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Interaction of Selected Commercial Antiseptics with Natural Products against Methicillin-Resistant *Staphylococcus aureus* Strain

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Abstract: The carriage of methicillin-resistant *S. aureus* (MRSA) strains may determine the subsequent occurrence of infection, which mainly affects patients after surgeries. Therefore, its eradication with the use of antibiotics or antiseptics is a crucial method of preventing staphylococcal self-infections. The observed increase in bacterial resistance to known antibacterial substances poses a huge challenge to scientists. The aim of this study was to analyze the interaction of selected commercial antiseptics (Braunol, CITROclorex 2%, Microdacyn 60 Wound Care, Octenisept and Prontoderm Solution) with natural products (carvacrol, β-citronellol, geraniol, eugenol, farnesol, (-)-menthone, eucalyptol, limonene, linalyl acetate and *trans*-anethole) against the MRSA standard strain. The antistaphylococcal activity of commercial antiseptics in combination with natural products against MRSA was determined using the checkerboard method. The obtained results showed that most combinations decreased the MIC level of the commercial antiseptic and natural compounds. A statistically significant decrease in the MIC value of the antiseptic and natural constituent was observed for the Braunol–β-citronellol combination. Such a significant decrease in the MIC value of the natural compound against the analyzed strain was also detected for the Octenisept–β-citronellol and Prontoderm Solution–β-citronellol combinations. The interaction analysis showed that out of all 20 combinations of individual antiseptics with substances of natural origin, two combinations showed a synergistic effect (Braunol–β-citronellol, Braunol–carvacrol), and three combinations showed an additive effect (Braunol–eugenol, Braunol–geraniol, Prontoderm Solution–β-citronellol). The use of Braunol in combination with β-citronellol or carvacrol may become an alternative method of eradicating MRSA strains. However, further studies are needed to determine the toxicity of the analyzed combinations.

Keywords: MRSA; natural products; essential oil compounds; antiseptics; checkerboard method; synergistic activity

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1. Introduction

Staphylococcus aureus is responsible for many different infections of varying severity, including skin and soft tissue infection, bacteremia, endocarditis and pleurisy [1]. It is worth emphasizing that more and more scientific reports reveal increasing resistance of bacteria (including *S. aureus*) not only to antibiotics and chemotherapeutics, but also to currently available antiseptics [2]. The observed increase in bacterial resistance to known

antibacterial substances poses a huge challenge to scientists and has led them them seek new solutions. An interesting way to fight against resistant strains of *S. aureus* (e.g., in the treatment and care of infected postoperative wounds or in the eradication of bacterial carriage) may involve using an antiseptic in combination with natural products, including essential oils (EOs) and their main compounds, which, according to the available literature, are characterized by antimicrobial properties [3]. These substances often act synergistically with antiseptics and reduce doses or concentrations to effective levels which are necessary to achieve therapeutic effects [4]. The carriage of methicillin-resistant *S. aureus* (MRSA) strains may determine the subsequent occurrence of infection (so-called autoinfection), which mainly affects patients after surgeries, including cardiac and orthopedic ones. Therefore, their eradication with the use of antibiotics (e.g., intranasal mupirocin) or antiseptics (e.g., washing the entire body with chlorhexidine) is a crucial method of preventing staphylococcal self-infections in such patients [5]. It is worth emphasizing that infections caused by the same strain whose carriage was detected before the occurrence of *S. aureus* in the blood constitute the vast majority of staphylococcal bacteremias in carriers of this pathogen [6]. Infections of *S. aureus* etiology also include those occurring in postoperative patients, in whom the focus of infection depends on the surgical site (so-called surgical site infection—SSI). Interestingly, the nasopharyngeal carriage of *S. aureus* has been shown to be a significant risk factor for SSI. *S. aureus* is considered the most common etiological factor of postoperative mediastinitis [7].

Antiseptics are widely used in medicine to prevent the transmission of microorganisms and SSI and limit the colonization of patients and medical staff. They are available in various preparations, such as liquids with atomizers, gels for topical use, body washes or irrigation fluids. To ensure safety, these preparations should be highly effective and have low toxicity. A number of different preparations bear specific indications for use [8]. Povidone-iodine (PVP-I) is an aqueous solution of free iodine and polyvinylpyrrolidone [9]. PVP-I exhibits killing properties against Gram-positive (including MRSA strains) and Gram-negative bacteria, fungi, spores and viruses [10]. The bactericidal effect of PVP-I is achieved by adding free iodine to the amino group in amino acid and nucleic acid compounds. The thiol group in cysteine, which is an important sulfur amino acid, is also modified. Changes are also observed in the phenolic groups of amino acids and structures of fatty acids. In consequence, key enzymes are inhibited and structural proteins of the bacteria are destroyed, which, in turn, leads to the death of the microorganism. So far, no increasing resistance to PVP-I has been reported [11]. This compound is considered safe and is routinely used as an antiseptic in medical facilities (e.g., in the preparation called Braunol). Chlorhexidine digluconate (CHG) is a mixture of chlorhexidine and D-gluconic acid. Chlorhexidine, demonstrating antiseptic properties, is the main substance contained in the preparation. However, D-gluconic acid, which acts as a buffer, also exhibits antiseptic properties [12]. CHG is a positively charged molecule. Its mechanism of action involves interaction with negatively charged phospholipids of bacterial membranes, which results in the release of the main intracellular components, the occurrence of structural changes in proteins (precipitation) and cytolysis. Its activity is pH-dependent (the optimal pH for its action is 5.5–7.0). It has a killing effect against Gram-positive and Gram-negative bacteria, viruses and fungi, but it is not active against fungal spores [13]. There are scientific literature reports on the relationship between antibiotic resistance and CHG resistance. It has been proven that Gram-negative bacteria showing resistance to some antibiotics (e.g., imipenem, ciprofloxacin) may be resistant to CHG [14]. This compound is used in skin antiseptics (e.g., in the preparation called CITROclorex 2%) as well as in dentistry. It is considered the gold standard among mouthwashes with antibacterial properties [12]. Octenidine dihydrochloride (*N*,*N*′-(1,10 decanediyldi-1[4*H*]-pyridinyl-4-lidene) bis-(1-octanamino) dihydrochloride) (OCT) is a non-volatile and surface-active substance. OCT is a stable compound, operating at a pH range of 1.6–12.2 [15]. The mechanism of action of OCT is to bind to negatively charged structures of microorganisms, e.g., the bacterial envelope, the cell membrane, polysaccharides and phospholipids. A result of this combination is

cytolysis of the cell. OCT has a strong killing effect against Gram-positive and Gram-negative bacteria, fungi and enveloped viruses [16]. For a long time, bacteria have been susceptible to this compound; however, recent reports indicate possible adaptation of microorganisms to its high concentrations [17–19]. It is widely used to disinfect skin, mucous membranes and wounds, e.g., in the Octenisept preparation [20]. Polyhexanide biguanide (1-(diaminmethylidene)-2-hexylguanidine) (PHMB) is a positively charged compound that interacts with negatively charged bacterial structures. It also affects the operation of ion pumps, enzymes and cell receptors of microorganisms. PHMB is a colorless and odorless compound which is highly soluble in water and alcohol [21]. The killing spectrum of PHMB includes Gram-positive and Gram-negative bacteria, fungi, protozoa and the human immunodeficiency virus. However, the latest scientific data indicate gradual resistance of fungi and protozoa to PHMB [22]. This compound is considered safe and to have low toxicity compared to other antiseptics [23]. It is commonly used as an antiseptic in the medical and cosmetic industries (e.g., in Prontoderm Solution) and in the sanitization of recreational water [15]. Hypochlorous acid and other compounds, being sources of chlorine, are widely used in medicine as antiseptics (e.g., in Microdacyn 60 Wound Care) [24,25]. These compounds are commonly used in endodontics, but they are quite toxic to aquatic organisms [26]. EOs, being volatile compounds, characterized by a strong aroma, constitute a source of substances with antiseptic properties. They are secondary plant metabolites that can be obtained by various methods, e.g., steam distillation, extrusion, solvent extraction or cold absorption in fats [27]. EOs consist of mixtures of various chemical compounds, including ketones, alcohols, aldehydes, esters and terpenes. These compounds exhibit specific properties that are used in various industries [28]. The main ingredients of EOs include carvacrol, citronellol, geraniol, eugenol, farnesol, menthone, eucalyptol (1,8-cineole), limonene, linalyl acetate and *trans*-anethole [29–32].

The aim of this study was to analyze the interaction of selected commercial antiseptics (Braunol, CITROclorex 2%, Microdacyn 60 Wound Care, Octenisept and Prontoderm Solution) with natural products (carvacrol, β-citronellol, geraniol, eugenol, farnesol, (-)-menthone, eucalyptol, limonene, linalyl acetate and *trans*-anethole) against the *S. aureus* ATCC 43300 (MRSA) strain.

2. Materials and Methods

2.1. Bacterial Strain and Culture Conditions

In the current study, the *S. aureus* ATCC 43300 (MRSA) strain was used. The reference strain was cultivated in Columbia agar with 5% sheep blood (bioMérieux, Warsaw, Poland) and incubated at 37 °C for 18 h under aerobic conditions.

2.2. Tested Substances

The following five commercial antiseptics were used: Braunol (B. Braun Medical AG, Melsungen, Germany; PVP-I = 7.5 g/100 mL aqueous solution), CITROclorex 2% (Ecolab sp. z o.o., Cracow, Poland; CHG = 2 g/100 mL aqueous solution and ethyl alcohol = 73.6 g/100 mL aqueous solution), Microdacyn 60 Wound Care (Kikgel, Ujazd, Poland; hypochlorous acid = 40 ppm and sodium hypochlorite = 40 ppm), Octenisept (Schülke & Mayr GmbH, Norderstedt, Germany; OCT = 0.1 g/100 mL aqueous solution and phenoxyethanol = 2 g/100 mL aqueous solution) and Prontoderm Solution (B. Braun Medical AG, Melsungen, Germany; PHMB = 0.11% of liquid volume).

Moreover, the ten following natural products purchased from Merck KGaA (Darmstadt, Germany) were analyzed: carvacrol (≥98.0%), β-citronellol (≥95.0%), geraniol (≥98.0%), eugenol (≥98.0%), farnesol (≥95.0%), (-)-menthone (≥96.0%), eucalyptol (≥99.0%), limonene (≥97.0%), linalyl acetate (≥96.0%) and *trans*-anethole (≥99.0%). All tested substances were stored at 4 °C in dark glass bottles.

2.3. Dilutions of the Tested Substances

Mueller-Hinton broth (MHB, Merck KGaA, Darmstadt, Germany) was used to prepare dilutions of the tested substances. Due to hydrophobic properties of natural products, the tested compounds were dissolved in a non-ionic detergent—Tween 80 (Merck KGaA, Darmstadt, Germany)—at a concentration of 1% (*v*/*v*), which, according to the available literature, does not affect the growth of microorganisms [4]. Finally, concentrations from 50.0% to 0.02% (expressed in *v*/*v*, from 500 µL/mL down to 0.002 µL/mL) for all tested substances were obtained.

2.4. Determination of Effectiveness of the Tested Substances against MRSA Standard Strain

The MIC of the tested substances was determined using the microdilution method in a 96-well microplate in accordance with recommendations of the Clinical and Laboratory Standards Institute (CLSI) [33]. Briefly, after an 18 h incubation period at 37 °C, *S. aureus* colonies were suspended in phosphate-buffered saline solution (PBS) and adjusted to a concentration of 10^8 colony-forming units/mL (cfu/mL). Following CLSI guidelines, the suspension was diluted again in fresh MHB (1:150), obtaining a 10^6 cfu/mL concentration. Then, 50 µL of prepared microbial suspension was introduced into all the wells. The microplates were incubated for 18 h at 37 °C under aerobic conditions. After incubation, 20 µL of a resazurin sodium salt solution (Merck KGaA, Darmstadt, Germany), at a concentration of 0.02% (*w*/*v*), reduced by live bacteria to a resorufin [34], was added to all the wells. The plates were re-incubated for 2–3 h at 37 °C, and then, the obtained results were qualitatively assessed with the naked eye. A blue color in a particular well with the lowest active substance dilution corresponded to the MIC value. The study included a positive control (bacteria in MHB) and a negative control (MBH). The MIC of active substances was determined twice.

The MBC value (the lowest concentration of the active substance killing approx. 99.9% of microorganisms) was determined by transferring 50 µL of *S. aureus* culture at concentrations higher than MIC to plates containing 50 µL of fresh MHB. The plates were incubated for 18 h at 37 °C under aerobic conditions [35]. The absence of bacterial growth (no turbidity) in a particular well with the lowest active substance concentration was equivalent to the MBC value. The MBC of active substances was determined twice. In order to demonstrate the effectiveness of the commercial antiseptics and natural products, the MBC/MIC ratio was calculated. An MBC/MIC ratio ≤ 4 and an MBC/MIC ratio > 4 were defined as bactericidal and bacteriostatic activity, respectively [36].

2.5. Determination of the Effectiveness of Combined Tested Substances against MRSA Standard Strain

Determination of the antistaphylococcal activity of commercial antiseptics in combination with natural products (with MIC values $\leq$ 50.0%) against the *S. aureus* ATCC 43300 strain was performed using the checkerboard method in a 96-well microplate, according to the procedure described by Yap et al. [37] and modified by Kwiatkowski et al. [35]. For this purpose, 25 µL of appropriate dilutions of commercial antiseptics and 25 µL of appropriate dilutions of natural products were mixed. Next, 50 µL of microbial suspension (10^6 cfu/mL) was added to all wells. The microplates were incubated for 18 h at 37 °C under aerobic conditions. After incubation, the effectiveness of the mixed substances was assessed using resazurin and the results were qualitatively assessed with the naked eye. A blue color in a particular well with the lowest active dilution combinations corresponded to the MIC value of the combination. The effectiveness of the commercial antiseptic–natural product combination was determined to obtain the value of the fractional inhibitory concentration (FIC) and the fractional inhibitory concentration index of the mixture (FICI). For this purpose, the following formulas were applied:

$$\text{FIC of antiseptic} = \frac{\text{MIC of the antiseptic in combination}}{\text{MIC of the antiseptic alone}}, \quad (1)$$

$$\text{FIC of natural product} = \frac{\text{MIC of the natural product in combination}}{\text{MIC of the natural product alone}}, \quad (2)$$

$$\text{FICI} = \text{FIC of the antiseptic} + \text{FIC of the natural product}. \quad (3)$$

The results were interpreted as synergistic (FICI $\leq$ 0.5), additive (0.5 < FICI $\leq$ 1.0), no-effect (1.0 < FICI < 4.0) or antagonistic (FICI $\geq$ 4.0) [38].

2.6. Statistical Analysis

After performing the tests, data were analyzed by ANOVA followed by Dunn's multiple comparisons test using GraphPad Prism 8.0.1 (GraphPad Software Inc., San Diego, CA, USA). Differences with $p < 0.05$ were considered significant.

3. Results

3.1. Effectiveness of Tested Substances against MRSA Standard Strain

Based on the results, it was confirmed that the *S. aureus* ATCC 43300 strain was susceptible to four out of five tested commercial antiseptics. Among all substances, CITROclorex 2% and Microdacyn 60 Wound Care exhibited the best and the weakest antibacterial properties, respectively. Furthermore, it was observed that three out of four antiseptics which were active against the reference strain had a bacteriostatic effect.

It was also noted that the *S. aureus* ATCC 43300 strain was susceptible to five out of ten natural products. Of all the compounds, farnesol exhibited the best antistaphylococcal properties. Five out of ten tested natural agents (eucalyptol, limonene, linalyl acetate, (-)-menthone and *trans*-anethole) showed no antimicrobial properties (MIC > 50.0%). Furthermore, four out of five active substances of natural origin demonstrated a bactericidal effect against the reference strain of *S. aureus*. Detailed data regarding MIC, MBC, the MBC/MIC ratio and the effectiveness of the tested substances against the *S. aureus* ATCC 43300 strain are listed in Table 1.

Table 1. Analysis of results of the minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC), the MBC/MIC ratio and the effectiveness of tested substances against the *Staphylococcus aureus* (ATCC 43300) strain.

Substance	MIC (%)	MBC (%)	MBC/MIC Ratio	Effectiveness
Commercial antiseptics				
Braunol	6.25 ± 0.00	12.5 ± 0.00	2	BB
CITROclorex 2%	0.01 ± 0.00	0.10 ± 0.00	8	BS
Octenisept	0.20 ± 0.00	1.56 ± 0.00	8	BS
Prontoderm Solution	0.20 ± 0.00	1.17 ± 0.55	6	BS
Microdacyn 60 Wound Care	>50.0	>50.0	-	-
Natural products				
β-citronellol	6.25 ± 0.00	6.25 ± 0.00	1	BB
Eugenol	3.13 ± 0.00	3.13 ± 0.00	1	BB
Eucalyptol	>50.0	>50.0	-	-
Farnesol	0.10 ± 0.00	0.78 ± 0.00	8	BS
Geraniol	3.13 ± 0.00	3.13 ± 0.00	1	BB
Carvacrol	0.78 ± 0.00	0.78 ± 0.00	1	BB
Limonene	>50.0	>50.0	-	-
(-)-Menthone	>50.0	>50.0	-	-
Linalyl acetate	>50.0	>50.0	-	-
trans-Anethole	>50.0	>50.0	-	-

BB—bactericidal activity; BS—bacteriostatic activity.

3.2. Analysis of Interaction of Commercial Antiseptics with Natural Products against MRSA Standard Strain

The obtained results showed that four out of five Braunol–natural product combinations decreased the MIC level of the commercial antiseptic and natural compounds,

respectively. This was most noticeable in the Braunol–β-citronellol combination, for which a statistically significant decrease in the MIC value of the antiseptic (8-fold) and natural constituent (16-fold) was observed. Four out of five CITROclorex 2%–natural agent combinations contributed to a 2- or 5-fold increase in the MIC value of the antiseptic. Nevertheless, these combinations variably influenced the MIC value of natural ingredients, for which increased (n = 2/5) and decreased (n = 3/5) antibacterial activity was recorded, respectively. For three out of five Octenisept–natural agent combinations, a 2-fold increase in the MIC value of Octenisept in combination with eugenol, farnesol and carvacrol was found. Interestingly, in most cases (n = 4/5) of interactions, phytochemicals were characterized by higher antibacterial activity. This was most visible in the Octenisept–β-citronellol combination, where a statistically significant reduction (16-fold) in the MIC value of the natural ingredient against the analyzed strain was noted. The highest decrease (31-fold) in the MIC value of the natural ingredient was reported in the Prontoderm Solution–β-citronellol combination; these results were statistically significant ($p < 0.05$). It is also worth noting that in all commercial antiseptic–β-citronellol/geraniol/carvacrol combinations, a decrease in the MIC value of the natural substance was observed. Detailed data on the MIC assessment of antiseptics and natural agents analyzed alone and in combination are presented in Figure 1.

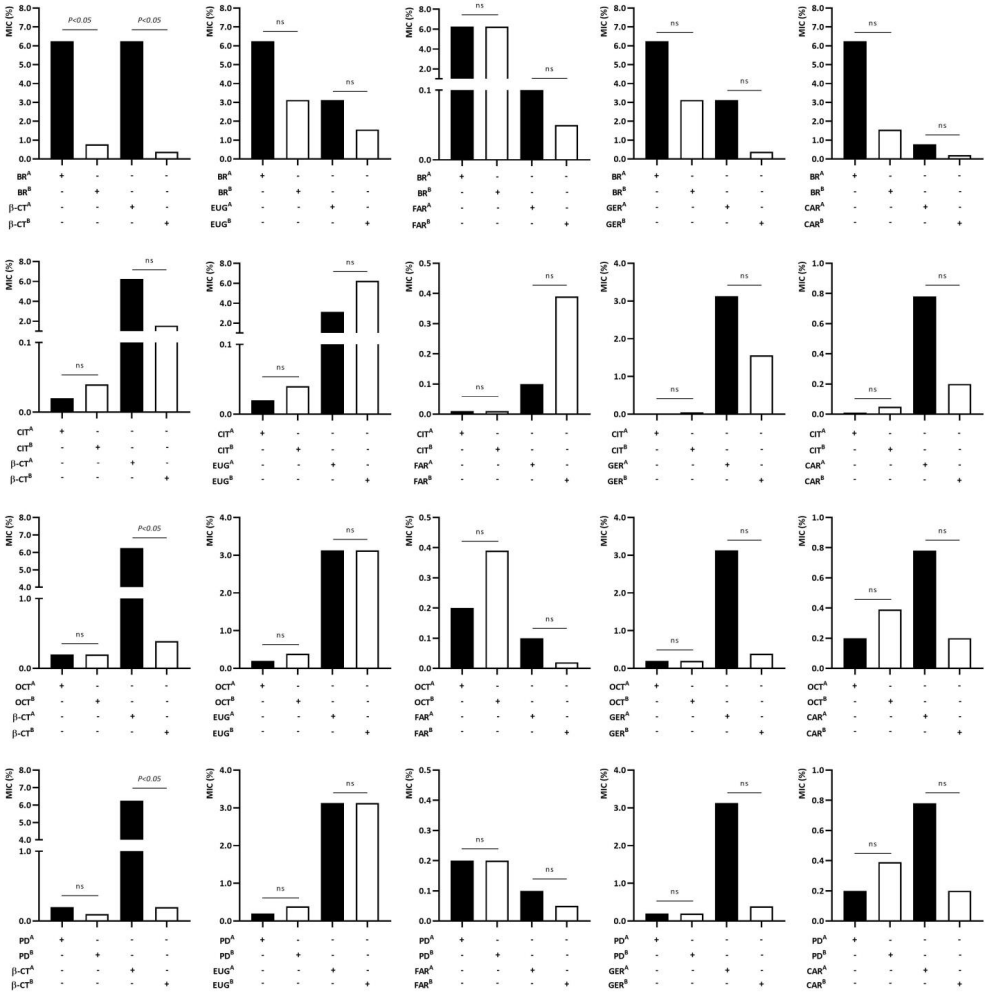

Figure 1. Evaluation of the minimum inhibitory concentration (MIC) of selected antiseptics and natural products analyzed alone (A, black bars) and in combination (B, white bars). Braunol—BR; CITROclorex 2%—CIT; Octenisept—OCT; Prontoderm Solution—PD; β-citronellol—β-CT; eugenol—EUG; farnesol—FAR, geraniol—GER; carvacrol—CAR; ns—not significant.

An interaction analysis showed that out of all 20 combinations of individual antiseptics with substances of natural origin, two combinations showed a synergistic effect (Braunol–β-citronellol, Braunol–carvacrol), three combinations showed an additive effect (Braunol–eugenol, Braunol–geraniol, Prontoderm Solution–β-citronellol), eleven combinations showed no effect (Braunol–farnesol, CITROclorex 2%–β-citronellol, Octenisept–β-citronellol, Octenisept–eugenol, Octenisept–farnesol, Octenisept–geraniol, Octenisept–carvacrol, Prontoderm Solution–eugenol, Prontoderm Solution–farnesol, Prontoderm Solution–geraniol, Prontoderm Solution–carvacrol) and four combinations showed antagonistic effects (CITROclorex 2%–eugenol, CITROclorex 2%–farnesol, CITROclorex 2%–geraniol, CITROclorex 2%–carvacrol). Detailed data on the FICI values for individual combinations are presented in Figure 2.

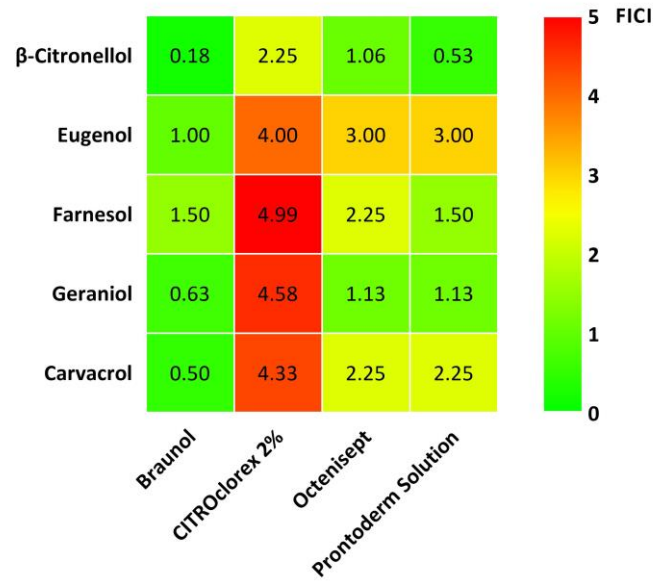

Figure 2. Fractional inhibitory concentration index (FICI) of combinations of selected commercial antiseptics and natural agents. FICI $\leq$ 0.5—synergistic effect; 0.5 < FICI $\leq$ 1.0—additive effect; 1.0 < FICI < 4.0—no effect; FICI $\geq$ 4.0—antagonistic effect.

4. Discussion

MRSA strains, which are also characterized by multidrug resistance, may develop cross-resistance to antiseptics [2]. Scientific literature reports mainly focus on the occurrence of cross-resistance between CHG and benzalkonium chloride [39]. However, recently, there have been reports of bacteria acquiring resistance to other antiseptics, e.g., PHMB or OCT, which were considered to be the so-called golden remedies" in wound antisepsis [17,40,41]. Antiseptics are widely used in medical care. They are used by hospitals, clinics, private specialist medical practices, dental offices and also beauty salons. Therefore, emerging antiseptic resistance should be constantly monitored. It is necessary to develop new formulations with antimicrobial activity, including natural-based compounds exhibiting other beneficial properties, such as immunostimulatory or anti-inflammatory.

Our studies confirmed that the *S. aureus* ATCC 43300 (MRSA) strain was susceptible to four (Braunol, CITROclorex 2%, Octenisept and Prontoderm Solution) out of five tested antiseptics. MIC testing, using the microdilution method, did not reveal susceptibility of the tested strain to Microdacyn 60 Wound Care, which contains hypochlorous acid (40 ppm) and sodium hypochlorite (40 ppm). This lack of antibacterial activity does not necessarily mean bacterial resistance to the active compounds in the analyzed preparation, but may be related to its killing activity at higher concentrations (>50.0%). Similar observations were described in a study by Dydak et al. [42], who confirmed that Microdacyn 60 Wound Care was inactive against *S. aureus* at concentrations ranging between 50% and 0.1%. Moreover, literature reports reveal that the antibacterial effect of chlorine-releasing compounds depends on the concentration of Cl ions in the antiseptic [43]. Severing et al. [44] proved

a lack of antimicrobial activity in compounds releasing chlorine at a concentration of <670 ppm. It is worth emphasizing that Microdacyn 60 Wound Care is characterized by a total concentration of Cl ions of 80 ppm.

There are many literature reports on the effect of PVP-I, whose MIC against MRSA strains is varied and usually ranges between 256 µg/mL and 5000 µg/mL [45–47]. However, there are few reports on the effects of Braunol itself, which contains 7.5 g of povidone iodine per 100 mL of solution. Studies conducted by Hirsch et al. [48] showed that the MIC of Braunol against the methicillin-susceptible *S. aureus* ATCC 29213 (MSSA) strain was 4%. Their result was similar to that obtained in this study, where the MIC and MBC values against the MRSA strain were 6.25% and 12.5%, respectively.

There are no reports in the scientific literature on the MIC value of CITROclorex 2% (which contains CHG at a concentration of 2 g/100 mL of solution and ethyl alcohol at a concentration of 73.6 g/100 mL of solution) against *S. aureus* strains. Information on the action of this preparation against bacteria (including strains with the MRSA phenotype) is only provided by producers and distributors of this antiseptic in the product information sheet. Based on the conducted research, it was found that this preparation was characterized by the best antistaphylococcal properties (MIC = 0.01%, MBC = 0.10%), which may indicate the effective action of the combination of CHG with ethyl alcohol against the tested MRSA strain. Interestingly, this observation was also confirmed in a study conducted by Mulberry et al. [49]. The authors noted that the combination of 1% CHG and 61% ethyl alcohol contributed to a more significant reduction in microorganisms than 4% CHG (without ethyl alcohol) or 61% ethyl alcohol (without CHG). Nevertheless, it is worth pointing out that CHG demonstrates highly effective antibacterial properties against MRSA strains, and its MIC values range from ≤0.01% to 0.8% [50–52]. Unfortunately, due to the increasing and significant resistance of bacteria to this compound, its use should be constantly monitored [53]. Ethyl alcohol is the second most active substance in CITROclorex 2%. It has been proven that this compound achieves the best antibacterial effect at a concentration of 60–85% [54]. Moreover, Rozman et al. [55] observed in their study that the MIC value of an antiseptic preparation (containing ethanol at a concentration of 45 g/100 g of solution) against staphylococci was 225 µg/mL, which confirms its weaker properties alone than in combination with CHG.

OCT, being a moderately toxic antiseptic, is used in the local treatment of infections (e.g., postoperative wounds, bacterial vaginosis in pregnant women, nail infections or acne) [56]. The quick onset of action (as soon as after 60 s) is its great advantage. However, there are literature reports indicating its increasing resistance and toxicity. One such study was carried out by Bock et al. [17], who proved that *Pseudomonas aeruginosa* can develop resistance to high concentrations of OCT (even 16× or 256× higher MIC values than the initial concentrations), which is associated, among other factors, with mutations in the *smvR*, *psssA* and *pgsA* genes. Moreover, research conducted by Hirsch et al. [48] suggests that Octenisept exhibits strong toxicity (at 10.0% and 12.5% dilutions) towards keratinocytes and fibroblasts isolated from human skin. Nevertheless, such high dilutions of Octenisept may play an important killing role against bacterial biofilm, the destruction of which usually requires higher doses of the active agent. This was noticed, among other studies, in the research of Krasowski et al. [43], which revealed that a solution based on OCT at a dilution of 12.5% destroyed the biofilm produced by *S. aureus*. However, in the professional literature, there are a lot of data on much lower MIC and MBC values obtained for Octenisept and its main compounds (OCT and phenoxyethanol) against *S. aureus*. These values usually range between 0.20% and 0.78% [47,48,51] and are similar to the results obtained in the present study (MIC = 0.20 ± 0.00%, MBC = 1.56 ± 0.00%).

Prontoderm Solution is an antiseptic used mainly in wound care, especially in the treatment of second-degree burns. Its main component is PHMB, the mechanism of which involves binding to negatively charged structures of bacteria. It affects many cellular elements, such as the cell wall and membrane, ion pumps and enzymes [23]. According to the scientific literature, PHMB has antistaphylococcal properties (including against MSSA

and MRSA strains), at MIC values ranging from 0.1% to 0.2% [47,57]. These results are similar to those obtained in the present study, in which the MIC and MBC values against the MRSA strain were 0.20 ± 0.00% and 1.17 ± 0.55%, respectively.

EOs are commonly used all over the world. According to the World Health Organization, some nations use plant-based treatments as the main therapy, and developing countries take advantage of this trend and the benefits of natural compounds for therapeutic purposes [58,59]. The selection of essential oil constituents in this study was not accidental. They represent the predominant ingredients of thyme (carvacrol), clove (eugenol), eucalyptus (eucalyptol), peppermint (menthone), lavender (linalyl acetate, citronellol), geranium (geraniol, citronellol), rose (farnesol, citronellol) and fennel (*trans*-anethole, limonene) EOs, which are described in the European Pharmacopoeia and the Polish Pharmacopoeia. Farnesol demonstrated the best antibacterial properties against the analyzed strain (MIC = 0.10 ± 0.00%; MBC = 0.78 ± 0.00%). Yet, only this compound exhibited bacteriostatic properties. Slightly higher MIC results (2.6%) of farnesol were obtained in a study carried out by Bonikowski et al. [60]. The researchers analyzed farnesol and its antibacterial properties against the *S. aureus* ATCC 43300 strain. However, due to differences in the applied methodology, their results may differ from those obtained in this study. In a study conducted by Bonikowski et al. [60], the initial bacterial concentration was 10^8 cfu/mL, while in this study, the concentration was 10^6 cfu/mL (according to the CLSI recommendations). With regard to eugenol (MIC and MBC = 3.13%) and geraniol (MIC and MBC = 3.13%), similar antibacterial properties were recorded against the analyzed standard strain. According to scientific literature, the MIC and MBC values for these compounds against *S. aureus* strains (including MRSA strains) are similar and range from 1.0% to 3.5% [61–64]. Slightly higher and lower MIC and MBC values were observed for the antibacterial activity of β-citronellol and carvacrol. They were 6.25% and 0.78%, respectively. However, these results differ from those obtained by other researchers, i.e., the MIC and MBC against the tested MRSA strains were estimated to be higher than 12.5% [65–68].

A synergistic effect against the analyzed reference strain was noticed for Braunol with β-citronellol (FICI = 0.18) or carvacrol (FICI = 0.50). In turn, an additive effect was found for the combination of Braunol with eugenol (FICI = 1.00) or geraniol (FICI = 0.63) and Prontoderm Solution with β-citronellol (FICI = 0.53). There are no literature studies on the potential synergistic and additive effects of Braunol (including PVP-I) or Prontoderm Solution (including PHMB) with phytochemicals. These data mainly concern the synergistic impact of, among others, PVP-I with antiseptics (e.g., CHG [69]) or antibiotics (e.g., vancomycin [70]), as well as PHMB with biocides (e.g., bronopol or chlorocresol, i.e., compounds used as preservatives in cosmetics) [71]. The mechanism of action of PVP-I involves the release of free iodine, which then binds to the amino groups of amino acids and disrupts the protein structure [72]. In turn, PHMB acts on ion pumps, enzymes and cell receptors [21]. β-citronellol and carvacrol disrupt the structure of proteins and impair the fluidity of the cell membrane, its permeability and its osmoregulatory ability [68]. Eugenol inhibits lipid peroxidation [73], while geraniol penetrates the cell membrane and interacts with intracellular proteins [74]. Therefore, the observed synergistic and additive effects of these compounds may enhance their destructive nature towards proteins. Nevertheless, further research is required to prove the molecular mechanism of action of these compounds in combination. These studies confirm the fact that the substances of origin have antibacterial properties against MRSA strains, and their combination with selected antiseptics may enhance each other. The direct treatment or care of infected wounds with Braunol in combination with β-citronellol or carvacrol may become an alternative way to fight MRSA infections. This is extremely important, considering the fact that wound infections are also recurrent, e.g., in patients with diabetic foot or after surgery. Such combinations may inhibit resistance to commonly used biocides. However, further studies should be carried out to prove the safety of these substances in combination and a possible molecular mechanism of action against MRSA strains.

Among the ingredients of EOs, the strongest effects are those of phenolic compounds such as thymol, carvacrol and eugenol. Eugenol is used in dentistry where it may come into contact with mucous membranes through its release from root canal sealers [75]. Slamenova et al. [76] found that the mild cytotoxic effect of carvacrol and thymol (inhibitory concentration—$IC_{20–40}$) on human hepatoma or colonic cells had no DNA-damaging effect; moreover, it reduced the level of DNA lesions. Thus, in the next study, we also need to examine the cytotoxic effects of the proposed combinations of biocides and EO components. Additionally, the obtained results support the search for the synergistic effect of substances of natural origin with antiseptics not only against Gram-positive bacteria, but also against Gram-negative bacteria. The latter, compared to Gram-positive bacteria, far more frequently demonstrate resistance to antibacterial agents.

5. Conclusions

CITROclorex 2%, containing CHG and ethyl alcohol solutions, exhibited the best antibacterial activity against the *S. aureus* ATCC 43300 (MRSA) strain. Of the ten natural agents, β-citronellol, eugenol, farnesol, geraniol and carvacrol were effective against the MRSA strain. A synergistic effect against the MRSA strain was found for the Braunol–β-citronellol and Braunol–carvacrol combinations. The application of Braunol in combination with β-citronellol or carvacrol may become an alternative method of eradicating MRSA strains. Yet, further research is required to confirm this solution against a representative group of clinical MRSA strains from difficult-to-treat infections. It is also necessary to carry out tests regarding the cytotoxic and allergenic effects of the proposed combinations.

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