire Fujian Province. Meantime the sample size is relatively small which could lead to the deviation of results in our research. And the prospective experiment with more adequate sample size and longer follow-up time will be conducted in the future.

In conclusion, this present study analyzes the pathogens of the hospitalized patients with LRTI, and showed the epidemiological and clinical characteristics in Fujian Province which could be used to guide clinical drug use. It aims at providing the basis for the preliminary judgment of the pathogens of respiratory tract infection from the admission of the patients to the time before obtaining the results of laboratory pathogen detection. Moreover, it provides evidence for the use of antibiotics and antiviral therapy, which plays an important role in man-

aging hospitalized patients with respiratory tract infections, the prevention of abuse of antibiotics, and the spread of the virus.

Disclosure of conflict of interest

None.

Address correspondence to: Yusheng Chen, Department of Respiratory and Critical Care Medicine, Fujian Provincial Hospital, Fujian Provincial Medical College, Fujian Medical University, Fuzhou, Fujian Province, China; Provincial Clinical Medical College of Fujian Medical University, No.134 East Street, Gulou District, Fuzhou 350001, Fujian Province, China. Tel: +86-0591-88217531; E-mail: chenyusheng2003@sina.com

References

[1] Millett ER, Quint JK, Smeeth L, Daniel RM and Thomas SL. Incidence of community-acquired lower respiratory tract infections and pneumonia among older adults in the United Kingdom: a population-based study. PLoS One 2013; 8: e75131.

[2] Hoshina T, Nanishi E, Kanno S, Nishio H, Kusuhara K and Hara T. The utility of biomarkers in differentiating bacterial from non-bacterial lower respiratory tract infection in hospitalized children: difference of the diagnostic performance between acute pneumonia and bronchitis. J Infect Chemother 2014; 20: 616-20.

[3] Fournier PE, Drancourt M, Colson P, Rolain JM, La Scola B, Raoult D. Modern clinical microbiology: new challenges and solutions. Nat Rev Microbiol 2013; 11: 574-85.

[4] Zheng Q, Mikš-Krajnik M, Yang Y, Xu W and Yuk HG. Real-time PCR method combined with immunomagnetic separation for detecting healthy and heat-injured salmonella typhimurium on raw duck wings. Int J Food Microbiol 2014; 186: 6-13.

[5] Chen YS, Li HR, Zhang W, Hua ZD, Lin XH, Lin MQ, Huang WS, Huang LP, Yu XL, Xu NL, Lin M, Xie BS, Shen XN, Xie JF, Wang Y, Huang M, Wu YA and Hu XL. Development of a bead-based suspension array for the detection of pathogens in acute respiratory tract infections. Exp Biol Med (Maywood) 2016; 241: 1551-1558.

[6] Turhan V, Kilic A, Mert G, Li H, Sefers SE, Stratton CW and Tang YW. Investigation of clinical relevance of bacterial colonization in patients with suspected viral respiratory tract infection by using multiplex PCR method. Dis Mol Med 2013; 1: 2-7.

[7] Arnott A, Vong S, Rith S, Naughtin M, Ly S, Guillard B, Deubel V and Buchy P. Human bocavirus amongst an all-ages population hospitalised with acute lower respiratory infections in Cambodia. Influenza Other Respir Viruses 2013; 7: 201-210.

[8] Ryu K, Takayanagi N, Ishiguro T, Kanauchi T, Kawate E, Kagiyama N and Sugita Y. Etiology and outcome of diffuse acute infectious bronchiolitis in adults. Ann Am Thorac Soc 2015; 12: 1781-1787.

[9] Alnimr AM. Streptococcus pseudopneumoniae: prospective detection among community lower respiratory isolates in Eastern Saudi Arabia. Br J Biomed Sci 2014; 71: 139-144.

[10] Ruopp M, Chiswell K, Thaden JT, Merchant K and Tsalik EL. Respiratory tract infection clinical trials from 2007 to 2012. A systematic review of clinicaltrials.gov. Ann Am Thorac Soc 2015; 12: 1852-1863.

[11] Chen Y, Wang D, Li H, Chen W, Zhang X, Shao H, Xu N, Lin M, Wang C, Yan S, Hu X and Gao Z. [The application of loop-mediated isothermal amplification for detection of common pathogenic bacteria in lower respiratory tract infections]. Zhonghua Jie He He Hu Xi Za Zhi 2014; 37: 270-273.

[12] Kang Y, Deng R, Wang C, Deng T, Peng P, Cheng X, Wang G, Qian M, Gao H, Han B, Chen Y, Hu Y, Geng R, Hu C, Zhang W, Yang J, Wan H, Yu Q, Wei L, Li J, Tian G, Wang Q, Hu K, Wang S, Wang R, Du J, He B, Ma J, Zhong X, Mu L, Cai S, Zhu X, Xing W, Yu J, Deng M and Gao Z. Etiologic diagnosis of lower respiratory tract bacterial infections using sputum samples and quantitative loop-mediated isothermal amplification. PLoS One 2012; 7: e38743.

[13] Melot B, Colot J and Guerrier G. Bacteremic community-acquired infections due to Klebsiella pneumoniae: clinical and microbiological presentation in New Caledonia, 2008-2013. Int J Infect Dis 2015; 41: 29-31.

[14] Farida H, Severin JA, Gasem MH, Keuter M, van den Broek P, Hermans PW, Wahyono H and Verbrugh HA. Nasopharyngeal carriage of Klebsiella pneumoniae and other Gram-negative bacilli in pneumonia-prone age groups in

Semarang, Indonesia. J Clin Microbiol 2013; 51: 1614-1616.

[15] Nor Azizah A, Fadzilah MN, Mariam M, Anis Siham ZA, Ariza A, Noor Shafina MN and Anita Kaur A. Community-acquired bacteremia in paediatrics: epidemiology, aetiology and patterns of antimicrobial resistance in a tertiary care centre, Malaysia. Med J Malaysia 2016; 71: 117-121.

[16] Vong S, Guillard B, Borand L, Rammaert B, Goyet S, Te V, Lorn Try P, Hem S, Rith S, Ly S, Cavailler P, Mayaud C and Buchy P. Acute lower respiratory infections in ≥ 5 year-old hospitalized patients in Cambodia, a low-income tropical country: clinical characteristics and pathogenic etiology. BMC Infect Dis 2013; 13: 97.

[17] Parker J, Fowler N, Walmsley ML, Schmidt T, Scharrer J, Kowaleski J, Grimes T, Hoyos S and Chen J. Analytical sensitivity comparison between singleplex real-time PCR and a multiplex PCR platform for detecting respiratory viruses. PLoS One 2015; 10: e0143164.

[18] Feng L, Li Z, Zhao S, Nair H, Lai S, Xu W, Li M, Wu J, Ren L, Liu W, Yuan Z, Chen Y, Wang X, Zhao Z, Zhang H, Li F, Ye X, Li S, Feikin D, Yu H and Yang W. Viral etiologies of hospitalized acute lower respiratory infection patients in China, 2009-2013. PLoS One 2014; 9: e99419.

[19] Simpson RJ, Bigley AB, Spielmann G, LaVoy EC, Kunz H and Bollard CM. Human cytomegalovirus infection and the immune response to exercise. Exerc Immunol Rev 2016; 22: 8-27.

[20] Siciliano RF, Castelli JB, Randi BA, Vieira RD and Strabelli TM. Cytomegalovirus colitis in immunocompetent critically ill patients. Int J Infect Dis 2014; 20: 71-73.

[21] Cebey-Lopez M, Herberg J, Pardo-Seco J, Gomez-Carballa A, Martinon-Torres N, Salas A, Martinon-Sanchez JM, Gormley S, Sumner E, Fink C and Martinon-Torres F. Viral co-infections in pediatric patients hospitalized with lower tract acute respiratory infections. PLoS One 2015; 10: e0136526.

[22] Ruuskanen O, Lahti E, Jennings LC and Murdoch DR. Viral pneumonia. Lancet 2011; 377: 1264-1275.

[23] Zhao T, Liu Y, Cao B, Wang H, Chen L, She D, Liang Z, Sun T, Li Y, Tong Z, Wang Z, He B, Yang W, Qu J, Li X, Chen R, Ye F, Kang J, Li E, Chen P, Zheng Y, Ma Z, Ren J, Shi Y, Sun W, Feng Y, Fan H, Jiang S, Zhang S, Xiong S, Zuo P and Wang Z. Prospective multicenter study of pathogen distributions in early-onset and late-onset hospital-acquired pneumonia in china. Antimicrob Agents Chemother 2013; 57: 6404-6405.

[24] Mullerova H, Lu C, Li H and Tabberer M. Prevalence and burden of breathlessness in patients with chronic obstructive pulmonary disease managed in primary care. PLoS One 2014; 9: e85540.

[25] Gallego M, Pomares X, Espasa M, Castaner E, Sole M, Suarez D, Monso E and Monton C. Pseudomonas aeruginosa isolates in severe chronic obstructive pulmonary disease: characterization and risk factors. BMC Pulm Med 2014; 14: 103.

[26] Perotin JM, Dury S, Renois F, Deslee G, Wolak A, Duval V, De Champs C, Lebargy F and Andreoletti L. Detection of multiple viral and bacterial infections in acute exacerbation of chronic obstructive pulmonary disease: a pilot prospective study. J Med Virol 2013; 85: 866-873.

[27] Polverino E, Bothamley GH, Goletti D, Heyckendorf J, Sotgiu G and Aliberti S. The best of respiratory infections from the 2015 European respiratory society international congress. ERJ Open Res 2016; 2.

[28] Eusebio N, Amorim AA, Gamboa F and Araujo R. Molecular identification and genotyping of Pseudomonas aeruginosa isolated from cystic fibrosis and non-cystic fibrosis patients with brochiectasis. Pathog Dis 2015; 73: 1-7.

[29] Rynda-Apple A, Robinson KM and Alcorn JF. Influenza and bacterial superinfection: illuminating the immunologic mechanisms of disease. Infect Immun 2015; 83: 3764-3770.

[30] Diavatopoulos DA, Short KR, Price JT, Wilksch JJ, Brown LE, Briles DE, Strugnell RA and Wijburg OL. Influenza a virus facilitates streptococcus pneumoniae transmission and disease. Faseb J 2010; 24: 1789-1798.

[31] Damasio GA, Pereira LA, Moreira SD, Duarte dos Santos CN, Dalla-Costa LM and Raboni SM. Does virus-bacteria coinfection increase the clinical severity of acute respiratory infection? J Med Virol 2015; 87: 1456-1461.

[32] Chen K, Jia R, Li L, Yang C and Shi Y. The aetiology of community associated pneumonia in children in Nanjing, China and aetiological patterns associated with age and season. BMC Public Health 2015; 15: 113.