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Bacterial Distribution and Resistance in 3002 Phlegm Samples

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The pathogenic bacterial distributions and resistance states in patients with lower respiratory infections were analyzed to provide evidence for clinical therapy. Bacteria were cultured and separated according to clinical routine. A bacteria measuring medical software was used for statistical analysis. A drug sensitivity test was performed using the minimal inhibitory concentration (MIC) method. The detection rate of pathogenic bacteria in phlegm samples was 38.61%. Gram-negative bacilli comprised 94.31% of the total bacterial population. The four common bacteria were *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli*. The drug sensitivity test showed that the pathogenic bacteria, which can cause pulmonary infections, had different degrees of resistance to many antibiotics. Only cefoperazone/sulbactam achieved better sterilizing effects on the four common Gram-negative bacilli than the other antibiotics. The resistance rates were below 10%. Pathogenic bacterial resistance was poor in upper respiratory infections. Thus, antibiotic use should be standardized to control the variation in bacterial drug resistance.

Key words: phlegm sample, pathogenic bacteria, drug resistance.

A lower respiratory tract infection is defined as a respiratory tract infection under the glottis, including the main category of diseases, such as acute broncho-bronchitis, chronic bronchitis with concurrent infection, pneumonia, bronchiectasis with concurrent infection, and acute exacerbation period of chronic obstructive pulmonary disease.[1-3] It is the most common disease-causing infection in China, and is found in 12% and 16% of inpatients from city and urban areas, respectively.[4] Given that drug application (e.g., broad-spectrum antibiotics and immunodepressants) has increased in recent years, the bacterial spectrum structure causing lower respiratory tract infections continuously changes and drug-resistant bacteria increase each year.[5-8] In different areas, the distribution and drug resistance of pathogenic bacteria causing respiratory tract infections vary because of different medication habits.[9,10] These reasons make the therapy of lower respiratory tract infections difficult. Thus, understanding the type of pathogenic bacteria causing lower respiratory tract infection and drug resistance is important, and can serve as a guide for rational clinical administration.

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MATERIALS AND METHODS

Samples

A total of 3002 phlegm samples were collected from outpatients and ward patients of the Traditional Chinese Medicine Hospital of Zhengzhou from January to December 2012.

Bacterial isolation assessment and drug sensitivity test

According to the *Nationwide Clinical Laboratory Operation Manual*, separation culture was performed. Bacterial assessment and drug sensitivity test were carried out by a bacteria

measuring system with a random diagnostic reagent plate in vitro and DL-96 bacteria measuring system (Zhuhai Medical Biotech Co., Ltd).

Quality control standard bacteria

Escherichia coli (ATCC25922), *Klebsiella pneumoniae* (ATCC70060), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC25923), and *Candida albicans* (ATCC90029) were all purchased from the Clinical Laboratory Center of the Ministry of Public Health.

Statistical analysis

A bacteria measuring medical software (Zhuhai DL Medical Biotech Company) was used for statistical analysis.

RESULTS

Pathogenic bacterial distribution

A total of 1159 bacterial strains were separated in 3002 phlegm samples. The detection rate was 38.61%. Gram-negative bacteria, fungi, and Gram-positive bacteria had 1093 (94.31%), 40 (3.45%), and 26 (2.24%) strains, respectively. Table 1 shows the detailed constituent ratio of bacteria.

Table 1. Constituent ratio of pathogenic bacteria(%)

Pathogenic bacteria	Strains number	(%)
G-bacilli	1093	94.31
Pseudomonas aeruginosa	298	25.71
Acinetobacter baumannii	240	20.71
Klebsiella pneumoniae	214	18.46
Escherichia coli	103	8.89
Enterobacter cloacae	41	3.54
Acinetobacter lwoffi	34	2.93
Stenotrophomonas maltophilia	27	2.33
Serratia marcescens	23	1.98
Acinetobacter calcoaceticus	16	1.38
Enterobacter agglomerans	15	1.29
Serratia plymuthica	13	1.12
Klebsiella oxytoca	11	0.95
Other	58	5.00
Fungi	40	3.45
Candida albicans	27	2.33
Candida tropicalis	11	0.95
Other	2	0.17
G+coccus	26	2.24
Enterococcus faecium	7	0.60
Staphylococcus intermedius	4	0.35
Staphylococcus haemolyticus	3	0.26
Staphylococcus epidermidis	3	0.26
Other	9	0.78
Total	1159	100%

Drug sensitivity test results

The drug sensitivity test results of the four bacteria, which had the highest detection rates, are shown in Table 2. The resistance rate of *P. aeruginosa* for trimethoprim/sulfamethoxazole and minocin was the highest (100%), whereas that for piperacillin/tazobactam sodium and polymyxin B was the lowest (6.3%). The resistance rates of *Acinetobacter baumannii* for norfloxacin and polymyxin B were 100% and 0%, respectively. The resistance rates of *K. pneumoniae* and *E. coli* for ampicillin were the highest, whereas those for meropenem and imipenem were the lowest.

ESBL detection rate

ESBL was detected in 59 (57.28%) of 103 *E. coli* strains. ESBL was detected in 57 (26.64%) of 214 *K. pneumoniae* strains.

DISCUSSION

The detection rate of pathogenic bacteria in phlegm samples was 38.61%. Gram-negative bacilli comprised 94.31% in 1159 tested strains of pathogenic bacteria. This result was in accordance with the entire report of Kaya et al.[11] *P. aeruginosa*, *A. baumannii*, *K. pneumoniae*, and *E. coli* were the most common pathogens.

Drug sensitivity test showed that the pathogenic bacteria causing pulmonary infections had different degrees of resistance for many antibiotics. Only cefoperazone/sulbactam exhibited better sterilizing effects on the four common Gram-negative bacilli than the other antibiotics. The resistance rate was lower than 10%, which was in accordance with previous reports.[12,13] However, the resistance rate for trimethoprim/sulfamethoxazole was adverse, which exceeded 60%. The resistance rate for cefotaxime exceeded 40%. This phenomenon may be associated with drug abuse.

A. baumannii demonstrated a multidrug resistance phenomenon. Minocin (2.5%), cefoperazone/sulbactam (3.3%), and polymyxin B (0.00%) exhibited resistant rates below 20% among the 18 antibiotics. Higher drug resistance was found for aminoglycosides, tert-cephalosporin, and bibasic carbostyril. Moreover, the drug

Table 2 Resistance rate of main gram negative bacilli for antibacterials (%)

Aantibacterials	PAE (n = 298)	ABA (n = 240)	KPN (n = 214)	ECO (n = 103)
Gentamicin	55.70	68.33	37.38	76.70
Amikacin	17.11	65.00	15.42	12.62
Trimethoprim/ sulfamethoxazole	100	70.42	60.75	91.26
Ciprofloxacin	22.82	70.83	16.82	71.84
Norfloxacin	25.50	100	16.82	71.84
Levofloxacin	17.11	33.33	12.62	67.96
Meropenem	10.07	50.83	2.80	0.00
Imipenem	12.42	49.17	2.80	0.00
Minocin	100	2.50	33.64	25.24
Piperacillin	38.59	70.00	42.52	83.50
Piperacillin/ tazobactam sodium	6.38	59.17	7.01	9.71
Ampicillin	-	-	97.20	93.20
Ampicillin/ sulbactam	-	22.50	38.32	53.40
Mmoxicillin/ clavulanic acid	-	-	46.73	84.47
Cephazolin	-	-	47.66	83.50
Cefuroxime	-	-	42.06	77.67
Cefotaxime	45.64	68.75	42.06	77.67
Ceftriaxone	-	70.42	42.06	77.67
Ceftazidime	20.81	67.50	20.56	55.34
Cefepime	16.11	43.33	24.30	49.51
Cefoperazone/ sulbactam	6.71	3.33	6.54	2.91
Polymyxin B	6.38	0.00	-	-
Aztreonam	17.45	-	23.36	59.22

resistance rates for meropenem and imipenem reached approximately 50%. Despite the strong effect of cefoperazone/sulbactam in treating bacterial infection, resistance increases with wide clinical application.[14,15] *A. baumannii* was notably sensitive to polymyxin B and resistant to carbapenem antibiotics. Clinical therapy methods should be selected according to specific conditions.

The resistance rate of *P. aeruginosa*, whose detection rate was the highest for piperacillin/tazobactam, was the lowest (6.38%), followed by that of cefoperazone/sulbactam (6.71%). The resistance rates for meropenem, imipenem, amikacin, levofloxacin, cefepime, and aztreonam were below 20%. *K pneumoniae* and *E. coli* belong to the Enterobacteriaceae. They showed higher sensitivity for carbapenem antibiotics, such as meropenem and imipenem. However, the drug resistance of *E. coli* was higher than that of *K. pneumoniae*. This result agreed with those of previous reports.[16,17] Drug sensitivity test showed that *E. coli* was insensitive to penbritin, and the resistance rates for antibiotics, such as cephalosporins and quinolones, exceeded 50%, which was consistent with previous reports.[18,19] When patients with three types of bacterial infections are treated, piperacillin/ tazobactam sodium and cefoperazone/sulbactam should be used as the first line of treatment. Meanwhile, the administration of meropenem and imipenem should be reduced as low as possible to avoid inducing high drug-resistant strains.

This study used sputum culture for the etiological diagnosis of lower respiratory tract infections. However, the inevitable contamination of phlegm samples with upper respiratory tract normal flora possibly caused inaccurate results.

Given the unreasonable application of antibiotics and many prophylactic medications, the resistance state of pathogenic bacteria causing lower respiratory tract infections has worsened. Moreover, treating serious patients has become highly difficult. Thus, drug administration guidelines for disease-causing infections should be formulated according to the local drug

resistance of bacteria. Furthermore, clinical bacteriological analysis workers should improve communication with doctors. Doctors should not only understand the transition of pathogenic bacteria causing respiratory tract infections, but also follow the principles of medication administration when antibiotics are used. Experienced medication should be avoided, which can effectively control drug resistance variation in bacteria and decrease medical costs.

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