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The Clinical Application Value of a Novel Chip in the Detection of Pathogens in Adult Pneumonia: A Multi-Centre Prospective Study in China

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Purpose: The detection of pathogenic microorganisms plays a significant role in the diagnosis and management of pneumonia that are responsible for a substantial number of deaths worldwide. However, conventional microbiological tests (CMT) have low accuracy and are time-consuming. In this study, we aim to evaluate the clinical value of Chips for Complicated Infection Detection (CCID) in detecting pneumonia pathogens.

Patients and Methods: This study was conducted at nine hospitals in China from January 2021 to September 2022. Respiratory samples from adult pneumonia patients were collected from each patient. CMT and CCID were performed in parallel to identify the pathogens.

Results: A total of 245 patients were included, with 73% being elderly. CCID identified pathogenic microbes in 78.0% of patients and conventional microbiological tests (CMT) in 57.1% of the patients ($p<0.001$). The overall positive and negative percent agreements between CCID and CMT for pathogen detection were 90.07% and 38.46%, respectively. 38.8% of patients were diagnosed with mixed infections with at least two pathogens by CCID. Bacterial infections identified by CCID accounted for 60.0% of 245 patients, with the top 3 being Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterococcus faecium, respectively. K. pneumoniae was the most common pathogen in elderly patients, with a significantly higher prevalence compared to non-elderly patients ($p = 0.0011$). Among the 197 patients who had used antibiotics before sample collection, the positive rate of CCID was significantly higher than that of CMT ($p < 0.001$).

Conclusion: This study indicates that compared to CMT, this novel chip has significant advantages in detecting pathogens in pneumonia patients, especially in the elderly.

Keywords: pneumonia, elderly, pathogens, chip, LAMP

Introduction

Pneumonia is the leading cause of morbidity and mortality worldwide, with clinical manifestations ranging from mild pneumonia, characterized by fever and sputum, to severe pneumonia, characterized by respiratory distress and sepsis. Data from the United States showed that approximately 650 adults are hospitalized with community acquired pneumonia (CAP) every year per 100,000 population, corresponding to 1.5 million unique CAP hospitalizations each year.[1] The overall incidence of CAP was 7.13 per 1000 person-years in urban China.[2] In older

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adults, pneumonia presents differently, with higher rates of hospitalization, rehospitalization, and mortality than in younger patients.[3] In the absence of a clear microbiological diagnosis, the treatment of pneumonia is inevitably empirical, which can lead to the overuse of broad-spectrum antibiotics and the selection of drug-resistant pathogens. Timely identification of pathogens and initiation of antibiotic therapy as soon as possible is crucial for pneumonia patients.

Detection and identification of pathogenic microorganisms often rely on conventional microbiological tests (CMT), which have poor sensitivity and are time-consuming. During most waiting periods for culture-based diagnostic results, inappropriate antibiotic treatment intervals of 48 to 72 hours have been proven to be associated with adverse outcomes in CAP.[4] It is necessary to adopt accurate and rapid methods for pathogen detection and diagnosis confirmation, guiding targeted treatment and improving prognoses.

The loop-mediated isothermal amplification (LAMP) technology is an emerging isothermal nucleic acid amplification method developed by Notomi et al in 2000[5]. The basic principle of LAMP is auto-cycling strand displacement DNA synthesis in the presence of Bst DNA polymerase. The reaction yields between 10^6 and 10^9 copies of the target DNA within a duration of 30 to 60 minutes, conducted under isothermal conditions at temperatures ranging from 60 to 65 °C.[6] Since LAMP is a new detection technology, it has been widely used in medicine, biology, environmental monitoring, and food industries.[7–9] Microfluidic chips, also known as lab-on-A-chip, integrate sample pretreatment, biological separation, biochemical reactions, and signal analysis into chips of a few square centimeters. Their micrometer-scale structures allow precise control of fluid flow in microchannels, enabling the miniaturization and automation of comprehensive analyses.[10] Isothermal amplification methods have recently accelerated the development of microfluidic chips.[11] The microfluidic chip-based isothermal amplification method combines the advantages of isothermal amplification for nucleic acid detection, such as high specificity and sensitivity and short detection time, with the advantages of microfluidic chips, such as controllable liquid flow, efficient use of samples and reagents, increased analysis speed and low cost.[10,12] Based on these advantages, the combination of LAMP and microfluidic detection for multiple pathogens provides a good application technology for the diagnosis and analysis of pathogens of infectious diseases.

CCID (Chips for Complicated Infection Detection), based on LAMP and microfluidics chips, is a technology for the rapid identification of complicated, critical disease-infected pathogens within 4–8 hours. In this study, CCID was applied to samples from adult pneumonia patients, and the sensitivity and clinical benefits of this testing method were evaluated in comparison with conventional microbiological methods.

Material and Methods

Sample Collection

The prospective study was carried out between January 2021 and September 2022 in nine centers in China (Peking University First Hospital, Anhui Chest Hospital, Beijing Chest Hospital, Beijing Shijitan Hospital, Beijing Jishuitan Hospital, Aerospace Central Hospital, Beijing Jingmei Group General Hospital, Beijing Tsinghua Changgung Hospital, and Xuanwu Hospital Capital Medical University). Patients diagnosed with pneumonia were eligible for enrollment if they met the following criteria: (1) were at least 18 years of age; (2) both CCID and CMT were performed to detect pathogens; (3) had samples available for standard procedures; and (4) the clinical and imaging data were complete. Pneumonia is defined according to the Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of pneumonia in adults.[13,14] The enrolled patients were divided into the elderly group (≥65 years) and the non-elderly group elderly (< 65 years).

The exclusion criteria were pregnant women, patients unable to give written informed consent, patients unable to produce sputum spontaneously, and non-infectious interstitial lung diseases, pulmonary edema, atelectasis, pulmonary embolism, pulmonary eosinophil infiltration, or pulmonary vasculitis. Samples were collected from patients according to standard procedures and were divided into two parts, one for conventional microbiological tests (CMT) and the other for CCID tests.

Conventional Microbiological Testing Methods

All of the above samples were subjected to a series of laboratory tests immediately. Conventional microbiological testing included bacterial, mycobacterial, and fungal cultures and smears of samples, and PCR assays and serological antibody detection for *Chlamydia pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila, Epstein–Barr virus* (EBV), *Cytomegalovirus*, and other herpes simplex viruses. Additionally, (1,3)-*β*-D-glucan, galactomannan, and Cryptococcus antigen tests were performed for *Candida, Aspergillus*, and *Cryptococcus*, respectively. T-SPOT.TB and Xpert MTB/RIF testing was done for *Mycobacterium tuberculosis*.

CCID Detection and Analysis

The samples for CCID were sent to a testing company (CapitalBio Technology, Beijing, China) for nucleic acid extraction, sample loading, isothermal amplification, data analysis, and result presentation. Samples were transported at 4 degree and delivered to the laboratory within 72 hours. The workflow of CCID detection and analysis is shown in Figure 1. The list of pathogens detected by CCID is described in Supplemental Table 1.

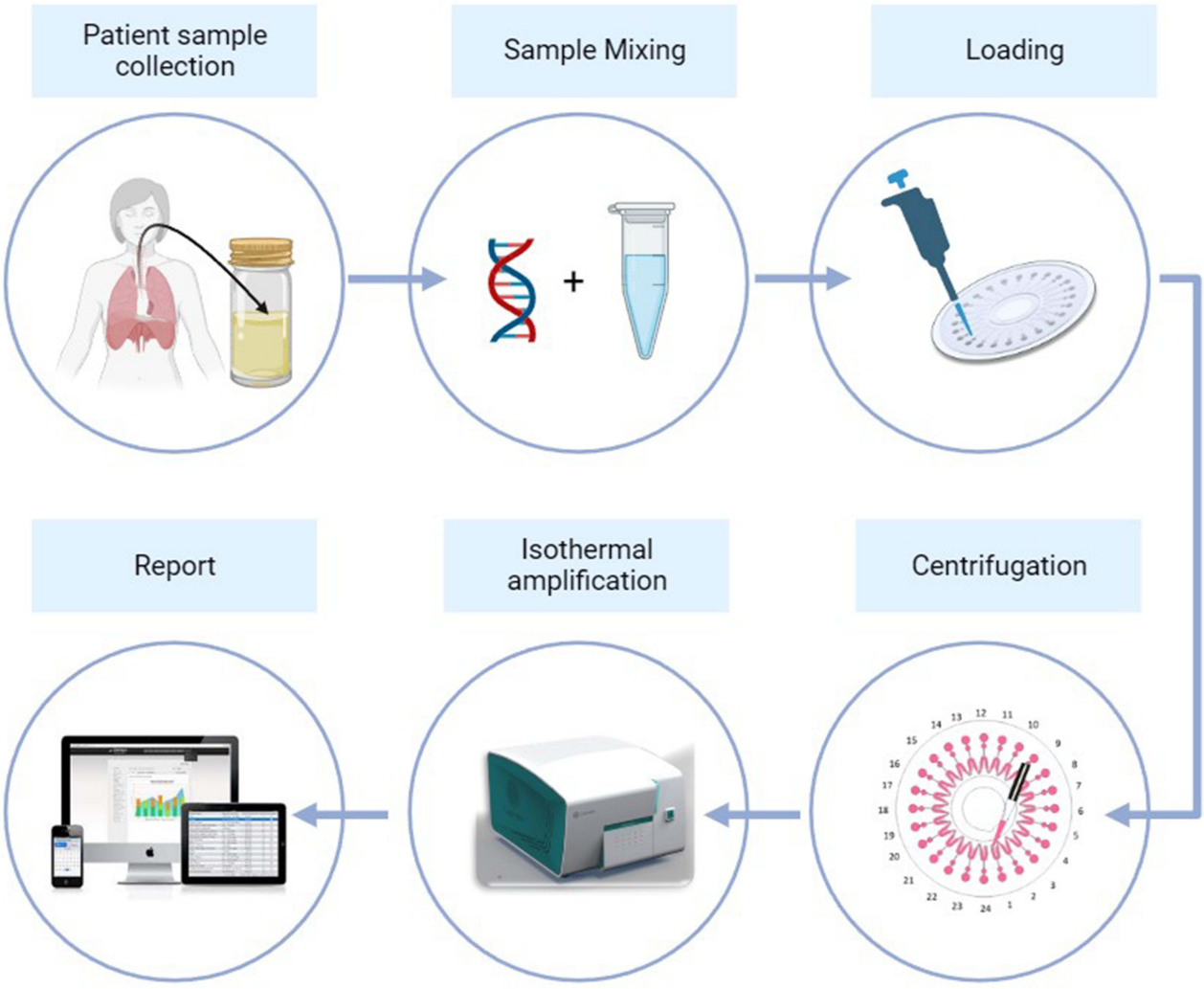

Figure 1 Workflow of CCID detection. This figure was created with the aid of Biorender (https://biorender. com/).

Statistical Analysis

Continuous variables are presented as median and interquartile range (IQR), whereas categorical variables are presented as counts and percentages. *T*-test and Wilcoxon signed-rank test were used for continuous variables. The chi-square test or Fisher's exact test was used to analyze categorical variables. The positive percent agreement and negative percent agreement of CCID compared with CMTs were used. A two-tailed P value of less than 0.05 was considered statistically significant. Data analyses were performed using SPSS version 23.0 software and GraphPad Prism version 8.0 software.

Results

Baseline Characteristics

Between January 2021 and September 2022, a total of 245 patients with pneumonia were enrolled from nine medical institutions in China (Figure 2). The median age was 71 (IQR, 61–82) years, with 73.06% of the population being elderly. Eighty-two (33.47%) patients were female. Additionally, a significant portion of the patients, 231 in total, had at least one comorbidity, involving respiratory diseases (115, 46.94%), circulatory diseases (159, 64.90%), metabolic diseases (92, 37.55%), digestive diseases (83, 33.88%), tumors (59, 24.08%), autoimmune diseases (11, 4.49%), neurological diseases (81, 33.06%), hematological disease (39, 15.92%), renal diseases (37, 15.10%) (Table 1). A total of 245 eligible respiratory specimens, including 133 cases of BALF, 111 cases of sputum, and 1 tracheal aspirate were collected in the study. The names of the participating centers and the number of cases submitted for testing are shown in Supplemental Table 2. All positive results were included in further aetiological evaluation.

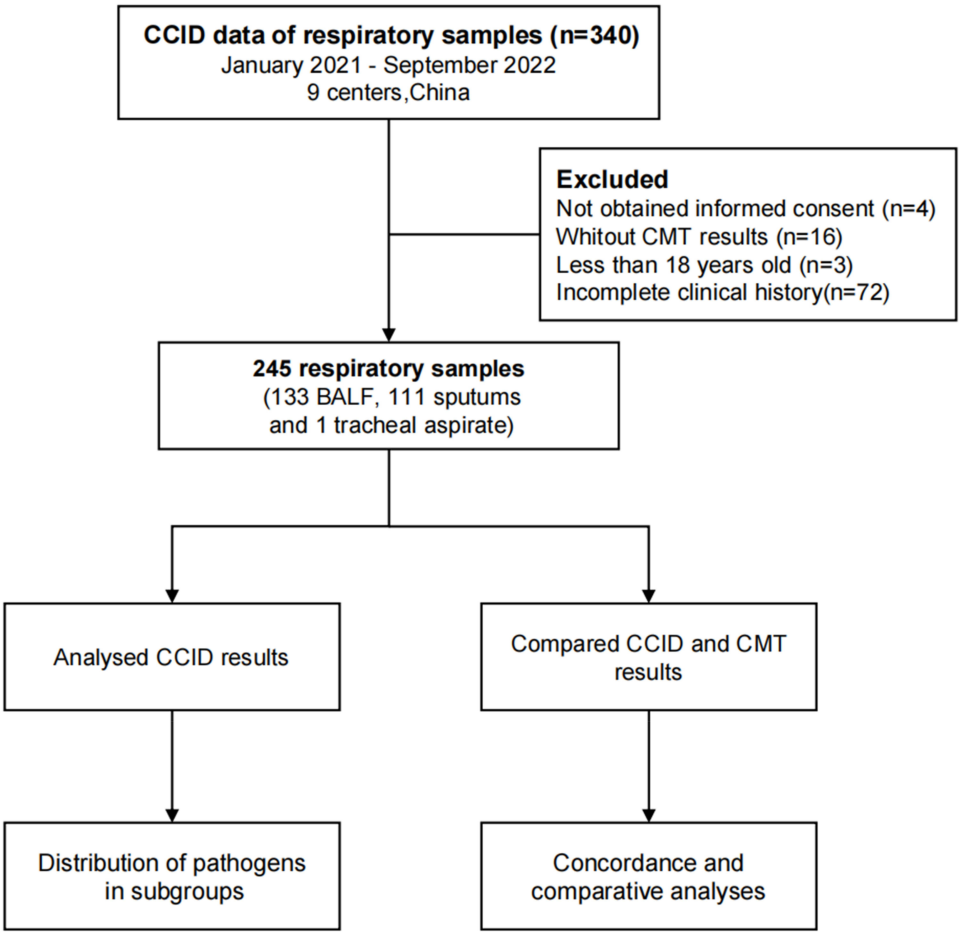

Figure 2 Flow chart of this study.

Table 1 Baseline Characteristics of 245 Patients

Characteristics	All cases	Elderly	Non-elderly
Total	245	179	66
Gender, no. (%)			
Male	163(66.53%)	130(72.63%)	33(50%)
Female	82(33.47%)	49(27.37%)	33(50%)
Age, median (IQR)			
Median	71(61–82)	78(70–84)	50(39–58)
Sample type, no. (%)			
BALF	133(54.29%)	88(49.16%)	45(68.18%)
Sputum	111(45.31%)	90(50.28%)	21(31.82%)
Airway secretions	1(0.41%)	1(0.56%)	0
Comorbidities, no. (%)			
Respiratory diseases	115(46.94%)	87(48.60%)	28(42.42)
Circulatory diseases	159(64.90%)	138(77.10%)	21(31.82)
Metabolic diseases	92(37.55%)	75(41.90%)	17(25.76%)
Digestive diseases	83(33.88%)	68(37.99%)	15(22.73%)
Tumors	59(24.08%)	46(25.70%)	13(19.70%)
Autoimmune diseases	11(4.49%)	9(5.03%)	2(3.03%)
Neurological diseases	81(33.06%)	73(40.78%)	8(12.12%)
Hematological disease	39(15.92%)	28(15.64%)	11(16.67%)
Renal diseases	37(15.10%)	33(18.44%)	4(6.06%)

Abbreviation: BALF, Bronchoalveolar lavage fluid.

Concordance of CCID and CMT

The results of CCID and CMT are presented in Figure 3A. CCID identified pathogens in 191 (77.96%) patients, while CMT identified pathogens in 140 (57.14%) patients. The positive rate was significantly higher in CCID compared to

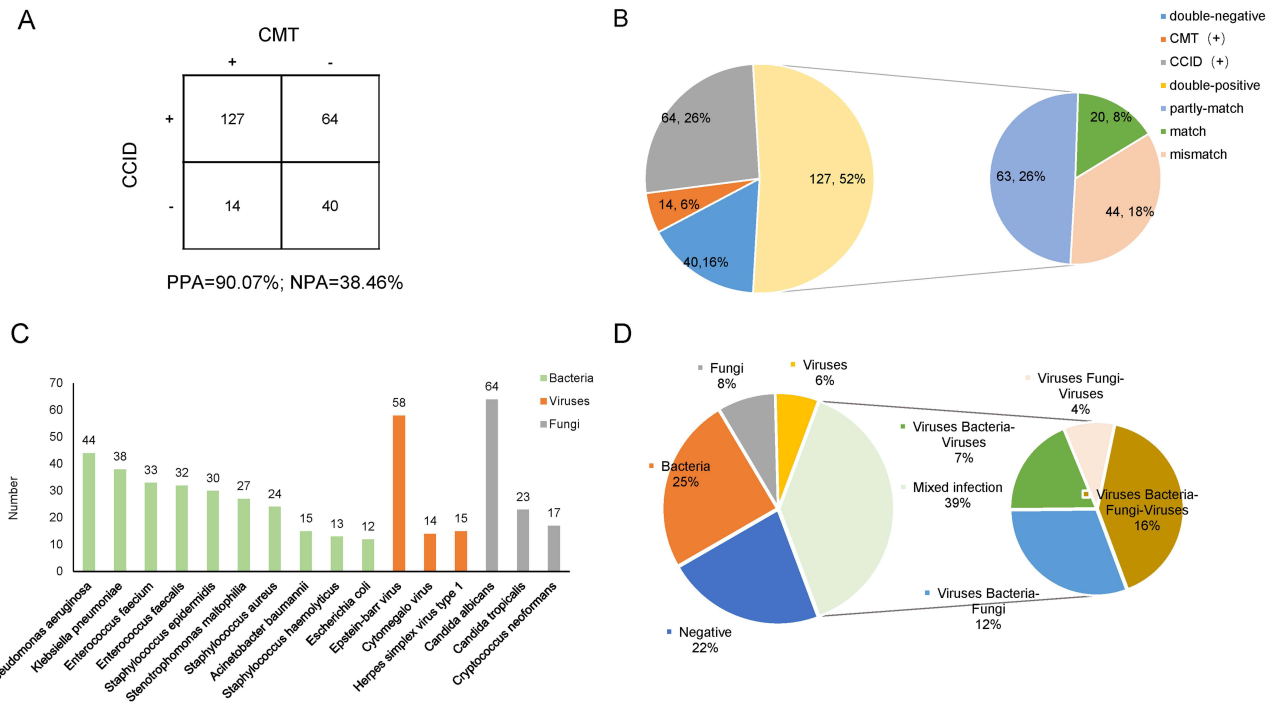

Figure 3 Concordance of diagnosis between CCID and CMT. (**A**) Contingency table for CCID and CMT. PPA: positive percentage agreement, NPA: negative percentage agreement. (**B**) The results of CCID and CMT were positive in 127 (51.84%) cases. Among the double-positive cases, 20 (8%) were matched and 65 (26%) were partly-matched. (**C** and **D**) Composition of pathogens in patients with CCID results.

CMT ($\chi^2 = 28.36$, $p<0.001$). The overall positive and negative percent agreements between CCID and CMT for pathogen detection were 90.07% and 38.46%, respectively.

Among the 245 patients, CCID and CMT were both positive in 127 (51.84%) patients and both negative in 40 (16.33%) patients. Sixty-four samples were positive by CCID only (26.12%) and 14 were positive by CMT only (5.71%). Among the double-positive samples, there were 20 (8.16%), 63 (25.71%), and 44 (17.96%) samples that matched, partly-matched, and mismatched between CCID and CMT results, respectively (Figure 3B). The value of the Kappa statistic was 0.305, indicating a significant agreement ($p<0.001$).

Distribution of Pathogens Identified by CCID

A total of 191 samples were detected pathogens by CCID, and 54 samples were negative. Of the 191 samples, bacteria were the most common pathogens (147/191, 77.0%). The most common bacteria were *Pseudomonas aeruginosa* (44/147, 29.9%), followed by *Klebsiella pneumoniae* (38/147, 25.8%), *Enterococcus faecalis* (33/147, 22.4%), *Enterococcus faecalis* (32/147,21.8%), and *Staphylococcus epidermidis* (30/147, 20.4%). Among the detected fungi and viruses, the most common were *Candida albican*s and EBV, with 64 (33.5%) and 58 (30.4%) cases, respectively (Figure 3C). In addition, mixed infections account for 38.8% of all cases (95/245), of which bacteria - fungi - virus co-infections account for 15.9% (39/245), bacteria combined with fungi infections account for 11.8% (29/245), bacteria combined with virus infections account for 7.3% (18/245), and fungi combined with virus accounts for 3.7% (9/245) (Figure 3D). Except for *Mycobacterium tuberculosis* complex, the percentage of CCID-positive samples was significantly higher than that of culture-positive samples in terms of bacteria (Figure 4).

Comparison Analysis at the Sample-Type Level

We conducted a differential analysis of pathogens detected by CCID in different sample types. Among the 133 BALF cases, the most common pathogen was *P. aeruginosa* (26/133, 19.5%), followed by *K. pneumoniae* (18/133, 13.5%), *E. faecalis* (10/133, 7.5%), and *E. faecium* (10/133, 7.5%). Among the 111 sputum samples, the most detected pathogen was *S. epidermidis* (24/111, 21.6%), followed by *E. faecalis* (22/111, 19.8%), *E. faecium* (22/111, 19.8%), and *K. pneumoniae* (20/111, 18.02%) (Figure 5A and Supplemental Table 3). In the sputum samples, we found

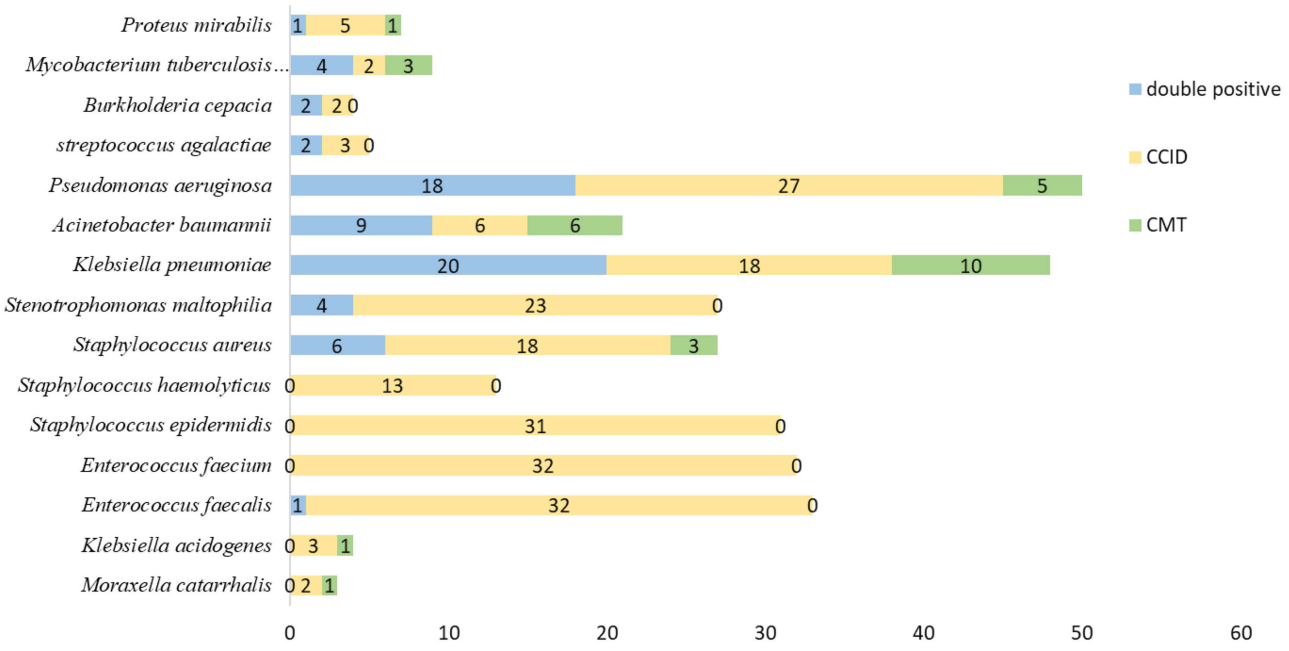

Figure 4 Overlap of common bacteria detected by CCIID and CMT. Detection efficiency of CCID and CMT for different bacteria.

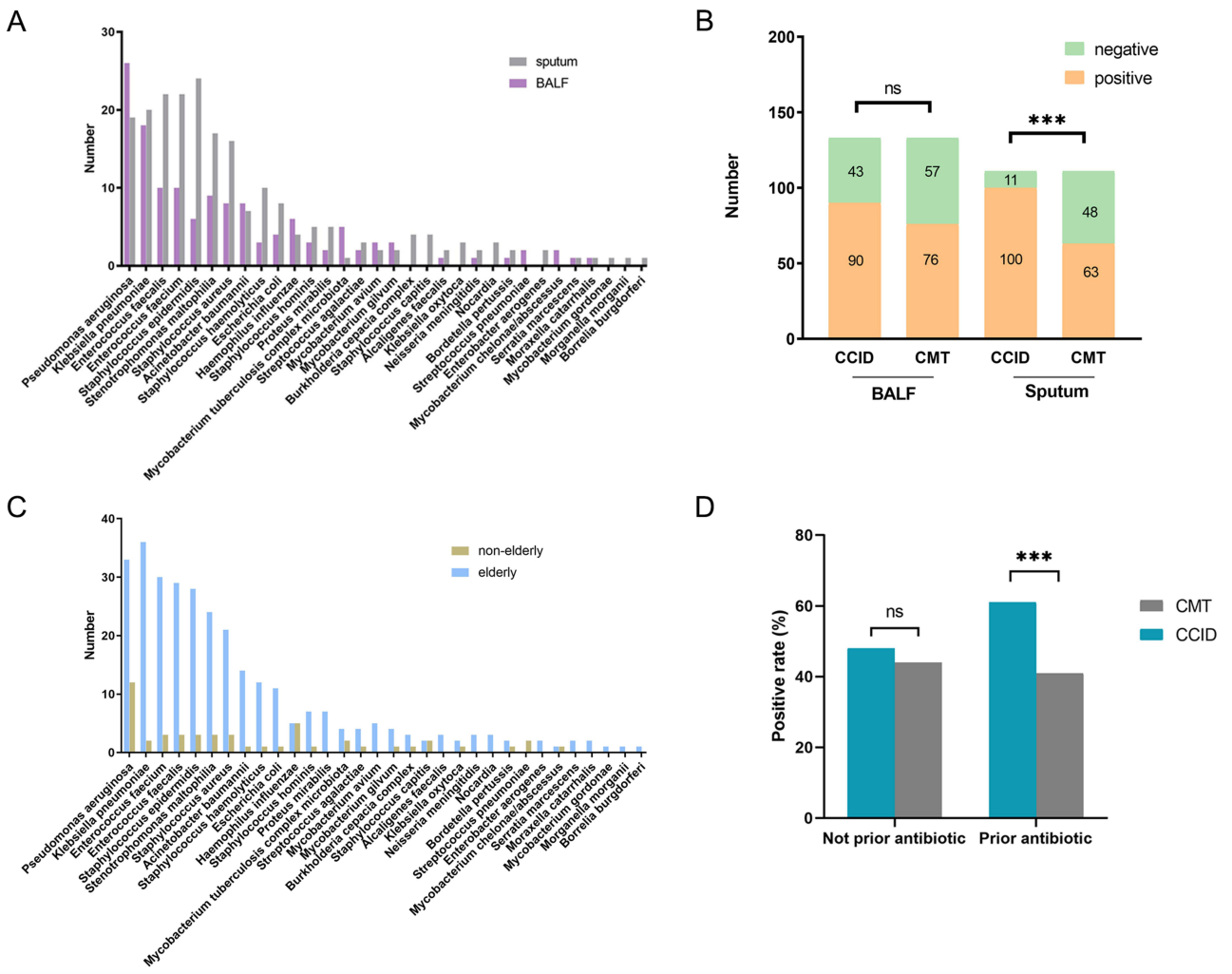

Figure 5 Pathogen analysis in the subgroups and effect of antibiotic exposure on pathogen detection. (**A**) Different pathogenic organisms between sputum and BALF samples. (**B**) Positive numbers of CCID and CMTs in BALF and sputum samples. (**C**) Pathogen analysis detected by CCID in elderly and non-elderly. (**D**) CCID positivity and CMT positivity in samples with prior antibiotic exposure. ns, no significant difference; ***, $p < 0.001$.

a significantly higher sensitivity in detection by CCID vs CMT ($p<0.001$), whereas the detection rate of CCID did not differ from that of CMT in the BALF samples (Figure 5B).

Distribution of Pathogens Detected by CCID in Elderly and Non-Elderly

To identify the pathogens differences between the elderly and the non-elderly, we divided patients into two groups: the elderly group (≥65 years) and the non-elderly group (<65 years) to compare the frequency of positive pathogen detection. Among the elderly group, the most commonly detected pathogens identified by CCID were *K. pneumoniae* (36/179, 20.1%), followed by *P. aeruginosa* (33/179, 18.4%) and *E. faecalis* (29/179, 16.2%). The top 10 most frequently detected pathogens were consistently observed in both groups (Figure 5C). The elderly group had significantly higher proportions of *K. pneumoniae, E. faecium, E. faecalis, S. epidermidis*, and *S. maltophilia* compared to the non-elderly group ($p < 0.05$) (Supplemental Table 4).

Effect of Antibiotic Exposure on Pathogen Detection

Among the 224 patients with available antibiotic exposure data, 197 (87.9%) patients had been treated with antibiotics prior to the tests for CCID and CMT, while 27 (12.1%) patients had not received any antibiotic treatment. The most commonly used antibiotics were β-lactams, prescribed to 187 patients (94.9%), followed by quinolones in 29 patients

(14.7%), macrolides in 5 patients (2.5%), and aminoglycosides in 5 patients (2.5%) (Supplemental Table 5). The positivity rate of CCID was higher than that of CMT (120/197 vs 80/197) and the difference was statistically significant ($p < 0.001$) in antibiotic-exposure patients. There was no significant difference in the positivity rate between CCID and CMT in non-exposed patients (13/27 vs 12/27; $p = 0.09$) (Figure 5D).

Discussion

Pneumonia is a leading infectious cause of hospitalization and death among adults.[15] Delayed etiological diagnosis can lead to inappropriate empiric broad-spectrum antibiotic treatment, resulting in poor treatment outcomes, longer hospital stays, and increased medical costs.[16] Therefore, early microbial identification is very important for the timely diagnosis and treatment of pneumonia patients. In this multi-center study, CCID as a novel chip detected pathogenic microbes in 78% of pneumonia patients even though most of the patients had already been treated with antibiotics before testing. In addition, two or more pathogens were detected in 39% (95/245) patients according to CCID. The data also showed that the positive detection rate of CCID was superior to that of CMTs for sputum samples. Finally, we found that pathogenic characteristics of pneumonia in elderly and non-elderly patients were different in subgroup analysis. The elderly group had significantly higher proportions of *K. pneumoniae, E. faecium, E. faecalis, S. epidermidis*, and *S. maltophilia* than the non-elderly group.

Due to the limitations of traditional pathogen detection methods, pathogens were detected in only 38% of community-acquired pneumonia adults.[15] In recent years, microbiological diagnosis with the use of rapid multiplexed molecular platforms has developed rapidly. According to this study, the detection rate of CCID reached 78%, which was approximately 20% higher than that of traditional pathogen tests. Considering that healthy lungs have a large amount of microbial colonization,[17] clinicians should be cautious in distinguishing between colonizing bacteria and infectious bacteria when explaining the results of CCID. However, for each pathogen, a higher frequency of each pathogen was found by using CCID compared to CMT. Notably, the positivity rate of CCID was 60.91% in antibiotic-exposure patients. The result of this method was less affected by antibiotic exposure than CMT. The price of a CCID chip that covers 20 indicators was only 150 RMB (~$20). Therefore, we concluded the LAMP microfluidic chip for detecting common bacterial infections has a high detection rate and can detect a variety of pathogens simultaneously, which is more advantageous than traditional detection methods.

In terms of the types of pathogens detected by our chip, bacteria overwhelmingly surpass fungi and viruses as the main pathogens. The most commonly detected bacteria included *P. aeruginosa* and *K. pneumoniae*, in agreement with extensive research findings. *P. aeruginosa* was the most commonly detected bacterial pathogen by CCID testing in the current study. The prevalence *of P. aeruginosa* pneumonia has been increasing over the past decades. Although *P. aeruginosa* is a rare cause of CAP, a systematic review of 50 studies from 2010 to 2014 in mainland China estimated *P. aeruginosa* accounted for 19.4% and 17.8% of all isolates of VAP and HAP, which is roughly consistent with our result (18.37%).[18] It is worth noting that isolation of *P. aeruginosa* from respiratory secretions may indicate colonization rather than infection, especially in patients having pre-existing structural abnormalities.[19,20] *P. aeruginosa* has been isolated from sputum in 4% ~ 15% of adults with COPD patients without pneumonia.[21] In this study, 46.75% of patients had respiratory disease and 24.08% of patients had neoplastic disease. These may lead to a high detection rate of *P. aeruginosa* detection.

Our data showed that *K. pneumoniae* was detected in 38 samples of adult pneumonia, which was similar to previous studies.[22] *K. pneumoniae* was described as one of the core respiratory pathogens and the three leading bacterial pathogens in patients with acute respiratory infection in China.[23,24] Additionally, the proportion of *K. pneumoniae* detected in older adults was significantly higher than that in the non-elderly. Another study in China also reported that high frequencies of *K. pneumoniae* ($p < 0.001$) were found in the group aged ≥65 years.[25] These findings indicated that *K. pneumoniae* should be seriously considered as a priority screening pathogen in older adults.

The difficulty in distinguishing colonization from infection is one of the challenges in the molecular diagnosis of respiratory pathogens. CCID results showed the most common fungi were Candida albicans. It is a colonizing bacterium in the immune normal population, but it may be a pathogenic pathogen in the immunocompromised population. In addition, given molecular techniques can detect multiple pathogens, determining the true pathogen that causes the disease

becomes more complicated. Mixed infections were identified in 39% of the patients in this study. In clinical practice, CCID may not be an independent diagnostic tool, and clinical doctors need to distinguish between colonized bacteria and pathogenic bacteria based on clinical symptoms, signs, and imaging findings. However, it must be acknowledged that CCID provides clinical doctors with advantageous diagnostic criteria, especially when dealing with patients with mixed or complex infections. Future research on pneumonia thus needs to address the conundrum of polymicrobial respiratory disease and their influence on the pathogenesis of pneumonia.

Our study was not without limitations. First, the study described the presence of pathogenic microorganisms detected by CCID in the lower respiratory tract, leaving the task of determining whether they belong to pathogenic or colonizing bacteria to clinicians. Secondly, sputum and tracheal aspirate samples may be more affected by oropharyngeal colonizing bacteria than BALF. Due to poorly tolerated invasive tests that are usually not prescribed on elderly patients with severe illness, it is difficult to obtain high-quality sample types for clinical detection. However, there is growing evidence of an association between bacterial colonization levels and the onset of pneumonia.[26,27] Moreover, the types of pathogens detected by CCID are limited, precluding the detection of rare pathogens as they were not included in the chip. Finally, because of the particularity of RNA virus, the primer target of the chip does not include RNA virus. In the future, a chip that covers the majority of respiratory pathogens will be designed for pathogen diagnosis in pneumonia patients.

Conclusion

In conclusion, we systematically compared the detection performance of CCID and CMT in patients with pneumonia, and further clarified the pathogen spectrum in adult pneumonia. The results showed that CCID was effective in detecting pneumonia pathogens, especially for the distribution of pathogens against bacterial targets, making it a promising microbiological detection and diagnostic method. In addition, we found that CCID has high clinical application potential in the detection of pathogens in mixed infections.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Peking University First Hospital (2021-keyan132). Informed consent was obtained from all participants in this study.

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Disclosure

The authors report there are no competing interests to declare.

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