ISSN 2307-8960 (online)

World Journal of *Clinical Cases*

World J Clin Cases 2023 June 16; 11(17): 3932-4209

Published by Baishideng Publishing Group Inc

World Journal of Clinical Cases

Contents

Thrice Monthly Volume 11 Number 17 June 16, 2023

REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Case Control Study

Retrospective Cohort Study

Retrospective Study

World Journal of Clinical Cases

Thrice Monthly Volume 11 Number 17 June 16, 2023

Contents

World Journal of Clinical Cases

Thrice Monthly Volume 11 Number 17 June 16, 2023

Contents

NAME OF JOURNAL
World Journal of Clinical Cases

ISSN
ISSN 2307-8960 (online)

LAUNCH DATE
April 16, 2013

FREQUENCY
Thrice Monthly

EDITORS-IN-CHIEF
Bao-Gan Peng, Jerzy Tadeusz Chudek, George Kontogeorgos, Maurizio Serati, Ja Hyeon Ku

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2307-8960/editorialboard.htm

PUBLICATION DATE
June 16, 2023

COPYRIGHT
© 2023 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Clinical Cases

Submit a Manuscript: https://www.f6publishing.com

World J Clin Cases 2023 June 16; 11(17): 4026-4034

DOI: 10.12998/wjcc.v11.i17.4026

ISSN 2307-8960 (online)

ORIGINAL ARTICLE

Retrospective Study

Anti-bacterial mechanism of baicalin-tobramycin combination on carbapenem-resistant *Pseudomonas aeruginosa*

Li-Min Jin, Hui Shen, Xing-Ying Che, Ye Jin, Chun-Mei Yuan, Neng-Hua Zhang

Specialty type: Methodology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Douglas P, Canada; Keluskar J, United States

Received: March 28, 2023
Peer-review started: March 28, 2023
First decision: April 11, 2023
Revised: April 21, 2023
Accepted: May 12, 2023
Article in press: May 12, 2023
Published online: June 16, 2023

Li-Min Jin, Hui Shen, Xing-Ying Che, Ye Jin, Chun-Mei Yuan, Neng-Hua Zhang, Laboratory Department, Jiaxing Hospital of Traditional Chinese Medicine, Jiaxing 314001, Zhejiang Province, China

Corresponding author: Neng-Hua Zhang, Doctor, Associate Chief Physician, Laboratory Department, Jiaxing Hospital of Traditional Chinese Medicine, No. 1501 Zhongshan East Road, Jiaxing 314001, Zhejiang Province, China. znhshzyxwx@163.com

Abstract

BACKGROUND

Pseudomonas aeruginosa (*P. aeruginosa*) is an important cause of nosocomial infections, and contributes to high morbidity and mortality, especially in intensive care units. *P. aeruginosa* is considered a 'critical' category bacterial pathogen by the World Health Organization to encourage an urgent need for research and development of new antibiotics against its infections.

AIM

To investigate the effectiveness of baicalin combined with tobramycin therapy as a potential treatment method for carbapenem-resistant *P. aeruginosa* (CRPA) infections.

METHODS

Polymerase chain reaction (PCR) and RT-PCR were used to detect the expression levels of drug-resistant genes (including *VIM*, *IMP* and *OprD2*) and biofilm-related genes (including *algD*, *pslA* and *lasR*) in CRPA that confer resistance to tobramycin, baicalin and tobramycin combined with baicalin (0, 1/8, 1/4, 1/2 and 1MIC).

RESULTS

There was a correlation between biofilm formation and the expression of biofilm-related genes. In addition, *VIM*, *IMP*, *OprD2*, *algD*, *pslA* and *lasR* that confer biofilm production under different concentrations in CRPA were significantly correlated. The synergistic effect of baicalin combined with tobramycin was a significant down-regulation of *VIM*, *IMP*, *algD*, *pslA* and *lasR*.

CONCLUSION

Baicalin combined with tobramycin therapy can be an effective treatment method for patients with CRPA infection.

Key Words: Baicalin; *Pseudomonas aeruginosa*; Tobramycin; Carbapenem-resistant *Pseudomonas aeruginosa*; Therapy

Core Tip: Baicalin combined with tobramycin therapy shows potential as an effective treatment method for patients with carbapenem-resistant *Pseudomonas aeruginosa* infection, as it significantly down-regulates drug-resistant and biofilm-related genes.

Citation: Jin LM, Shen H, Che XY, Jin Y, Yuan CM, Zhang NH. Anti-bacterial mechanism of baicalin-tobramycin combination on carbapenem-resistant *Pseudomonas aeruginosa*. *World J Clin Cases* 2023; 11(17): 4026-4034
URL: https://www.wjgnet.com/2307-8960/full/v11/i17/4026.htm
DOI: https://dx.doi.org/10.12998/wjcc.v11.i17.4026

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is an important cause of nosocomial infections, and contributes to high morbidity and mortality, especially in intensive care units[1,2]. *P. aeruginosa* is considered a 'critical' category bacterial pathogen by the World Health Organization (WHO) to encourage an urgent need for research and development of new antibiotics against its infections. It is an opportunistic pathogen that naturally exists in the human skin, respiratory tract, and gastrointestinal tract. As *P. aeruginosa* is a common cause of respiratory and urinary tract infections, as well as bloodstream infections, its infections are priority healthcare-associated infections in some hospitals. According to data collected by the Global Drug Resistance Surveillance Network from 1996 to 2016, *P. aeruginosa* bloodstream infections accounted for 5.3% of all bloodstream infections in more than 200 medical centers in 45 countries.

With the widespread and irrational use of carbapenem antibiotics to treat *P. aeruginosa* infections, carbapenem-resistant *P. aeruginosa* (CRPA) strains have gradually appeared, presenting a new challenge to existing clinical treatments. In 2017, WHO listed CRPA as one of the three major bacteria requiring urgent development of new drugs to control infection[3]. Although aminoglycoside antibacterial drug-tobramycin has shown strong efficacy against CRPA, they are dose-dependent, have greater renal and ear toxicity, and are prone to drug resistance[4,5]. At the same time, recent research has increasingly focused on the antibacterial effect of compounds found in traditional Chinese medicine. Among them, baicalin has been shown to have a strong effect on *P. aeruginosa* in some studies[6].

In vivo experiments showed that baicalin (100 mg/kg) could reduce mortality in mice infected with drug-resistant *P. aeruginosa*, indicating its potent bacteriostatic effect on this strain[7,8]. Therefore, in this study, the anti-bacterial effect of baicalin combined with tobramycin on CRPA infection was investigated, including the potential synergistic effect of the two drugs against this pathogen. Our previous study also demonstrated the synergistic effect of combining baicalin with tobramycin against CRPA. The biofilm formation ability and biofilm activity of CRPA decreased under the combined effect of baicalin and tobramycin in a non-dose-dependent manner. In this study, RT-polymerase chain reaction (PCR) was used to investigate the synergistic effect of combining baicalin with tobramycin on the expression of carbapenem resistance genes *VIM*, *IMP* and *OprD2* and biofilm-related genes *algD*, *pslA* and *lasR* in CRPA. In addition, tobramycin combined with baicalin medication group was compared with tobramycin alone medication group to study the effect of baicalin combined with tobramycin on CRPA.

MATERIALS AND METHODS

Bacteria and medicines

A total of 30 tobramycin-susceptible CRPA strains were collected from inpatients in our hospital from 2018 to 2020. Repeated strains isolated from the same part of the same patient were excluded from this count. All the 30 strains screened met the "Regulations on the Administration of Medical Institutions" of the State Council. The quality control strain ATCC27853 was provided by Mérieux (France). Both baicalin and tobramycin were purchased from Shanghai Maclean Biochemical Technology Co., Ltd (China).

Reagents and equipment

The following reagents were used: Columbia blood agar plate (Mérieux, France), lysostaphin (Shanghai Yuanye Biotechnology Co., Ltd., China), quantitative PCR reagent SG Fast qPCR Master Mix (ABI) and absolute ethanol (Jiangxi Grass Coral Disinfection Products Co., Ltd.). The following equipment were used: Ezup column bacterial genomic DNA extraction kit (Shanghai Sangon Bioengineering Co., Ltd.), 96-well cell culture plate (NEST company), common bacteria incubator (Shanghai Boxun Biotechnology Co., Ltd., China), VITEK turbidimeter (BioMérieux China Co., Ltd., China), Micropipette (Xingchuang Experimental Instrument Co., Ltd., China), Micro Vortex Mixer (Shanghai Huxi Analytical Instrument Factory Co., Ltd., China), Electrothermal Thermostat (Shanghai Yuejin Medical Instrument Co., Ltd., China), Small low-speed centrifuge (Thermo company), SW-CJ-1D clean workbench (Jiangsu Sujie equipment factory), PCR reaction amplifier (BIO company), StepOne fluorescence quantitative PCR instrument (ABI) and Stepone plus fluorescence Quantitative PCR instrument (ABI).

CRPA DNA extraction

Briefly, the reserved experimental strains were inoculated on Columbia blood plates and cultured overnight at 35°C. After 18-24 h, a single pure colony was inoculated into 4 mL sterile LB broth with shaking at 35°C for 18-24 h. The concentration was then adjusted to 0.5 McDonnell's with sterile physiological saline. A certain amount of bacterial solution was diluted 100 times with sterile solution. To prepare bacterial culture, bacteria containing a certain concentration of tobramycin, baicalin, and tobramycin solution (1, 1/2, 1/4, 1/8 MIC) in LB broth (equivalent to 5×10^5 cfu/mL) was incubated at 35°C for 24 h. For DNA extraction, 1 mL of this bacterial culture was added to a 1.5 mL centrifuge tube and centrifuged at 8000 rpm for 1 min at room temperature, with the supernatant discarded and the precipitate retained as DNA.

Tobramycin resistance gene and biofilm gene detection

PCR and RT-PCR were used to detect the expression of CRPA genes resistant to tobramycin, baicalin, and tobramycin combined with baicalin and biofilm-related genes, including carbapenem resistance genes *VIM*, *IMP* and *OprD2*, and biofilm-related genes *algD*, *pslA*, and *lasR*. With 16SrRNA as the reference gene, the differences in the expression profile of drug resistance genes in different groups were analyzed in comparison with the blank control group. The primers were adapted from previous publications. The primer sequences are shown in Table 1.

From 2016 to 2020, patients admitted to our hospital with CRPA bloodstream infection were included. Their clinical and microbiological data were comprehensively collected. The inclusion criteria were as follows: (1) Inpatients aged ≥ 18 years; (2) having complete clinical data; and (3) ≥ 1 blood culture specimen of *P. aeruginosa* with positive culture and clinical evidence of corresponding bloodstream infection and multiple cultures of *P. aeruginosa* strains collected from the same patient. This study analyzed the first cultured strains.

Patients were divided into death group and survival group according to whether the patients died during hospitalization or not. Univariate analysis was performed on the clinical characteristics, laboratory indicators and treatments of the two groups of patients. Subsequently, univariate analysis results with $P < 0.05$ were included in the multivariate analysis to explore independent risk factors for patient mortality.

Statistical analysis

The SPSS version 26.0 statistical software was used to analyze and process the results. Chi-square test was used to analyze count data, and *t*-test was used for comparison between two samples. Logistic regression analysis was used for multivariate analysis, and the odds ratio and 95%CI were calculated. Values with $P < 0.05$ were considered statistically significant.

RESULTS

Determination of the relative expression levels of CRPA resistance genes and biofilm-related genes using RT-PCR

The relative expression levels of CRPA resistance genes and biofilm-related genes varied under different drug concentrations, as shown in Table 2.

As shown in the Table 2, the drug resistance genes *VIM* and *IMP* were significantly down-regulated in both the single-use group and the combination group. Similarly, most of the biofilm-related genes, including *algD*, *pslA* and *lasR* were significantly down-regulated. In contrast, the *OprD2* combination and baicalin and tobramycin alone were up-regulated. *Mycin* was down-regulated, whereas *lasR* was slightly up-regulated in the baicalin-alone group.

Table 1 Polymerase chain reaction primers used

Gene	Sequence	Tm (℃)
VIM-1	RTF:GTTTG GTCGC ATATC GCAAC	60
VIM-2	RTR:AATGC GCAGC ACCAG GATAG	60
IMP-1	RTF:GAAGG YGTTT ATGTT CATAC	60
IMP-2	RTR:GTAMG TTTCA AGAGT GATGCC	60
OprD-1	RTF:ATGAA AGTGA TGAAG TGGAG CG	60
OprD-2	RTR:TTACA GGATC GACAG CGGAT AG	60
algD-1	RTF:CGAGAAGTCCGAACGCCACAC	60
algD-2	RTR:ATCGGCGGGAAGTCGTA	60
pslA-1	RTF:GGCCTGTTTCCCTACCT	60
pslA-2	RTR:GCGGATGTCGTGGTTG	60
lasR-1	RTF:GAAGATGGCGAGCGACCTTGGATTC	60
lasR-2	RTR:CTCGTGCTGCTTTCGCGTCTGGTAG	60
16sRNA	RTF:ACTCCTACGGGAGGCAGCAG	60
16sRNA	RTR:ATTACCGCGGCTGCTGG	60

Correlation between CRPA biofilm formation and related gene expression

Table 3 shows the Pearson correlation between the amount of biofilm formation and the expression of related genes.

The experimental data revealed a certain correlation between the biofilm formation of the strain and the expression of related genes (Table 3). Significant correlations were found between biofilm volume and *VIM* (PCC = 0.862, $P < 0.01$), *IMP* and *VIM* (PCC = 0.761, $P < 0.05$), *OprD2* and *IMP* (PCC = 0.811, $P < 0.05$), *pslA* and *OprD2* (PCC = 0.683, $P < 0.01$) and *lasR* and *pslA* (PCC = 0.935, $P < 0.01$).

Patient clinical data

After applying the inclusion criteria, a total of 57 patients were enrolled in this study, including 22 in the tobramycin combined with baicalin group and 35 in the tobramycin-only group, with a mean age of 58 years. The baseline data for all patients with CRPA bloodstream infections are shown in Table 4. Among the 57 patients, 36 were admitted to the intensive care unit, and 12 were mechanically ventilated. During follow-up, 17 patients died and the remaining patients survived.

Therapeutic effect of baicalin combined with tobramycin

This paper analyzed clinical variables associated with patient outcomes. The results of univariate analysis showed that APACHE II score, albumin, combined surgical treatment and baicalin combined with tobramycin were significantly associated with the prognosis of patients. As can be seen from the multivariate analysis results shown in Table 5, patients receiving baicalin in combination with tobramycin had a significant improvement in prognosis. Table 5 presents the multivariate logistic regression analysis of death group and survival group in *P. aeruginosa* bloodstream infection.

DISCUSSION

P. aeruginosa is a non-fermenting gram-negative bacillus that is a common opportunistic pathogen[9]. It is highly adaptable to external environments and can survive in a humid environment for a long time [10]. When *P. aeruginosa* contaminates medical water or medical equipment, it easily forms biofilms that are difficult to remove, which often leads to hospital-acquired infections[11]. In recent years, with the widespread use of antibiotics, *P. aeruginosa* has acquired resistance genes, developing multidrug resistance, pan-drug resistance and even complete resistance[5]. CRPA is resistant strain of *P. aeruginosa* that poses a great challenge to current clinical treatment[12].

Studies have shown that the current CRPA resistance mechanisms mainly include carbapenemase production, outer membrane permeability protein deletion, and active efflux pump mechanisms[13]. Metallo-β-lactamases (MBLs) are a group of carbapenemases that can extensively hydrolyze β-lactam antibiotics[14]. At present, the MBLs produced by *P. aeruginosa* are detected in all clinical settings, including the *VIM* and *IMP* families, especially *VIM*-2[15]. In contrast, the domestic *P. aeruginosa*

Table 2 Differences in relative expression levels between related genes

Index	Group	Assay name	ΔCt	ΔΔCT	Fold change	Up/down
1	Baicalin	*VIM*	15.94	2.63	0.16	Down
	Tobramycin	*VIM*	16.81	3.50	0.09	Down
	Baicalin + tobramycin	*VIM*	18.09	4.78	0.04	Down
	Control	*VIM*	13.31		1.00	
2	Baicalin	*IMP*	13.73	2.58	0.17	Down
	Tobramycin	*IMP*	13.49	2.34	0.20	Down
	baicalin+ tobramycin	*IMP*	13.56	2.41	0.19	Down
	Control	*IMP*	11.15		1.00	
3	Baicalin	*OprD2*	10.13	-0.11	1.08	Up
	Tobramycin	*OprD2*	11.40	1.16	0.45	Down
	Baicalin + tobramycin	*OprD2*	9.64	-0.60	1.52	Up
	Control	*OprD2*	10.24		1.00	
4	Baicalin	*algD*	1.88	0.37	0.77	Down
	Tobramycin	*algD*	2.23	0.72	0.61	Down
	Baicalin + tobramycin	*algD*	3.44	1.93	0.26	Down
	Control	*algD*	1.51		1.00	
5	Baicalin	*pslA*	3.29	0.63	0.65	Down
	Tobramycin	*pslA*	3.11	0.45	0.73	Down
	Baicalin + tobramycin	*pslA*	3.17	0.51	0.70	Down
	Control	*pslA*	2.66		1.00	
6	Baicalin	*lasR*	1.18	-0.09	1.06	Up
	Tobramycin	*lasR*	1.43	0.16	0.90	Down
	Baicalin + tobramycin	*lasR*	1.66	0.39	0.76	Down
	Control	*lasR*	1.27			

Table 3 Pearson correlation between the amount of biofilm formation and the expression of related genes

	RB	*VIM*	*IMP*	*OprD2*	*algD*	*pslA*	*lasR*
RB	1	**0.862**	0.373	-0.3548	0.178	0.223	-0.001
VIM		1	**0.761**	0.287	0.319	0.1321	0.007
IMP			1	**-0.811**		0.0505	0.471
OprD2				1	0.3155	**0.683**	0.214
algD					1	0.4772	0.691
pslA						1	**0.935**
lasR							1

Significant correlations were found between biofilm volume and *VIM* ($P < 0.01$), *IMP* and *VIM* ($P < 0.05$), *OprD2* and *IMP* ($P < 0.05$), *pslA* and *OprD2* ($P < 0.01$) and *lasR* and *pslA* ($P < 0.01$).

detections are mainly *VIM*, *IMP* and *SPM* types[16]. Channel proteins are embedded in the lipid bilayer of *P. aeruginosa*, which is a non-specific, water-soluble diffusion channel that spans the cell membrane. Among them, the outer membrane proteins OprC, OprD2 and OprE show strong pore activity[17]. Scoffield *et al*[18] and Wang *et al*[19] reported that *OprD2* gene deletion is the main mechanism of *P. aeruginosa* resistance to imipenem. Quorum sensing is a general mechanism of bacterial cell-to-cell

Table 4 Baseline characteristics of patients with carbapenem-resistant *Pseudomonas aeruginosa* bloodstream infection

Clinical variables	Patients (*n* = 57)
Age (yr)	58 ± 23
Length of hospital stay before infection (d)	24 ± 5
Combeites	
Blood system disease	12
Solid tumor	11
Admission to intensive care unit	36
APACHE II	19 ± 7
TTP	18 ± 6
Albumin < 30 g/L	23
Underwent surgery	18
Invasive mechanical ventilation	12
Antibiotic application ≥ 7 d	37
Outcome	
Sepsis and shock	22
Death	17

Table 5 Multivariate logistic regression analysis of death group and survival group in *Pseudomonas aeruginosa* bloodstream infection

Clinical variables	Odds ratio	95%CI	*P* value
APACHE II	1.27	1.02-1.58	0.033
Albumin < 30 g/L	6.72	1.18-32.57	0.035
Underwent surgery	3.56	1.03-6.22	0.048
The baicalin in combination with tobramycin	0.56	0.42-0.73	0.027

information transfer, which controls the behavior of entire bacterial populations by synthesizing and secreting signaling molecules (also known as autoinducing molecules)[20]. When signal molecules reaches a certain concentration threshold in bacterial population density, the expression of some specific genes is activated, which regulates the adaptive function of bacterial populations. *P. aeruginosa* strains are characterized by quorum sensing systems, mainly Las quorum sensing systems[21].

The Las system consists of *LasR* and *LasI* genes. The Las system is associated with *P. aeruginosa* infection and can increase its resistance to certain antibiotics, such as imipenem and ciprofloxacin[22]. The *algD* gene is the first gene encoding an alginate biosynthesis enzyme operon. The transcriptional activation of this gene is associated with the synthesis of alginic acid[23]. It has been reported that acid salts play an important role in the formation of *P. aeruginosa* biofilms[24]. As a highly conserved sequence, *pslA* is the first gene encoding glucotransferase on the *P. aeruginosa psl* operon. It has been reported in the literature that both of these two-polysaccharide synthesis-related genes play key roles in *P. aeruginosa* adhesion and biofilm formation[25]. Therefore, in this study, the biofilm-related genes *LasR*, *algD* and *pslA* and drug resistance genes *VIM*, *IMP* and *OprD2* were detected in different groups of gene changes, and the association between these genes and the synergistic effect of the combination drug were analyzed.

This experiment showed that baicalin, tobramycin and baicalin combined with tobramycin group had different effects on gene expression. Pearson's correlation analysis results revealed a correlation between the amount of biofilm formation and the expression of *VIM*, *IMP*, *OprD2*, *algD*, *pslA* and *lasR* genes. The most significant correlations were as follows: the amount of biofilm and *VIM*, *OprD2* and *IMP*, *pslA* and *OprD2*, *lasR* and *pslA*. The drug resistance genes *VIM* and *IMP* were significantly down-regulated in the single-use group and the combined drug group. Similarly, most of the biofilm-related genes *algD*, *pslA*, and *lasR* were significantly down-regulated, indicating that the three groups of drugs were all effective against CRPA, with better efficacy in combination than as single medication. The up-regulation of *OprD2* combined with baicalin alone was consistent with the results of Scoffield *et al*[18]. However, the down-regulation of tobramycin alone and the slight up-regulation of *lasR* in the baicalin group was different, probably due to individual differences among strains, including their inhibition ratios. Other

drug resistance mechanisms may also account for this difference. Therefore, the mechanism of action of *P. aeruginosa* is complex and requires further research.

CONCLUSION

The fatality rate among patients with *P. aeruginosa* bloodstream infection is high[26]. Many factors account for the poor prognosis of patients. These can be divided into host including advanced age, APACHE II score, underlying diseases such as hematological diseases and malignant tumors, and septic shock, and iatrogenic factors such as irrational initial antibiotic treatment[27]. The multivariate logistic regression analysis of death in this study showed that APACHE II score, combined surgery, and albumin < 30 g/L were independent risk factors for death[28]. A foreign study involving 187 cases of hospital-acquired *P. aeruginosa* bloodstream infection showed that the combined use of empiric antibiotics could reduce the mortality of infected patients[29]. Further multivariate analysis showed that empiric sensitive antibiotic therapy could reduce the risk of infection. For patients with suspected infection, sensitive drugs should be empirically used early. Our results showed that patients treated with baicalin combined with tobramycin had significantly better prognosis compared with those treated with tobramycin alone. Therefore, baicalin combined with tobramycin therapy shows potential as an effective treatment for patients with CRPA infection.

ARTICLE HIGHLIGHTS

Research background

Pseudomonas aeruginosa (*P. aeruginosa*) is an important cause of nosocomial infections, and contributes to high morbidity and mortality, especially in intensive care units. *P. aeruginosa* is considered a 'critical' category bacterial pathogen by the World Health Organization to encourage an urgent need for research and development of new antibiotics against its infections.

Research motivation

In this study, the anti-bacterial effect of baicalin combined with tobramycin on carbapenem-resistant *P. aeruginosa* (CRPA) infection was investigated, including the potential synergistic effect of the two drugs against this pathogen.

Research objectives

The objective of this research is to analyze the distribution of CRPA in a specific hospital over a period of time, and to investigate the effectiveness of baicalin combined with tobramycin therapy as a potential treatment method for CRPA infections. The study aims to explore the correlation between biofilm formation and the expression of drug-resistant and biofilm-related genes in CRPA, with the goal of identifying potential targets for effective treatment.

Research methods

The expression levels of drug-resistant genes and biofilm-related genes in CRPA that confer resistance to tobramycin, baicalin and tobramycin combined with baicalin were detected.

Research results

There was a correlation between biofilm formation and the expression of biofilm-related genes. In addition, *VIM*, *IMP*, *OprD2*, *algD*, *pslA* and *lasR* that confer biofilm production under different concentrations in CRPA were significantly correlated. The synergistic effect of baicalin combined with tobramycin was a significant down-regulation of *VIM*, *IMP*, *algD*, *pslA* and *lasR*.

Research conclusions

Baicalin combined with tobramycin therapy can be an effective treatment method for patients with CRPA infection.

Research perspectives

Future research can focus on further investigating the effectiveness of baicalin combined with tobramycin therapy in the treatment of CRPA infections, including exploring optimal dosage and administration methods. Additionally, further studies can explore the mechanisms underlying the down-regulation of drug-resistant and biofilm-related genes by baicalin and tobramycin, as well as potential side effects or limitations of this treatment method. Moreover, more research could be conducted to investigate other potential treatments for CRPA infections and to evaluate their clinical efficacy. Finally, efforts can be made to develop new approaches for preventing and controlling the

spread of CRPA in hospitals and healthcare settings.

FOOTNOTES

Author contributions: Jin LM is mainly responsible for experimental design and writing articles; Shen H is mainly responsible for collecting data and samples; Chen XY conducted experimental operations and conducted statistical analysis of articles; Jin Y and Yuan CM are responsible for specimen selection and experimental operations; Zhang NH is responsible for experimental guidance and important revisions to the article, and all authors read and approved the final version.

Supported by Jiaxing Science and Technology Planning Project, No. 2019AY32006 and No. 2020AY30004.

Institutional review board statement: The study was reviewed and approved by the Jiaxing Hospital of Traditional Chinese Medicine Institutional Review Board.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: There is no conflict of interest.

Data sharing statement: No additional data are available.

Country/Territory of origin: China

ORCID number: Li-Min Jin 0000-0002-1636-7013; Hui Shen 0009-0007-3982-5850; Xing-Ying Che 0009-0009-8872-1999; Ye Jin 0009-0008-1176-2883; Chun-Mei Yuan 0009-0004-3744-9386; Neng-Hua Zhang 0000-0001-7182-2720.

S-Editor: Wang JL
L-Editor: A
P-Editor: Yu HG

REFERENCES

1 **García-Reyes S**, Soberón-Chávez G, Cocotl-Yanez M. The third quorum-sensing system of Pseudomonas aeruginosa: Pseudomonas quinolone signal and the enigmatic PqsE protein. *J Med Microbiol* 2020; **69**: 25-34 [PMID: 31794380 DOI: 10.1099/jmm.0.001116]
2 **Sharma G**, Rao S, Bansal A, Dang S, Gupta S, Gabrani R. Pseudomonas aeruginosa biofilm: potential therapeutic targets. *Biologicals* 2014; **42**: 1-7 [PMID: 24309094 DOI: 10.1016/j.biologicals.2013.11.001]
3 **Mittal R**, Lisi CV, Gerring R, Mittal J, Mathee K, Narasimhan G, Azad RK, Yao Q, Grati M, Yan D, Eshraghi AA, Angeli SI, Telischi FF, Liu XZ. Current concepts in the pathogenesis and treatment of chronic suppurative otitis media. *J Med Microbiol* 2015; **64**: 1103-1116 [PMID: 26248613 DOI: 10.1099/jmm.0.000155]
4 **Becker B**, Cooper MA. Aminoglycoside antibiotics in the 21st century. *ACS Chem Biol* 2013; **8**: 105-115 [PMID: 23110460 DOI: 10.1021/cb3005116]
5 **Pang Z**, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. *Biotechnol Adv* 2019; **37**: 177-192 [PMID: 30500353 DOI: 10.1016/j.biotechadv.2018.11.013]
6 **Ahmadian L**, Haghshenas MR, Mirzaei B, Norouzi Bazgir Z, Goli HR. Distribution and Molecular Characterization of Resistance Gene Cassettes Containing Class 1 Integrons in Multi-Drug Resistant (MDR) Clinical Isolates of Pseudomonas aeruginosa. *Infect Drug Resist* 2020; **13**: 2773-2781 [PMID: 32848427 DOI: 10.2147/IDR.S263759]
7 **Pannewick B**, Baier C, Schwab F, Vonberg RP. Infection control measures in nosocomial MRSA outbreaks-Results of a systematic analysis. *PLoS One* 2021; **16**: e0249837 [PMID: 33826678 DOI: 10.1371/journal.pone.0249837]
8 **Entenza JM**, Moreillon P. Tigecycline in combination with other antimicrobials: a review of in vitro, animal and case report studies. *Int J Antimicrob Agents* 2009; **34**: 8.e1-8.e9 [PMID: 19162449 DOI: 10.1016/j.ijantimicag.2008.11.006]
9 **Al-Tawfiq JA**, Bazzi AM, Rabaan AA, Okeahialam C. The effectiveness of antibacterial curtains in comparison with standard privacy curtains against transmission of microorganisms in a hospital setting. *Infez Med* 2019; **27**: 149-154 [PMID: 31205037]
10 **Alpers K**, Vatareck E, Gröbe L, Müsken M, Scharfe M, Häussler S, Tomasch J. Transcriptome Dynamics of Pseudomonas aeruginosa during Transition from Overlapping To Non-Overlapping Cell Cycles. *mSystems* 2023; **8**: e0113022 [PMID: 36786632 DOI: 10.1128/msystems.01130-22]

11 **Loveday HP**, Wilson JA, Kerr K, Pitchers R, Walker JT, Browne J. Association between healthcare water systems and Pseudomonas aeruginosa infections: a rapid systematic review. *J Hosp Infect* 2014; **86**: 7-15 [PMID: 24289866 DOI: 10.1016/j.jhin.2013.09.010]

12 **Horcajada JP**, Montero M, Oliver A, Sorlí L, Luque S, Gómez-Zorrilla S, Benito N, Grau S. Epidemiology and Treatment of Multidrug-Resistant and Extensively Drug-Resistant Pseudomonas aeruginosa Infections. *Clin Microbiol Rev* 2019; **32** [PMID: 31462403 DOI: 10.1128/CMR.00031-19]

13 **Jean SS**, Harnod D, Hsueh PR. Global Threat of Carbapenem-Resistant Gram-Negative Bacteria. *Front Cell Infect Microbiol* 2022; **12**: 823684 [PMID: 35372099 DOI: 10.3389/fcimb.2022.823684]

14 **Pal A**, Dhara L, Tripathi A. Contribution of acrB upregulation & OmpC/Ompk36 loss over the presence of bla(NDM) towards carbapenem resistance development among pathogenic Escherichia coli & Klebsiella spp. *Indian J Med Res* 2019; **149**: 528-538 [PMID: 31411177 DOI: 10.4103/ijmr.IJMR_716_17]

15 **Soberón-Chávez G**, González-Valdez A, Soto-Aceves MP, Cocotl-Yañez M. Rhamnolipids produced by Pseudomonas: from molecular genetics to the market. *Microb Biotechnol* 2021; **14**: 136-146 [PMID: 33151628 DOI: 10.1111/1751-7915.13700]

16 **Ruiz-Roldán L**, Rojo-Bezares B, de Toro M, López M, Toledano P, Lozano C, Chichón G, Alvarez-Erviti L, Torres C, Sáenz Y. Antimicrobial resistance and virulence of Pseudomonas spp. among healthy animals: concern about exolysin ExlA detection. *Sci Rep* 2020; **10**: 11667 [PMID: 32669597 DOI: 10.1038/s41598-020-68575-1]

17 **Zhao C**, Li K, Mou X, Zhu Y, Chen C, Zhang M, Wang Y, Zhou K, Sheng Y, Liu H, Bai Y, Li X, Zhou C, Deng D, Wu J, Wu HC, Bao R, Geng J. High-fidelity biosensing of dNTPs and nucleic acids by controllable subnanometer channel PaMscS. *Biosens Bioelectron* 2022; **200**: 113894 [PMID: 34973563 DOI: 10.1016/j.bios.2021.113894]

18 **Scoffield JA**, Wu H. Oral streptococci and nitrite-mediated interference of Pseudomonas aeruginosa. *Infect Immun* 2015; **83**: 101-107 [PMID: 25312949 DOI: 10.1128/IAI.02396-14]

19 **Wang D**, Li J, Wang L. Comprehensive study of instable regions in Pseudomonas aeruginosa and Mycobacterium tuberculosis. *Biomed Eng Online* 2018; **17**: 133 [PMID: 30458797 DOI: 10.1186/s12938-018-0563-8]

20 **Wang J**, Meng M, Li M, Guan X, Liu J, Gao X, Sun Q, Li J, Ma C, Wei L. Integrin α5β1, as a Receptor of Fibronectin, Binds the FbaA Protein of Group A Streptococcus To Initiate Autophagy during Infection. *mBio* 2020; **11** [PMID: 32518187 DOI: 10.1128/mBio.00771-20]

21 **Veetilvalappil VV**, Manuel A, Aranjani JM, Tawale R, Koteshwara A. Pathogenic arsenal of Pseudomonas aeruginosa: an update on virulence factors. *Future Microbiol* 2022; **17**: 465-481 [PMID: 35289684 DOI: 10.2217/fmb-2021-0158]

22 **González-Alsina A**, Mateu-Borrás M, Doménech-Sánchez A, Albertí S. Pseudomonas aeruginosa and the Complement System: A Review of the Evasion Strategies. *Microorganisms* 2023; **11** [PMID: 36985237 DOI: 10.3390/microorganisms11030664]

23 **Satarian F**, Hosseini HM, Ghadaksaz A, Amin M, Fooladi AAI. Multi-Drug Resistant Clinical Pseudomonas aeruginosas Inhibited by Ferula gummosa Boiss. *Recent Pat Antiinfect Drug Discov* 2018; **13**: 89-99 [PMID: 29701161 DOI: 10.2174/1574891X13666180426163427]

24 **Ma LZ**, Wang D, Liu Y, Zhang Z, Wozniak DJ. Regulation of Biofilm Exopolysaccharide Biosynthesis and Degradation in Pseudomonas aeruginosa. *Annu Rev Microbiol* 2022; **76**: 413-433 [PMID: 35655342 DOI: 10.1146/annurev-micro-041320-111355]

25 **Kim S**, Li XH, Hwang HJ, Lee JH. Thermoregulation of Pseudomonas aeruginosa Biofilm Formation. *Appl Environ Microbiol* 2020; **86** [PMID: 32917757 DOI: 10.1128/AEM.01584-20]

26 **GBD 2019 Antimicrobial Resistance Collaborators**. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 2022; **400**: 2221-2248 [PMID: 36423648 DOI: 10.1016/S0140-6736(22)02185-7]

27 **Gellatly SL**, Hancock RE. Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. *Pathog Dis* 2013; **67**: 159-173 [PMID: 23620179 DOI: 10.1111/2049-632X.12033]

28 **Karatuna O**, Yagci A. Analysis of quorum sensing-dependent virulence factor production and its relationship with antimicrobial susceptibility in Pseudomonas aeruginosa respiratory isolates. *Clin Microbiol Infect* 2010; **16**: 1770-1775 [PMID: 20132256 DOI: 10.1111/j.1469-0691.2010.03177.x]

29 **Zhao Y**, Lin Q, Liu L, Ma R, Chen J, Shen Y, Zhu G, Jiang E, Mi Y, Han M, Wang J, Feng S. Risk Factors and Outcomes of Antibiotic-resistant Pseudomonas aeruginosa Bloodstream Infection in Adult Patients With Acute Leukemia. *Clin Infect Dis* 2020; **71**: S386-S393 [PMID: 33367574 DOI: 10.1093/cid/ciaa1522]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com