Scientific Research Publishing

Advances in Microbiology, 2023, 13, 223-236
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Study on the Effect of Shuanghuanglian Powder Needle Combined with Cefoperazone and Sulbactam Sodium on *Pseudomonas aeruginosa in Vitro*

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How to cite this paper: Li, J.M., Wei, M.D., Wang, L.L., Wu, Y.B., Kang, L., Su, C.C, Yang, F.L. and Su, G.S. (2023) Study on the Effect of Shuanghuanglian Powder Needle Combined with Cefoperazone and Sulbactam Sodium on *Pseudomonas aeruginosa in Vitro*. *Advances in Microbiology*, **13**, 223-236.
https://doi.org/10.4236/aim.2023.135014

Received: April 19, 2023
Accepted: May 27, 2023
Published: May 30, 2023

Abstract

Objective: To explore the antibacterial activity of combined use of Shuanghuanglian and cefoperazone sulbactam sodium on resistant strains of *Pseudomonas aeruginosa*. **Methods:** The *Pseudomonas aeruginosa* strains which were sensitive and resistant to cefoperazone sulbactam sodium were selected to prepare different test bacterial solutions respectively; The experimental liquid of Shuanghuanglian and Cefoperazone Sulbactam Sodium were prepared separately and set as different test groups and control groups; The Drug Sensitivity Tests of Shuanghuanglian and cefoperazone sulbactam sodium at different concentration gradients which were used alone or used in combination were carried out for different strains with sensitivity and resistance, And use standard entry as a reference control. **Result:** The results of drug sensitivity test of Shuanghuanglian combined with Cefoperazone-Sulbactam sodium against the resistant strains of *Pseudomonas aeruginosa* were compared with the results of drug sensitivity test of the two separately used, and the difference was statistically significant ($P < 0.05$) [The drug sensitivity test results of Shuanghuanglian and cefoperazone sulbactam sodium to *Pseudomonas aeruginosa* resistant strains were statistically significant compared with the drug sensitivity test results of Shuanghuanglian and Cefoperazone

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Sulbactam Sodium used separately ($P < 0.05$)]; There was a dependence between strains and concentration in the effect of the combination of the two drugs. **Conclusion**: The combination of Shuanghuanglian and cefoperazone sulbactam sodium has synergistic antibacterial or bactericidal effect on *Pseudomonas aeruginosa* resistant strains.

Keywords

Shuanghuanglian, Cefoperazone-Sulbactam Sodium, Use in Combination, *Pseudomonas aeruginosa*, Drug Resistance, Bacteriostasis

1. Introduction

The *Pseudomonas aeruginosa* (PAE) which belongs to Gram-negative bacteria is a kind of non-fermentative bacteria, and it has no buds and capsule, also it has flagella and motor ability, and it easy to colonize, mutate and produce multiple drug resistance. *Pseudomonas aeruginosa* is mainly distributed in water, air, and soil in the natural environment, and often colonizes or parasitizes the gastrointestinal tract, respiratory tract, mucosa, and skin of healthy individuals, as well as adheres to the surface of medical devices; It is a common opportunistic pathogen in clinical practice and the main pathogen causing hospital and community infections [1] [2] [3], especially in intensive care units (ICUs) and respiratory wards of hospitals. In recent years, due to the improper use of antibiotics in China, as well as the use of various immunosuppressants and tumor chemotherapy drugs, there has been a significant increase in the sources, routes, and susceptible populations of hospital infections, and the trend of hospital infections is gradually increasing or worsening [4] [5]; The latest data from the National Bacterial Resistance Monitoring Network (CARSS) shows that the clinical isolation rate of *Pseudomonas aeruginosa* ranks third, second only to *Escherichia coli* and *Klebsiella pneumoniae* [6]. The incidence of infectious diseases caused by PAE remains high, meanwhile, the characteristics of drug resistance, mutation and colonization of PAE become more and more complex and serious, which brings great difficulties to clinical treatment. In 2017, the World Health Organization listed PAE as a "key" pathogen in urgent need of research and development of new antibiotics, and the U.S. Center for Disease Control and Prevention listed multi-drug resistant PAE as one of the pathogens leading to global hospital-acquired infection (HAI) [7]; Therefore, how to reduce the occurrence rate of hospital infection and seek for new ways for effective treatment of PAE resistant strains are the common concern and urgent topic of the majority of medical workers. In addition, numerous studies have reported that traditional Chinese medicine has its unique role in the treatment of bacterial infections, and has advantages such as less drug resistance, less toxic side effects, and abundant resources [8] [9] [10] [11] [12]. At the same time, clinical anti-infection experience has shown that the treatment of *Pseudomonas aeruginosa* with a single medica-

tion is not ideal and is prone to drug resistance; Many authoritative institutions advocate for combined medication.

In this paper, the broth dilution method was used to prepare different Concentration Gradients of Shuanghuanglian powder injection solution, Cefoperazone-Sulbactam Sodium (SCF) solution and the mixed solution of Shuanghuanglian injection and SCF for *in vitro* antibacterial tests on SCF-sensitive bacteria and drug-resistant bacteria, respectively; To explore the *in vitro* effect of Shuanghuanglian powder injection combined with SCF on drug-resistant PAE of SCF, and to seek a new way of combining traditional Chinese and Western medicine to treat resistant PAE, providing a certain reference basis for clinical trials; The research content is as follows.

2. Materials and Methods

2.1. Materials

2.1.1. Source and Isolation of Strains

The PAE standard strain (ATCC 27853) used in this study was provided by the Microbiology Laboratory of our clinical laboratory. SCF sensitive and resistant strains are mainly isolated from clinical samples such as blood, secretions, sputum, and urine submitted by various departments. The American BD phoenix M50 microbial identification system was adopted for drug-sensitive test; Refer to the 2016 edition of the American Society for Clinical and Laboratory Standards (CLSL) for the determination of SCF sensitive and drug-resistant strains [13].

2.1.2. Drugs and Reagents

Shuanghuanglian (freeze-dried) for injection is produced and provided by Harbin Pharmaceutical Group's Second Traditional Chinese Medicine Factory, with a specification of 600 mg/piece and batch number of 1804008; SCF for injection is produced and provided by Pfizer Pharmaceutical Co., Ltd., the specification is cefoperazone 0.5 g + sulbactam 0.25 g/bottle, and the batch number is X59694; M-H broth culture medium is produced and provided by Hangzhou Binhe Microbial Reagent Co., Ltd.; Dextran standard solution is produced and provided by the China Institute for the Control of Pharmaceutical and Biological Products.

2.1.3. Instruments

The instruments and equipment used in this study are mainly biosafety cabinets produced and provided by Jinan Xinbeixi Company, model BHC-1300 IIA/B3, BD Phoenix M50 microbial identification system produced and provided by BD Company in the United States, self thermostatic incubator (SP) provided by China Biotechnology Co., Ltd., enzyme marker model THERMO FISHER Multiskan FC provided by THERMO Company, etc.

2.2. Methods

2.2.1. Isolation and Identification of Bacteria

The samples submitted for clinical examination were inoculated onto MAC

plates using the plate marking method and incubated at 37˚C for 18 - 24 hours. Strictly follow the National Clinical Laboratory Operating Regulations [14] to cultivate and isolate PAE strains, and were identified and confirmed by the Clinical and Laboratory Standards Institute (CLSI)-2019 of the United States; At the same time, the BD phoenix M50 microbial identification system of the United States was used for drug sensitive test to extract the sensitive strains and resistant strains of PAE respectively.

2.2.2. Preparation of Experimental Bacterial Solution [15]

Select an appropriate amount of PAE colonies and grind them evenly in sterile M-H broth culture medium. Incubate at 37˚C for 18 - 24 hours, a turbidimeter was used to correct the concentration of bacterial suspension, Obtain a concentration of 1×10^8 cfu/ml of bacterial liquid mother liquor. Dilute with M-H broth culture medium before use to obtain bacterial suspension for later use.

2.2.3. Preparation of Test Solution [15]

Accurately weighed 2 g of freeze-dried Shuanghuanglian powder, then dissolved it in 9 ml of sterilized purified water, and fixed the volume to 10 ml to obtain Shuanghuanglian mother liquor (200 mg/ml). Dissolved 0.75 g of SCF powder for injection (0.5 g of cefoperazone + 0.25 g of sulbactam) in sterile purified water to prepare 1 mg/ml SCF mother solution. Principle of drug-sensitive dilution of Shuanghuanglian: the initial concentration was 1/2 (100 mg/ml), and then the drug was diluted with sterilized purified water in multiple ratio; When diluted to a concentration of 1/128 (0.78125 mg/ml) in the 7th tube, the concentration range of Shuanghuanglian was obtained from 1/128 (0.78125 mg/ml) to 1/2 (100 mg/ml); After the multiple dilution of SCF solution by the same method, the final concentration range of SCF is 1/128 (0.00390625 mg/ml) - 1/2 (0.5 mg/L).

2.2.4. Bacterial Culture Using Shuanghuanglian Alone and SCF Alone [16] [17]

According to NCCLS microdilution principle, 10 ml sterile glass test tube was used for drug sensitivity test. Experimental group (7 concentration groups): Take 7 sterile glass test tubes, number them and add 1 ml of sterile pure water to each tube, Add 1 ml of Shuanghuanglian or SCF mother liquor to the first tube, mix well, take 1 ml to the second tube, fully mix the second tube, take 1 ml to the third tube, dilute in this ratio and mix well to the seventh tube, then, take 1 ml and discard it; After 1 ml M-H broth medium was added to each tube, 50 μl bacterial mother liquor was added (PAE sensitive and drug resistant bacterial mother liquor tests were conducted successively); Meanwhile, quality control group, negative control group and positive control group were set up (6 concentration groups were set in each group). Quality control group: Add 1 ml of sterilized purified water and 1 ml of M-H broth culture medium respectively; Negative control group: Add 1 ml of Shuanghuanglian or SCF and 1 ml of M-H broth culture medium respectively; Positive control: Add 1 ml of sterilized purified water, 1 ml of M-H broth culture medium, and 50% respectively μ Cultivate the

mother liquid of bacterial solution at 37˚C for 20 hours.

2.2.5. Combined use of Shuanghuanglian and SCF Drugs for Bacterial Culture [18] [19]

According to NCCLS microdilution principle, 10 ml sterile glass test tube was used for drug sensitivity test. Experimental group (7 concentration groups): 7 sterile glass test tubes were taken, numbered and 1 ml sterile purified water was added into each tube; 0.5 ml Shuanghuanglian and 0.5 ml SCF mother liquor were added into tube No. 1; The other operations were the same as the drug sensitivity test of using Shuanghuanglian alone or SCF alone (the test of PAE sensitive and drug-resistant bacteria mother liquor should also be carried out in the drug combination test), and cultivated at 37˚C for 20 hours.

2.2.6. Drug Sensitivity Test [20] [21]

Comply with the relevant operating procedures of automatic microbiological analyzer; Sterile medium was used as blank with enzyme label instrument (sterile medium with the same dilution ratio was used as blank for each test tube); Set the wavelength to 600 nm, measure the optical density of each group at 0 hours of initial cultivation and 20 hours after cultivation at 37˚C, and determine the effect of drug concentration on bacterial growth through changes in optical density (*i.e.* OD value).

2.2.7. Statistical Methods

Statistical analysis was conducted using SPSS 24.0 statistical software. The test results are based on X. The mean ± S is represented, and the comparison between groups is performed using one-way ANOVA LSD-t-test. $P < 0.05$ meant the difference was statistically significant.

3. Results

3.1. Drug Sensitivity Test Results of SCF-Sensitive Strains

Both the use of Shuanghuanglian alone or SCF alone, or the combination of Shuanghuanglian and SCF drugs, have certain antibacterial or bactericidal effects on SCF sensitive strains (there was no significant change in absorbance after initial cultivation and cultivation at 37˚C for 20 hours, indicating that the bacteria were inhibited or grew slowly after cultivation at 37˚C for 20 hours. Both the use of drugs alone or in combination had certain antibacterial or bactericidal effects.), There is a certain correlation and dependence between its antibacterial or bactericidal effect and drug concentration, and after being cultured at 37˚C for 20 hours, there is no significant difference in the OD value of the drug sensitivity test results between the two drugs alone or in combination, and the difference is not statistically significant ($P > 0.05$). The photo density of initial culture at 0 h and the drug sensitivity test results to sensitive strains after culture at 37˚C for 20 h for the drugs used alone and in combination are shown in Table 1 below.

3.2. Results of Drug Sensitivity Test for SCF-Resistant Strains

Both the use of Shuanghuanglian alone and the use of SCF alone have no antibacterial or bactericidal effects on SCF resistant strains (*i.e.*, there is a significant change in absorbance after initial cultivation and cultivation at 37˚C for 20 hours, with a significant increase in OD value, indicating that bacteria have grown after cultivation at 37˚C for 20 hours, and the drug has no antibacterial effect or reduced antibacterial effect); However, the combined use of Shuanghuanglian and SCF drugs has certain bacteriostatic or bactericidal effects on SCF-resistant strains (*i.e.*, the absorbance changed significantly and OD value decreased significantly after the initial culture and 37˚C culture for 20 h, indicating that bacteria are inhibited or grow slowly after 37˚C culture for 20 h, and the combined use of drugs has certain bacteriostatic or bactericidal effect); The effect is also related and dependent on the drug concentration; And after 20 hours of cultivation at 37˚C, there was a significant difference in optical density between the combination of drugs and the use of drugs alone, and the difference was statistically significant ($P < 0.05$). The optical density at 0 hours of initial cultivation and the drug sensitivity test results for SCF resistant strains after 20 hours of cultivation of drugs used in combination and alone are shown in Table 2 below.

Table 1. Comparison of absorbance between different drugs and standard PAE sensitive strains in initial culture and culture at 37˚C for 20 h.

Bacterial Strain	Cultivation Time	Relative drug concentration						
		1/2	1/4	1/8	1/16	1/32	1/64	1/128
SCF	0 h	0.201	0.182	0.161	0.126	0.090	0.061	0.050
	20 h	0.198	0.178	0.158	0.120	0.086	0.053	0.048
Shuanghuanglian	0 h	0.221	0.222	0.203	0.165	0.135	0.093	0.071
	20 h	0.213	0.218	0.194	0.155	0.121	0.086	0.068
SCF + Shuanghuanglian	0 h	0.218	0.208	0.190	0.153	0.126	0.090	0.073
	20 h	0.209	0.191	0.183	0.141	0.109	0.077	0.063

Table 2. Comparison of absorbance between different drugs and standard PAE resistant strains in initial culture and culture at 37˚C for 20 h.

Bacterial strain	Cultivation time	Relative drug concentration						
		1/2	1/4	1/8	1/16	1/32	1/64	1/128
SCF	0 h	0.201	0.188	0.168	0.136	0.098	0.068	0.046
	20 h	0.661	0.698	0.762	0.806	0.841	0.890	0.982
Shuanghuanglian	0 h	0.229	0.226	0.210	0.171	0.136	0.098	0.070
	20 h	0.731	0.768	0.802	0.841	0.889	0.922	0.995
SCF + Shuanghuanglian	0 h	0.218	0.208	0.190	0.165	0.121	0.093	0.072
	20 h	0.072	0.080	0.088	0.153	0.186	0.223	0.302

4. Discussion

PAE is a kind of non-fermented gram-negative bacilli widely distributed in nature, which is one of the main pathogens causing nosocomial infections, as well as the most common opportunistic pathogens in clinical practice, mainly respiratory system infection, and has now become one of the main pathogens of hospital infections [22] [23]. PAE is a normal parasitic bacterium in the human body; Generally, due to the inhibitory effect of the host's normal flora, it does not cause disease; It is only when the parasitic environment changes, the host's normal flora is dysregulated, and other factors change that it causes disease; The main antibiotics available for clinical use in human infections include β-Lactam Antibiotics (Ceftazidime, Cefoperazone, Aztreonam, Imipenem, Meropenem), Aminoglycoside Antibiotics (such as tobramycin, amikacin, gentamicin), quinolone antibiotics (ciprofloxacin, ofloxacin, levofloxacin, clinfloxacin), etc. SCF is a compound antibacterial drug of β-lactam + β-lactamase inhibitor; Sulbactam is a broad-spectrum enzyme inhibitor with certain antibacterial activity, which is produced by common pathogenic bacteria such as Staphylococcus aureus and a variety of negative bacteria β-Lactam enzyme has strong and irreversible bacteriostasis, which has a strong and irreversible inhibitory effect, it can inactivate a variety of class A β-lactam enzymes, so as to synergistically enhance the antibacterial effect of cefoperazone sodium , but for Class B, C and D, the hydrolysis of β-lactamase is relatively poor [24], and the β-lactamases mediated by some negative bacillus chromosomes are also inactive [25]. Cefoperazone belongs to the third generation cephalosporin preparations, and its antibacterial mechanism is mainly to inhibit the synthesis of bacterial cell walls, but its stability to β-lactamase is relatively poor. Sulbactam, Sulbactam uses the function of enzyme inhibitor to effectively protect cefoperazone from β. The hydrolysis of lactam enzyme is conducive to enhancing the antibacterial activity of cefoperazone [26]. The combined application of cefoperazone and sulbactam not only exerts positive synergistic antibacterial activity against Gram-negative bacilli, but also its antibacterial effect is several times of that of single drug [27]. Due to its stability on β-lactamase and its good antibacterial activity against gram-negative bacilli such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, etc., it can be used to treat respiratory tract diseases, urinary tract diseases, and reproductive system diseases caused by these bacteria [28], and it is widely used in the treatment of moderate and severe bacterial infections in clinical practice, especially the treatment of some drug-resistant bacteria [29]. Because SCF has a good bacteriostatic or bactericidal effect on PAE and a low drug resistance rate [30], it is often used as one of the preferred treatment drugs for nosocomial infections caused by PAE in clinic.

Shuanghuanglian powder injection is mainly a pure traditional Chinese medicine preparation prepared from extracts of three Chinese herbal medicines: Honeysuckle, *Scutellaria baicalensis*, and *Forsythia suspensa*; The honeysuckle in the prescription has antibacterial and antiviral effects, can activate T lympho-

cytes and increase their conversion rate, improve the phagocytic ability of patients' white blood cells, and has anti endotoxin, anti infection, and antipyretic and detoxifying effects; *Scutellaria baicalensis* has antibacterial, antiviral, and anti-inflammatory effects, as well as liver protection, cholagogic, and antioxidant effects; *Forsythia suspensa* has extremely strong resistance to pathogenic microorganisms and can inhibit various types of Gram-positive bacteria, Gram-negative bacteria and influenza virus; The combination of these drugs can play a role in clearing heat and detoxifying and anti-infection [31] [32]. Modern pharmacological studies have shown that Shuanghuanglian drugs have inhibitory effects on various pathogens, which is beneficial for thoroughly eliminating pathogenic microorganisms in patients' bodies [33]. The components of honeysuckle in Shuanghuanglian have a wide antibacterial range and have varying degrees of inhibitory effects on *Pseudomonas aeruginosa*, Pneumococcus, and other bacteria; At the same time, the water extract of honeysuckle has significant antiviral activity, which can prevent viral induced cell damage; When used in combination with azithromycin and forsythia, it plays an enhanced and complementary role, which can improve the antiviral infection ability of patients and promote the reduction of inflammation; Thus, serum levels of TNF-α, IL-6 and CRP can be effectively reduced [34] [35]. In addition, the drug can also enhance the phagocytic ability of leukocytes and increase the concentration of interleukin, ultimately achieving the purpose of enhancing the non-specific immune ability of the body [36] [37].

In recent years, traditional Chinese medicine and its extracts have increasingly attracted the attention of scholars both domestically and internationally in terms of their anti infection, antiviral, and immune enhancement effects; Traditional Chinese medicine antibacterial therapy has the advantages of broad antibacterial spectrum, not easy to induce bacterial resistance, low residue, etc. The application of traditional Chinese medicine antibacterial is expected to delay, inhibit, and even reverse the resistance of PAE. *Scutellaria baicalensis*, Honeysuckle and *Forsythia suspensa* in Shuanghuanglian preparation can inhibit the activity of microbial enzymes and the release of bacterial endotoxin by destroying the structure of PAE cell wall and membrane; And by inhibiting and clearing the cell biofilm (BBF) and interfering with the formation of BBF, antibacterial purposes or reversing the resistance of PAE can be achieved [38]. For example, many previous studies have found that *Scutellaria baicalensis*, Honeysuckle, *Forsythia suspensa*, etc. have inhibitory or killing effects on PAE; Many literature studies [39] have also shown that the antibacterial mechanism of *Scutellaria baicalensis* on PAE mainly includes inhibiting the formation of PAE biofilm and its ability to adhere to solids, and damaging the structure of biofilm, improving the susceptibility of the bacteria to drugs, and improving the antibacterial effect of the body. However, the antibacterial effect of Shuanghuanglian, like most Chinese herbal medicines, also has some shortcomings, mainly due to the low content of active ingredients in Chinese herbal medicines, which makes it difficult to reach the blood drug concentration. Compared with existing antibiotics, the effective-

ness of active ingredients is often lower, resulting in relatively limited antibacterial effect [40].

Over the years, due to the non-standard and unreasonable use of antibiotics, the resistance rate of PAE to various antibiotics, especially carbapenems, has been continuously increasing. According to a survey conducted by the China Antibiotics Monitoring Network Center in 2017, the resistance rate of PAE to SCF is 24.8% [41]; Even the third generation of cephalosporin has a high resistance rate, which may be related to the widespread clinical use of cephalosporin antibacterial drugs; Because PAEs that produce cephalosporin enzymes can hydrolyze first-generation to third-generation cephalosporin drugs and cephalomycin drugs, and it is resistant to most β-lactam antibiotics, which makes their resistance more prominent and serious [42] [43]. The resistance mechanisms of *Pseudomonas aeruginosa* are both complex and diverse. Currently, the main discovered resistance mechanisms include: 1) the bacteria produce inactivating enzymes, which alter the active structure of antibiotics; 2) Gene mutations cause changes in channel proteins and outer membrane permeability; 3) Gene mutations or changes in coding enzymes cause changes in the target sites of antibacterial drugs; 4) Formation of bacterial biofilm; 5) Obtaining exogenous drug resistance genes; 6) Other drug resistance mechanisms, such as integron action [44] [45]. The phenomenon of drug resistance of PAE is becoming more and more serious, and the characteristics of traditional Chinese medicine in anti infection treatment, such as not easily leading to dysbiosis of the bacterial community, enhancing the body's immunity, and reducing bacterial resistance, have prompted people to consider the combination of the two to achieve complementary advantages; For example, Shuanghuanglian combined with antibiotics in treating acute respiratory infections can not only promote the recovery of the illness, but also enhance the body's ability to resist pathogenic bacteria and regulate the function of patients' immune system [46]; Wang Linjing *et al.* found that *Scutellaria baicalensis*, honeysuckle and Prunella can make ciprofloxacin antagonism to PAE by promoting the expression of efflux pump gene, and honeysuckle, *Andrographis paniculata* and Chinese gallnut can also make gentamicin antagonism to PAE [47], indicating that the combined use of Chinese and western medicines has obvious synergistic antibacterial and additive effects on PAE, and the combined antibacterial effect *in vitro* is better. At present, the combined use of Chinese and Western medicines is the focus and hot spot in the field of clinical pharmacy [48], and Shuanghuanglian powder injection is a typical representative of the traditional Chinese medicine injection of heat clearing and detoxification, and has become one of the first choice drugs of traditional Chinese medicine for treating respiratory infectious diseases; It is often used in combination with antibiotics in clinical practice [49]. The results of this study show that neither SCF nor Shuanghuanglian has bacteriostatic/bactericidal effect on SCF resistant strains, but the combination of SCF and Shuanghuanglian has certain bacteriostatic/bactericidal effect on SCF resistant strains, and the effect is

related to and dependent on the drug concentration; Although the mechanism of the combined use of drugs on PAE resistant to SCF is not clear, it may be related to the change of bacterial cell wall, membrane permeability, inhibition of biofilm formation, and change of bacterial efflux [50]; The relevant mechanisms still need to be further studied, but the synergistic antibacterial effect of traditional Chinese medicine and antibiotics, as well as the changes in the internal composition and physicochemical properties of drug-resistant strains after treatment with traditional Chinese medicine, thus restoring their sensitivity to corresponding antibiotics, indicate that traditional Chinese medicine has the ability to reverse the resistance of drug-resistant bacteria to some extent, and its mechanism of reversing bacterial resistance may be the elimination of drug-resistant plasmids, inhibiting the expression of drug-resistant genes, reducing the activity of drug-resistant enzymes, and inhibiting biofilm synthesis, etc. It is precisely because Shuanghuanglian may have the ability to reverse the resistance of PAE resistant strains to some extent, thereby restoring the sensitivity of SCF to resistant strains and exerting the synergistic effect of combined use of drugs [51].

5. Conclusion

To sum up, the combination of Shuanghuanglian and cefoperazone sulbactam sodium can produce certain synergistic antibacterial or bactericidal effects on *Pseudomonas aeruginosa* resistant strains. This discovery can provide a new clue and experimental reference for the effective use of a combination of Chinese and Western medicine in the treatment of drug-resistant bacterial infections and the development of new antibacterial drugs in clinical practice. On the other hand, it can effectively guide the prevention and treatment of PAE resistance in clinical practice, thus having important practical significance.

Limitations of the Study

The research object of this study is the PAE antibacterial test *in vitro*. Due to different in vivo and *in vitro* environments, the number of pathogenic bacteria infected by patients and the source of specimens are different, and further clinical studies are needed on the efficacy of combined drugs in vivo and whether there are compatibility contraindications or adverse reactions. Specimens in this study are collected from patients in the municipal jurisdiction, with regional differences; the next step is to collect specimens from more regions and extract more *Pseudomonas aeruginosa* strains for research, so as to reduce regional differences.

Acknowledgements

During the research process of this topic, we received the help of many departments and individuals as well as other personnel who were not involved in the research of this topic. All members of the project express our heartfelt gratitude to the departments and individuals who have provided support and assistance to

the research of this project, and wish them good health and all the best.

Fund Projects

Research Project Supported by the Health Commission of Guangxi Zhuang Autonomous Region (Z20190417).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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