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Prospective profile of bacterial pathogens in hospitalized adult patients with community-acquired pneumonia: insights from multiplex real-time PCR and traditional culture techniques

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Abstract

Introduction: Hospitalized community-acquired pneumonia (CAP) is mainly caused by bacteria and plays its role as primary bacteria alone or combined. The study aimed to determine the proportion of bacterial pathogens causing CAP in hospitalized adult patients and to examine the combination of these bacteria.

Methods: This study was a cross-sectional descriptive design in prospect conducted on 341 adult patients with CAP hospitalized at the Respiratory Department of Nguyen Tri Phuong Hospital, Nhan Dan Gia Dinh Hospital, and University Medical Center from April 2021 to March 2023. Sputum samples were collected, assessed for reliability (according to the Barlett scale), and transported to Nam Khoa Company's laboratory to perform traditional culture techniques and multiplex real-time PCR (MPL-rPCR).

Results: Male sex and age over 60 were 62.5% and 73.0%, respectively. Bacterial pathogens were detected by MPL-rPCR and traditional culture techniques at rates of 67.7% and 46.0%, respectively ($p<0.001$). More than one strain of bacteria was commonly found in each sputum. *Klebsiella pneumoniae* was detected by multiplex real-time PCR and traditional culture techniques at a high rate (18.5% & 13.5%), *Acinetobacter baumannii* (17.3% & 12.9%), *Streptococcus pneumoniae* (16.4% & 0.6%), *H. influenzae* (14.1% & 0.6%) and *P. aeruginosa* (4.4% & 3.8%). Atypical bacteria were only *Mycoplasma*, with 6.2%, and occurred as a combined bacteria. The rate of bacterial combination was 77.1%, and two or more combined bacteria was 58.4%.

Conclusions: Bacterial pathogens are detected at 67.7% by MPL-rPCR and 46.0% by traditional culture techniques ($p<0.001$). Bacterial pathogens are multiform and increase in Gram-negative bacilli.

Keywords: community-acquired infections; polymerase chain reaction; cross-sectional studies; bacteria

1. INTRODUCTION

Community-acquired pneumonia (CAP) is a common infectious disease that can affect anyone at any age. Prior authors have shown that bacterial pathogens causing CAP can play their role as primary or as combined bacteria at different prevalence rates due to bacterial strains [1]–[4]. Therefore, traditional cultural techniques are limited for many reasons [5]. As most patients often have the habit of using antibiotics before hospitalization, the bacteria could still exist in alveolar

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or bronchial epithelial fluid but could have already deceased in the sputum. Besides, several subjective causes from the laboratory could reduce the ability to culture pathogens successfully, including a lack of an appropriate environment to isolate the primary pathogens while they are often difficult to culture, the culture period lasts about 2 or 3 days, or technicians are inadequately trained to choose the proper pathogenic colonies on agar to isolate the primary pathogenic bacteria.

We used the multiplex real-time PCR technique with high sensitivity and specificity to overcome such difficulties and correctly detect bacterial pathogens. The method can simultaneously clone and find specific nucleic acid sequences of bacteria and determine the number of copies to permit the realization of the primary pathogenic bacteria and combined bacteria [5]. Studying the characteristics of the pathogen in CAP is necessary, helping clinicians choose empiric treatment when the causative agents still need to be identified. In particular, this multicenter study that used the multiplex real–time PCR technique, so the results have high practical application value.

The study aimed to determine the proportion of bacterial pathogens causing CAP in hospitalized adult patients and to examine the combination of bacteria in hospitalized patients with CAP.

2. METERIALS AND METHODS

2.1. Study design

This study was a cross-sectional descriptive design in prospect conducted on adult patients with CAP hospitalized at the Respiratory Department of Nguyen Tri Phuong Hospital, Nhan Dan Gia Dinh Hospital, and University Medical Center from April 2021 to March 2023.

Sample selection criteria: sputum of hospitalized CAP patients according to the standards of the Ministry of Health in Decision No. 4815/QD-BYT was transported to Nam Khoa Company's Laboratory. After that, the authors and technicians implemented techniques to identify the pathogens in those sputum samples.

Exclusion criteria: sputum of hospitalized CAP patients with lung cancer, advanced tuberculosis, HIV infection or on immunosuppressive therapy or sputum samples taken next time on the same patient during the same course of treatment.

Deviation control: Strictly implement the criteria for diagnosis and classification of underlying diseases; choose a standard sample on the Barlett scale; take the exclusion standard seriously; and perform the standard procedure at the Laboratory of Nam Khoa Biotek Company.

During the effectuation process, we only conducted patients' sputum sample tests in the laboratory to discover bacterial pathogens causing CAP due to the demand of clinical doctors. The researcher had no contact with patients or interfered with clinical doctors' treatments. This research was also approved by the Independent Ethics Committee (IEC) of the University of Medicine and Pharmacy HCMC at Decision No 330/DHYD-HDDD, issue: June 14th, 2019. This manuscript was prepared and written following the strengthening the reporting of observational studies in epidemiology (STROBE) guidelines [6]. The STROBE checklist of the manuscript is described in the supplementary document.

2.2. Collection of sputum samples

We only perform tests on collected sputum samples that have been evaluated for reliability based on the Barlett scale.

The sputum samples were transported to Nam Khoa Company's laboratory (ISO 15189, ISO 17025, ISO 13485) to carry out by traditional culture technique as well as by multiplex real-time PCR with King Fisher FLEX machine and PCR CFX 96^{TM} real-time system (testing NKDNAR-NAprep-MAGBEAD based on the principle of using beads from coated silica (manufactured and validated by Nam Khoa company)). Bacteria detected by the MPL-rPCR technique with a quantity≥100,000 copies were identified as the causative agent of CAP. Since there was no standard for atypical bacteria, we decided on pathogenic agents (regardless of quantity) when detecting them. Bacteria with the highest number of copies were believed to be primary pathogens, and others were combined agents [5],[7]. With the traditional culture technique, all isolated bacteria and fungi were recorded.

Study size :

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

In which :

Z = 1,96 (standard distribution table)

p = 0,69 (based on REAL study 2016–2017) [8]

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

So,

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

Hospitalized CAP patients are indicated for sputum collection: Nguyen Tri Phương hospital: 101 sputum samples, Nhan Dan 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

Rejection of patients' sputum samples that violate the selection criteria.

Data collection was solved using Microsoft Excel 2020 (Microsoft, Redmond, WA, USA) to describe patient characteristics and proportions of detected bacteria and SPSS 20.0 software (IBM, Chicago, IL, USA) to analyze patient characteristics and compare ratios.

3. RESULTS

Our study was executed on 341 sputum samples from 341 patients that met all the criteria. The results of bacterial detection by using MPL-rPCR and traditional culture techniques are shown in the following tables: Tables 1–4.

For statistical analysis of 341 hospitalized adult patients with community-acquired pneumonia, data collection was performed as follows:

Table 1 shows that male sex and age over 60 were the most prevalent (62.5% and 73.0%). CAP patients with chronic obstructive pulmonary disease (COPD) accounted for 26.7% of total CAP patients. The rates of bacterial pathogens (positive rates) detected by multiplex real-time PCR technique and by traditional culture technique were 67.7% and 46.0%, respectively. These differences were statistically significant (p<0.001).

Table 1. Characteristics of CAP patients

Characteristics	n (%)	p-value
Gender		
Female	128 (37.5)	p<0.001
Male	213 (62.5)	
Age		
16–60 years	92 (27.0)	p<0.001
>60 years	249 (73.0)	
CAP		
With COPD	91 (26.7)	p<0.001
Without COPD	250 (73.3)	
Patients with positive rate		
By PCR technique	231 (67.7)	p<0.001
By culture technique	157 (46.0)	

p-values were obtained by Chi-square test.
CAP, community-acquired pneumonia; COPD, chronic obstructive pulmonary disease.

By analyzing bacteria causing CAP in 341 hospitalized patients, we found bacterial pathogens detected in 231 patients by multiplex real-time PCR and in 157 patients by traditional culture, as shown Table 2.

Table 2 shows the list of bacterial pathogens that caused CAP in hospitalized.

Multiplex real-time PCR and traditional culture techniques detected *K. pneumoniae* at a high rate (18.5% and 13.5%), followed by *A. baumannii* (17.3% and 12.9%). Although *S. pneumoniae* was found by multiplex real-time PCR at a rate of 16.4%, by traditional culture techniques, it was detected only at 0.6%. Atypical bacteria were found, but only *Mycobacteria* was found at a frequency of 6.2%. There were many cases in which more than one bacterial strain was detected in one sputum.

Based on quantitative measurement of copies by multiplex real-time PCR, data collection in combination with bacterial pathogens causing CAP in 231 hospitalized patients was performed, as shown in Table 3.

Of 231 patients, only 96 (41.6%) were infected with primary bacteria alone, and 135 patients (58.4%) were infected with two or more combined bacteria. The rate of bacterial

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Table 2. Bacterial pathogens detected in sputum samples (n=341)

Multiplex real-time PCR			n (%)	Traditional culture	n (%)
Gram-positive	*Streptococcus pneumoniae*		56 (16.4)	*Streptococcus pneumoniae*	2 (0.6)
	Streptococcus agalactiae		2 (0.6)	*Streptococcus agalactiae*	0 (0.0)
	Staphylococcus aureus (MRSA)		7 (2.1)	*Staphylococcus aureus (MRSA)*	1 (0.3)
	Staphylococcus aureus (MSSA)		1 (0.3)	*Staphylococcus aureus (MSSA)*	11 (3.2)
	Coagulase negative *Staphylococcus*		5 (1.5)	*Coagulase* negative *Staphylococcus*	5 (1.5)
	Staphylococcus epidermidis (MRSE)		21 (6.2)	*Staphylococcus epidermidis (MRSE)*	1 (0.3)
	Enterococcus faecalis		7 (2.1)	*Enterococcus faecalis*	0 (0.0)
	Enterococcus faecium		9 (2.6)	*Enterococcus faecium*	4 (1.2)
Gram-negative	Enterobacteriaceae	*Escherichia coli*	33 (9.7)	*Escherichia coli*	16 (4.7)
		Klebsiella pneumoniae	63 (18.5)	*Klebsiella pneumoniae*	47 (13.5)
		Enterobacter cloaceae	1 (0.3)	*Enterobacter cloaceae*	0 (0.0)
		Morganella morganii	12 (3.5)	*Morganella morganii*	0 (0.0)
		Providencia sp.	11 (3.2)	*Providencia* sp.	0 (0.0)
		Citrobacter freundii	0 (0.0)	*Citrobacter freundii*	1 (0.3)
		Proteus mirabilis	5 (1.5)	*Proteus mirabilis*	3 (0.9)
	Others	*Acinetobacter baumannii*	59 (17.3)	*Acinetobacter baumannii*	44 (12.9)
		Burkholderia cepacia	9 (2.6)	*Burkholderia cepacia*	2 (0.6)
		Burkholderia pseudomallei	0 (0.0)	*Burkholderia pseudomallei*	1 (0.3)
		Pseudomonas aeruginosa	15 (4.4)	*Pseudomonas aeruginosa*	13 (3.8)
		Moraxella catarrhalis	4 (1.2)	*Moraxella catarrhalis*	1 (0.3)
		Haemophilus influenzae	48 (14.1)	*Haemophilus influenzae*	2 (0.6)
		Haemophilus influenzae type B	1 (0.3)	*Haemophilus influenzae* type B	0 (0.0)
		Stenotrophomonas maltophilia	29 (8.5)	*Stenotrophomonas maltophilia*	3 (0.9)
Atypical bacteria	*Mycoplasma* sp.		21 (6.2)	*Mycoplasma* sp.	0 (0.0)

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*.

combination was 77.1% (323/419), and *E. coli* often occurred as a combined bacteria in 78.8% (26/33). *P. aeruginosa*, *S. maltophilia*, *M. catarrhalis*, *M. morganii*, *Providencia*, and *Mycoplasma* were the only combined bacteria.

Based on quantitative measurement of copies by multiplex real-time PCR, we analyzed the top 5 bacterial pathogens that combined with other bacteria causing CAP in hospitalized patients, as shown in Table 4.

Table 4 shows that *K. pneumoniae*, *A. baumannii*, *S. pneumoniae*, *Haemophilus influenzae*, and *Escherichia coli* in the top five bacterial pathogens were found as primary bacteria as well as combined bacteria, in which *E. coli* played the role of only combined bacteria.

4. DISCUSSION

In total, there were 91/341 (26.7%) COPD patients in our study, similar to the prevalence rates of previous authors: Pascual-Guardia et al. [9] (21%), Gómez-Junyent et al. [10] (23.9%), Sharaf Khaneh et al. [11] (32.7%) and Sogaard et al. [12] (33.3%).

Our study found CAP patients of male sex and age over 60 years at rates of 62.5% and 73.0%, respectively. These rates were similar to those reported by Li et al. [13] (71.0% and 75.3%), Gómez-Junyent et al. [10] (61.0% and 67.0%), and Pasquale et al. [14] (70.2% and 70.5%) but lower than those by Dang et al. [15] (85.5% and 87.6%). Previous studies have shown that male sex and old age are mainly associated

Table 3. The proportion of primary pathogens and combined bacteria detected by multiplex real-time PCR

Pathogens	Primary		Combined		Total	
	N	%	N	%	n	%
Streptococcus pneumoniae	12	3.5	44	12.9	56	16.4
Streptococcus agalactiae	0	0	2	0.6	2	0.6
Staphylococcus aureus (MRSA)	1	0.3	6	1.8	7	2.1
Staphylococcus aureus (MSSA)	1	0.3	0	0	1	0.3
Coagulase negative *Staphylococcus*	2	0.6	3	0.9	5	1.5
Staphylococcus epidermidis (MRSE)	1	0.3	20	5.9	21	6.2
Enterococcus faecalis	2	0.6	5	1.5	7	2.1
Enterococcus faecium	2	0.6	7	2.1	9	2.6
Escherichia coli	7	2.1	26	7.6	33	9.7
Klebsiella pneumoniae	15	4.4	48	14.1	63	18.5
Enterobacter cloaceae	0	0	1	0.3	1	0.3
Morganella morganii	0	0	12	3.5	12	3.5
Providencia sp.	0	0	11	3.2	11	3.2
Proteus mirabilis	0	0	5	1.5	5	1.5
Acinetobacter baumannii	15	4.4	44	12.9	59	17.3
Burkholderia cepacia	3	0.9	6	1.8	9	2.6
Pseudomonas aeruginosa	5	1.5	10	2.9	15	4.4
Moraxella catarrhalis	2	0.6	2	0.6	4	1.2
Haemophilus influenzae	24	7.0	24	7.0	48	14.1
Haemophilus influenzae type B	0	0	1	0.3	1	0.3
Stenotrophomonas maltophilia	4	1.2	25	7.3	29	8.5
Mycoplasma sp.	0	0	21	6.2	21	6.2
Total	96		323		419	

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*.

Table 4. The combination of the top five bacterial pathogens

Pathogens[1)]	Primary alone	Primary in combination	Combined only	Combined bacteria mainly common
Klebsiella pneumoniae (63)	9	16	38	*A. baumannii, P. aeruginosa, E. coli, S. maltophilia*
Acinetobacter baumannii (59)	10	13	36	*K. pneumoniae, S. pneumoniae, H. influenzae, E. coli*
Streptococcus pneumoniae (56)	17	18	21	*K. pneumoniae, H. influenzae, M. catarrhalis*
Haemophilus influenzae (48)	19	10	19	*S. pneumoniae, A. baumannii, Mycoplasma* sp.
Escherichia coli (33)	2	8	23	*A. baumannii, M. catarrhalis, M. morganii, Providencia* sp.

[1)] Bacterial pathogens can be the primary agents alone, as agents in combination with other bacteria, or as only combined bacteria.

with an increased rate of CAP [10],[16]–[18].

Bacterial pathogens detected by MPL-rPCR and by traditional culture technique were 67.7% and 46.0%, respectively, and these different rates were statistically significant in bacterial pathogen detection between the two methods ($p<0.001$). Using the multiplex real-time PCR technique, Ly and Pham [2], and Ly and Ly [3] detected bacterial pathogens causing CAP at 69% and 65.5%, similar to our study (67.7%) (Table 1). The bacterial detection rate in CAP patients by culture technique was 46.0% in this study; it varied and depended on the participants of patients, date and place in different studies, such as 53.0% by Li et al. [13], 53.8% by Dao [19], 57.4% by Jain et al. [20], 60.36% by Zhang et al. [21], 39.4% by Assefa et al. [22], and 33.5% by Gebre et al. [23].

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Bacterial pathogens causing CAP in our study were multiform and increased in Gram-negative bacilli, similar to reports by previous authors [12],[15],[18],[22]–[24]. *K.pneumoniae* was found by multiplex real-time PCR and traditional culture techniques at high rates (18.5% and 13.5%), followed by *A. baumannii* (17.3% and 12.9%) (Tables 2 and 3). Gram-negative bacilli, especially *K. pneumoniae* and *A. baumannii*, have increased in causing hospitalized CAP in recent days. In our study, *S. pneumoniae* was detected by multiplex real-time PCR at a rate of 16.4%; however, it was 0.6% in isolation by traditional culture techniques. There were significant differences in the isolation rate of *S. pneumoniae* between the two methods and between our study and previous reports by authors such as Ly and Pham [1] (28.3%), Gómez-Junyent et al. [10] (36.5%), Li et al. [13] (25%), Purba et al. [25] (29.2%), and Temesgen et al. [26] (35.9%).

Pseudomonas aeruginosa was critical in causing hospitalized CAP, especially serious CAP or CAP with COPD. However, in our study, its isolation rate detected by multiplex real-time PCR was 4.4%, lower than those of Ly and Pham [1] (5.5%), Ly and Ly [3] (6.3%) but similar to Cillóniz et al. [27] (4.2%). Although *P. aeruginosa* was found at a low rate, it was serious because of antibiotic resistance, mortality, and outcomes [2],[13],[18],[24],[28]–[32], especially severe CAP with COPD, older age, and regular oral corticosteroid therapy [30]–[33].

Atypical bacteria were found only by multiplex real-time PCR. In our study, *Mycoplasma* was detected at a proportion of 6.2% (Table 2), similar to previous reports of Liu et al. [34] (6.5%) but lower than those of Shoar and Musher [35] (8.8%). *Mycoplasma* rarely causes infection independently [9] and is often associated with other pathogenic agents [36]–[38]. We found more than one bacterial strain commonly in one sputum of CAP patients [10],[23].

In bacterial combination, our study showed that the rate of CAP patients infected with primary bacteria alone was 41.6%. CAP patients infected with two or more combined bacteria was 58.4%, in which *E. coli* often occurred as a combined bacteria in 78.8% (Table 3). Among 419 detected pathogenic agents, 323 bacterial agents played the role of combination at a proportion of 77.1% (323/419) (Table 3), higher than those from previous reports by authors: Ly and Pham [1] 38.3% (62/162), Ly and Ly [3] 39.2% (118/301) and Ta [39] 37.5% (33/88). We may have found more combined bacteria than those in the past because of multiplex real-time PCR with 72 primers used in this study. By analyzing the top five bacterial pathogens causing CAP in hospitalized patients, we found that bacterial pathogens can be the primary agents alone, as agents in combination with other bacteria, or as only combined bacteria. *K.pneumoniae*, *A. baumannii*, *S. pneumoniae*, *H. influenzae*, and *E. coli* played the role of primary bacteria as well as combined bacteria. In contrast, *P. aeruginosa*, *Stenotrophomonas maltophilia*, *Moraxella catarrhalis*, *Morganella morganii*, *Providencia*, and *Mycoplasma* commonly occurred as only combined bacteria (Table 4).

5. CONCLUSION

CAP patients of male sex and age over 60 years are at high proportions of 62.5% and 73.0%, respectively. Bacterial pathogens are detected at 67.7% by multiplex real-time PCR and 46.0% by traditional culture techniques ($p<0.001$). Bacterial pathogens are multiform and increased in Gram-negative bacilli. *K. pneumoniae* and *A. baumannii* are found more commonly, while *P. aeruginosa* is found at a low proportion of 4.4%. Atypical bacteria are only *Mycoplasma*, with a proportion of 6.2%. More than one bacterial strain is commonly found in each sputum. The rate of bacterial combination is 77.1%, the rate of CAP patients infected with two or more combined bacteria is 58.4%, and *E. coli* often occurs as a combined bacteria in 78.8%.

This work was conducted only on CAP patients at three central hospitals in Ho Chi MInh city, therefore data may not reflect cases of less severity that were diagnosis at small hospitals in rural area or in other proviences. Furthermore, with 341 cases collected, our finding may not be representive of entire hospitalized CAP. In addition, serological techniques were not available at the study setting that could not identify more atypical bacteria.

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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 the Independent Ethics Committee (IEC) of the University of Medicine and Pharmacy HCMC at Decision No 330/DHYD-HDDD, issue: June 14th, 2019.

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