

DETECTION OF COMMON BACTERIAL AND VIRAL PATHOGENS CAUSING COMMUNITY ACQUIRED PNEUMONIA USING RT-PCR AND ITS CORRELATION WITH SOME CLINICAL AND DEMOGRAPHIC PARAMETERS

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ABSTRACT

Background: Pneumonia is the most serious infectious disease and one of the most common causes of death in the world. It clinically divided into community-acquired pneumonia (CAP) and nosocomial pneumonia. Pneumonia can be caused by bacteria, viruses, fungi and protozoa. Although culture considered as standard gold method for diagnosis of bacterial causes of pneumonia but still characterized by low sensitivity and time consuming. Because data concerning etiology of CAP are limited in our area. The aim of the study was to detect common bacterial and viral causes of CAP using multiplex real time PCR technique as well as to study their association with demographic and clinical characteristics.

Methods: A total of 50 sputum samples were collected from hospitalized community acquired pneumonic adult patients aging from 18 to more than 65 years old in Azadi teaching and emergency hospitals in Duhok city from a period of from June 2022 to June 2023. Bosphore RPP Basic Bundle Kit of RT-PCR (Anatolia, Turkey) was used.

Results: From 50 samples, 42 (84%) were positive by RT-PCR technique. The most predominant detected pathogens were *Streptococcus pneumoniae* 23 (46%) followed by *Klebsiella pneumoniae* 14 (28%), *Staphylococcus aureus* 3 (6%), *Mycoplasma pneumoniae* and *Legionella pneumophila* with similar results 1(2%), while *Moraxella catarrhalis*, *Bordetella pertussis*, *Pneumocystis jirovecii*, *Legionella pneumophila llongbeachae*, *Haemophilus influenzae* type b and *Chlamydia pneumoniae* were negative. All samples were negative for Influenza B, Adenovirus, Parechovirus and Influenza C viruses.

Conclusion: From the results of this study, it can be concluded that among bacterial causes of adult CAP patients, *Streptococcus pneumoniae* was the predominant followed by *Klebsiella pneumoniae*, while among atypical bacteria both *Mycoplasma pneumoniae* and *Legionella pneumophila* were detected. All samples were negative for Adenovirus, Influenza B, C and Parechovirus. CAP was more commonly found among young aged adult, males, patients with comorbidities and with CURB-65 score-2.

KEYWORD: community acquired pneumonia, RT-PCR, adults

1. INTRODUCTION

Community acquired pneumonia (CAP) is a clinical syndrome where acute lungs infection develops in individuals not recently admitted to hospital and do not have regular exposure to the health care system (Afroz *et al.*, 2023).

Community acquired pneumonia continues to be the main threat, particularly among children, the elderly and compromised hosts such as people with chronic diseases (Afroz *et al.*, 2023).

The incidence rate of pneumonia varies according to the season, region and population characteristics and it is also higher male than in female (Prina *et al.*, 2015).

Therefore, rapid diagnostic techniques and

pathogen-directed treatment are important (Serin *et al.*, 2014). Traditionally, clinicians have utilized X-rays in combination with clinical symptoms as a standard tool to diagnose CAP, however, numerous other causes can lead to the same outcomes. Furthermore, until recent years, the gold standard for diagnosing pneumonia etiology was blood and sputum culture and Gram-stained sputum (Joelons *et al.*, 2023). However, the etiology of CAP can be established in only (30-50%) cases utilizing conventional techniques (Serin *et al.*, 2014).

Molecular techniques such as PCR has been shown to be more sensitive diagnostic methods for detecting CAP etiology (Gadsby *et al.*, 2016). Multiplex PCR can identify several various agents in the same tube and offer rapid diagnosis.

Moreover, it is not affected by the usage of antibiotic (Serin *et al.*, 2014).

The CAP bacterial causes vary between countries and alters with time within the same country, which is probably owing to frequent utilize of antibiotics, increased awareness of the disease, changes in environmental pollution, and changes in life expectancy (Shah *et al.*, 2010). The problem is much greater in the developing countries where pneumonia is the most common cause of hospital attendance in adults (Akter *et al.*, 2014).

Community acquired pneumonia presentation may range from mild pneumonia characterized by fever and productive cough to severe pneumonia leading to respiratory distress and sepsis syndrome need treatment in Intensive care unit (ICU). Any delay in admission to ICU has been shown to be associated with increased mortality (Farooqui *et al.*, 2015).

Furthermore, unnecessary admission to ICU rises the management cost and cause depletion of precious hospital resources. (Patil *et al.*, 2020).

several serum biomarkers and multiple established risk scores such as CURB 65 have been utilized to assess CAP severity to optimize the treatment of patients with CAP (Patil *et al.*, 2020).

The aim of this study was to detect bacterial, viral and fungi among hospitalized CAP adult patients and to correlate the causative agents with clinical and demographical parameters.

2. MATERIALS AND METHODS

2.1. Study design and sample collection

A cross-sectional study was conducted at both Azadi teaching and emergency hospitals in Duhok city from June 2022 to June 2023. A total of 50 patients with community acquired pneumonia were enrolled in the study with ages ranging from 18 to > 65 years (55.26 ± 17.06). Sputum Samples were collected by expectoration from each patient before taking antibiotics and transferred immediately in cool box to the central laboratory, Duhok General Health Directorate. All samples were stored at $-20\text{ }^{\circ}\text{C}$ for nucleic acid extraction. All patients were interviewed and a special questionnaire format was prepared and filled. The questionnaires were included age, gender, underlying diseases, signs and symptoms, SpO_2 , respiratory rate, blood pressure.

2.2. statistical analysis

All data pertaining to demographic information, and laboratory results were entered

and edited using Microsoft Word 2016 for windows 10. Data analysis was conducted with the Statistical Package for Social Science (SPSS) software version 23 IBM. Descriptive statistics was provided for the demographic data and questionnaire of the study. Chi-square test was utilized to estimate the association of the studied variables with CAP pathogens. $P\text{-Values} \leq 0.05$, was considered statistically significant.

2.3. Ethical approval

The study was approved by the Research Ethics Committee of the Directorate General of Health in Duhok Governorate No. 04102023-8-8

2.4. Inclusion and Exclusion criteria

2.4.1. Inclusion criteria

Patients included in the study were with age ≥ 18 years, have one or more of the following signs & symptoms (cough, dyspnea, fever and chest pain), agreed to be enrolled in our study and before initiation antibiotic treatment.

2.4.2. Exclusion criteria

Patients excluded from this study were patients < 18 , did not allow to be part of the study and who were under antibiotic treatments.

2.5. Real time – Polymerase chain reaction (RT-PCR) method

Nucleic acids were extracted from sputum samples using addprep viral nucleic acid extraction kit (Bio-Tech, South Korea) according to the manufacturing instructions supplied by the kit.

Bosphore RPP Basic Bundle Kit (Anatolia, Turkey) was used for detecting of the following respiratory pathogens: Influenza C, Influenza B, *Mycoplasma pneumoniae*, *Klebsiella pneumoniae*, Adenovirus, *Bordetella pertussis*, *Moraxella catarrhalis*, *Haemophilus influenzae* (B), *Staphylococcus aureus*, Parechovirus, *Legionella pneumophila*, *Pneumocystis jirovecii*, *Chlamydia pneumoniae*, *Streptococcus pneumoniae* and *Legionella pneumophila /longbeachae*.

3. RESULTS AND DISCUSSION

3.1. Identification of the causative agents by RT-PCR

Among 50 sputum, 42 (84%) samples were positive. The predominant pathogens were *Streptococcus pneumoniae* and *Klebsiella pneumoniae* which detected in 23/50 (46%) and 14/50 (28%) respectively followed by *Staphylococcus aureus* 3 (6%) and 1 (2%) for both *Mycoplasma pneumonia* and *Legionella pneumophila*. All samples were negative for

viruses: Influenza B, Adenovirus, Parechovirus and Influenza C as well as for *Haemophilus influenzae* type B, *Chlamydia trachomatis* and *Pneumocystis jirovecii* as shown in Table 3.1.

These results were higher than results of Joelsons *et al* (2023) who found 61.7% of the sputum were positive, while similar to the results of Serin *et al* (2014) who found that 90% of the sputum samples were positive for different bacteria by PCR technique. This discrepancy of the results could be attributed to several factors like methodology, sample size, ages, environment and seasonal variation.

In the current study, *S. pneumoniae* was the most predominant pathogen in which 46% of the samples were positive. Similar results were obtained from different countries such as studies by Afroz *et al* (2023) in Bangladesh, Serin *et al* (2014) in Turkey, Batool *et al* (2021) in Saudi Arabia and Temesgen *et al* (2019). On the other hand, studies from Iraq by Jaaffar *et al* (2019) and El-Sokkary *et al* (2018) in Egypt found that *Klebsiella pneumoniae* were the predominant pathogen. These dissimilarities of the results can be attributed to sample size, methodology, study population and seasons.

Low percentages (2%) of atypical bacteria like *M. pneumoniae* and *Legionella pneumophila* were detected in this study. Similar results were obtained by Herrera *et al* (2016). for low level of atypical bacteria in this study either due to their

absence in respiratory tract or in low concentration that below the detection levels of the technique.

There is another explanation of PCR negativity which is that PCR results are more useful during the early stages of infection when more organisms are likely to be present. However, the likelihood of positivity in PCR result diminishes overtime because the sensitivity decreases significantly in the interval from symptom onset to specimen collection increases or since no consensus exists regarding which molecular target should be amplified to achieve higher sensitivity and specificity neither does a clearly defined standard protocol exist (Afroz *et al.*, 2023).

In the current study, Viruses such as Influenza B, Adenovirus, Parechovirus and Influenza C were not detected. These results disagreed with the results of others like Serin *et al* (2014) in Turkey who found viruses in 4%, Joelsons *et al* (2023) in Brazil found 42.01%.

Different reasons could be behind that no viral pathogens were detected in the current study such as the number of viruses was limited in the kit, seasonal variation, small sample size due to the high cost or kind of the sample because most studies carried out on the respiratory viral detection were depend on nasopharyngeal swab rather than sputum (Lee *et al.*, 2018).

Table (3.1): Community acquired pneumonia pathogens detected by RT-PCR

Causative agent	Freq (%)	
Bacteria	Not detected	13 (26)
	<i>Klebsiella pneumoniae</i>	14 (28)
	<i>Streptococcus pneumoniae</i>	23 (46)
	<i>Staphylococcus aureus</i>	3 (6)
	<i>Mycoplasma pneumonia</i>	1 (2)
	<i>Legionella pneumophila</i>	1 (2)
	<i>M. catarrhalis</i>	0 (0)
	<i>B. pertussis</i>	0 (0)
	<i>L. pneumophila /longbeachae</i>	0 (0)
	<i>C. pneumoniae</i>	0 (0)
	<i>H. influenza type B</i>	0 (0)
Viruses	Influenza B	0 (0)
	Adenovirus	0 (0)
	Parechovirus	0 (0)
	Influenza C	0 (0)
Fungi	<i>P. jirovecii</i>	0 (0)

3.2. Association of demographical characteristics with the results of Rt-PCR

The results of RT-PCR according to age, gender and smoking are shown in Table 3.2. In the current study, the highest number of the pathogens were 24 (64.9%) in adult patients with 26-65 years old followed by 11 (29.7%) among elderly group (>65 year), while the lowest percentage was 5.4% among young patients (18-25) years old. The results of the current study were disagreed with the study in Baghdad, Iraq by (Jaaffar *et al.*, 2019) who showed that most of the patients were young between (10-30) years, while similar to the results of Akter *et al* (2014) in Bangladesh who found that positive case were among adult patients than young .Although the high percentage of positivity was found in adult patients than young and elderly patients but statistically no significant differences were detected by Fisher Exact test ($p= 0.856$). We expected that elderly patients will be more positive than young ages due to the weakened immunity but small sample size could play a major factor.

Regarding gender, males were more positive 25/34 (67.6%) than female 12/16 (32.4%). A significant difference was found with gender ($p<$

0.01) in which males were more positive than females. Similar findings were also found by Serin *et al* (2014) in Turkey, Joelsons *et al* (2023) and Assunção *et al* (2019) in Brazil

and Akter *et al* (2014) in Bangladesh, On the other hand, our results were in contrarily with those by Jaaffar *et al* (2019) from Iraq, Baghdad and Batoool *et al* (2021) in Saudi Arabia who found that females were more suffered from CAP than female. Generally, males are more suffered from CAP than female because smoking is more common among males than females which is the main predisposing factor for developing pneumonia.

Based on smoking more positive cases were detected among non-smoker patients than smoker patients in which 43.2% of smokers were positive, while 56.8% of non- smoker patients were positive. No statistical differences were found among smoker and non-smoker CAP patients. These results were in oppositive of others like Lupisan *et al* (2019) and El-Sokkary *et al* (2018) who found that pneumonia was more common among smoking patients than non-smokers. One possibility of our unexpected results that the size of the samples was small.

Table (3.2): Results of culture and RT-PCR in association with demographic characteristics

Demographic variable		RT-PCR		P-Value
		Negative (%)	Positive (%)	
Age	Young adult (18-25)	0 (0.0)	2 (5.4)	0.856**
	Adult (26-65)	8 (61.5)	24 (64.9)	
	Seniors (> 65)	5 (38.5)	11 (29.7)	
Gender	Male	9 (69.2)	25 (67.6)	1.000**
	Female	4 (30.8)	12 (32.4)	
Smoking	Yes	3 (23.1)	16 (43.2)	0.320**
	No	10 (76.9)	21 (56.8)	

* Chi-square

** Fisher exact test

on of positive results with patient clinical characteristics

Distribution of positive and negative cases according to clinical characteristics are shown in Table 3.3. High percentages of positivity were detected in patients with chest pain, cough, dyspnea and fever which were 94.6%, 89.2%, 81.1% and 59.4% respectively. No significant association was found between positivity and clinical characteristics by Fisher Exact test ($p> 0.05$).

Chest pain is one of the most common symptoms of pneumonia. Chest pain is caused by

the membranes in the lungs filling with fluid. This creates pain that can feel like a heaviness or stabbing sensation and usually worsens with coughing, breathing (Ticona *et al.*, 2021).

The outcomes were different from the results conducted by Batoool *et al* (2021) who found that Cough was the most common clinical signs, while El-Sokkary *et al* (2018) from Egypt found that fever was the most common clinical symptom and Pipali (2017) in India also found fever was the most clinical symptoms of fever. Clinical signs and symptoms vary from person to person and depend on the type of the causative agents

whether bacterial or viral and on the severity of infection.

Regarding the severity of the disease, the percentage of positivity was higher (56.8%) among patients with score 2 compared to 43.2% among patients with score 3-5. The possible explanation for opposite expected results that patients with score 3-5 are more severely infected than patients with score 2, therefore admitted directly to ICU and received high doses of antibiotics for prolonged times.

These results agreed with the results of El-Sokkary *et al* (2018) in Egypt and Kasamatsu *et al* (2012) in Japan, found that patients with CURB-65 score 2 were more positive for pathogens than patients with score 3-5. The majority of the positive cases were among patients with chronic diseases 31 (83.8%) compared to 6 (16.2%) among patients without chronic diseases but statistically without any differences ($p=1.00$). This is due to the fact that the incidence of CAP increases with age and is

associated with an elevated morbidity and mortality due to the physiological changes associated with aging and a greater presence of chronic diseases. Similar to our results were found by Naucler *et al* (2019) in Sweden, Assefa *et al* (2022) in Ethiopia, El-Sokkary *et al* (2018) in Egypt and Batool *et al* (2021) in Saudi Arabia who found that patients with chronic underlying diseases are more commonly suffered from pneumonia than those without chronic comorbidities. Regarding the duration of hospitalization, majority of the positive cases were recorded for one week 27 (73%) followed by two and 3 weeks which were 6 (16.2%) and 4 (10.8%) respectively. This may be logic because of receiving antibiotics which decreased the amount of positivity.

The main drawbacks of the study were low number of cases and limited period of the study as well as highly expensive detection kit that covered relatively small number of cases which adversely affects the data analysis.

Table (3.3): Clinical characteristics with culture and RT-PCR

Clinical Manifestation		RT-PCR		P-Value
		Negative (%)	Positive (%)	
Chest pain	Yes	12 (92.3)	35 (94.6)	1.000**
	No	1 (7.7)	2 (5.4)	
Cough	Yes	13 (100)	33 (89.2)	0.561**
	No	0 (0)	4 (10.8)	
Dyspnea	Yes	11 (84.6)	30 (81.1)	1.000**
	No	2 (15.4)	7 (18.9)	
Fever	Yes	6 (46.2)	22 (59.5)	0.406**
	No	7 (53.8)	15 (40.5)	
CURB-65	(2)	8 (61.5)	21 (56.8)	0.764*
	(3-5)	5 (38.5)	16 (43.2)	
Chronic	With	11 (84.6)	31 (83.8)	1.000**
	Without	2 (15.4)	6 (16.2)	
Duration of Hospitalization	1 Week	11 (84.6)	27 (73)	0.425**
	2 Week	2 (15.4)	4 (10.8)	
	3 Week	0 (0)	6 (16.2)	

* Chi-square

** Fisher exact test

4. CONCLUSION

From the results of this study, it can be concluded that among bacterial causes of adult CAP patients, *Streptococcus pneumoniae* was the predominant followed by *Klebsiella pneumoniae*, while among atypical bacteria both *Mycoplasma pneumoniae* and *Legionella pneumophila* were detected. All samples were negative for Adenovirus, Influenza B, C and Parechovirus.

CAP was more commonly found among young aged adult, males, patients with comorbidities and with CURB-65 score-2.

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پوختە

پیشینه: هه وکردنی سییه کان مەترسیدارتترین نەخۆشییە درمیە و یەکیکە لە هۆکارە باوەکانی مردن لە جیهاندا. لە رووی کلینیکیە وە دابەش بوو بە سەر هه وکردنی سییه کان کۆمەلایەتی [CAP] و هه وکردنی سییه کان نەخۆشخانە. هه وکردنی سییه کان بە هۆی بەکتیریا و ڤایرۆس و قارچک و پرتوتۆزۆاوه دروست دەبێت. هەرچەندە کشتوکال وەک شپۆازی زێری ستاندارد بۆ دەستنیشانکردنی هۆکارە بەکتیریاکانی هه وکردنی سییه کان دادەنرێت بەلام هیشتا بە ههستیاری کەم و کات بەفەرۆدان تاییەتمەندە. چونکە زانیارییەکان سەبارەت بە هۆکاری CAP لە ناوچەکەماندا سنووردان. ئامانجی توێژینە وەکە دیاریکردنی هۆکارە بەکتیریا و ڤایرۆسییە باوەکانی CAP بوو بە بەکارهێنانی تەکنیکەکانی PCR لە کاتی راستەقینەدا و هەر وەها لیکۆلینە وە لە پەيوەندییەکانیان لە گەل تاییەتمەندییە دیمۆگرافی و کلینیکییەکان.

شپۆازەکان: کۆی گشتی 50 نمونە بەلغەم لە نەخۆشە گەورەسەلەکانی سییه کان و هەرگیرا و لە کۆمەلگە نەخۆشخانە و هەرگیرا کە تەمەنیان لە 18 بۆ زیاتر لە 65 سال بوو لە نەخۆشخانەکانی فیرکاری و فیراکەوتنی ئازادی لە شاری دھۆک لە ماوەی مانگی حوزەیرانی 2022 تا حوزەیرانی 2023. کیتی باندلی بنەرەتی بۆسڤۆر RPP لە RT-PCR (ئەنادۆل، تورکیا) بەکارهێنرا.

دەرەنجامەکان: لە 50 نمونە، 42 (84%) بە تەکنیکەکانی RT-PCR پۆزەتیف بوون. زۆرتین ماددە نەخۆشخوایە دۆزراوەکان بریتی بوون لە سترپیتۆکۆکۆسی سییه کان ۲۳ (۴۶%) و دواتر کلیبسییلا نۆمۆنیا ۱۴ (۲۸%)، ستافیلۆکۆکۆسی ئاورپۆس ۳ (۶%)، هه وکردنی سییه کان میکۆپلازما و لیجیۆنیا پنۆمۆفیل بە ئەنجامە هاوشیوەکان ۱ (۲%)، لە کاتیکدا مۆراکسیلا کاتارالیس، بۆردیتیل کۆکە، سییه کان جیرۆڤتسی، لیجیۆنیا پنۆمۆفیل/لۆنگیچ، هیمۆفیلۆس ئەنفلۆنزا جۆری [] و کلایدیای سییه کان نیگەتیف بوون. سەرچەم نمونەکان نیگەتیف بوون بۆ ڤایرۆسی ئەنفلۆنزا بی، ئەدینۆڤایرۆس، پاریکۆڤایرۆس و ئەنفلۆنزا سی. ئەنجام: لە ئەنجامی ئەم توێژینە وەیەدا، دەتوانرێت بلین کە لە نیوان هۆکارە بەکتیریاییەکانی نەخۆشانی CAP گەورەسالدا، سترپیتۆکۆکۆسی سییه کان زال بوو و دواتر کلیبسییلا پنۆمۆنیا، لە کاتیکدا لە نیوان بەکتیریا نااساییەکان هەردوو میکۆپلازما پنۆمۆنیا و لیجیۆنیا پنۆمۆفیلیا دۆزراونەتەو. هەموو نمونەکان نیگەتیف بوون بۆ ڤایرۆسی ئەدینۆ، ئەنفلۆنزا بی، سی و پاریکۆڤایرۆس. CAP زیاتر لە نیوان گەنجان تەمەن پیگەیشتوو، نیر، نەخۆشانی تووشبوو بە نەخۆشی هاوبەش و بە CURB-65 [] دۆزرایەو.

الخلاصة

المقدمة : الالتهاب الرئوي هو أخطر الأمراض المعدية وأحد أسباب الوفاة الأكثر شيوعاً في العالم. ينقسم سريريًا إلى الالتهاب الرئوي المكتسب من المجتمع [CAP] والالتهاب الرئوي المكتسب من المستشفيات . يمكن أن يحدث الالتهاب الرئوي بسبب البكتيريا والفيروسات والفطريات والأوالي. على الرغم من أن طريقة الزرع تعتبر طريقة ذهبية قياسية لتشخيص الأسباب البكتيرية للالتهاب الرئوي إلا أنها لا تزال تتميز بحساسية منخفضة وتستغرق وقتًا طويلاً. لأن البيانات المتعلقة بمسببات CAP محدودة في منطقتنا. الهدف من الدراسة هو للكشف عن الأسباب البكتيرية والفيروسية الشائعة لـ CAP باستخدام تقنية PCR [1][2] بالإضافة إلى دراسة ارتباطها بالخصائص الديموغرافية والسريية.

الطرق: تم جمع 50 عينة من البلغم من المرضى البالغين المصابين بالالتهاب الرئوي المكتسب العمر من 18 إلى أكثر من 65 سنة في مستشفيات آزادي التعليمية و الطوارئ في مدينة دهوك خلال الفترة من حزيران 2022 إلى حزيران 2023 (Bosphore RPP Basic Bundle Kit of RT-PCR). (الأناضول، تركيا) كان مستعملاً.

النتائج: من 50 عينة، 42 (84%) كانت إيجابية بتقنية RT-PCR. كانت أكثر مسببات الأمراض المكتشفة شيوعاً هي *pneumoniae* [3][4][5][6][7][8][9][10][11][12][13][14] 23 (46%) تليها *Klebsiella pneumoniae* 14 (28%)،

و *Mycoplasma pneumonia* [15][16][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46][47][48][49][50] 3 (6%) ، مع نتائج مماثلة من *Legionella pneumophila* ، *Bordetella pertussis* ، *Moraxella catarrhalis* ، *Legionella pneumophila /longbeachaej* ، *Pneumocytis jirovecii*

من النوع b و *Haemophilus influenzae* كانت سلبية. وكانت جميع العينات سلبية بالنسبة لفيروسات الأنفلونزا [51][52][53][54][55][56][57][58][59][60][61][62][63][64][65][66][67][68][69][70][71][72][73][74][75][76][77][78][79][80][81][82][83][84][85][86][87][88][89][90][91][92][93][94][95][96][97][98][99][100] ، *Parechovirus* والأنفلونزا C.

الاستنتاج: من نتائج هذه الدراسة يمكن استنتاج أنه من بين الأسباب البكتيرية لمرضى CAP البالغين، كانت *Streptococcus pneumoniae* هي السائدة تليها *Klebsiella pneumoniae* ، ومن ضمن البكتيريا اللانمطية تم الكشف عن كل من *Mycoplasma pneumoniae* و *Legionella pneumophila*. كانت جميع العينات سلبية لـ *influenza B,C* ، *Adenovirus* و *Parechovirus*. تم العثور على CAP بشكل أكثر شيوعاً بين الشباب البالغين والذكور والمرضى الذين يعانون من أمراض متزامنة والذين حصلوا على درجة CURB65-2.