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Short Communication

Bacteriostatic Effect of Royal Jelly on Methicillin-Resistant *Staphylococcus aureus*

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Bacteriostatic, Effect, Royal jelly, MRSA, Infection, SDS-PAGE

ABSTRACT

This study aimed to investigate the bacteriostatic effect of royal jelly (RJ) on methicillin-resistant *Staphylococcus* (MRSA) and to elucidate the underlying mechanism. MRSA cultures were exposed to varying concentrations of royal jelly (RJ), and MRSA absorbance at 600 nm was measured every 10 hours to assess the impact of RJ on MRSA proliferation. SDS-PAGE electrophoresis and Coomassie blue staining were employed to assess the impact of RJ treatment on MRSA protein expression. A 5% concentration of RJ exhibited substantial inhibition of MRSA proliferation ($P < 0.01$), with a concentration-dependent increase in inhibitory potency. SDS-PAGE electrophoresis and Coomassie Blue staining results suggested a potential link between royal jelly and its impact on MRSA protein expression, contributing to its inhibitory effect. RJ can disrupt the normal MRSA protein expression and impede MRSA proliferation, though it doesn't completely eradicate MRSA.

Royal jelly (RJ) is secreted by the mandibular and hypopharyngeal glands of nursing worker honeybees in *Apis mellifera*, which is solely fed to larvae and the adult queen honeybee (Chi *et al*., 2021). RJ is widely used in traditional medicine and is an important functional food and health enhancer (Fratini *et al*., 2016). It serves distinct biological roles in supporting human health, including immune regulation, lifespan extension, memory enhancement, digestion promotion, blood glucose regulation, as well as antibacterial and anticancer properties, among others (Guo *et al*., 2021). Studies have shown that RJ contains major RJ proteins (MRJPs) with antibacterial effects (Li *et al*., 2021; Park *et al*., 2019). The use of drugs such as antibiotics to treat bacterial infections has many side effects, so it is necessary to find new and safe treatments. Natural bee products, such as honey, RJ, and bee venom, can be promising alternatives for treating bacterial infections (Otręba *et al*., 2021).

Staphylococcus aureus is a Gram-positive bacterium in community and health care-related infections and may be resistant or sensitive to the commonly used antibiotics (oxacillin or cefoxitin) used to treat it, namely methicillin-resistant *Staphylococcus aureus* (MRSA) (Dadashi *et al*., 2018; Zhen *et al*., 2020). MRSA was initially documented in the United Kingdom in 1961. In the ten years following its initial description, MRSA led to hospital outbreaks in numerous regions worldwide. MRSA has now become widespread in healthcare facilities worldwide, making it a central target of global infection control initiatives (Lee *et al*., 2018). Globally, the occurrence and mortality rate of MRSA infections have been increasing significantly on an annual basis (Dadashi *et al*., 2018). MRSA carries the drug resistance gene *mecA*, which encodes the drug-resistant protein PBP2a. This protein binds to the active sites of methicillin and β-lactam antibiotics, leading to drug resistance (Alkharsah *et al*., 2018). RJ and garlic extracts exhibit a significant enhancement in wound healing in an *in vivo* mouse model infected with MRSA (El-Gayar *et al*., 2022). RJ samples gathered in northern Thailand demonstrated strong inhibitory effects on MRSA, a bacterium responsible for skin ailments (Uthaibutra *et al*., 2023).

The purpose of this study was to evaluate the

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inhibitory effect of RJ on MRSA, elucidate its mechanism, and provide a new approach for the treatment of MRSA infection.

Materials and methods

Fresh RJ, provided by Shanxi Agricultural University, was kept in the refrigerator at -20°C. The strain used in the test was MRSA (ATCC43300) purchased from Beijing Baioubowei Biotechnology Co., Ltd. The liquid medium was nutritious broth medium and the solid medium was nutritious Agar medium, which was purchased from Hangzhou Binhe microbial Reagent Co., Ltd and Qingdao Binde Biotechnology Co., Ltd. Gram staining kit was purchased from Anhui Chaohu Hongci Medical Equipment Co., Ltd.

For identification of test strains, the colony growth and hemolysis were observed when the blood agar plate was cultured at 37°C for 24 h. The tested strains were stained with Gram staining kit and identified by PCR amplification of drug resistance gene *mec A*. The primer sequence is shown in Table I. The amplification conditions were as follows: pre-denaturation at 94°C for 5 min; followed by 25 cycles at 94°C for 30 s, 46°C for 30 min, and 72°C for 2 min; extension at 72°C for 10 min.

For preparation of bacterial suspension, the strain was inoculated on solid medium, cultured for 18 h, single colony was inoculated on liquid medium and incubated in shaker at 37°C for 24 h. The bacterial culture was diluted with PBS to produce the bacterial liquid with the concentration of 10^8 CFU/ml.

For bacteriostatic test liquid medium and different amounts of RJ were added to the centrifuge tube, and the same amount of MRSA was inoculated to ensure the same amount of MRSA in each tube. The final concentration of RJ in the centrifuge tube is 0%, 5%, and 10%. The bacteria in all centrifuge tubes were cultured in 200 rpm shaker and incubator at 37°C. Bacterial proliferation was assessed by measuring the absorbance at 600 nm, following the approach outlined by Ibarlucea *et al.* (2017).The absorbance of the culture medium at 600 nm was measured every 10 h. The experiment was repeated three times.

The soluble proteins of MRSA bacteria were rdn on SDS-PAGE according to the method of Lihong *et al.* (2010), MRSA was cultured in the medium containing 0%, 5%, and 10% RJ for 50 h. The supernatant was discarded after 1000 rpm centrifugation and the bacteria were retained. RIPA lysate was used to split precipitation before being centrifuged for 10 min at 13800 rpm. The supernatant was collected and the sample buffer was added. Samples were then boiled for 10 min and separated by 10% SDS-PAGE. After electrophoresis, the gel was dyed with 0.25% Coomassie brilliant blue dye and decolorized with eluent until the band was clearly visible.

One-way analysis of variance (ANOVA) was utilized to compare various groups, with data analysis performed using SPSS 20.0. Statistically significant results were defined by P-values below 0.05.

Results

Blood agar plate culture showed that yellow grape bead-like colonies were formed on the culture medium, and a clear hemolytic ring was formed around the colonies (Fig. 1A). It was purple after Gram staining, indicating that the strain was gram-positive bacterium (Fig. 1B). After PCR, *16S rRNA* and *mec A* gene bands were amplified by agarose gel electrophoresis, and the position of the bands was consistent with the expected length (Fig. 1C). Based on the above results, the strain were determined as MRSA.

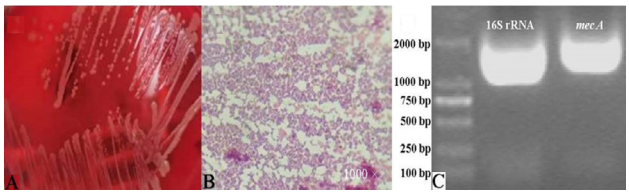

Fig. 1. Results of strain identification (A) Blood Agar culture colony (B) Gram staining (C) Results of agarose gel electrophoresis of *16s rRNA* and *mec A* genes.

Figure 2 shown the effects of different concentrations of RJ on MRSA in the first 30 h, the absorbance of 0% RJ group at 600 nm was significantly higher than that of the other two groups. The addition of 5% and 10% RJ to the culture medium could significantly decrease the absorbance value at 600nm ($P<0.01$). The results showed that RJ has a strong inhibitory effect on MRSA.

Table I. *mec A* and *16s rRNA* primer sequences.

Gene	Primer	Sequence (5'-3')	Size(bp)
mec A	*mec A-F*	CGGGATCCAAAGATAAAGAAATTAATAATACTA	1948 bp
	mec A-R	GGGGTACCTTATTCATCTATATCGTATTTTTTA	
16s rRNA	*16s rRNA-F*	AAAGATGGCATCATCATTCAAC	1372 bp
	16s rRNA-R	TACCGTCATTATCTTCCCCAAA	

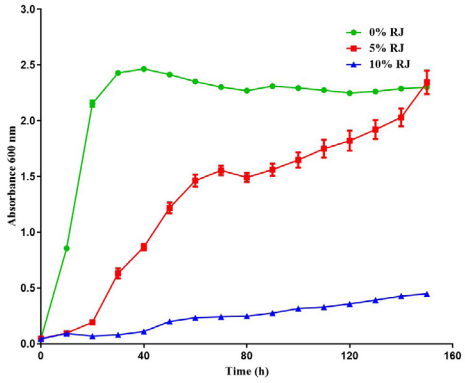

Fig. 2. Effects of different concentrations of RJ on the proliferation of MRSA, $^{**}P<0.01$ vs. 0% RJ group.

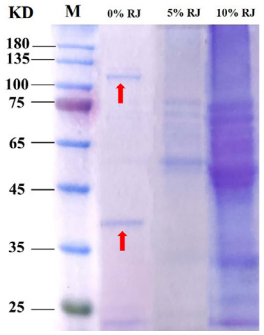

Fig. 3. SDS-PAGE electrophoresis of MRSA soluble protein after royal jelly treatment.

Figure 3 shown the effect of RJ on the expression of MRSA protein. It was found that after 5% RJ and 10% RJ processing, the two bands between 35 kD-45 kD and 100 kD-135 kD disappeared (red arrow marked position). The results showed that RJ could inhibit the expression of MRSA protein.

Discussion

The problem of bacterial drug resistance is becoming more and more serious. In addition to methicillin, MRSA has been found to be resistant to a variety of antibiotics, such as β-lactam, quinolone, sulfamethoxazole, erythromycin, clindamycin, linazolone, and daptomycin (Lai *et al*., 2018). At present, vancomycin is the first choice for the treatment of MRSA infection, but with the emergence of vancomycin-mediated *Staphylococcus aureus* (VISA) and heterogeneous vancomycin-mediated *S. aureus* (hVISA), the efficacy of vancomycin in the treatment of MRSA decreases (Bhise *et al*., 2018). In recent years, due to the unreasonable use of vancomycin in the treatment of MRSA infection, the sensitivity of vancomycin weakens year by year, resulting in the emergence of vancomycin-resistant *S. aureus* (VRSA) (Ramazoni *et al*., 2018). Lack of monitoring of vancomycin dosage leads to side effects such as red man syndrome and nephrotoxicity caused by overuse of vancomycin (Meaney *et al*., 2014; Nagahama *et al*., 2018). The emergence of these problems brings great challenges to the treatment of MRSA infection, so it is urgent to find new methods for the treatment of MRSA infection.

RJ is a nutritional secretion produced by worker bees. It has been found that the components of RJ include many antimicrobial peptides, such as royalactin, apisimin, jeleines I, II, III, IV, apalbumin α and 10-HAD (Bărnuțiu *et al*., 2011). Antimicrobial peptides are considered to be natural antibiotics, which can kill broad-spectrum pathogens and drug-resistant bacteria, and have a very low risk of drug resistance (Zasloff, 2002). Antimicrobial peptides provide new methods and ideas for solving the problem of bacterial drug resistance (Boukraâ, 2015). A variety of antimicrobial peptides in RJ can resist gram-positive bacteria and prevent skin from being infected by pathogenic bacteria (Boukraâ and Sulaiman, 2009). Eshraghi and Seifollahi (2003) found that RJ has an inhibitory effect on six kinds of bacteria, including *S. aureus*. *In vitro* studies have found that RJ can effectively inhibit MRSA and has a certain effect on MRSA infection caused by spinal implantation in rabbits (Gunaldi *et al*., 2014). This study found that RJ can inhibit the proliferation of MRSA *in vitro*, but was unable to eradicate MRSA completely. The results are basically consistent with the previous experimental results based on in vivo studies (Gunaldi *et al*., 2014). The results of Coomassie brilliant blue dye showed that MRSA treated with RJ could affect the expression of MRSA protein. RJ is a mixture of water (more than 60%), protein, lipids, sugars, vitamins, minerals and free amino acids. The composition of RJ is affected by many factors, such as season, type of collector, flower variety, geographical environment, and so on (Seven *et al*., 2014). The complexity of its components determines its various biological functions. This study found that RJ is not sufficient to kill MRSA, but can affect the expression of MRSA protein. RJ was found to totally eliminate MRSA and enhance wound healing in animals (El-Gayar *et al*., 2016). This conclusion is not consistent with the results of this study. The antibacterial activity of RJ and rape honey combination was greater than that of rape honey (Dinkov *et al*., 2016). The antibacterial activity of RJ may be attributed to the components contained in it. Its anti-MRSA effect needs to be further verified in clinical practice. Consequently, it is imperative to isolate and purify the active compounds within RJ that are effective against MRSA, in order to generate novel approaches for MRSA infection treatment.

Conclusions

RJ has a certain inhibitory effect on MRSA,

which may be related to its inhibition of MRSA protein expression. But RJ was unable to kill MRSA completely. It is found that the effective component of inhibiting MRSA in RJ is of great significance to the prevention and treatment of MRSA.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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