ORIGINAL ARTICLE

Analysis of the Aetiological Distribution and Drug Resistance of Pathogens in Hospitalized Patients with Acute Exacerbation of Chronic Obstructive Pulmonary Disease

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ABSTRACT

Objective: *To explore the aetiological distribution and drug resistance of pathogens in hospitalized patients with chronic obstructive pulmonary disease (COPD) to enable a reasonable anti-infective treatment therapy and timely and effective control of the disease.*

Methods: *Data from a retrospective study of 245 patients who were admitted to the respiratory ward of Beijing Shijitan Hospital, Capital Medical University (Beijing, China) were analysed. Those patients diagnosed with acute exacerbation (AE) of COPD were enrolled in the study from October 2010 to October 2013. Among them, 58 patients tested positive for bacteria, and 86 positive sputum samples were identified and tested for pathogen susceptibility to drugs using Vitek-II. Extended spectrum beta-lactamase (ESBL) was detected and analysed for bacterial identification and susceptibility to drugs; the process of ESBL testing was completed automatically by Vitek-II.*

Results: *Eight-six bacteria were isolated comprising 54 Gram-negative bacilli (62.79%), nine Gram-positive cocci (10.47%), and 23 fungi (26.74%). Among them, 88.9% were methicillin-resistant Gram-positive cocci, while ESBLs of* Klebsiella pneumoniae *and* Escherichia coli *were 5.56% each. Patients with poor lung function were more susceptible to drug-resistant bacteria.*

Conclusions: *Patients hospitalized with AECOPD were infected mainly with Gram-negative bacilli and with a higher rate of resistant strains of infection; however, because the pathogens had some regional distribution, a drug should be chosen for treatment based on their local distribution.*

Keywords: Acute exacerbation, aetiology, chronic obstructive pulmonary disease, drug resistance

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an airflow-limiting disease that is both preventable and treatable. Chronic obstructive pulmonary disease has become the third most common cause of hospitalization and the fourth most common cause of death worldwide, and has become the only leading cause of death that continues to increase (1). Acute exacerbation (AE) of COPD is associated with hospitalization in respiratory wards, and the condition has become an important factor in the poor prognosis and death of COPD patients. Research shows that the majority of AECOPD is as a result of bacterial or viral infections (2); therefore, antibiotic therapy plays an important role in the treatment of the condition. It is essential in the prognosis of patients hospitalized with AECOPD to assess and understand the aetiological distribution and drug-resistant characteristics of the pathogens and to choose reasonable antimicrobial drug treatment. To this end, data were collected on patients who were hospitalized with AECOPD in the respiratory ward of Beijing Shijitan Hospital, Capital Medical University (Beijing, China) from October 2010 to October 2013, and 58 patients with sputum cultures that tested positive for pathogens were enrolled in the study. Eighty-six strains of isolated pathogens were analysed for aetiological distribution and drug-resistant characteristics, which provided a reference for future anti-infection treatment of AECOPD.

SUBJECTS AND METHODS

Baseline data from a retrospective study of 58 AECOPD patients hospitalized in the respiratory ward from October 2010 to October 2013 were analysed. Those testing positive

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for pathogens were consecutively enrolled in the study. The diagnoses of both COPD and AECOPD were defined according to the standards set by the respiratory branch of the Chinese Medical Association [revised in 2007] (3).

Sputum specimens

After the patients gargled with water three times in the morning and coughed deeply, sputum samples were collected. The sputum specimens were immediately sent to a bacteriology laboratory. Sputum specimens were collected for three consecutive days after patient admission.

Identification of bacteria

Specimens were inoculated with Chinese blue tablet (Guangzhou Detgerm Microbiology Technology Co., Ltd.) and grown on blood agar plates (Guangzhou Detgerm Microbiology Technology Co., Ltd.) at 37 °C for 18–72 hours. Merieux Vitek-II was used to identify bacteria and test for drug susceptibility; *Escherichia coli* anhydrotetracycline (ATC), *Staphylococcus aureus* ATC and *Pseudomonas aeruginosa* ATC were used as quality controls to select for antibiotics. Resistance to cefoxitin was used as the criteria by which to identify methicillin-resistant *Staphylococcus* (MRS). The process followed the standards of the Clinical and Laboratory Standards Institute (publication version M100-S23) and the methods of operation were according to the National Guide to Clinical Laboratory Procedures.

Extended spectrum beta-lactamase detection

Extended spectrum beta-lactamases (ESBLs) were completely filtered automatically by Vitek-II while identifying bacteria and testing for susceptibility to drugs.

Pathogen criteria

The criteria used for identifying a dominant pathogen were as follows: a) a bacterial culture or cultures of morning sputum represented the only dominant bacteria for two or more consecutive days; b) the concentration of bacteria in one sputum specimen was $\geq 10^7$cfu/mL.

RESULTS

Aetiological distribution

Eighty-six bacteria were isolated comprising 54 Gram-negative bacilli (62.79%), nine Gram-positive cocci (10.47%), and 23 fungi (26.74%). Among Gram-negative bacilli, *Acinetobacter baumannii* was predominant (38.89%), while methicillin-resistant *Staphylococcus aureus* was the major organism among Gram-positive cocci (88.89%). *Candida albicans* was the major fungi [47.83%] (Table 1).

Table 1: Distribution of pathogens of 86 hospitalized patients with AECOPD

	Number of strains	Ratio
Gram-negative bacilli	54	62.79%
Acinetobacter baumannii	21	38.89%
Pseudomonas aeruginosa	8	14.81%
Haemophilus influenzae	6	11.11%
Klebsiella pneumoniae ssp	3	5.56%
ESBL *K pneumoniae*	3	5.56%
ESBL *Escherichia coli*	3	5.56%
Stenotrophomonas maltophilia	3	5.56%
Enterobacter cloacae	2	3.70%
Xylose oxidation *Alcaligenes*	1	1.85%
Calcium acetate *Acinetobacter*	1	1.85%
Serratia marcescens	1	1.85%
Denitrification *Achromobacter*	1	1.85%
Acinetobacter haemolysis	1	1.85%
Gram-positive cocci	9	10.47%
Methicillin-resistant *Staphylococcus aureus*	8	88.89%
S haemolyticus	1	11.11%
Fungi	23	26.74%
Candida albicans	11	47.83%
C tropicalis	5	21.74%
C glabrata	3	13.04%
C krusei	3	13.04%
Unnamed *Candida*	1	4.35%

AECOPD: acute exacerbation of chronic obstructive pulmonary disease; ESBL: extended spectrum beta-lactamase

Table 2: Drug-resistance analysis in main strains of Gram-negative bacilli

	Acinetobacter baumannii **(n/%[1])**	***Pseudomonas aeruginosa*** **(n/%)**	***Haemophilus influenza*** **(n/%)**	***Klebsiella pneumoniae*** **ESBLs (n/%)**
Ampicillin	14/93.33%	3/100%	4/66.67%	3/100%
Piperacillin/tazobactam	18/90%	0/0%	N[2]	2/66.7%
Cefuroxime	10/100%	3/100%	1/16.67%	0/0%
Cefepime	19/90.48%	3/30%	0/0%	1/33.3%
Cefoperazone/sulbactam	4/19.05%	1/20%	N	2/66.7%
Levofloxacin	8/40%	3/50%	0/0%	1/33.3%
Amikacin	0/0%	1/16.67%	N	0/0%
Imipenem	18/85.71%	3/37.5%	0/0%	2/66.7%
Co-trimoxazole	10/47.62%	3/100%	3/60%	2/66.7%

[1]n refers to the number of drug-resistant strains; % indicates the proportion of drug-resistant strains tested for drug susceptibility.
[2]The strains that had not been tested for sensitivity to this drug
ESBL: extended spectrum beta-lactamase

Drug-resistance analysis

The *in vitro* susceptibility test showed that *Haemophilus influenzae* was still sensitive to β-lactam antibiotics, but *A baumannii*, *P aeruginosa*, and *Klebsiella pneumoniae* had different levels of resistance to β-lactamases and cephalosporins, while the rate of drug resistance to β-lactamase was > 90% (Table 2). *Staphylococcus aureus* was resistant to drugs such as β-lactamases, levofloxacin and imipenem (100%); however, it showed no resistance to vancomycin or linezolid (Table 3). In fungi, *C albicans* and *C tropicalis* were sensitive to fluconazole and itraconazole (Table 4). In this study, patients with poor lung function were more susceptible to drug-resistant bacteria (Table 5).

Table 3: Drug-resistant analysis in main strains of Gram-positive cocci

Methicillin-resistant *Staphylococcus aureus* (n/%[1])	
Penicillin	8/100%
Levofloxacin	8/100%
Clindamycin	7/87.5%
Imipenem	2/100%
Vancomycin	0/0%
Linezolid	0/0%

[1]n refers to the number of drug-resistant strains; % indicates the proportion of drug-resistant strains tested for drug susceptibility

DISCUSSION

With the widespread use of broad-spectrum antibiotics, an ageing population, increased COPD morbidity, increased length of hospitalization, and prolonged survival of COPD patients, the AECOPD pathogens have dramatically changed. This study found that among bacterial culture results of hospitalized AECOPD patients, the incidence of Gram-negative bacilli and fungal infections were 62.79% and 26.74%, respectively. In Gram-negative bacilli infections, the rate of *Acinetobacter baumannii* infection (38.89%) was significantly higher than that of *P aeruginosa* (14.81%), which differed from national results in recent years (4–6). The reasons for this difference were as follows: a) the distribution of pathogens had regional and epidemiological characteristics and b) the majority of patients enrolled in this study were those with severe COPD and hospitalized because of exacerbations of the disease. After repeatedly receiving empiric antibiotics and corticosteroids, the bacteria series in the patients changed. When patients with AECOPD were hospitalized, the pathogenic bacteria with which they were infected were mostly hospital bacteria. In addition to the changes in the anatomy of the respiratory tract, decreased immunity in the natural host, exposure to a pathogenic environment, and empiric therapy gradually filtered out the more toxic bacterial series that survived in patients with severe COPD (7, 8). This might be the reason that the isolation rate of *A baumannii* was higher than that of *P aeruginosa* in this study. The Gram-positive coccus was mainly methicillin-resistant *S aureus* (88.89%) in this study, which was similar to that reported by Luo *et al* in 2010 (4). Candida showed a higher rate than that reported by Luo *et al* (4) and He (9). The reasons for this higher rate might be as follows: a) these patients were elderly and had chronic illness, immune dysfunction and poor nutritional status, and thus were more susceptible to fungal infection and other opportunistic infections; b) the majority of patients had severe COPD and when admitted to the hospital because of AECOPD, often received empiric broad-spectrum antibiotics leading to an increased risk of fungal infections; c) most of these patients were treated with inhalable corticosteroids, which could have increased the probability of oral fungal

Table 4: Drug-resistant analysis in main strains of fungi

	***Candida albicans* (n/%[1])**	***Candida tropicalis* (n/%)**	***Candida glabrata* (n/%)**
Fluconazole	0/0%	0/0%	3/100%
Itraconazole	0/0%	0/0%	1/100%
Voriconazole	0/0%	0/0%	0/0%
Amphotericin B	0/0%	0/0%	0/0%

[1]n refers to the number of drug-resistant strains; % indicates the proportion of drug-resistant strains tested for drug susceptibility

Table 5: Relationship of drug-resistant pathogens and lung function

	Lung function of patients	
	FEV1[1] ≥ 50 (n/%[2])	**FEV1 < 50% (n/%)**
Acinetobacter baumannii	2/9.52%	19/90.48%
Pseudomonas aeruginosa	2/25%	6/75%
Haemophilus influenzae	1/16.67%	5/83.33%
Klebsiella pneumoniae ssp and ESBL strains	1/16.67%	5/83.33%
ESBL *E coli*	0/0%	3/100%
Methicillin-resistant Staphylococcus aureus	2/25%	6/75%

[1]FEV1 = forced expiratory volume in one second
[2]n refers to the number of drug-resistant strains; % indicates the proportion of drug-resistant strains tested for drug susceptibility
ESBL: extended spectrum beta-lactamase

infections; although the patients had gargled before the sputum specimens were taken, there was still some oral bacterial contamination and colonization that moved into the lower respiratory tract; and d) some patients received systemic corticosteroids during treatment, which led to suppression of immune function and greatly increased the probability of fungal infection.

The *in vitro* susceptibility test showed that *Haemophilus influenzae* was still sensitive to β-lactam antibiotics, but *A baumannii*, *P aeruginosa*, and *Klebsiella pneumoniae* had different levels of resistance to β-lactamases and cephalosporins, while the rate of drug resistance to β-lactamase was > 90%. Most patients with Gram-negative bacterial infections, such as those from *A baumannii*, *P aeruginosa* and *K pneumoniae,* had severe COPD; therefore, they could be given empirical anti-infection treatment according to their lung function. To reduce the use of β-lactams and cephalosporins for patients with AECOPD who were repeatedly hospitalized, the first empiric treatment was β-lactamase enzyme/inhibitor, or imipenem. *Staphylococcus aureus* was resistant to drugs such as β-lactamases, levofloxacin, and imipenem; however, it showed no resistance to vancomycin or linezolid, which was consistent with the results in the literature (10–12). During the clinical diagnosis and treatment process of patients with severe or very severe COPD who are repeatedly hospitalized, clinicians must be aware of drug-resistant Gram-positive bacteria and fungi when aggressive broad-spectrum anti-infection therapy shows poor results. It is important to timely administer an antibiotic therapy of vancomycin or linezolid. The *in vitro* susceptibility test also found that *C albicans* and *C tropicalis* were sensitive to fluconazole and itraconazole, although *C glabrata* had natural resistance to both drugs. This result was also consistent with recent reports from domestic studies (13).

With increasing age, the forced expiratory volume in one second (FEV1) shows a natural tendency to decrease. The normal structure of pulmonary alveoli is destroyed in COPD patients so that the decrease in FEV1 is more rapid. The lower or more quickly the decrease in the FEV1, the worse the prognosis and the shorter the life expectancy (14). Impaired lung function can also lead to a decline in the reserve capacity of lung function and increased infection by more toxic bacteria (14, 15). The more severely damaged the pulmonary function, the stronger the invasion by infectious bacteria (14, 16). If bacterial infection appears in the airway, it can induce inflammatory factors that evoke oxidative stress, which can then destroy the balance of the proteinase system in the pulmonary tissue and airways, resulting in emphysema. Antigens in the respiratory tract can cause an anaphylactic reaction, exacerbating airway hyper-responsiveness and inflammation of eosinophils (17). As is known, airway hyper-responsiveness is one of the risk factors of COPD; therefore, a bacterial infection can accelerate the rate of the decline in FEV1. This study showed that with the decline in lung function and the increase in disease severity, the rate of bacterial isolation in patients with AECOPD was significantly higher. Through analysis of the bacteria strains, severe and very severe COPD patients who were hospitalized were often accompanied by infections of *Acinetobacter*, *Pseudomonas*, and resistant Gram-positive cocci, such as *S aureus*, and the isolation rate of these bacterial species was significantly higher than that of other species. In contrast, the bacterial isolation rate was low in patients with relatively good lung function, and through clinical anti-infective treatment, these patients were mostly infected after acute exacerbation with atypical pathogens, such as mycoplasma and chlamydia. This observation was similar to that reported by Xu *et al* (15); therefore, it was presumed that there was a close relationship between the pathogens that infected hospitalized AECOPD patients and their lung function and the severity of their diseases.

There were some limitations to this study. Because the sample was small, the results might be biased; however, the results might still prompt the study of the aetiological distribution of the pathogens and major kinds of antibiotics for the drug-resistant strains of pathogens in hospitalized AECOPD patients in our region. This would provide clinicians with references for reasonable anti-infection treatment to timely and effectively control the disease.

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