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# In Vitro Evaluation of the Combination Activity of Carvacrol and Oxacillin against Methicillin-Resistant *Staphylococcus aureus* Strains

Elif ODABAŞ KÖSE [1], Özlem KOYUNCU ÖZYURT [2]

**ABSTRACT**

**Aim:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most resistant bacteria to antibiotics. Many antibiotics are known to be insufficient to treat infections caused by these bacteria. Plant-derived antibacterials have drawn more attention as a source of new therapeutics. Carvacrol is a monoterpenic phenol compound found in various essential oils and shows antimicrobial activity against many pathogens. In this study, the combination activity of carvacrol and oxacillin against ten MRSA clinical strains was evaluated.

**Material and Methods:** To determine Minimum Inhibitory Concentrations (MIC) of carvacrol and oxacillin against ten MRSA clinical strains, broth microdilution method was performed. The combination activity of carvacrol and oxacillin was determined with checkerboard synergy test. Whether both antimicrobials and their combinations caused membrane damage in MRSA-6 strain was detected by measuring the amount of nucleic acid leaking out of the cell across membrane with UV spectrophotometer.

**Results:** Carvacrol showed antibacterial activity against all MRSA strains with MIC values in the range of 64-256 µg/ml. The synergistic effect (FICI≤0.5) between carvacrol and oxacillin was determined against seven strains. Carvacrol caused a membrane damage on MRSA-6 strain. As a result of combination with oxacillin, the increase in the membrane damage of MRSA-6 strain was found to be statistically significant ($p<0.001$).

**Conclusion:** According to the results of this study, carvacrol increased the antibacterial effect of oxacillin against MRSA strains. Thus, carvacrol can be used in combination with oxacillin against MRSA as a novel antimicrobial agent. However, the results of this study should be supported by further studies.

**Keywords:** Methicillin-resistant *Staphylococcus aureus*; plant-drug interactions; synergy.

# Metisilin Dirençli *Staphylococcus aureus* Suşlarına karşı Karvakrol ve Oksasilinin Kombinasyon Aktivitesinin İn Vitro Değerlendirilmesi

**ÖZ**

**Amaç:** Metisilin dirençli *Staphylococcus aureus* (MRSA) antibiyotiklere en dirençli bakterilerden biridir. Birçok antibiyotiğin bu bakterilerin neden olduğu infeksiyonları tedavi etmede yetersiz kaldığı bilinmektedir. Bitki türevli antibakteriyeller yeni terapötiklerin kaynağı olarak dikkat çekmektedirler. Karvakrol, çeşitli uçucu yağlarda bulunan monoterpenik fenol bileşiğidir ve birçok patojene karşı antimikrobiyal aktivite göstermektedir. Bu çalışmada on klinik MRSA suşuna karşı karvakrol ve oksasilinin kombinasyon aktivitesi değerlendirilmiştir.

**Gereç ve Yöntemler:** MRSA suşlarına karşı karvakrol ve oksasilinin Minimum İnhibitör Konsantrasyonlarını (MİK) saptamak için broth mikrodilüsyon metodu uygulanmıştır. Karvakrol ve oksasilinin kombinasyon aktivitesi checkerboard sinerji testi ile belirlenmiştir. Her iki antimikrobiyalin ve kombinasyonlarının MRSA-6 suşunda membran hasarına neden olup olmadığı ise membran boyunca hücre dışına sızan nükleik asit miktarının UV spektrofotometre ile ölçülmesiyle saptanmıştır.

**Bulgular:** Karvakrol 64-256 µg/ml aralığındaki MİK değerleri ile tüm MRSA suşlarına karşı antibakteriyel aktivite göstermiştir. Karvakrol ve oksasilinin yedi suşa karşı sinerjistik etki gösterdiği saptanmıştır (FICI≤0,5). Karvakrol, MRSA-6 suşunda membran hasarına neden olmuştur. Oksasilin ile kombinasyonu sonucunda, MRSA-6 suşunun

1 Akdeniz University, Vocational School of Health Sciences, Antalya, Türkiye
2 Akdeniz University, Faculty of Medicine, Department of Medical Microbiology, Antalya, Türkiye

Sorumlu Yazar / Corresponding Author: Elif ODABAŞ KÖSE, e-mail: elifkose@akdeniz.edu.tr
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membran hasarındaki artış istatistiksel olarak anlamlı bulunmuştur (p<0,001).
**Sonuç:** Bu çalışmanın sonuçlarına göre, karvakrol MRSA suşlarına karşı oksasilinin antibakteriyel aktivitesini arttırmıştır. Bu yüzden karvakrol oksasilin ile birlikte MRSA suşlarına karşı yeni bir antimikrobiyal ajan olarak kullanılabilir. Bununla birlikte, bu çalışmanın sonuçları daha ileri çalışmalar ile desteklenmelidir.
**Anahtar Kelimeler:** Metisilin dirençli *Staphylococcus aureus*; bitki-ilaç etkileşimleri; sinerji.

## INTRODUCTION

*Staphylococcus aureus* (*S. aureus*) is an important pathogen responsible for most of the bacterial infections worldwide. In addition to skin and soft tissue infections, it can cause very serious life-threatening infections such as pneumonia and meningitis. *S. aureus* is also a commensal organism and lives asymptomatically in the nose of approximately 30% of healthy people (1-4).

The main problem about the treatment of diseases caused by *S. aureus* is the resistance of this bacteria to antibiotics. *S. aureus* produces penicillinase enzyme which inactivates the antibiotic, thus penicillin resistance occurs. Penicillinase hydrolyzes the β-lactam ring at the center of penicillin and penicillin-derived antibiotics. Methicillin, a penicillin-derived semi-synthetic antibiotic, has been developed for the treatment of penicillin-resistant *S. aureus* infections and it is resistant to β-lactamase inactivation (1,5). Methicillin-resistant *S. aureus* (MRSA) strains were reported within two years after the use of this antibiotic (2).

Infections caused by MRSA were initially restricted to hospitals. However, in the 1990s, MRSA started to cause infections in healthy populations outside the hospitals. These strains, currently referred as community-associated MRSAs, are more virulent than hospital-associated strains and are capable of spreading faster (3,6).

Treatment of MRSA infections is quite difficult, because resistance is not only seen against β-lactams but also against many other antimicrobial. It is therefore important to develop new drugs or alternative therapies that are effective against MRSA (7,8). One of the basic approaches in alternative therapies is the combination of plant-derived compounds to increase the effectiveness of currently used antibiotics. A herbal compound that shows synergism with an antibiotic can be used as an option in combination therapies (9).

It has been known that some essential oils isolated from plants and their components have antimicrobial properties. In particular, the antimicrobial activities of these essential oils depends on the presence of phenolic compounds in their contents (10). Carvacrol (2-methyl-5-[1-methylethyl] phenol), a phenolic monoterpenoid, is one of the most important components of essential oils produced by a large number of aromatic plants. The main antibacterial mechanism of carvacrol is the damage of the cell membrane. It causes deterioration of the membrane integrity and leakage of vital intracellular components (11,12). In addition, the presence of the hydroxyl group and a delocalized electron system in its structure are the other possible reasons for its antibacterial activity (11).

In our previous study about the antimicrobial activity of *Origanum bilgeri* essential oil and its major constituent carvacrol, we found that carvacrol showed high antimicrobial activity against two strains of *S. aureus* (13). Based on our previous results, we planned to test the combination activity of carvacrol with oxacillin against ten MRSA clinical strains. It was also investigated whether these antimicrobial agents cause cell membrane damage.

## MATERIAL AND METHODS

### Bacterial Strains and Antimicrobial Agents

This study was conducted with the 2019/1089 numbered permission of the ethics committee. It was carried out in accordance with the rules of scientific research and publication ethics. Ten MRSA clinical strains used in the study were obtained from the Microbiology Department of the Central Laboratory of Akdeniz University Hospital. Stock solutions of *S. aureus* strains which were isolated from clinical samples were cultured on Blood Agar (Becton Dickinson, USA). Following incubation at 35±2°C for 18-24 hours, the colonies were identified by MALDITOF MS (Bruker Biotyper Daltonik, Germany) method. Antibiotic susceptibilities of colonies which identified as *S. aureus* were analyzed by BD Phoenix automated system (Becton Dickinson, USA). Ten strains identified as MRSA were included in the study. *S. aureus* ATCC 43300 (methicillin-resistant strain) and *S. aureus* ATCC 29213 (methicillin-sensitive strain) were used as quality control strains.

512 µg/ml oxacillin sodium salt (28221, Sigma-Aldrich) stock solution was prepared in distilled water. Carvacrol (W224511, Sigma-Aldrich) was dissolved in pure ethanol to prepare 10 mg/ml stock solution.

### Broth Microdilution Method

Broth microdilution method was used to detect MIC values of oxacillin and carvacrol (14). 50 µl of antimicrobial agent was added to the first wells of 96-well microdilution plates which contains 50 µl of cation-adjusted Mueller Hinton Broth (CAMHB, Merck KGaA, Darmstadt, Germany). Serial dilutions were then performed. The concentration range of oxacillin was 128-0.0625 µg/ml, whereas the range of carvacrol was 512-0.25 µg/ml. 50 µl of bacterial suspension was added to each well ($5x10^5$ cfu/ml). In addition, bacterial growth control (CAMHB+bacteria) and medium sterility control (CAMHB) for each microdilution plate were studied. The microdilution plates were incubated at 35±2°C for 16-20 hours in an incubator. MIC values were determined by comparing the growth density in the wells containing antibiotics with the growth density in the control wells (without antibiotics). MIC is the lowest antimicrobial drug concentration that completely inhibits the growth of bacteria in microdilution wells and can be determined by the naked eye. Each experiment was repeated three times.

### Checkerboard Synergy Test

In order to investigate the combination activity of oxacillin and carvacrol, checkerboard synergy test was performed. This method is one of the synergy tests based on microdilution method. The efficacy of the combination of the two antimicrobial agents was tested on a 96-well microplate for each strain. CAMHB was used as medium. The combination activity of two antimicrobial agents was tested in the dilution range of 4xMIC and 0.03125xMIC.

**Table 1.** The pattern of the checkerboard panel format of MRSA-6 strain

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | OXA 2 + CAR 4 | OXA 2 + CAR 8 | OXA 2 + CAR 16 | OXA 2 + CAR 32 | OXA 2 + CAR 64 | OXA 2 + CAR 128 | OXA 2 + CAR 256 | OXA 2 + CAR 512 | | OXA 2 | CAR 4 | |
| B | OXA 4 + CAR 4 | OXA 4 + CAR 8 | OXA 4 + CAR 16 | OXA 4 + CAR 32 | OXA 4 + CAR 64 | OXA 4 + CAR 128 | OXA 4 + CAR 256 | OXA 4 + CAR 512 | | OXA 4 | CAR 8 | |
| C | OXA 8 + CAR 4 | OXA 8 + CAR 8 | OXA 8 + CAR 16 | OXA 8 + CAR 32 | OXA 8 + CAR 64 | OXA 8 + CAR 128 | OXA 8 + CAR 256 | OXA 8 + CAR 512 | | OXA 8 | CAR 16 | |
| D | OXA 16 + CAR 4 | OXA 16 + CAR 8 | OXA 16 + CAR 16 | OXA 16 + CAR 32 | OXA 16 + CAR 64 | OXA 16 + CAR 128 | OXA 16 + CAR 256 | OXA 16 + CAR 512 | | OXA 16 | CAR 32 | |
| E | OXA 32 + CAR 4 | OXA 32 + CAR 8 | OXA 32 + CAR 16 | OXA 32 + CAR 32 | OXA 32 + CAR 64 | OXA 32 + CAR 128 | OXA 32 + CAR 256 | OXA 32 + CAR 512 | | OXA 32 | CAR 64 | |
| F | OXA 64 + CAR 4 | OXA 64 + CAR 8 | OXA 64 + CAR 16 | OXA 64 + CAR 32 | OXA 64 + CAR 64 | OXA 64 + CAR 128 | OXA 64 + CAR 256 | OXA 64 + CAR 512 | | OXA 64 | CAR 128 | |
| G | OXA 128 + CAR 4 | OXA 128 + CAR 8 | OXA 128 + CAR 16 | OXA 128 + CAR 32 | OXA 128 + CAR 64 | OXA 128 + CAR 128 | OXA 128 + CAR 256 | OXA 128 + CAR 512 | | OXA 128 | CAR 256 | Growth Control |
| H | OXA 256 + CAR 4 | OXA 256 + CAR 8 | OXA 256 + CAR 16 | OXA 256 + CAR 32 | OXA 256 + CAR 64 | OXA 256 + CAR 128 | OXA 256 + CAR 256 | OXA 256 + CAR 512 | | OXA 256 | CAR 512 | Sterility Control |

CAR: Carvacrol, OXA: Oxacillin, MIC values of carvacrol and oxacillin were found to be 128 µg/ml and 64 µg/ml, respectively. The dilutions of oxacillin were made horizontally whereas the dilutions of carvacrol were made vertically in a 96 well plate. MIC values of both agents were repeated in the $10^{th}$ and $11^{th}$ vertical columns. Numbers show the substance concentrations as µg/ml.

Oxacillin was added vertically while carvacrol was added horizontally to the wells. The MIC values of both drugs were repeated simultaneously with the checkerboard test, on the same plaque. The bacterial suspension was prepared to produce a final inoculum of $5x10^5$ cells/ml and was added to each well. In addition, bacterial growth control (CAMHB+bacteria) and medium sterility control (CAMHB) for each plate were studied. The plates were incubated at 35±2°C for 16-20 hours in an incubator. Each experiment was repeated three times. The pattern of the checkerboard panel format is presented in Table 1.

Fractional Inhibition Concentration (FIC) index of both antimicrobial agents was calculated for interpretation of the results. According to formulas:

$FIC_A$=(MIC of A in combination/MIC of A alone)

$FIC_B$=(MIC of B in combination/MIC of B alone)

FIC index (FICI)=$FIC_A$+$FIC_B$

FICI≤0.5 was interpreted as synergism, 0.5<FICI≤4 was interpreted as indifference, and FICI> 4 was interpreted as antagonism (15).

**Measurement of Cell Membrane Damage**

Cell membrane damage was studied according to the method described by Devi et al. (16) with slight modifications. In this method, the presence of membrane damage is determined by measuring the amount of nucleic acid leaking through the membrane by UV-VIS spectrophotometer. Membrane damage measurements were performed on the MRSA-6 strain which the lowest MIC value of carvacrol was obtained. Initially, MRSA-6 strain was incubated overnight at 35±2°C in MHB. The bacterial culture was then centrifuged at 4000 g for 15 minutes, and the pellet was washed two times with PBS. The concentrations of 1xMIC, ½xMIC, ¼xMIC, 1/8xMIC of oxacillin and carvacrol, and the concentrations which determined to have synergistic effect while both agents were combined (1/4xMIC for carvacrol, 1/8xMIC for oxacillin) were added to the bacteria suspensions. The suspension containing only PBS and bacteria was used as control. All samples were incubated at 35±2°C for three hours. At the end of this period, all samples were centrifuged at 13400 g for 15 minutes and then the supernatant was removed. $OD_{260}$ of supernatant was measured using a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) to determine the amount of nucleic acids released from the cytoplasm. Measurements were performed in three replicates.

**Statistical Analysis**

The data was presented as the mean±SEM. Analysis was performed using a professional statistics software program (Graph Pad Software, San Diego, CA, USA). ANOVA with Bonferroni post-test for intergroup comparisons. The graphs were drawn using Sigma Plot version 10.0 (SPSS Inc., Chicago, IL, USA) software. $p<0.05$ was considered to be statistically significant.

## RESULTS

**Determination of MIC Values of the Antimicrobial Agents**

The broth microdilution test was performed to evaluate the antimicrobial activity of carvacrol on MRSA strains. Carvacrol showed antimicrobial activity against all tested *S. aureus* strains. The MIC values of carvacrol and oxacillin against ten MRSA clinical strains were found in the range of 64-256 µg/ml. The oxacillin MICs against *S. aureus* ATCC 43300 and *S. aureus* ATCC 29213 were determined as 64 µg/ml and 1 µg/ml, respectively. These values were found in the range of the MIC values determined by Clinical and Laboratory Standards Institute (CLSI). All MIC values obtained as a result of the broth microdilution test are given in Table 2.

**Table 2.** The results of the antibacterial activities of carvacrol, oxacillin and their combination against MRSA strains.

| Strains | MIC Results | | Checkerboard Synergy Test Results | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CAR (µg/ml) | OXA (µg/ml) | CAR in combination (µg/ml) | OXA in combination (µg/ml) | FIC CAR | FIC OXA | FICI | Interpretation |
| MRSA 1 | 128 | 128 | 32 | 16 | 0.25 | 0.125 | 0.375 | SYN |
| MRSA 2 | 128 | 128 | 32 | 32 | 0.25 | 0.25 | 0.5 | SYN |
| MRSA 3 | 256 | 128 | 128 | 32 | 0.5 | 0.25 | 0.75 | IND |
| MRSA 4 | 128 | 128 | 32 | 32 | 0.25 | 0.25 | 0.5 | SYN |
| MRSA 5 | 128 | 128 | 32 | 16 | 0.25 | 0.125 | 0.375 | SYN |
| MRSA 6 | 64 | 128 | 16 | 16 | 0.25 | 0.125 | 0.375 | SYN |
| MRSA 7 | 128 | 256 | 64 | 32 | 0.5 | 0.125 | 0.625 | IND |
| MRSA 8 | 256 | 128 | 128 | 16 | 0.5 | 0.125 | 0.625 | IND |
| MRSA 9 | 128 | 256 | 64 | 64 | 0.5 | 0.25 | 0.75 | IND |
| MRSA 10 | 128 | 128 | 32 | 16 | 0.25 | 0.125 | 0.375 | SYN |
| *S. aureus* ATCC 43300 | 128 | 64 | 32 | 8 | 0.25 | 0.125 | 0.375 | SYN |
| *S. aureus* ATCC 29213 | 128 | 1 | | | | | | |

MRSA: Meticillin resistance *S. aureus*, MIC: Minimum Inhibitory Concentration, FIC: Fractional Inhibition Concentration, FICI: Fractional Inhibition Concentration Index, CAR: Carvacrol, OXA: Oxacillin, SYN: Synergy, IND: Indiference

**Table 3.** Evaluation of the checkerboard test result of MRSA-6 strain.

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| | | CAR 4 | CAR 8 | CAR 16 | CAR 32 | CAR 64 | CAR 128 (MIC) | CAR 256 | CAR 512 |
| A | OXA 2 | | | | | | IND | | |
| B | OXA 4 | | | | | IND | | | |
| C | OXA 8 | | | | **SYN** | | | | |
| D | OXA 16 | | | | SYN | | | | |
| E | OXA 32 | | | IND | | | | | |
| F | OXA 64 (MIC) | IND | IND | IND | | | | | |
| G | OXA 128 | | | | | | | | |
| H | OXA 256 | | | | | | | | |

MIC: Minimum Inhibitory Concentration, CAR: Carvacrol, OXA: Oxacillin, SYN: Synergy, IND: Indiference, Shaded squares indicate wells showing bacterial growth. The synergy result in which the lowest FICI value calculated was evaluated.

**Evaluation of the Checkerboard Test Results**

The combination activity of carvacrol and oxacillin was studied against 11 MRSA strains including *S.aureus* ATCC 43300. The results of checkerboard synergy test are given in Table 2. According to the results, synergistic effect was found in seven of eleven MRSA strains with FICI values in the range of 0.375-0.5 and indifference effect was determined in four of them with FICI values in the range of 0.625-0.75. Carvacrol caused a four- to eight-fold reduction in oxacillin MICs against all strains. These results showed that the combination of carvacrol with oxacillin increased inhibition of MRSA strains. Evaluation of the checkerboard test result of MRSA-6 strain is presented in Table 3.

**Results of Membrane Damage Measurements**

The amounts of nucleic acids that leaked through the membrane were measured by UV-VIS spectrophotometer to determine if carvacrol causes cell membrane damage. The absorbance values of the bacterial supernatant is shown in Figure 1. As a result of measurements performed at different concentrations of carvacrol (1/8xMIC, 1/4xMIC, 1/2xMIC, 1xMIC), the absorbance

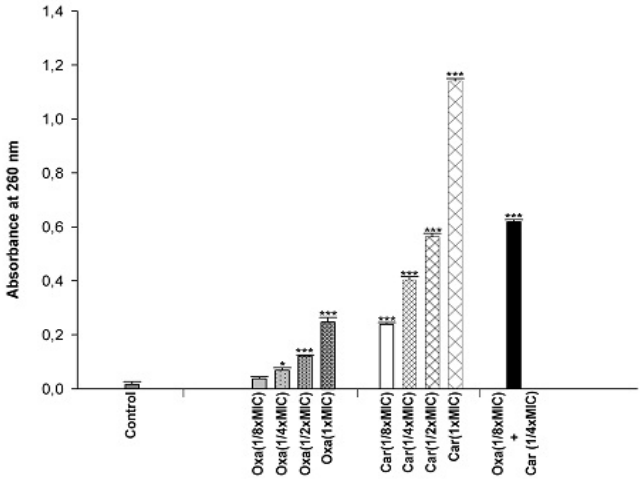


**Figure 1.** The presence of 260 nm absorbing materials in supernatants of MRSA-6 strain treated with different concentrations of carvacrol and oxacillin alone or in combination. The data is the avarage triplicates and *, ** and *** significance at the level of $p<0.05$, $p<0.01$ and $p<0.001$ respectively.

values were obtained as 0.26, 0.41, 0.56 and 1.14, respectively. When the concentration of carvacrol increases, $OD_{260}$ values were significantly increased ($p<0.001$).

OD values of oxacillin were lower than carvacrol (0.068, 0.12 and 0.24, respectively). The absorbance values of carvacrol (¼xMIC) plus oxacillin (1/8xMIC) combination were obtained as 0.62. When these values are compared with the OD values obtained at the same concentrations when both substances are alone, cell membrane damage was found to be significantly increased ($p<0.001$).

## DISCUSSION

The synergistic effect between plant-derived compounds and antibiotics can lead to the reuse of an antibiotic that is insufficient in treatment alone. Therefore, the importance of combination studies against resistant bacteria is increasing. *S. aureus* is one of the most investigated bacteria since it has recently become resistant to many antibiotics and therefore causes widespread serious infections worldwide. Many herbal compounds whose antibacterial activity and synergistic effect in combination with antibiotics against MRSA have been investigated to find a solution to the resistance problem (17-20). In these studies, it was found that herbal compounds such as epigallocatechin gallate, galangine, curcumin and punicalagin showed synergistic activity against MRSA. When epigallocatechin gallate was combined with β-lactam group antibiotics such as benzylpenicillin, ampicillin, oxacillin, methicillin and cephalexin, synergistic and additive effect was obtained with FICI values in the range of 0.126 to 0.625 (8). As a result of the combination of galangine and gentamicin, synergism against MRSA strains was detected with FICI values in the range of 0.19-0.25 (21). In another study, oxacillin, ampicillin, norfloxacin and ciprofloxacin were combined with curcumin and the MICs of the four antibiotics were determined to reduce by 2 to 128-fold (4). The combination of punicalagin and oxacillin showed high antimicrobial activity against MRSA strains and it was found that punicalagin caused four to eight times decrease in oxacillin MIC values (22).

Carvacrol is generally the major component in the essential oils of plants such as thyme and majoram. The high antimicrobial activities of these essential oils are usually attributed to their high carvacrol content. Carvacrol has been found to be effective against many bacteria including *S. aureus* in many studies (23-25). In addition, there are several studies investigating the combination activity of carvacrol in the literature. Carvacrol was combined with erythromycin against erythromycin-resistant Group A Streptococci and synergistic activity was detected in 21 of the 32 strains (26). The combination of carvacrol and nalidixic acid against nalidixic acid-resistant bacteria showed synergistic activity against five of the eight bacterial species (27). It was determined that the combination of colistin and carvacrol showed synergistic activity against 5 of the 8 colistin resistant *Acinetobacter baumannii* strains. (28). The combinations of ampicillin, penicillin and bacitracin with carvacrol demonstrated a synergistic activity against *S. aureus* (29). Based on the literature, we have not found any other study evaluating the combination activity of carvacrol with oxacillin against MRSA. As a result of our study, carvacrol has been found to exhibit synergistic activity against the majority of MRSA clinical strains when combined with oxacillin, and cause a decrease in oxacillin resistance.

Carvacrol was shown to cause membrane damage in MRSA-6 strain. Many studies have determined that carvacrol acts by causing membrane damage in the bacterial cell (23,25,30). In this study, the mechanism of antibacterial effect of carvacrol was confirmed once more. In addition, while carvacrol and oxacillin were combined, membrane damage was also increased. Due to the hydrophobic properties of carvacrol, it causes an icrease in the permeability and fluidity of the membrane structure by interacting with fatty acids (24). In addition, inhibition of ATPase activity, leakage of cell ions and reduction of proton motility are other mechanisms of its action (31). Beta-lactam antibiotics such as methicillin and oxacillin is known to inhibit bacterial cell-wall synthesis and the resistance of MRSA to these antibiotics occur through certain known mechanisms. (1,32). Combination of carvacrol with oxacillin may have caused synergistic effect due to its different targets. According to Langeveld et al. (31), most antibiotics have specific targets and in most cases it seems likely that synergy is due to multiple target effects. Palaniappan et al. (29) also say that the mechanism of natural antimicrobials for reducing the antibiotic resistance is not known precisely, but is probably due to some structural changes in resistant bacteria. For example, natural antimicrobials may have been effective by facilitating the drug's penetration through the outer layers of the bacterial cell wall or by blocking the inhibitory effects of protective enzymes or by interfering with single or multiple metabolic targets of the antibiotic.

## CONCLUSION

In conclusion, this study showed that carvacrol had antibacterial activity against MRSA strains and caused cell membrane damage. Oxacillin was found to be more effective against MRSA strains when combined with carvacrol. Carvacrol may be used in therapy to reduce oxacillin resistance in the future. But further studies are needed on this subject.

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