MEDPHARMRES
pISSN : 1859-1779 | eISSN : 2615-9139

Original Article
MedPharmRes 2024;8(3):153-161
https://doi.org/10.32895/UMP.MPR.8.3.17

Bacterial and viral co-infections in community-acquired pneumonia in adults: a prospective study of multiple hospital centers

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Abstract

Introduction: Community-acquired pneumonia (CAP) is mostly caused by bacteria and viruses. Identifying pathogenic bacteria and viruses using traditional culture techniques is challenging. Therefore, multiplex real-time PCR (MPL-rPCR) has the capacity not only to concurrently identify the causative bacteria, atypical bacteria, and viruses but also to quantify their load and detect co-infections.

Method: This study was carried out on patients with CAP who were admitte to the Respiratory departments of Nguyen Tri Phuong Hospital, Nhan Dan Gia Dinh Hospital and University Medical Center, from April 2021 to March 2023, using a cross-sectional descriptive design in prospect. Sputum samples, evaluated by the Barlett scale, were collected and processed using the MPL-rPCR technique at Nam Khoa Company's laboratory.

Results: Bacterial pathogens and viruses were detected at rates of 67.7% and 57.5% ($p<0.05$). Gram – negative bacteria included *Klebsiella pneumoniae* at 18.5%, *Acinetobacter baumannii* at 17.3%, and *Haemophilus influenzae* at 14.1%. Among Gram-positive bacteria, *Streptococcus pneumoniae* was found at 16.4%. The *Epstein–Barr virus* was the most frequently identified virus at 34.9%, followed by *Cytomegalovirus* at 16.7%, and *Influenza virus* type A at 10.3%. One sputum sample showed the presence of more than one bacterium or virus, with high rates observed for *Epstein–Barr virus* and *Cytomegalovirus*.

Conclusions: Gram – negative bacteria are found in high proportions, and viruses were predominant, particularly Epstein–Barr virus, Cytomegalovirus, Influenza virus types A and B. Almost all viruses were co-infected with pathogenic bacteria, and multiple bacteria or viruses were identified in one sputum sample.

Keywords: community-acquired infections; real-time polymerase chain reaction; coinfection; pneumonia

1. INTRODUCTION

Hospitalized community acquired pneumonia (CAP) is a widespread disease that could affect all ages and genders, which is mostly caused by bacteria and viruses [1,2]. However, defining the pathogenic bacteria and viruses responsible for CAP is challenging because the patients' sputum (or phlegm) is easily contaminated when passing through oropharynx.

Received: Aug 18, 2023 / Revised: Dec 15, 2023 / Accepted: Dec 18, 2023
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Therefore, traditional culture technique has several limitations [3]. Traditional culture technique cannot detect atypical bacteria as well as viruses. In addition, patients often using antibiotics before hospitalization, potentially leading to the destruction of bacteria in the sputum samples while they may still exist in alveolar or bronchial epithelial fluid; a lack of suitable environment to isolate primary bacteria, particularly *Streptococcus pneumoniae*, *Haemophilus influenzae*. To overcome those difficulties, we implemented multiplex real-time PCR (MPL-rPCR) technique, known for its high sensitivity and specificity. MPL-rPCR not only enables simultaneous detection of causative bacteria and viruses but also quantifies their quantity to define the primary, thereby delineating and the combined pathogenic agents (co-infection).

Our aims were: (1) to determine the proportion of bacteria and viruses causing CAP in hospitalized adult patients. (2) to determine rate of bacterial and viral combinations.

2. METHODS

2.1. Study design

This study utilized a prospective cross-sectional descriptive design, conducted on adult patients with CAP hospitalized at Respiratory department of Nguyen Tri Phuong Hospital, Nhan Dan Gia Dinh Hospital and University Medical Center from 04/2021 to 03/2023.

Sample selection criteria involved the collecting sputum samples from hospitalized CAP patients diagnosed by clinical doctors according to the Ministry of Health standards specified in Decision No. 4815/QD-BYT. These sputum samples were then transferred to Nam Khoa Company's Laboratory, where the authors conducted analyses to identify the causative agents. Exclusion criteria included sputum samples from hospitalized CAP patients with lung cancer, advanced tuberculosis, human immunodeficiency virus (HIV) infection, or undergoing treatment with immunosuppressive drugs. Sputum samples collected from the same patient during the treatment period were also excluded.

Deviation control: Strictly comply with diagnostic standards and classification of underlying diseases; select samples based on the Barlett scale (≥2 point); strictly implement exclusion criteria and perform testing procedures according to the standard procedures of Nam Khoa Biotek Company's Laboratory. For ethical considerations, we only worked with patients' sputum samples at Nam Khoa Biotek's Laboratory. The researcher did not get in touch with patients or interfered with the doctors' treatment process. The Independent Ethics Committee (IEC) of the University of Medicine and Pharmacy HCMC approved our study at Decision No 330/DHYD-HDDD, issue: June 14th, 2019.

This manuscript was prepared and written in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [4]. The STROBE check list of the manuscript is described in the supplementary document.

2.2. Collection of sputum samples

We exclusively examined sputum samples which rated at or above 2 points on the Barlett scale. These samples were transported to Nam Khoa Company's laboratory for analysis using both traditional culture technique as well as by MPL-rPCR. With the MPL-rPCR method, the nucleic acid was extracted by DNARNAprep-MAGBEAD drug (belongs to Nam Khoa Co., Ltd, Ho Chi Minh City, Vietnam) and the King Fisher FLEX machine (from Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, these DNA extracts were combined with MPL-rPCR mixes specific for bacterial pathogens causing pneumonia (Nam Khoa Co., Ltd) and subject to detection and quantification of the target nucleic acid using CFX 96™ real-time PCR machine (from Biorad, Herculanes, CA, USA). All bacteria with numbers ≥100,000 copies were recognized as pathogens. If atypical bacteria and viruses are detected in sputum samples (regardless of quantity), they are recorded as pathogens. Whichever bacteria were counted with highest number was the main pathogen while the others are combined agents [2]. Bacteria identified by traditional culture technique were all identified as pathogens, regardless of whether they were the main agent or a combination agent.

Study size :

https://doi.org/10.32895/UMP.MPR.8.3.17

$$n = \frac{Z^2_{(1-\alpha/2)} p(1-p)}{d^2}$$

In which :

Z = 1.96 (Standard distribution table)

p = 0.69 (based on REAL study 2016–2017) [5]

d : is the error, with the expectation of a reliability of 95%, choose an error of 5% = 0.05

So,

$$n = \frac{(1.96)^2 \times 0.69 \times 0.31}{(0.05)^2} = 328.68$$

The number of sputum samples we collected at Nguyen Tri Phương hospital (101 sputum samples), Nhan dân Gia Định hospital (172 sputum samples) and University Medical Center (68 sputum samples). A total of 341 sputum samples (equal 341 patients) were analyzed.

2.3 Statistical analysis

Eliminate patients' sputum samples that did not agree the selection criteria.

We used software SPSS 20.0 and Microsoft Excel 2020 for statistical analysis.

3. RESULTS

There were 341 sputum samples from 341 CAP patients that met the criteria presented above.

The demographic data and the results in bacterial and viral detection by MPL-rPCR technique were shown in Table 1.

Table 1 above indicates that the proportion of causative bacteria in CAP was 67.7%, while the proportion of causative viruses was 57.5%. The difference of these percentages was found to be statistically significant (p<0.05). Furthermore, the relationships between females with males, between age group≤60 years with age group>60 years were also statistically significant (p<0.05).

The causative bacterial pathogens and viruses in CAP detected by MPL-rPCR from sputum samples of 341 hospitalized patients were shown in Table 2.

Table 1. The demographic data and the bacterial detection by multiplex real-time PCR

Characteristics	Bacteria detected n (%)[1)]	Virus detected n (%)[1)]	p-value
Gender			
Female	82 (24.0)	72 (21.1)	p<0.001
Male	149 (43.7)	124 (36.4)	
Age			
16–60 years	56 (16.4)	51 (15.0)	p<0.001
>60 years	175 (51.3)	145 (42.5)	
CAP patients	231	196	
Positive rate	67.7	57.5	p=0.0056

[1)] The percentage among 341 CAP patients.
CAP, community-acquired pneumonia.

In 341 sputum samples from CAP hospitalized adult patients, there were 231 sputum samples determined as having bacterial pathogens, reaching a rate of 67.7% and 196 sputum samples detected with viruses, the positive rate was 57.5%. In many cases where bacteria pathogens as well as viruses were detected, there were multiple pathogenic agents found in a single sputum sample of CAP patients.

The list of bacterial pathogens showed that, Gram-negative bacilli occurred in higher percentages than Gram-positive cocci (290 vs 108), in which *Klebsiella pneumoniae* 18.5%, *Acinetobacter baumannii* 17.3%, *H. influenzae* 14.1% and *Escherichia coli* 9.7% while *S. pneumoniae* was the highest percentage in Gram-positive cocci at 16.4%. *Mycoplasma*, atypical bacterium was detected at 6.2%.

From the list of viruses, this study showed that *Epstein – Barr virus* was found at the percentage of 34.9%, followed by *Cytomegalovirus* 16.7%, *Influenza virus* type A 10.3%, *Influenza virus* type B 4.4%, *Rhinovirus* and *Respiratory syncytial virus* were found at the rates of 3.5% and 2.9%.

Based on the quantity of bacterial pathogen discovered by the MPL-rPCR, we categorized the detected bacterial pathogens into main (primary) bacterial pathogens, which exhibited the highest copy numbers, and the co-infected (combined) bacterial pathogens, which had lower copy numbers. The co-infection of bacterial agents was shown in Table 3.

Table 3 illustrates that *S. pneumoniae* and *H. influenzae* were frequently detected as a primary bacterium alone, while

Table 2. The proportion of bacterial and viral pathogens detected by multiplex real-time PCR

Bacteria	n (%)[1)]	*Virus*	n (%)[1)]
Streptococcus pneumoniae	56 (16.4)	*Influenza virus* type A	35 (10.3)
Streptococcus agalactiae	2 (0.6)	*Influenza virus* type B	15 (4.4)
Staphylococcus aureus (MRSA)	7 (2.1)	*Influenza virus* type C	1 (0.3)
Staphylococcus aureus (MSSA)	1 (0.3)	*Parainfluenza virus type 3*	9 (2.6)
Coagulase negative staphylococcus	5 (1.5)	*Epstein – Barr virus* (EBV)	119 (34.9)
Staphylococcus epidermidis (MRSE)	21 (6.2)	*Cytomegalovirus* (CMV)	57 (16.7)
Enterococcus faecalis	7 (2.1)	*Rhinovirus*	12 (3.5)
Enterococcus faecium	9 (2.6)	*Respiratory cyncytial virus*	10 (2.9)
Escherichia coli	33 (9.7)	*Human metapneumovirus*	8 (2.3)
Klebsiella pneumoniae	63 (18.5)	*Adenovirus*	1 (0.3)
Enterobacter cloacae	1 (0.3)	*Bocavirus*	1 (0.3)
Morganella morganii	12 (3.5)	*SARS CoV-2*	12 (3.5)
Providencia sp.	11 (3.2)		
Proteus mirabilis	5 (1.5)		
Acinetobacter baumannii	59 (17.3)		
Burkholderia cepacia	9 (2.6)		
Pseudomonas aeruginosa	15 (4.4)		
Moraxella catarrhalis	4 (1.2)		
Haemophilus influenzae	48 (14.1)		
Haemophilus influenzae type B	1 (0.3)		
Stenotrophomonas maltophilia	29 (8.5)		
Mycoplasma sp.	21 (6.2)		
Total	419		280
Positive	231 (67.7)		196 (57.5)

[1)] The percentage was over 100% since in many cases, more than one bacteria or virus were found in one sputum of CAP patients.
MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; CAP, community-acquired pneumonia.

K. pneumoniae, *A. baumannii*, *Pseudomonas aeruginosa* often acted as primary bacteria in combination with other bacteria. *M. morganii* and *Providencia* sp. were exclusively found as combined bacteria.

Analyzed the results showed in Table 4, except *Human metapneumovirus*, the remain viruses were all in combination with bacterial pathogens causing CAP at the percentages about 60%, in which *Epstein-Barr virus*, *Cytomegalovirus*, *Influenza virus* type A, B had the higher percentages in combination with bacterial pathogens. The primary viruses causing CAP occurred in low percentage 30.6% (60/196). The co-infected bacteria in combination were most common with *S. pneumoniae*, followed by *H. influenzae*, *K. pneumoniae*, *A. baumannii*.

4. DISCUSSION

There were 341 CAP patients who met the inclusion criteria of our study, and infections by pathogenic bacteria and viruses were predominance in male and in individuals over 60 years of age, consistent with reports by previous authors such as Tao [1], Li [6], Gómez-Junyent [7], Cavallazzii [8], Dang [9], Voiriot [10]. The significant increase in age of CAP patients in previous decades was likely due to the aging population [11]. In this study, pathogenic bacteria were detected at the rate of 67.7%, similar to previous reports by Ly & Pham [12] (69%), Ly & Ly [13] (65.5%), while pathogenic viruses were found at the rate of 57.5%, higher than reports by Tao [1] (23.4%), Voiriot [10] (28%), Self

https://doi.org/10.32895/UMP.MPR.8.3.17

Table 3. The combination of bacterial pathogens causing CAP in hospitalized adult patients

Pathogens[1)]	Primary alone	Primary in combination	Combined	The main combined bacteria
Klebsiella pneumoniae (63)	15	16	32	*A. baumannii*, *P. aeruginosa*, *E. coli*, *S. maltophilia*
Acinetobacter baumannii (59)	15	13	31	*K. pneumoniae*, *S. pneumoniae*, *H. influenzae*, *E. coli*
Streptococcus pneumoniae (56)	12	18	26	*K. pneumoniae*, *H. influenzae*, *M. catarrhalis*
Haemophilus influenzae (48)	24	10	14	*S. pneumoniae*, *A. baumannii*, *Mycoplasma* sp.
Escherichia coli (33)	7	8	18	*A. baumannii*, *M. catarrhalis*, *M. morganii*, *Providencia* sp.
Stenotrophomonas maltophilia (29)	4	12	13	*A. baumannii*, *K. pneumoniae*, *M. morganii*, *Providencia* sp.
Staphylococcus epidermidis (21)	1	7	13	*S. pneumoniae*, *S. maltophilia*, *A. baumannii*, *K. pneumoniae*
Pseudomonas aeruginosa (15)	5	7	3	*K. pneumoniae*, *S. maltophilia*, *H. influenzae*
Morganella morganii (12)	0	0	12	*E. coli*, *S. pneumoniae*, *A. baumannii*, *S. maltophilia*
Providencia sp. (11)	0	0	11	*E. coli*, *K. pneumoniae*, *S. pneumoniae*, *A. baumannii*
Enterococcus faecium (9)	2	1	6	*A. baumannii*, *K. pneumoniae*, *S. maltophilia*
Burkholderia cepacia (9)	3	2	4	*A. baumannii*, *K. pneumoniae*, *S. maltophilia*, *Providencia* sp., *Mycoplasma*
Staphylococcus aureus (*MRSA*) (7)	1	1	5	*K. pneumoniae*, *E. coli*, *S. pneumoniae*, *H. influenzae*
Enterococcus faecalis (7)	2	2	3	*A. baumannii*, *S. maltophilia*, *K. pneumoniae*, *E. coli*, *Mycoplasma*
Coagulase negative Staphylococcus (5)	2	0	3	*K. pneumoniae*, *B. cepacia*, *A. baumannii*, *Mycoplasma*
Proteus mirabilis (5)	0	0	5	*K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *Providencia* sp., *S. pneumoniae*
Moraxella catarrhalis (4)	2	1	1	*S. pneumoniae*, *K. pneumoniae*
Streptococcus agalactiae (2)	0	1	1	*E. coli*, *K. pneumoniae*
Staphylococcus aureus (*MSSA*) (1)	1	0	0	
Enterobacter cloaceae (1)	0	0	1	*K. pneumoniae*
Haemophilus influenzae type B (1)	0	0	1	*S. pneumoniae*
Mycoplasma (21)	0	0	21	*K. pneumoniae*, *A. baumannii*, *E. coli*, *S. maltophilia*, *B. cepacia*, *Providencia*, *P. aeruginosa*, *H. influenzae*
Total (419)	96	99	224	

[1)] Bacterial pathogens can act as primary bacteria alone or as primary bacteria in co-infection or as only co-infected bacteria.
CAP, community-acquired pneumonia; MRSA, Methicillin-resistant *Staphylococcus aureus*; MSSA, Methicillin-susceptible *Staphylococcus aureus*.

[14] (24.5%), Kim [15] (17.7%), Radovanovic [16] (28.4%), Alimi [17] (22.0%), Ruuskanen [18] (29.0%).

Table 2 showed that, among 231 positive sputum samples from CAP patients, there were 419 bacteria detected by MPL-rPCR, in which Gram-negative bacilli occurred in higher percentages than those in Gram-positive cocci, likely reports by previous authors [8,9,19–21]. Perhaps Gram-negative bacilli, especially *A. baumannii* and *K. pneumoniae* has become more prevalent in causing CAP patient in recent days. *S. pneumoniae* was found at the highest prevalent 16.4% in Gram-positive cocci, which is lower than reported by authors such as Gómez-Junyent [7] (36.5%), Purba [22] (29.2%), Temesgen [23] (35.9%). However, some studies indicated that, although *S. pneumoniae* occurred less common in recent day but it still plays an significant role in causing CAP in adult patients [8,9,19–21].

In our study, *P. aeruginosa* causing CAP was counted at the low rate 4.4% (Table 2), but it holds significance in causing CAP, particularly severe CAP, due to its rick factors such as antibiotic resistance and mortality [6,11,21,24–28]. Furthermore, previous studies have reported that *P. aeruginosa* remained important for patients with severe chronic obstructive pulmonary disease (COPD), especially among the elderly who are receiving regular oral corticosteroid therapy [28–30].

Mycobacteria was the only atypical bacteria detected in

Table 4. The combination of viruses with bacterial pathogens in causing CAP

Virus	Primary virus n (%)	Combined with bacteria[1] n (%)	Combined bacteria (n)
Influenza virus type A (35)	7 (20.0)	28 (80.0)	*S. pneumoniae* (9), *A. baumannii* (4), *H. influenzae* (3), *E. coli* (2), *MRSE* (2), *K. pneumoniae* (2), *S. maltophilia* (2), *P. aeruginosa* (2), *B. cepacia* (1), *Mycoplasma* (1)
Influenza virus type B (15)	4 (26.7)	11 (73.3)	*S. pneumoniae* (6), *MRSA* (1), *MRSE* (1), *E. coli* (1), *H. influenzae* (1), *S. maltophilia* (1)
Influenza virus type C (1)	1 (100)	0 (0)	-
Parainfluenza virus type 3 (9)	0 (0)	9 (100)	*K. pneumoniae* (2), *B. cepacia* (2), *S. pneumoniae* (1), *S. agalactiae* (1), *P. aeruginosa* (1), *H. influenzae* (1)
Epstein-Barr virus (EBV) (119)[2]	28 (23.5)	79 (66.4)	*H. influenzae* (17), *A. baumannii* (14), *K. pneumoniae* (14), *S. pneumoniae* (8), *E. coli* (5), *S. maltophilia* (5), *MRSE* (3), *B. cepacia* (3), *MRSA* (2), *E. faecalis* (2), *P. aeruginosa* (2), *E. faecium* (2), *Mycoplasma* (1), *CoNS* (1)
Cytomegalovirus (CMV) (57)[3]	1 (1.8)	44 (77.2)	*A. baumannii* (12), *H. influenzae* (9), *K. pneumoniae* (6), *S. maltophilia* (5), *S. pneumoniae* (4), *MRSE* (2), *E. coli* (2), *P. aeruginosa* (2), *E. faecalis* (2), *B. cepacia* (1)
Rhinovirus (12)	5 (41.7)	7 (58.3)	*S. pneumoniae* (3), *K. pneumoniae* (2), *A. baumannii* (1), *H. influenzae* (1)
Respiratory syncytial virus (10)	4 (40.0)	6 (60.0)	*K. pneumoniae* (3), *H. influenzae* (2), *P. aeruginosa* (2)
Human metapneumo virus (8)	5 (62.5)	3 (37.5)	*H. influenzae* (2), *K. pneumoniae* (1)
Adenovirus (1)	0 (0)	1 (100)	*S. pneumoniae* (1)
Bocavirus (1)	0 (0)	1 (100)	*A. baumannii* (1)
SARS CoV-2 (12)	5 (4.7)	7 (58.3)	*E. coli* (2), *K. pneumoniae* (2), *A. baumannii* (2), *H. influenzae* (1)
Total	60	196	

[1] In some cases, virus can combine with 2 or more bacteria.
[2] 12 cases (10.1%) infected in combination with fungi or other viruses.
[3] 12 cases (21.1%) infected in combination with fungi or other viruses.
CAP, community-acquired pneumonia; MRSE, methicillin-resistant *Staphylococcus epidermidis*; MRSA, methicillin-resistant *Staphylococcus aureus*.

low frequency 6.2%, similar to previous research of Liu [31] (6.5%). Some recent authors have commented that atypical bacteria causing CAP were rarely detected and often occurred as co-bacteria with other bacterial pathogens [32–34].

In this study, *Epstein-Barr virus* was detected at the rate of 34.4%, followed by *Cytomegalovirus* 16.7% (Table 2). These rates were higher than those of *Influenza virus* type A 10.3%, *Influenza virus* type B 4.4%, *Rhinovirus* 3.5% and *Respiratory syncytial virus* 2.9%.We were surprised by the high percentages of *Epstein-Barr virus* and *Cytomegalovirus*, and we were left wondering if they were co-infections or opportunistic agents, necessitating further careful and thorough examination. Apart from the report by Voiriot [10], where the percentages for *Influenza virus* type A were at 18.4% , *Rhinovirus* at 12.6%, almost all reports by previous authors showed that *Influenza virus* type A, *Influenza virus* type B, *Rhinovirus*, *Respiratory syncytial virus*, *H. metapneumovirus* were detected at percentages ranging from 2% to 8%, which typically corresponds to the seasons when outbreaks of respiratory viruses are occurring [1,14–18,35] In many cases, more than one pathogenic bacteria and virus were detected in a single sputum sample. Our study, as shown in Table 3, revealed that among 231 sputum samples detected with pathogenic bacteria by MPL-rPCR, there were 96 sputum samples detected with only one % andial pathogens (primary bacteria alone) at the rate of 28.2% (96/341) and 135 sputum samples detected with 2 or more bacterial pathogens at the rate of 39.6% (135/341), similar to previous reports by Ly & Pham [12] 38.3%, Ly & Ly [13] 39.2% and Ta [36] 37.5%. *S. pneumoniae* and *H. influenzae* were discovered frequently as a primary bacterium alone while *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa* were often found as primary bacteria in combination with other bacteria (co-infection). *M. morganii* and *Providencia* sp. were found as only combined

https://doi.org/10.32895/UMP.MPR.8.3.17

bacteria (bacterial co-infection).

Analyzing the results in Table 3, almost viruses were in combination with bacterial pathogens causing CAP at the rates from 60% to 80%, in which, *Epstein-Barr virus*, *Cytomegalovirus*, *Influenza virus* type A, B, having the highest percentages. *S. pneumoniae*, *H. influenzae*, *K. pneumoniae* were the most frequent bacteria in viral co-infection [1,10,16,35]. Detections of *Influenza virus*, *Respiratory syncytial virus*, *H. metapneumovirus* in adult patients with CAP likely indicate an etiology role, whereas detections of *Epstein-Barr virus* and *Cytomegalovirus* should require further careful and thorough examination [17,18,37].

5. CONCLUSION

Bacterial pathogens and viruses were detected at positive rates of 67.7% and 57.5%, respectively ($p<0.05$), in which bacterial pathogens extend to Gram-negative bacilli such as *K. pneumoniae*, *A. baumannii*, *H. influenzae*, while predominent viruses occur included *Epstein-Barr virus*, *Cytomegalovirus*, *Influenza virus* type A, B. More than one pathogenic bacteria and viruses are found in one sputum sample. *S. pneumoniae*, *K. pneumoniae*, *H. influenzae* are the most common bacteria in viral co-infections and almost all viruses are co-infected with pathogenic bacteria. *Epstein-Barr virus* and *Cytomegalovirus* should require further scrutiny examination.

Acknowledgements

Not applicable.

Funding sources

Not applicable.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics Approval

All procedures in this study were approved by Independent Ethics Committee (IEC) of the University of Medicine and Pharmacy HCMC at Decision No 330/DHYD-HDDD, issue: June 14th, 2019.

REFERENCES

1. Tao RJ, Luo XL, Xu W, Mao B, Dai RX, Li CW, et al. Viral infection in community acquired pneumonia patients with fever: a prospective observational study. J Thorac Dis. 2018;10(7):4387-95.
2. Le TH, Ly KV, Hoang TM, Phan TCL. Combination of bacteria and virus causing community-acquired pneumonia in hospitalized adult patients. Vietnam Med J. 2023;256(2):267-72.
3. Pham HV. Technique for taking and examining clinical microbiology of different specimens. Teach Mater Microbiol Stud. 2005:54-62.

4. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the reporting of observational studies in epidermiology (STROBE): explanation and elaboration. Ann Intern Med. 2007;147(8):W163-94.
5. Van PH, Thanh NV, Ngoc TV, Duy ND. Pathogens causing hospitalized community-acquired pneumonia results from REAL study 2016–2017. J Med Times. 2018:51-63.
6. Li XJ, Li Q, Si LY, Yuan QY. Bacteriological differences between COPD exacerbation and community-acquired pneumonia. Respir Care. 2011;56(11):1818-24.
7. Gómez-Junyent J, Garcia-Vidal C, Viasus D, Millat-Martínez P, Simonetti A, Salud Santos M, et al. Clinical features, etiology and outcomes of community-acquired pneumonia in patients with chronic obstructive pulmonary disease. PLOS ONE. 2014;9(8):e105854.
8. Cavallazzi R, Ramirez J. Community-acquired pneumonia in chronic obstructive pulmonary disease. Curr Opin Infect Dis. 2020;33(2):173-81.
9. Dang QGV, Le TV. Clinical characteristics and outcomes of pneumonia in patients with chronic obstructive palmonary disease [Internet]. HCMC Res Society. 2018 [cited 2023 Aug 10]. https://www.hoihohaptphcm.org/index.php/chuyende/copd/469-dac-diem-lam-sang-va-ket-cuc-cua-viem-phoi-o-benh-nhan-benh-phoi-tac-nghen-man-tinh
10. Voiriot G, Visseaux B, Cohen J, Nguyen LBL, Neuville M, Morbieu C, et al. Viral-bacterial coinfection affects the presentation and alters the prognosis of severe community-acquired pneumonia. Crit Care. 2016;20(1):375.
11. Cillóniz C, Cardozo C, García-Vidal C. Epidemiology, pathophysiology, and microbiology of community-acquired pneumonia. Ann Res Hosp. 2018;2(1):1-11.
12. Ly Khanh V, Van PH. Pathogens causing hospitalized community-acquired pneumonia. J Med Ho Chi Minh City. 2018;22(2):238-43.
13. Ly KV, Ly VX. Bacteria causing hospitalized community-acquired pneumonia detected by multiplex real-time PCR. J Med Ho Chi Minh City. 2019;23(suppl 1):66-74.
14. Self WH, Williams DJ, Zhu Y, Ampofo K, Pavia AT, Chappell JD, et al. Respiratory viral detection in children and adults: comparing asymptomatic controls and patients with community-acquired pneumonia. J Infect Dis. 2016;213(4):584-91.
15. Kim MA, Park JS, Lee CW, Choi WI. Pneumonia severity index in viral community acquired pneumonia in adults. PLOS ONE. 2019;14(3):e0210102.
16. Radovanovic D, Sotgiu G, Jankovic M, Mahesh PA, Marcos PJ, Abdalla MI, et al. An international perspective on hospitalized patients with viral community-acquired pneumonia. Eur J Intern Med. 2019;60:54-70.
17. Alimi Y, Lim WS, Lansbury L, Leonardi-Bee J, Nguyen-Van-Tam JS. Systematic review of respiratory viral pathogens identified in adults with community-acquired pneumonia in Europe. J Clin Virol. 2017;95:26-35.
18. Ruuskanen O, Lahti E, Jennings LC, Murdoch DR. Viral pneumonia. Lancet. 2011;377(9773):1264-75.
19. Søgaard M, Madsen M, Løkke A, Hilberg O, Sørensen HT, Thomsen RW. Incidence and outcomes of patients hospitalized with COPD exacerbation with and without pneumonia. Int J Chron Obstruct Pulmon Dis. 2016;11(1):455-65.
20. Braeken D, Franssen F, Schütte H, Pletz M, Bals R, Martus P, et al. Microbial aetiology of community-acquired pneumonia and it's relation with ICS use in patients with COPD – results from the German competence network CAPNETZ. Eur Respir J. 2014;44(58):2476.
21. Bordon J, Slomka M, Gupta R, Furmanek S, Cavallazzi R, Sethi S, et al. Hospitalization due to community-acquired pneumonia in patients with chronic obstructive pulmonary disease: incidence, epidemiology and outcomes. Clin Microbiol Infect. 2020;26(2):220-6.
22. Purba AKR, Ascobat P, Muchtar A, Wulandari L, Rosyid AN, Purwono PB, et al. Multidrug-resistant infections among hospitalized adults with community-acquired pneumonia in an Indonesian tertiary referral hospital. Infect Drug Resist. 2019;12:3663-75.
23. Temesgen D, Bereded F, Derbie A, Biadglegne F. Bacteriology of community acquired pneumonia in adult patients at Felege Hiwot Referral Hospital, Northwest Ethiopia: a cross-sectional study. Antimicrob Resist Infect Control. 2019;8(1):101.
24. Ly Khanh V, Van PH. Pathogens causing hospitalized

https://doi.org/10.32895/UMP.MPR.8.3.17

community-acquired pneumonia in COPD patients. J Med Ho Chi Minh City. 2018;22(2):210-5.
25. Liapikou A, Polverino E, Ewig S, Cillóniz C, Marcos MA, Mensa J, et al. Severity and outcomes of hospitalised community-acquired pneumonia in COPD patients. Eur Respir J. 2012;39(4):855-61.
26. Lewis PO. Risk factor evaluation for methicillin-resistant S*taphylococcus aureus* and *Pseudomonas aeruginosa* in community-acquired pneumonia. Ann Pharmacother. 2021;55(1):36-43.
27. Sando E, Suzuki M, Ishida M, Yaegashi M, Aoshima M, Ariyoshi K, et al. Definitive and indeterminate *Pseudomonas aeruginosa* infection in adults with community-acquired pneumonia: a prospective observational study. Ann Am Thorac Soc. 2021;18(9):1475-81.
28. Restrepo MI, Babu BL, Reyes LF, Chalmers JD, Soni NJ, Sibila O, et al. Burden and risk factors for *Pseudomonas aeruginosa* community-acquired pneumonia: a multinational point prevalence study of hospitalised patients. Eur Respir J. 2018;52(2):1701190.
29. Ko FWS, Ip M, Chan PKS, Ng SSS, Chau SS, Hui DSC. A one-year prospective study of infectious etiology in patients hospitalized with acute exacerbations of COPD and concomitant pneumonia. Respir Med. 2008;102(8):1109-16.
30. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, et al. Diagnosis and treatment of adults with community-acquired pneumonia. An official clinical practice guideline of the American Thoracic Society and Infectious Diseases Society of America. Am J Respir Crit Care Med. 2019;200(7):e45-67.
31. Liu DS, Han XD, Liu XD. Current status of community-acquired pneumonia in patients with chronic obstructive pulmonary disease. Chin Med J. 2018;131(9):1086-91.
32. Dueck NP, Epstein S, Franquet T, Moore CC, Bueno J. Atypical pneumonia: definition, causes, and imaging features. RadioGraphics. 2021;41(3):720-41.
33. Chaabane N, Coupez E, Buscot M, Souweine B. Acute respiratory distress syndrome related to *Mycoplasma pneumoniae* infection. Respir Med Case Rep. 2017;20:89-91.
34. Tejada S, Romero A, Rello J. Community-acquired pneumonia in adults: what's new focusing on epidemiology, microorganisms and diagnosis. Erciyes Med J. 2018;40(4):177-82.
35. Pavia AT. What is the role of respiratory viruses in community-acquired pneumonia?: what is the best therapy for influenza and other viral causes of community-acquired pneumonia. Infect Dis Clin North Am. 2013;27(1):157-75.
36. Ta TDN. Study on clinic, Para clinic and etiology in community-acquired pneumonia [Ph.D. dissertation]. Hanoi: University of Medicine Ha Noi; 2016.
37. Esposito S, Daleno C, Prunotto G, Scala A, Tagliabue C, Borzani I, et al. Impact of viral infections in children with community-acquired pneumonia: results of a study of 17 respiratory viruses. Influenza Other Respir Viruses. 2013;7(1):18-26.