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ARTICLE

Study on the mechanisms of the cross-resistance to TET, PIP, and GEN in *Staphylococcus aureus* mediated by the *Rhizoma Coptidis extracts*

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Abstract

The purpose of this study was focused on the mechanisms of the cross-resistance to tetracycline (TET), piperacillin Sodium (PIP), and gentamicin (GEN) in *Staphylococcus aureus* (SA) mediated by *Rhizoma Coptidis extracts* (RCE). The selected strains were exposed continuously to RCE at the sublethal concentrations for 12 days, respectively. The susceptibility change of the drug-exposed strains was determined by analysis of the minimum inhibitory concentration. The 16S rDNA sequencing method was used to identify the RCE-exposed strain. Then the expression of resistant genes in the selected isolates was analyzed by transcriptome sequencing. The results indicated that RCE could trigger the preferential cross-resistance to TET, PIP, and GEN in SA. The correlative resistant genes to the three kinds of antibiotics were upregulated in the RCE-exposed strain, and the mRNA levels of the resistant genes determined by RT-qPCR were consistent with those from the transcriptome analysis. It was suggested from these results that the antibacterial Traditional Chinese Medicines might be a significant factor of causing the bacterial antibiotic-resistance.

Introduction

Bacterial pathogens can cause infectious diseases and hence result in detrimental effects on human. To deal with the infections, more and more researchers have focused their attention on exploiting new antibiotics [1]. Hitherto, antibiotics have been developed into different classes including tetracyclines, β-lactam antibiotics, aminoglycosides, and so on. In addition, biocides (e.g., chlorhexidine diacetate, CHX) are also crucial antibacterial agents that are broadly used as antiseptics, disinfectants, or preservatives to eliminate microbes or prevent microbial growth [2, 3]. These antibacterial agents have been widely applied in healthcare for centuries. It is considered that bacteria could develop the antibiotic resistance through the abuse of antibiotics and biocides due to their strong adaptability and ability to acquire heritable resistance genes [4, 5]. Antibiotic resistance has raised the morbidity and mortality caused by bacterial infections and huge economic losses [6, 7]. Moreover, with the appearance of superbugs such as the methicillin-resistant *Staphylococcus aureus* (MRSA), NDM-1, and so on, we are confronted with noneffective drugs the "post-antibiotic era" [8–10].

It is urgent to devote more effort to research into the mechanisms of acquisition of antibiotic resistance to help in dealing with this situation with the serious situation. The relationship between the biocide use and the clinical antibiotic-resistance has been intensively investigated [11–14]. In terms of the academic literature, it was pointed out that antibiotics and biocides might share the same mode of action, which could lead to the cross-resistance to antibiotics in bacteria [2, 11]. Furthermore, the bacteria could be induced to resist antibiotics by antibacterial agents used at sublethal doses [12].

Some antibacterial Traditional Chinese Medicines (TCMs) have been observed to have positive effects for treating infectious diseases, and they have been applied widely for more than 4000 years in China [15–17]. Generally, there are two types of antibacterial TCMs, i.e., antibacterial Chinese herbs (ACHs) and antibacterial Chinese patent medicines. These antibacterial TCMs were considered unable to select for the bacterial drug-resistance, attributed to their complicated antibacterial components, which can act

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on various target sites in the bacterial cell [18–20]. Therefore, the antibacterial TCMs are regarded as the ideal drugs to solve the problem of drug-resistant bacteria [21, 22]. One of the most well-known ACHs is *Rhizoma Coptidis* (RC), whose active ingredients contain berberine, coptisine, palmatine, and other compounds [23–26].

In our previous research, we reported the selection of resistant bacteria by the RC extracts (RCE) with decreased susceptibility to CHX and with cross-resistance to other antibiotics in *S. aureus* (*SA*) [27]. The mechanism of the cross-resistance to specific antibiotics in SA driven by RCE was characterized in this study.

Materials and methods

Chemicals and strains

Amikacin (AMK), cefepime (FEP), Piperacillin Sodium (PIP), and meropenem (MEM) were purchased from Shanghai Aladdin Biochemical Polytron Technologies Inc. (Shanghai, China). Tetracycline (TET), ciprofloxacin (CIP), and gentamicin (GEN) were provided by Tianjin Silan Technology Co., Ltd (Tianjin, China). CHX was obtained from China Pharmaceutical Biological Products Analysis Institute (Beijing, China). RC was purchased from the first affiliated hospital of Guangxi University of Chinese Medicine (Nanning, China), and identified according to the standard Chinese herbal identification procedures [28]. *SA* ATCC 25923 was purchased from National Institutes for Food and Drug Control (Beijing, China). Three clinical isolates (SA czx, SA cp, and SA lqq) were kindly supplied and identified by the first affiliated hospital of Guangxi University of Chinese Medicine.

Preparation of TCM reagent

RCE was prepared by the aqueous extraction based on the previous reported methods [29, 30]. Further, the extract was concentrated to 1.0 g ml^{-1}, which corresponded to the dose of 1.0-g crude herb per milliliter.

Minimum inhibitory concentration (MIC) assays

A geometric micro-dilution in the Mueller-Hinton broth method was employed for determining the MIC value in accordance with ISO standard 20776-1, and the susceptibility categorization was assessed on the basis of the current susceptibility and resistance breakpoints of the European Committee on Antibiotic Susceptibility Testing [31]. The MIC determination was carried out at least in triplicate under the same condition, and the data were presented as the value with the maximum probability.

Drug selection of strains

The experiments were performed using previously reported methods with slightly modifications [27]. Bacteria were cultivated in medium with the corresponding drugs at half-MIC concentration for 12 days with daily subculturing at the same half-MIC concentration of the respective drug. The change in susceptibility of these treated bacteria was determined. Samples of each of the treated isolates were stored in 20% glycerol at −80 °C for the further analysis [17].

Identification of bacteria

The phenotypic variants of the drug-selected strains were identified by 16S rDNA sequencing, which was conducted in BGI Genomics Co., Ltd (Shenzhen, China). Briefly, after isolation of DNA from the strains, the universal 16S rDNA primers, i.e., 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) were used to amplify the 16S rDNA genes [32]. Then the sequenced results were blasted on the NCBI website for homology analysis.

Transcriptome analysis

Total RNA of the selected strains was extracted with the TRIzol Reagent kit, respectively. Agarose gel electrophoresis was used to detect whether the RNA samples were degraded or contained impurities. The concentration and purity of RNA samples were assayed by Nanodrop, and their integrity was detected by Agilent 2100 Bioanalyzer. The mRNA was enriched with the Ribo-Zero Magnetic Kit, and then the synthesis and further purification of cDNA were carried out. The gene library was constructed by PCR amplification. The amplified library was purified by AMPure XP beads, and quantified by Qubit and qPCR, as well as visualized in Agilent 2100 Bioanalyzer. The NovaSeq 6000, a high-throughput sequencing platform, was utilized for the gene sequencing, which was performed in Wuhan Benagen Tech Solutions Company Limited (Wuhan, China).

Differential expression analysis was calculated by EBSeq [33], an R/Bioconductor package. A gene was considered to be differentially expressed when the false discovery rate was <0.05 and the $\log_2$ fold change was >1 or <−1 ($\log_2$ fold change of >1 or $\log_2$ fold change of <−1).

Real-time quantitative PCR (RT-qPCR) validation

In order to validate the transcriptome data, RT-qPCR was performed to quantify the mRNA transcripts of 15 selected genes using the Light Cylcler 96 (Roche, Mannheim,

Table 1 The MIC of strains before drugs selection

	RCE (mg ml^{-1})	CHX (μg ml^{-1})	CIP (μg ml^{-1})	TET (μg ml^{-1})	GEN (μg ml^{-1})	PIP (μg ml^{-1})	AMK (μg ml^{-1})	FEP (μg ml^{-1})	MEM (μg ml^{-1})
Breakpoints	–	–	$R>1$	$R>2$	$R>1$	$R>4$	$R>16$	$R>4$	$R>4$
ATCC 25923	1.95	1	1	1	1	1	1	2	1
SA czx	1.95	1	1	1	1	64	1	2	1
SA cp	1.95	1	1	8	1	64	1	2	1
SA lqq	1.95	1	1	8	8	4	1	1	1

The experiments were performed in triplicate under the same conditions. The MIC was presented as the value with the maximum probability

Table 2 The fold change in MIC after RCE selection

		RCE (mg/ml)	CHX (μg/ml)	CIP (μg/ml)	TET (μg/ml)	GEN (μg/ml)	PIP (μg/ml)	AMK (μg/ml)	FEP (μg/ml)	MEM (μg/ml)
Test no.[a]\breakpoints		–	–	$R>1$	$R>2$	$R>1$	$R>4$	$R>16$	$R>4$	$R>4$
ATCC 25923	1	**4**[b] (1.95, 7.81)	1	1	**16**[c] (1, 16)	**8** (1, 8)	**8** (1, 8)	1	2	1
	2	**4** (1.95, 7.81)	2	2	**16** (1, 16)	**16** (1, 16)	**16** (1, 16)	1	2	1
	3	**4** (1.95, 7.81)	1	1	**16** (1, 16)	**32** (1, 32)	**4** (1, 4)	2	2	1
SA czx	1	**4** (1.95, 7.81)	2	2	1	2	1/2	1	1	1
	2	**4** (1.95, 7.81)	2	1	1	1	1	1	1	1
	3	**4** (1.95, 7.81)	1	1	1	1	1	1	1	1
SA lqq	1	**4** (1.95, 7.81)	1	2	2	**4** (8, 32)	2	1	2	1
	2	**4** (1.95, 7.81)	1	2	2	**4** (8, 32)	2	1	2	1
	3	**4** (1.95, 7.81)	1	1	2	**4** (8, 32)	**4** (4, 16)	2	2	1
SA cp	1	**4** (1.95, 7.81)	1	1	1/8[d] (8,1)	1	1	1	1	1
	2	**4** (1.95, 7.81)	1	1	1/8 (8,1)	1	1	1	1	1
	3	**4** (1.95, 7.81)	1	1	1/8 (8,1)	1	1	1	2	1

[a]The selection experiments were performed at least in triplicate under the same conditions

[b]Data related to the less susceptibility (≧4-fold MIC increase) were highlighted in bold. The data listed in brackets were the MIC values before and after drug-exposure, respectively

[c]Data related to antibiotic resistance were highlighted in bold and underline format. dData related to increased susceptibility (≦1/4-fold MIC decrease) were highlighted in italic bold and underline format

[d]Data related to increased susceptibility (≦1/4-fold MIC decrease) were highlighted in italic bold and underline format

Germany). Each RT-qPCR reaction was performed in a final volume of 20 μl. The thermal cycling profile was as follows: 95 °C for 10 min; 45 cycles of 95 °C for 15 s, 60 °C for 60 s; melting of 95 °C for 10 s, 65 °C for 60 s, 97 °C for 1 s; a final cooling of 37 °C for 30 s. The cycle threshold values (C_T) were determined and the relative fold differences were calculated by the $2^{-\Delta\Delta CT}$ method using *GAPDH* as the reference gene [34]. Each experiment was run in triplicate.

Results

Susceptibility of the selected strains

The selected strains involved the reference strain (ATCC 25923) and three clinical strains (SA czx, SA cp, and SA lqq). These isolates exhibited different sensitivity to these tested antibiotics. The MICs of all the experimental drugs were showed in Table 1. Amongst these isolates, ATCC 25923 was susceptible to all tested antibiotics, SA czx was resistant to PIP, while SA cp was resistant to TET and PIP, and SA lqq was resistant to TET and GEN.

RCE selection

After RCE exposure, the decreased susceptibility to RCE (greter than or equal to fourfold MIC increase) was observed in almost all tests of the treated isolates, while there was no obvious change in the susceptibility to CHX in all treated isolates. In addition, the treated ATCC 25923 showed notable cross-resistance to TET, GEN, and PIP with not less than eightfold increase in MIC (Table 2). However, the increased susceptibility to TET with an eightfold decrease in MIC was observed in all three parallel tests of the treated SA cp. Notably, none of these selected strains was cross-resistant to CIP, FEP, and MEM after RCE exposure. Thus, it was suggested that the cross-resistance selected by RCE targeted differently for TET, GEN, and PIP. The results were different from those from our previous research, where the cross-resistance was non-preferential to all tested antibiotics after CHX or TET selection [27].

Table 3 Differentially expression of resistant genes

Gene name (NCBI)	Gene ID	Blast function	Control	RCE induction	log_2FC	Fdr value	Regulate
smrB	SAOUHSC_02419	Multidrug resistance efflux pump SepA	0	753.672	16.7051	0	Up
bmr3	SAOUHSC_02420	MFS transporter	0	94.8631	15.71946	0	Up
nhoA	SAOUHSC_03034	Acetyltransferase	0	166.508	15.45539	0	Up
BTN44_15030	SAOUHSC_02797	MFS transporter	0	35.0625	14.81486	0	Up
clpL	SAOUHSC_02862	ATP-dependent Clp protease ATP-binding subunit	7.00026	4844.31	5.807097	0	Up
hisG	SAOUHSC_03014	ATP phosphoribosyltransferase	0	53.3087	5.594224742	3.33E − 16	Up
norA	SAOUHSC_00703	MFS transporter	8.65392	1758.55	4.478808	0	Up
proP	SAOUHSC_00556	MFS transporter	13.6544	1298.61	3.44374	5.28E − 12	Up
fmtA_1	SAOUHSC_00998	Beta-lactamase	0.270798	115.813	2.854417	9.52E − 08	Up
emrB_1	SAOUHSC_02418	MFS transporter	3.60729	132.643	2.393273	7.20E − 06	Up
BTN44_07590	SAOUHSC_00979	Acetyltransferase	19.7398	1545.72	2.149393	8.78E − 05	Up
paiA	SAOUHSC_02651	Acetyltransferase	4.36272	109.363	1.945975	0.001609	Up
icaR	SAOUHSC_03001	Biofilm operon *icaABCD* HTH-type negative transcriptional regulator *IcaR*	746.85	2513.79	1.692279	0.010057	Up

Identification of bacteria

ATCC 25923 and its RCE-exposed isolate (the parallel test no. 1 in Table 2) were selected for 16S rDNA sequencing. By BLAST algorithm of the results, it was determined that the isolates could be identified as SA. Moreover, after assaying by NCBI-Blast, it was indicated that the homology between the sequenced strains and SA CP042008.1 as well as SA MK809240.1 was no <99.51%.

Transcriptome analysis

The two selected strains for 16S rDNA sequencing were further utilized for transcriptome analysis. From the results, it was found that there were 433 differentially expressed genes, among which 261 genes were upregulated and 172 genes were downregulated. After literature retrieval and categorization of these upregulated genes, thirteen genes related to the antibiotic resistance were listed in Table 3. By analyzing the gene function, the upregulated gene *smrB*, which encoded the efflux pump SepA, might significantly contribute to the antibiotic-resistant phenotype of the mutant. The cross-resistance to TET might be mainly due to the over-expression of the MFS efflux pumps in the mutant [35]. In this experiment, these encoding genes of MFS, including *bmr3*, *norA*, *BTN44-15030*, and *proP*, were all remarkably upregulated, resulting in the TET-resistant phenotype of the mutant. In addition, the gene *fmtA-1* encoding β-lactamases was upregulated, resulting in the development of the resistance to β-lactam antibiotics. It was shown that the cross-resistance to PIP could be observed in the mutant with greater than or equal to fourfold increase in MIC. Besides, it was well known that the inactivation of aminoglycoside by modifying enzymes could be the main resistant mechanism in SA [36, 37]. The gene *hisG*, encoding ATP phosphoribosyltransferase (belongs to the family of glycosyltransferases), and three genes including *nhoA*, *BNT44-07590*, and *paiA*, encoding acetyltransferase, were all upregulated in this study. The four genes mentioned might be associated with the GEN-resistant phenotype in the mutant.

RT-qPCR validation

In addition to the genes related to antibiotic resistance in Table 3, we also selected another three upregulated genes (including *BTN44_13995*, *HMPREF0776_1664*, and *vraG*) with the highest log_2 (fold change) value for RT-qPCR verification. The relevant primers are shown in Table 4. The mRNA levels of the selected genes determined by RT-qPCR were consistent with those from the transcriptome analysis except *emrB_1* (Fig. 1).

Discussion

The reference strain ATCC 25923 selected by RCE was noted to foster the preferential cross-resistance to the specific antibiotics, including TET, PIP, and GEN, but these treated isolates showed no significant change in the susceptibility to CIP, AMK, FEP, and MEM. Further, it was noted that the antibiotic-resistance phenotype might be random and non-repeatable in the exposed isolates, which

Table 4 Primers for RT-qPCR

Genes	Primer sequences (5′-3′)	Product length (bp)	Tm (°C)
GAPDH (reference)	F: TGACACTATGCAAGGTCGTTTCAC R: TCAGAACCGTCTAACTCTTGGTGG	288	61.56 62.06
smrB	F: AACGTTGTTGCAACTGTGTAAG R: TCATCATATTTGCAGTCGAGCA	115	58.25 58.20
bmr3	F: ACCCCAAACACTGCCAACTA R: CGCGTGATACAAGGTTTTGGA	120	59.45 59.20
nhoA	F: GTGGCGGTCGTAGTCTGAA R: ATAGCGCGGAATGTACCACTG	169	59.42 60.54
BTN44_15030	F: TCAGAATATGCGCCACCGAA R: ACGTCGGAGTTGTTTTGTGC	150	59.82 59.62
clpL	F: GCTGGAACGCAATATCGTGG R:CTGTGGCACCTGAACCGATA	127	59.70 59.75
hisG	F: AGACACCTCCAAAGAGCGAA R: CGCTAAAAGCGAACGGACTG	131	58.95 59.91
norA	F: ATGTTTGCAGTTGGCCACAA R: AATCCACCAATCCCTGGTCC	197	59.17 59.37
fmtA_1	F: CATCGATTACAGACGAAGACACA R: ACGGCGCAACCTTTTCCTTA	109	58.59 60.54
emrB_1	F: TGCAGTTAAATGCGATGGCG R: GAAATCTCACATGGCACGGC	135	59.90
BTN44_07590	F: ATATGGCACGCCATTACCTG R: CTTTGTCGCGATGCCTCTAC	103	58.10 59.08
paiA	F: GCACGAGGATTATGCTCCCA R: TTTCAAGGTGGCGGAAGAGG	110	59.89 60.25
icaR	F: TTGCGAAAAGGATGCTTTCAA R: ACGCCTGAGGAATTTTCTGGA	177	57.28 59.65
BTN44_13995	F: TGACGTCCTCGAATTGCACC R: AGAGGCAATTGCAGCGAATA	111	60.67 57.96
HMPREF0776_1664	F: ATCCCTCTGAATTGTCTGGTGG R: TGCACCTGTTGGTTCGTCAG	101	59.76 60.81
vraG	F: GGAAGGCTCACAAGTCGGAA R: TGCAAGCTCATAACTTCGTCG	103	59.97 59.01

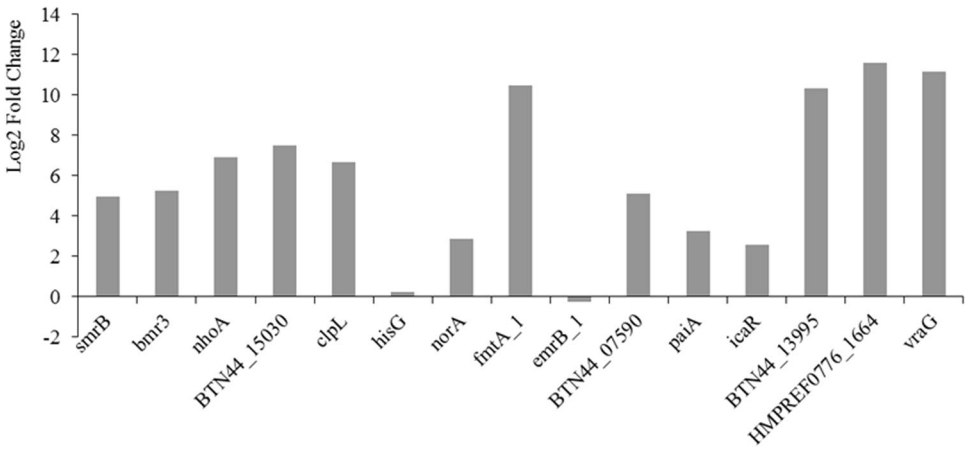

Fig. 1 RT-qPCR results of the RCE-exposed isolate vs. the reference strain. The RCE-exposed isolate was the treated strain of parallel test no. 1 in Table 2 and the reference strain was *Staphylococcus aureus* ATCC 25923

were unable to develop the same phenotype in multiple parallel tests.

The cross-resistant mechanisms were further analyzed by transcriptome analysis. Among the upregulated genes, thirteen resistant genes were related to the cross-resistant phenotypes of the mutant. The gene *smrB* encoding the SepA efflux pump, contributed to the antibiotic-resistant phenotype of the mutant. The TET-resistant phenotype was due to four upregulated genes including *bmr3*, *norA*, *BTN44-15030*, and *proP*. The resistance to PIP could be

related to the gene *fmtA-1*. In addition, the four genes including *hisG*, *nhoA*, *BNT44-07590*, and *paiA* might respond to the phenotype of the mutant who was resistant to GEN. Therefore, it is proposed that the cross-resistant mechanisms of SA to TET, PIP, and GEN after RCE selection were mainly due to the over-expression of efflux pumps and some modifying enzymes. In addition, for the increased susceptibility to TET with an eightfold decrease in MIC of the treated SA cp, it was speculated that some components in antibacterial Chinese medicines (e.g., RC) might have a reversal effect on a certain antibiotic-resistant strain or even several antibiotic cross-resistant strains.

Interestingly, *icaR* was one of the upregulated genes, which involved in the promoter that inhibits *icaADBC*, which is involved in the formation of bacterial biofilm. The extracellular polymer could be secreted from SA to form a dense barrier, blocking the penetration of antibiotics into the bacterial membrane and thus lessening the drug concentration inside the membrane. These results suggest that some components in RCE could inhibit the formation of bacterial biofilm by triggering the over-expression of *icaR*. On the other hand, we also found that 18 genes related to the virulence, including *tst, CJF57_00012*, *lukNF*, *isaB, BTN44_11020*, and other, else, were upregulated greatly (data not shown here), indicating that various virulence factors could be over-expressed in the RCE-selected isolate. Furthermore, there were three upregulated genes (*BTN44_13995*, *HMPREF0776_1664*, and *vraG*) with the highest $\log_2$ (fold change) value. It was suggested that they might play a key role in bacterial resistance or cell growth.

It is noted that the antibacterial TCMs are primarily used in China for the purpose of regulating the patients' homeostasis rather than eliminating the pathogens. Therefore, their therapeutic doses were less than the lethal doses of the pathogen in most cases. According to the results in this study, it was implied that the antibacterial TCMs might be a long-neglected significant factor for causing antibiotic resistance in bacteria. Moreover, the data calls for caution against unregulated use of antibacterial TCMs and the random discard of their residues into nature without proper treatment.

Data availability

The tables data used to support the findings of this study are included within the article.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Tishler PV. Production of penicillin. N Engl J Med. 2005;352:97.
2. Russell AD. Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. Lancet Infect Dis. 2003;3:794–803.
3. Thomas L, Russell AD, Maillard J-Y. Antimicrobial activity of chlorhexidine diacetate and benzalkonium chloride against Pseudomonas aeruginosa and its response to biocide residues. J Appl Microbiol. 2005;98:533–43.
4. Fernandes P, Ferreira BS, Cabral JM. Solvent tolerance in bacteria: role of efflux pumps and cross-resistance with antibiotics. Int J Antimicrob Agents. 2003;22:211–6.
5. Thomas L, Russell AD, Maillard J-Y. Antimicrobial activity of chlorhexidine diacetate and benzalkonium chloride against *Pseudomonas aeruginosa* and its response to biocide residues. J Appl Microbiol. 2005;98:533–43.
6. Langsrud S, Sidhu MS, Heir E, Askild LH. Bacterial disinfectant resistance a challenge for the food industry. Int Biodeterior Biodegrad. 2003;51:283–90.
7. Thabit AK, Crandon JL, Nicolau DP. Antimicrobial resistance: impact on clinical and economic outcomes and the need for new antimicrobials. Expert Opin Pharmacother. 2015;16:159–77.
8. Moremi N, Claus H, Mshana SE. Antimicrobial resistance pattern: a report of microbiological cultures at a tertiary hospital in Tanzania. BMC Infect Dis. 2016;16:756.
9. Owens RC Jr. Antimicrobial stewardship: concepts and strategies in the 21st century. Diagn Microbiol Infect Dis. 2008;61:110–28.
10. Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–10. Infect Control Hosp Epidemiol. 2013;34:1–14.
11. Davin-Regli A, Pagès JM. Cross-resistance between biocides and antimicrobials: an emerging question. Rev Sci Tech. 2012;31:89–104.
12. Lavilla Lerma L, Benomar N, Casado Muñoz Mdel C, Gálvez A, Abriouel H. Correlation between antibiotic and biocide resistance in mesophilic and psychrotrophic *Pseudomonas spp*. isolated from slaughterhouse surfaces throughout meat chain production. Food Microbiol. 2015;51:33–44.
13. Gilbert P, McBain AJ, Bloomfield SF. Biocide abuse and antimicrobial resistance: being clear about the issues. J Antimicrob Chemother. 2002;50:137–9.
14. Gilbert P, McBain AJ. Biocide usage in the domestic setting and concern about antibacterial and antibiotic resistance. J Infect. 2001;43:85–91.
15. Lau D, Plotkin BJ. Antimicrobial and biofilm effects of herbs used in traditional Chinese medicine. Nat Prod Commun. 2013;8:1617–20.
16. Zhang BL, Fan CQ, Dong L, Wang FD, Yue JM. Structural modification of a specific antimicrobial lead against *Helicobacter pylori* discovered from traditional Chinese medicine and a structure-activity relationship study. Eur J Med Chem. 2010;45:5258–64.
17. Wong RWK, Hagg U, Samaranayake L, Yuen MKZ, Seneviratne CJ, Kao R. Antimicrobial activity of Chinese medicine herbs

against common bacteria in oral biofilm. A pilot study. Int J Oral Maxillofac Surg. 2010;39:599–605.
18. Stermitz FR, Lorenz P, Tawara JN, Zenewicz LA, Lewis K. Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 50-methoxyhydno-carpin, a multidrug pump inhibitor. Proc Natl Acad Sci U S A. 2000;97:1433–7.
19. Betoni JE, Mantovani RP, Barbosa LN, Stasi LC, Junior AF. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. Mem Inst Oswaldo Cruz. 2006;101:387–90.
20. Sibanda T, Okoh AI. The challenges of overcoming antibiotic resistance: plant extracts as potential sources of antimicrobial and resistance modifying agents. Afr J Biotechnol. 2007;6:2886–96.
21. Choi UK, Kim MH, Lee NH. Optimization of antibacterial activity by Gold-Thread (Coptidis Rhizoma Franch) against Streptococcus mutans using evolutionary operation-factorial design technique. J Microbiol Biotechnol. 2007;17:1880–4.
22. Luo JY, Yan D, Yang MH. Study of the anti-MRSA activity of Rhizoma coptidis by chemical fingerprinting and broth microdilution methods. Chin J Nat Med. 2014;12:393–400.
23. Wang DW, Liu ZQ, Guo MQ, Liu SY. Structural elucidation and identification of alkaloids in Rhizoma coptidis by electrospray ionization tandem mass spectrometry. J Mass Spectrom. 2004;39:1356–65.
24. Yokozawa T, Ishida A, Kashiwada Y, Cho EJ, Kim HY, Ikeshiro Y. Coptidis rhizoma: protective effects against peroxynitrite-induced oxidative damage and elucidation of its active components. J Pharm Pharm. 2004;56:547–56.
25. Asai M, Iwata N, Yoshikawa A, Aizaki Y, Ishiura S, Saido TC, et al. Berberine alters the processing of Alzheimer's amyloid precursor protein to decrease Abeta secretion. Biochem Biophys Res Commun. 2007;352:498–502.
26. Wen SQ, Jeyakkumar P, Avula SR, Zhang L, Zhou CH. Discovery of novel berberine imidazoles as safe antimicrobial agents by down regulating ROS generation. Bioorg Med Chem Lett. 2016;26:2768–73.
27. Wu DM, Lu RC, Chen YQ, Qiu J, Deng CC, Tan Q. Study of cross-resistance mediated by antibiotics, chlorhexidine and Rhizoma coptidis in *Staphylococcus aureus*. J Glob Antimicrob Resist. 2016;7:61–6.
28. Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China (Part 2). Beijing, China: China Medical Science Press; 2010, p. 213–4.
29. Blaszczyk T, Krzyzanowska J, Lamer-Zarawska E. Screening for antimycotic properties of 56 traditional Chinese drugs. Phytother Res. 2000;14:210–2.
30. Kong WJ, Zhao YL, Xiao XH, Wang JB, Li HB, Li ZL, et al. Spectrum–effect relationships between ultra-performance liquid chromatography fingerprints and antibacterial activities of Rhizoma coptidis. Anal Chim Acta. 2009;634:279–85.
31. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0, 2019. http://www.eucast.org.
32. Dos Santos HRM, Argolo CS, Argolo-Filho RC, Loguercio LL. A 16S rDNA PCR-based theoretical to actual delta approach on culturable mock communities revealed severe losses of diversity information. BMC Microbiol. 2019;19:74.
33. Kanehisa M. The KEGG database. Novartis Found Symp. 2002;247:91–101.
34. Qian Y, Linglin G, Teng X, Yun C, Xin Y, Mengmeng H, et al. Amoxicillin administration regimen and resistance mechanisms of staphylococcus aureus established in tissue cage infection model. Front Microbiol. 2019;10:1–9.
35. Grossman TH. Tetracycline antibiotics and resistance. Cold Spring Harb Perspect Med. 2016. https://doi.org/10.1101/cshperspect.a025387.
36. Wencewicz TA. Crossroads of antibiotic resistance and biosynthesis. J Mol Biol. 2019;431:3370–99.
37. Favrot L, Blanchard JS, Vergnolle O. Bacterial GCN5-related *N*-acetyltransferases: from resistance to regulation. Biochemistry. 2016;55:989–1002.