

Analysis on constituents and drug resistance of pathogenic bacteria causing chronic obstructive pulmonary disease.

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Abstract

Objective: To separate the pathogenic bacteria of Chronic Obstructive Pulmonary Disease (COPD) and investigate their constituents and the resistance features of gram-negative *bacilli*, so as to provide some references for the clinical application of antibiotics in the respiratory department of Hunan Provincial People's Hospital.

Methods: A total of 710 COPD patients who were admitted in the respiratory department of Hunan Provincial People's Hospital from August 2013 to February 2017 were enrolled in the study. In order to get germ-free sputa, all of them had to rinse their mouth repeatedly with clean water or hydrogen peroxide. The sputa were assayed for drug sensitivity with the K-B methods drafted by National Committee for Clinical Laboratory Standards (NCCLS).

Results: A total of 813 strains of bacteria were isolated from 710 COPD patients, of which gram-negative *bacilli* were the major strains whose highlight was *Pseudomonas aeruginosa*. Gram-positive *bacilli* ranked second of which *Staphylococcus aureus* was the primary and fungi third. The strains and constituent ratio of gram-negative *bacilli* were significantly higher than that of gram-positive *bacilli* and that of fungi, and the difference was statistically significant ($P < 0.05$). Through drug sensitive tests, it was known that *Pseudomonas aeruginosa* and *Acinetobacter baumannii* had higher resistance rate to cefazolin, with a statistical difference ($P < 0.05$).

Conclusion: The highlight of bacteria-isolated is gram-negative bacteria which have very strong drug resistance, so that it is necessary to monitor unceasingly the resistance of bacteria, so as to guide reasonable medication in clinic.

Keywords: Chronic obstructive pulmonary disease, Pathogenic bacteria, Drug resistance rate, Drug sensitive test.

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Introduction

In recent years, as a result of antibiotics abuse, there is a big difference in the species, distribution, and resistance of pathogenic bacteria which give rise to lower respiratory tract infection [1], bringing great difficulties to clinical medication and treatment. As a common disorder of the lower respiratory tract infection, Chronic Obstructive Pulmonary Disease (COPD) is sorted into two broad categories: chronic bronchitis and pulmonary edema. Cough, even productive cough and asthma are the major manifestations of COPD in clinic which have a serious impact on the lives of the patients. The majority of the COPD patients are elderly people whose viscera functions and immunity degenerate gradually so that the disease is repeated and prolonged, increasing the difficulty of treatment. In order to fix such issues as complex constituents, mal-distribution, and resistance of the pathogenic bacteria and low immunity of the patients, we take some germ-free sputa with rational methods from 710 COPD patients who visited our hospital from August 2013 to February 2017 to be cultured and

analyse the constituents and resistance of pathogenic bacteria, to support clinical medication in reason. The details are following.

Materials and Methods

General materials

Based on the diagnosis standards of COPD of China, 710 COPD patients visited our hospital from August 2013 to February 2017 were enrolled in the study, 482 males, 228 females, aged from 45 to 80 y, with a mean age of (53.8 ± 8.1 y). They had various coughs, pyrexia, productive cough, even wheezing, and dyspnea, and some had lower extremity edema, varicose veins, cyanosis and so on. Among them, 395 cases had diabetes, hypertension, renal insufficiency, coronary heart disease, and cerebral hemorrhage, 279 chronic bronchitis, 19 bronchiectasis and 17 bronchial asthma.

Collection of samples

All patients were selected according to Diagnosis Criteria of Nosocomial Infection issued by Ministry of Health of China. The patients in accordance with the criteria, abandoning their first cough which was coughed out harder, coughed their second sputum into an aseptic box after gargled their mouth in the morning which were sent to laboratory immediately. The bacterial strains were isolated and identified in accordance with the national clinical testing procedures. The sputum samples collected in 1 h were inoculated in chocolate agar, goat blood agar, and McKinsey agar. Or sputum samples also could be obtained by bronchoscope, hatched at 35°C in an incubator with CO₂. After culture, the samples were identified and smeared according to rules, which were observed under microscope: sputum samples containing <10 squamous epithelial cells and >25 leukocytes, or squamous epithelial cells: leukocytes <1:2.5 per low-power field were acceptable ones with less contamination which could be cultured. The isolated sputum bacteria whose concentration $\geq 10^7$ cfu/ml were considered as pathogenic bacteria, and those whose concentrations $\leq 10^4$ cfu/ml as contaminated bacteria which were abandoned. If the concentration of the bacteria was between 10^4 and 10^7 cfu/ml, they should be repeatedly cultured until the same bacteria with a concentration of 10^5 - 10^6 cfu/ml were acquired twice in a row which could be regarded as pathogenic bacteria. The strains were appraised by the Vitek32 Bacteriometer from Bio Merieux, Franch.

Drug sensitive test

The isolated bacteria all were tested for drug sensitivity with K-B paper disk method. The culture medium for drug sensitive test consisted of resin and fibrin-free goat blood provided by Bio Merieux, and the strains included *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Drug sensitive disks included cefazolin, ampicillin, and gentamicin. And the sensitive test results were determined in accordance with the standards of American Clinical Laboratory Standardization Committee (CLSI/NCCLS).

Observation indexes

A total of 813 kinds of bacteria isolated were recorded which were mainly sorted into gram-negative *bacilli*, gram-positive *bacilli*, and fungus, of which the major species were listed in respective. The drugs which were resistant to the gram-negative *bacilli* and gram-positive *bacilli* were detected and noted, so did their resistance rate.

Statistical analysis

All data were analysed by software SPSS17.0, and the measurement data were expressed by mean \pm SD. After homogeneity test of variance and normal distribution test, the independent sample t-test used for the analysis among sample groups, and the enumeration data were compared using chi-square test.

Results

Constituent and distribution of pathogenic bacteria

A total of 813 strains were isolated from 710 COPD patients, including gram-negative *bacilli*, gram-positive *bacilli*, and fungus. The analysis on various strains was seen in Table 1. The species of gram-negative *bacilli* were much more than that of gram-positive *bacilli* and fungus, with a statistical difference ($P < 0.05$). The constituent ratio of gram-negative *bacilli* was much higher than that of the gram-positive *bacilli* and fungus ($P < 0.05$).

Table 1. Constituent ratio of pathogenic bacteria of 170 COPD patients infected.

Category	n	Constituent ratio/%
Gram-negative <i>bacilli</i>	554	68.1
<i>Pseudomonas aeruginosa</i>	125	15.3
<i>Escherichia coli</i>	118	14.5
<i>Acinetobacter baumannii</i>	82	10.1
<i>Klebsiella pneumoniae</i>	72	8.8
<i>Serratia</i>	65	8.0
<i>Enterobacter cloacae</i>	45	5.6
Other <i>bacilli</i>	47	5.8
Gram-positive <i>bacilli</i>	179	22.1
<i>Staphylococcus aureus</i>	59	7.2
<i>Streptococcus pneumoniae</i>	48	5.9
<i>Staphylococcus epidermidis</i>	38	4.7
<i>Enterococcus</i>	34	4.3
Fungus	80	9.8
<i>Candida albicans</i>	49	6.1
Other bacteria	31	3.7

Notes: compared with the gram-positive *bacilli* and fungus, the constituent ratio was much higher, $P < 0.05$.

Analysis on the drug resistance of the pathogenic bacteria

There were five common antibiotics put to use analyzing the drug resistance of major pathogenic gram-negative *bacilli* like *Pseudomonas aeruginosa*, *Escherichia coli*, and *Baumannii* of which findings were shown in Table 2. It was known that the resistance rate of *Escherichia coli* to piperacillin was much higher than that of *Pseudomonas aeruginosa* and *Baumannii*, with a statistical difference ($P < 0.05$); the resistance rate of *Baumannii* to amoxicillin higher than that of *Pseudomonas aeruginosa* and *Escherichia coli*, having a statistical significance ($P < 0.05$); the resistance rate of *Escherichia coli* to cefazolin lower than that of *Pseudomonas aeruginosa*, with a statistical significance ($P < 0.05$); the resistance rate of

Pseudomonas aeruginosa and *Escherichia coli* to cefotaxime higher than that of *Baumannii*, having a statistical significance, with a statistical difference ($P<0.05$); the resistance rate of *Baumannii* to ciprofloxacin lower than that of *Pseudomonas aeruginosa* and *Escherichia coli*, having a statistical significance ($P<0.05$); the resistance rate of *Escherichia coli* to ciprofloxacin higher than that of *Pseudomonas aeruginosa*, with a statistical significance ($P<0.05$).

Table 2. Resistance rate of the major gram-negative bacilli.

Antibiotic	<i>Pseudomonas aeruginosa</i>	<i>Baumannii</i>	<i>Escherichia coli</i>
	Number resistance rate	Number resistance rate	Number resistance rate
Piperacillin	38 30.6	13 15.9	95 80.3
Amoxicillin	39 30.7	53 73.7	21 17.9
Cefazolin	113 90.6	82 100	72 61.3
Cefotaxime	79 63.5	22 26.8	67 58.6
Ciprofloxacin	62 49.7	8 9.8	106 90.3

Discussion

Chronic Obstructive Pulmonary Disease (COPD) induced by airflow limitation is one of common and frequently-occurring diseases of lower respiratory tract infection. The patient has such manifestations in clinic as increased cough, increased expectoration, fever, even dyspnea, lower limb edema, and cyanosis. The majority of the patients with COPD are the aged because their hyp immunity, viscera degeneration and long-term use of various antibiotics [2] have pathogenic bacteria mutated and their drug resistance raised, increasing clinical treatment difficulty. Smoking is able to put on the prevalence rate of COPD [3] so that the amount of male COPD patients are more than that of the female and its prevalence rate gradually increases with age, being in accordance with the international reports [4]. COPD is mainly sorted into chronic bronchitis and pneumonedema. All bronchi of COPD patients are nearly infiltrated by inflammatory cells like neutrophils and lymphocytes, leading to repeated injury and repair of the bronchial line and finally an increase in collagen content in the bronchial wall to form a scar [5]. If the terminal pulmonary tissue contains too much gas, the alveolar septa would be destroyed, finally enlarging the lung volume and degenerating lung function [6].

In the study, we culture the sputa from 710 COPD patients in a germfree condition and obtain 813 pathogenic bacteria. The bacteria are cultured in chocolate agar or goat blood agar, placed in an aseptic container in case they are contaminated, enhancing the reliability. The germiculture paves the way for later drug sensitive test and can also direct the antibiotic therapy and the adjustment of treatment plan accurately. The pathogenic bacteria acquired are generally sorted into gram-negative bacilli, gram-positive bacilli, and fungus which are the major causes of lower respiratory tract infection [7].

Among them, there are 554 strains of gram-negative bacilli, accounting for 68.1% of the total. Among the gram-negative bacilli, there are 125 strains of *Pseudomonas aeruginosa* which is the largest part, being consistent with the international reports [8], accounting for 15.3% of the total; 118 strains of *Escherichia coli*, accounting for 14.5%; 82 strains of *Baumannii*, accounting for 10.1%. A total of 179 gram-positive bacilli are isolated, ranking second and accounting for 22.1% of the total; among the gram-positive bacilli, *Staphylococcus aureus* is the major one with 59 strains isolated, accounting for 7.2%; there are 48 strains of *Streptococcus pneumonia*, accounting for 5.9%. And there are 80 strains of fungus isolated, ranking third and accounting for 9.8% of the total, of which *Candida albicans* ranks first and *Aspergillus fumigatus* second. Both the amount of strains and the constituent ratio of the gram-negative bacilli are more than that of the gram-positive bacilli and fungus, with a statistical difference ($P<0.05$). This suggests that the pathogenic bacteria resulting in COPD are complicated and varied [9], so further study is needed for specific bacterium strains.

The prevalence rate of COPD has seen a growth year by year, and the treatment should focus on slowing down lung function decline and enhancing the patients' immunity and their lung function [10,11]. The ceaseless mutation of pathogenic bacteria gives rise to more complicate strains, boost drug resistance, and increase the difficulty of treatment in clinic. Therefore, as a key link, the infection of pathogenic bacteria isn't ignored during treatment [12]. In the study, we use dozens of antibiotics to test the drug sensitivity of 813 kinds of pathogenic bacteria according to K-B method drafted by NCCLS. At the same of ensuring that the bacteria are cultured in a sterile environment, the practitioner performing sensitive test should also pay attention to germfree operation and improve his aseptic sense, and all the experimental instruments should be thoroughly sterilized. In case of blind selection to increase treatment difficulties, the antibiotics should be reasonable determined based on the outcome of drug sensitive test [13]. We investigate the drug resistance of three gram-negative bacilli (*Pseudomonas aeruginosa*, *Escherichia coli*, and *Baumannii*) and find that the resistance rates of *Pseudomonas aeruginosa* and *Baumannii* to cefazolin are the highest, with a statistical difference ($P<0.05$). What's more, *Pseudomonas aeruginosa* has too strong drug resistance to be thoroughly cleared away by respiratory system, resulting in lung dysfunction and repeated illness [14]. *Escherichia coli* has the highest resistance rate to ciprofloxacin, and the difference is significant ($P<0.05$).

To sum up, COPD is the most common and important disease of respiratory tract infection whose prevalence rate and mortality has risen with years. The study catches the key point that pathogenic bacteria are the essential factor impacting on the patients and reveals such features of them as complicate constituent and strong drug resistance via germiculture and drug sensitive test. If some useful clinical data can predict disease and the change of strains, that would be benefited for proper application of antibiotics in clinic [15].

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