Comparison between the molecular diagnostic test and chest X-ray combined with multi-slice spiral CT in the diagnosis of lobar pneumonia

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Received August 1, 2021; Accepted October 2, 2021; Published November 22, 2021

Abstract: Lobar pneumonia is an inflammatory condition of the lung that mainly affects the lobes of the lungs and the alveoli, and it is usually caused by a bacterial infection. There are many ways to diagnosis this disease. But an early and accurate method for lobar pneumonia diagnosis has an important role in its treatment. Therefore, in this study, a comparison between the molecular diagnostic test and chest x-ray combined with multi-slice spiral CT was done to find out better diagnosis of lobar pneumonia. For this purpose, 122 individuals suspected of lobar pneumonia were studied by clinical examination, chest X-ray, and multi-slice spiral CT. For the molecular diagnostic test, the multiplex PCR was used for two main causes of the disease, Streptococcus pneumoniae and Klebsiella pneumoniae. Results showed that the specificity for Chest X-ray + Multi-slice Spiral CT had the highest amount (82.8%), but high sensitivity (100%) belonged to a molecular diagnostic test for both bacteria. On the other hand, the sensitivity and specificity of Streptococcus pneumoniae were better than Klebsiella pneumoniae and the possibility of error in Streptococcus pneumoniae was lower than Klebsiella pneumoniae. In general, although the Chest X-ray + Multi-slice Spiral CT method was better than the molecular diagnosis test, it could not identify the causative agent and did not show a difference between pathogens for better antibiotic treatment, and also the possibility of diagnosis is low at the beginning of the disease. Therefore, according to the results of the current study, the best way to diagnose lobar pneumonia is to use both methods, simultaneously.

Key words: Chest X-ray; Diagnosis; Lobar pneumonia; Molecular diagnosis test; Multi-slice spiral CT.

Introduction

Lobar pneumonia, as one of the three anatomic classifications for pneumonia, is known by the consolidation and solidification of inflammatory exudation in intra-alveolar space which affects a large and wide area of one or more lobes of a lung (1). The main cause of lobar pneumonia is bacteria (e.g. Streptococcus pneumoniae, Klebsiella pneumoniae, Moraxella catarrhalis, Mycobacterium tuberculosis, Haemophilus influenzae and Legionella pneumophila)(2, 3).

The gram-positive bacterium Streptococcus pneumoniae (pneumococcus) can be colonized asymptptomatically in the human nasopharynx (4). But this pathogen is one of the most important causes of invasive diseases such as pneumonia, sepsis and meningitis. In the world, this bacterium causes the death of more than 4.8 million people, every year. This mortality is more common in children under 5 years of age and the elderly (5, 6). This bacterium has more than 33 serotypes. The most important and main factor of pneumococcal virulence is its polysaccharide capsule, which is the basis of its serotyping (7).

Klebsiella pneumoniae is a gram-negative, immobile bacterium with a capsule, lactose fermenter, voluntary anaerobic, and cylindrical shape (8). Although this bacterium is found in the normal flora of the mouth, skin and intestines, it can cause dangerous pneumonia if aspirated (9-11). Klebsiella pneumonia is especially prevalent in alcoholics, hospitalized patients, and immunocompromised individuals such as those with AIDS (12).

Sometimes, pneumonia can be difficult to diagnose, especially in the first stage of disease, because its symptoms are very variable and are often very similar to the common cold or flu (13). Physical examination and checking the history of the disease is one of the first steps to diagnose pneumonia (14). For more detailed examinations, additional tests are taken from patients such as a blood test (to confirm infection and identify the germs that cause the disease), a chest radiography (to find the location and extent of pneumonia), pulse oximetry (to measure the level of oxygen in the blood), and mucosal sample (sputum) test (15). There are also more advanced diagnostic methods, such as chest CT scans (to better consider the lungs), arterial blood gas tests (to measure the amount of oxygen in the blood sample), pleural fluid culture (a small amount of fluids are removed from lung tissue for analysis and identification of pneumonia-causing bacteria), bronchoscopy, and molecular diagnostic tests (1, 16).

This study aimed to compare two diagnostic methods, molecular diagnostic test and chest X-ray combined with Multi-slice Spiral CT in lobar pneumonia patients. To molecular diagnosis test, Multiplex PCR was used for Streptococcus pneumoniae and Klebsiella pneumoniae.
Materials and Methods

Patient

122 individuals suspected of lobar pneumonia were selected as the object of study. Physical examination and clinical signs (e.g. cough with sputum, shortness of breath, and severe chest pain while breathing, etc.) were done to diagnose all subjects. Demographic information about patients is listed in Table 1.

Chest X-ray

The DICOM images included a heterogeneous set of x-ray images that were captured by Varian X-Ray (series HFG). The intensity of the window was optimized to visually enhance the lung tissue region.

Multi-slice Spiral CT

The scan was performed using a 16-piece spiral CT machine (Siemens, Germany) and patients were asked to hold their breath for 5 seconds. To collect data, a tenacious layer volume scan was used from the beginning of the trachea to the top of the diaphragm, and a 0.75 mm detector was performed. CT virtual endoscopy (CTVE), minimum intensity projection (MinIP), and other reconstruction techniques were used for post-image processing, mainly using shaded surface display (SSD), curve multi-planar reformation (CMPR), multiplanar reformation (MPR), volume rendering technique (VRT), minimum intensity projective (MinIP), and other reconstruction methods.

Bacterial diagnosis

Streptococcus pneumoniae

Blood, sputum, and urine samples were used to prepare the bacterial sample. These samples were grown on blood agar (Sigma-Aldrige, U.S.A), which were phenotypically similar to Streptococcus pneumoniae, and they were identified by gram staining, catalase test, bile solubility test, and optochin disc sensitivity. The standard strain of Streptococcus pneumoniae ATCC 63483 was used as quality control. For storage, the isolates were stored in the STGG medium (Merck, Germany) at a temperature of -73°C.

Klebsiella pneumoniae

For confirmation, clinical samples (Blood, sputum, and urine) were cultured on a blood agar medium (Sigma-Aldrige, U.S.A) and after regrowth, they were passed and kept at 37 °C for 24 hours. After incubation, direct smears were obtained from colonies suspected of Klebsiella pneumoniae, and if gram-negative bacilli were observed, the following diagnostic tests were performed: Conventional laboratory tests were used including oxidase test, culture in MR-VP (Methyl Red-Voges Proskauer broth), TSI (Triple Sugar Iron Agar), SIM (Simmons Citrate agar), lysine decarboxylase, and citrate and urea media. Isolated bacteria were then cultured in LB broth (Sigma-Aldrige, U.S.A) for long-term storage and after incubation at 37°C, stored in 50% glycerol at -73°C until further testing. As a quality control, the standard strain of Klebsiella pneumoniae ATCC 700603 was used.

DNA Extraction

Bacterial isolates were dissolved in 153μl of PBS saline phosphate buffer suspension and DNA extraction was performed according to the instructions of the Roche kit, German. The extracted DNA was examined by nano-drop and electrophoresis in 8% gel.

Multiplex PCR

In this study, the Multiplex PCR method was used to confirm Streptococcus pneumoniae and isolates Klebsiella pneumoniae. The cpsA gene, encoding polysaccharide capsule, was used to identify Streptococcus pneumoniae. And the SHV gene was used to identify Klebsiella pneumoniae (Table 2). HotStar Taq Master Mix kit (Sigma-Aldrige, U.S.A) was used to perform multiplex PCR reactions.

Statistical analysis

Data were analyzed by SPSS 19 software, and Wilcoxon and Chi-square tests were used and P <0.05 was considered significant.

Results

People with infectious pneumonia often experience cough with sputum, fever with severe chills, shortness of breath, stinging or severe chest pain while breathing, and increased breathing rate. In the elderly, confusion may be the most obvious symptom (17). In this study, Out of 122 cases related to lobar pneumonia, the highest rate of primary symptoms in patients was shortness of breath (78.29%), Cough with sputum (65.71%), and

Table 1. Demographic information in patients.

| Variable | Percent | P-value |
|----------|---------|---------|
| ≥20      | 31 (25.41%) |        |
| 20-40    | 29 (23.77%) |        |
| 40-60    | 21 (17.22%) | 0.001  |
| ≥80      | 16 (13.11%) |        |
| Gender   |         |         |
| Man      | 75 (61.47%) | 0.001  |
| Woman    | 47 (38.53%) |        |

Table 2. Information of primers of Streptococcus pneumoniae and Klebsiella pneumoniae.

| Gene | Sequence | Length | Melting Temperature |
|------|----------|--------|---------------------|
| cpsA | Forward  | 5´-CTCTATAGAATGGAGATATAAACTATGTTA-3´ | 280bp | 60.1°C |
|      | Reverse  | 5´-CCAAAGAAAAATACTACATTACATTACACATATGTC-3´ |        |        |
| SHV  | Forward  | 5´-GAAAAACACCTTGCGGACGG-3´ | 503bp | 59.4°C |
|      | Reverse  | 5´-ATTTGCTGATTTCCGCTCGGC-3´ |        |        |
Severe chest pain while breathing (73.81%).

As shown in Table 3, Chest X-ray + Multi-slice Spiral CT recognized 105 cases as positive which 101 of them had lobar pneumonia, so that the specificity of this test was 82%. To measure sensitivity, the specificity result was divided into positive diagnoses. The sensitivity for Chest X-ray + Multi-slice Spiral CT was 96.2%, and there was not any wrong diagnosis about healthy people with positive tests. Also, 4 patients had a negative test.

Since the annealing temperature for the two primers was close to each other, multiplex PCR was used. The results of multiplex PCR for *Streptococcus pneumoniae* showed that 96 cases had a positive test which 82 of them had lobar pneumonia and the specificity test was 67.2% (Table 3). The sensitivity for *Streptococcus pneumoniae* PCR test was 85.4%, and 7.37% of healthy People had positive tests, also 4% of patients had a negative test. *Klebsiella pneumoniae* test showed 84 cases had a positive test, the specificity test was 51.6%, the sensitivity was 75%, 10 healthy People had Positive Test, and 11 patients had a negative test. Also, the result of multiplex PCR showed that 12 cases had both bacteria. The specificity and sensitivity were 9.8% and 100%, respectively, and there were not any healthy people with positive tests and patients with negative tests.

### Discussion

Accurate and early diagnosis of lobar pneumonia can improve the effectiveness of treatment and prevent long-term complications for the patient (15). On the other hand, if it is not diagnosed, patients can subconsciously transmit the disease to others (18). On the other hand, misdiagnosis of the disease can lead to negative health consequences, psychological distress and financial costs. Therefore, it is very important to use a correct, accurate and timely diagnostic method for this disease (19).

Unfortunately, clinical criteria for pneumonia have a low diagnostic value in definitively detecting the presence of bacteria. The sensitivity of clinical criteria in the diagnosis of pneumonia is also low, as Bell et al. showed false-negative rates of 46% were reported for the clinical diagnosis of pneumonia (20). However, clinical examinations are necessary to begin diagnosing the disease and before prescribing antibiotics to treat lobar pneumonia.

There are several methods for a more accurate diagnosis of this disease. Because chest X-rays, like clinical examinations, had sensitivity and specificity problems (21, 22), so in this study, we decided to combine this method with Multi-slice Spiral CT and compare this diagnostic method with the molecular diagnosis method. The results of the present study showed that the specificity for Chest X-ray + Multi-slice Spiral CT had the highest amount (82.8%) which it means this method could diagnose lobar pneumonia better than a molecular diagnostic test. The specificity for the diagnosis of *Streptococcus pneumoniae* (67.2%) was higher than specificity for *Klebsiella pneumoniae*, which this result showed a revision for redesigning primer for *Klebsiella pneumoniae*. However the lowest specificity belonged to the molecular diagnostic test for both bacteria, but its sensitivity was the best with 100% sensitivity. This high sensitivity showed that all patients that were recognized by this method had truly lobar pneumonia. The sensitivity for Chest X-ray + Multi-slice Spiral CT, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae* were 96.2%, 85.4%, and 75%, respectively.

About the percentage of errors, in the current study, healthy people with a positive test were 0% for Chest X-ray + Multi-slice Spiral CT and molecular diagnostic test for both bacteria. It showed that these two tests did not have errors to show healthy people as lobar pneumonia patients. *Streptococcus pneumoniae* and *Klebsiella pneumoniae* multiplex PCR had 7.37% and 8.19% errors in diagnosis healthy people, respectively. Another considered error was patients with a negative test. Similar results have previously been reported in various studies of various diseases (23-25). The molecular diagnostic test for both bacteria with 0% did not have any error in diagnosing patients as healthy people. 3.2% of patients were recognized as healthy people by Chest X-ray + Multi-slice Spiral CT test. This was because these patients had lobar pneumonia in the early stages of infection, which was not detected by this method.

Several methods have been proposed for the diagnosis of lobar pneumonia (16). In this study, two methods of Chest X-ray + Multi-slice Spiral CT and molecular diagnostic test were compared. Each of these methods has its advantages and disadvantages. For example, although the molecular diagnosis method had a high error rate and low specificity, it is cheaper and more accessible than the Chest X-ray + Multi-slice Spiral CT method and requires less laboratory space. Also, this method is possible to use anywhere on the earth, but in the Chest X-ray + Multi-slice Spiral CT method, the possibility of transferring expensive and heavy equipment is low. On the other hand, although the Chest X-ray + Multi-slice Spiral CT method has more specificity, the possibility of diagnosis is low at the beginning of the disease. Another important point about the Chest X-ray + Multi-slice Spiral CT method is that it does not recognize the type of pathogen so that specific antibiotics can be used for treatment. Therefore, it can be concluded that the best way to diagnose is to use both methods simultaneously.

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