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ARTICLE

In Vitro Antibacterial Activity of *Galla Chinensis* Combined with Different Antibacterial Drugs against Carbapenem-Resistant E.Coli

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ABSTRACT

Objective: To evaluate the antibacterial effects of meropenem and levofloxacin respectively combined with *Galla chinensis* on carbapenem-resistant *Escherichia coli* in vitro. **Methods:** The protocol was designed with checkerboard method and the carbapenem-resistant *E.coli* was isolated in our hospital. The minimum inhibitory concentrations(MICs) of *G. chinensis* alone and combined with 2 antimicrobial agents against carbapenem-resistant *E.coli* were determined by broth dilution method and the fractional inhibitory concentration index (FICI) was calculated according to MICs results. Result: the combined use of *G. chinensis* and meropenem (or levofloxacin) significantly decreased both MIC50 and MIC90; After the combination of *G. chinensis* and meropenem, the synergistic effect was 86.7%, and the additive effect was 13.3%, no irrelevant and antagonistic effects. After combined use of *G. chinensis* and levofloxacin, the synergistic effect was 66.7%, and the additive effect was 33.3%. No irrelevant and antagonistic effects. **Conclusion:** *Galla chinensis* combined with meropenem or levofloxacin has synergistic and additive antibacterial effect, with certain combined antibacterial activity.

1. Introduction

Escherichia coli (*E. coli*) is a common Gram-negative pathogen in the hospital and is widely found in nature. It is a normal flora in the human and animal gut[1]. E. coli can cause a variety of infections when the body's immunity is reduced, the colony of the flora changes, or the flora is out of tune. According to the monitoring of bacterial resistance in China, E. coli is the highest-prevalence Gram-negative bacteria in the clinic, which can cause diarrhea, urinary tract infection, wound infection, bacteremia, meningitis and other diseases[2,3].

In recent years, with the increasing use of antibiotics, the detection rate of multidrug-resistant (MDR) E. coli in the clinic has increased year by year. It has posed a major challenge to clinical anti-infective treatment. In the face of this severe clinical anti-infective situation, carbapenem antibiotics are often used as clinically effective antibacterial drugs for clinical treatment[4]. However, with the increase in the clinical use of carbapenem antibiotics, more

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and more clinical detection of carbapenem-resistant E. coli has brought great challenges to clinical anti-infective treatment. With the continuous development and deepening of the research work on anti-infection of traditional Chinese medicine by domestic medical workers, the researchers found that some of the traditional Chinese medicines have effective antibacterial activity and can be used for antibacterial therapy or combined other antibacterial drugs for combined antibacterial therapy[5,6]. Studies have shown that *G. chinensis* in traditional Chinese medicine have good effective biological activities such as antibacterial, anti-caries, anti-mutation and anti-oxidation[6,7]. Therefore, this study will conduct a basic study on the antibacterial activity in vitro of *G. chinensis*s combined with different antibacterial drugs against carbapenem-resistant *E.coli*, in order to provide a basis for finding a reasonable and effective antibiotic treatment in the clinic.

2. Materials and Methods

2.1 Strain Specimens and Standard Strains

After removing duplicate strains isolated from the same site of the same patient, 30 strains of carbapenem-resistant *E.coli* isolated from Lingnan Hospital of the Third Affiliated Hospital of Sun Yat-sen University (June 2016 to June 2017). Identification and drug susceptibility test of isolated strains was performed using a fully automatic bacterial identification analyzer (MicroScan WalkAway-96 plus). 30 specimens of carbapenem-resistant *E.coli* were mainly derived from sputum, accounting for 50% (15/30), followed by blood 20% (6/30) and urine 10% (3/30), other specimens 20% (6/30). *E.coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used as quality control strains and provided by the Guangdong Center for Clinical Laboratory.

2.2 Instruments and Reagents

MicroScan Walk-Away 96 plus (Siemens, Germany), a fully automated bacterial identification and susceptibility analyzer (including supporting reagents and slats) was used. DR100 turbidimeter (Biomerieux, France); ultra-clean workbench (Beijing Donglian Haar Instrument Manufacturing Company, China)); 96-well plate (Corning, USA); MH (Mueller-Hinton) broth, sterile Saline, meropenem and levofloxacin standard (Wenzhou Kangtai Biological Company, China); Galla (Guangdong side pharmaceutical company, China).

2.3 Preparation of Bacterial Suspension

After the culture on the blood plate for 18~24h, pick up 3~5 pure colonies, use a sterile cotton swab to grind and mix in sterile physiological saline, and adjust the turbidity to 0.5 Mcfarland standard using DR100 type turbidimeter, and then use MH broth to dilute the turbidity-adjusted bacterial liquid by a factor of 100.

2.4 Preparation of Antibacterial Drugs

Weigh the appropriate amount of each drug with an analytical balance. *G. chinensis* was dissolved in ddH2O at a concentration of 10240 μg/mL. According to the requirements of the CLSI standard, the concentration of meropenem and levofloxacin was 5120 μg/mL.

2.5 The Micro Broth Dilution Method

Each antibacterial drug was diluted to 10 concentration gradients according to the dilution method by using MH broth sterilized in an autoclave. The concentration of *G. chinensis* was 10μg/ml~5120 μg/mL, the concentration of meropenem was 0.5μg/ml~2560μg/mL, and the concentration of levofloxacin was 2.5μg/ml~1280μg/mL. First, add 100 μL of different concentrations of antibiotics to each of the 1st to 10th holes of each horizontal row of the sterile 96-well culture plate, and then add 100 μL of bacterial suspension to each well. In addition, the negative control and the blank control were simultaneously used as quality control during the test. The culture plate with the added bacterial solution was placed in an incubator at 37°C for 16h to 20h. After the completion of the culture, the minimum inhibitory concentration (MIC) value of the different antibacterial drugs used alone was recorded (MIC_{alone}). The test was repeated 3 times.

2.6 The Micro Broth Checkerboard Dilution Method

According to the checkerboard method, the antibacterial combination design was carried out. First, different experimental antibacterial drugs were double diluted with MH broth to 8 different antimicrobial concentration gradients according to the 2 times MIC value of using the single drug. Take meropenem and levofloxacin respectively combined with *Galla chinensis*, and then add 50 μL of each antibiotic suspension to each well. Finally, add 100 μL of the diluted strain suspension, and incubate at 37 °C for 16 h to 20 h. After observing the test results and recording the MIC value of each antibacterial drug in the best combination of antibacterial drugs, in addition, the fractional inhibitory concentration index (FICI) value was calculated.

2.7 Calculation and Judgment of FICI Value[8]

The FICI value is considered the standard reference pa-

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rameter to quantify pairwise drug interactions in antimicrobial research. The FIC of drug A (FICA) is defined as the MIC of drug A in the presence of drug B divided by the MIC of drug A alone (FICA = [MICA(B)/MICA]); and vice versa (FICB = [MICB(A)/MICB]). The sum of FICA plus FICB gives the FICI (FICI = FICA + FICB), an indication of the degree of drug interaction. When FICI ≤ 0.5, it is synergistic. When 0.5 < FICI ≤ 1.0, it is additive. When 1.0 < FICI ≤ 2.0, it is irrelevant. When FICI > 2.0, it is antagonistic.

2.8 Statistical Analysis

Statistical analysis of the experimental data was performed using SPSS 22.0 statistical software. The synergistic rate between the two groups of antibiotics was compared by $\chi 2$ test. p Value <0.05 was considered to indicate statistical significance.

3. Results

3.1 Comparison of MIC Values of Antibacterial Drugs against Carbapenem-resistant E. coli When Used Alone or in Combination

The results showed that when *G. chinensis* was combined with meropenem, the MIC50(the MIC required for 50% of the bacteria to be inhibited) and the MIC90 (the MIC required for 90% of the bacteria to be inhibited) of the two antibacterial drugs were significant declined. The MIC50 and MIC90 of *G. chinensis* were 1/4 of that when the drug was used alone, and the MIC50 and MIC90 of meropenem were 1/32 and 1/4 of that of the drug was used alone; When *G. chinensis* was combined with levofloxacin, the MIC50 and MIC90 of *G. chinensis* were 1/4 of that when the drug was used alone, and the MIC50 and MIC90 of levofloxacin were 1/8 and 1/64 of that of the drug was used alone, as shown in Table 1.

Table 1. MIC values of *G. chinensis* with meropenem and levofloxacin alone or in combination against carbapenem-resistant *E.coli* (n=30, μg/mL)

Antibacterial drug		Alone			Combination	
	MIC_{50}	MIC_{90}	range	MIC_{50}	MIC_{90}	range
G. chinensis	640	2560	80~5120	160	640	40~640
Meropenem	640	1280	20~2560	20	320	5~320
G. chinensis	640	2560	80~5120	160	640	40~1280
Levofloxacin	40	640	10~1280	5	10	1.25~10

3.2 The FICI Distribution of *G. chinensis* against Carbapenem-resistant E. coli When Combined with Meropenem or Levofloxacin

When *G. chinensis* was combined with meropenem, the synergistic effect was 86.7%, the additive effect was 13.3%, and the irrelevance and antagonism were 0; When *G. chinensis* was used in combination with levofloxacin, the synergistic effect was 66.7%, the additive effect was 33.3%, and the irrelevance and antagonism also were 0,as shown in Table 2. The synergistic ratio (synergistic effect+additive effect) of the two different antimicrobial combinations was indicated no statistical significance. ($P > 0.05$).

Table 2. The FICI distribution of *G. chinensis* against thirty strains of carbapenem-resistant E. coli when combined with meropenem or levofloxacin

Drug combination	FICI ≤ 0.5	0.5 < FICI ≤ 1.0	1.0 < FICI ≤ 2.0	FICI > 2.0
G. chinensis+Meropenem	26(86.7%)	4(13.3%)	0(0%)	0(0%)
G. chinensis+Levofloxacin	20(66.7%)	10(33.3%)	0(0%)	0(0%)

4. Discussion

Galla chinensis, a nontoxic Chinese herbal medicine, is naturally formed when Rhus chinensis Mill is parasitized by *Melaphis chinensis* Bell. *G. chinensis* is considered to be a potential antibacterial agent. The main active ingredient of *G. chinensis* is *G. chinensis* tannin, which is formed by the condensation of 7 to 9 molecules of gallic acid and 1 molecule of glucose. The content is about 60% to 70%, even up to 80%; *G. chinensis* tannin can be made into nearly 100 kinds of fine chemical products by purification and synthesis. It has been widely used in medicine, chemical industry and food, and has become a research hotspot in the research field[7]. *G. chinensis* not only has the effect of clearing away heat and detoxification, but also has a significant broad-spectrum antibacterial effect. In the early studies, the MIC rsults of *G. chinensis* ethanol extract against 140 strains of Enterococcus showed that the MIC90 of *G. Chinensi* against *Enterococcus. faecalis*, *Enterococcus faecium* and other Enterococcus was 0.315mg/mL, 0.63mg/mL and 0.63 mg/mL, respectively, which suggesting that *G. chinensis* ethanol extract has strong antibacterial activity against Enterococcus[9]. Li Kaixuan et al. also found that *G. chinensis* has effective antibacterial activity by detecting the antibacterial activity of Chinese herbal extracts against multi-drug resistant Acinetobacter baumannii[6]. In this study, the bacteriostatic activity of *G. chinensis* against carbapenem-resistant *E.coli* was tested

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to show that the MIC value of 80~5120μg/mL has certain effective antibacterial activity.

In recent years, the detection rate of carbapenem-resistant *E.coli* in the clinic has gradually increased, which has brought great challenges to clinicians' anti-infective treatment. Faced with the increasingly severe drug resistance situation and the slow development of new effective antibacterial drugs, it is of great significance to carry out the deep excavation of traditional Chinese medicines and the combination of antibacterial drugs for anti-infective treatments[10,11]. Both meropenem and levofloxacin are commonly used antibacterial drugs in the clinic and as drug use increases, there is more drug resistance. Therefore, finding synergistic antibacterial drugs for anti-infective treatment plays an important role in the rational and effective use of clinical antibacterial drugs. In this study, the results indicated that *G. chinensis* has a certain combined effect when used in combination with meropenem or levofloxacin. When *G. chinensis* was combined with meropenem, the MIC50 and MIC90 of the two antibacterial drugs were significant declined. The MIC50 and MIC90 of *G. chinensis* were 1/4 of that when the drug was used alone, and the MIC50 and MIC90 of meropenem were 1/32 and 1/4 of that of the drug was used alone; When *G. chinensis* was combined with levofloxacin, the MIC50 and MIC90 of *G. chinensis* were 1/4 of that when the drug was used alone, and the MIC50 and MIC90 of levofloxacin were 1/8 and 1/64 of that of the drug was used alone. In addition, when *G. chinensis* was combined with meropenem, the synergistic effect was 86.7%, the additive effect was 13.3%, and the irrelevance and antagonism were 0; When *G. chinensis* was used in combination with levofloxacin, the synergistic effect was 66.7%, the additive effect was 33.3%, and the irrelevance and antagonism also were 0. These also indicated that the combination of *G. chinensis* and the above two antibacterial drugs has a certain combined effect. The results are different from those reported by Zhang Haiyue et al.[12] and Wang Lingjing[13], which may be related to the difference of strains.[14] In this study, the clinically isolated carbapenem-resistant E. coli were selected. Zhang Haiyue et al. used bovine levofloxacin-resistant E. coli, and Wang Lingjing et al. chosed multidrug resistant *Pseudomonas aeruginosa*. The mechanism of bacterial resistance is complex, and it can be resistant to antibiotics by producing β-lactamase, change in cell membrane permeability, active efflux pumps, change in drug targets, and biofilm formation[15]. Wang Lingjing et al. showed that *G. chinensis* alone or in combination with ciprofloxacin can promote the expression of multiple drug-resistant *Pseudomonas aeruginosa* (MDR-P A) efflux pump genes to different extents, suggesting that Chinese medicine passes promote efflux pump gene expression to play an antagonistic role in antibiotics. It is suggested that there may be different types of bacteria, and the mechanism of resistance is also different, resulting in differences in experimental results[16,17].

5. Conclusion

In summary, this study found that *G. chinensis* combined with meropenem or levofloxacin has a certain combined antibacterial effect on anti-infective treatment of carbapenem-resistant *E.coli*, and this treatment can be considered for anti-infective treatment in clinical practice. However, this study also has defects such as the small number of research strains and the small number of antibacterial drugs, and we will further study them in future research.

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