Journal of Research in Pharmacy

Research Article

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Investigation of antimicrobial and antibiofilm activity of *Curcuma longa* L. extracts against methicillin-resistant *Staphylococcus aureus* (MRSA) isolates

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Received: 25 July 2024 / Revised: 13 September 2024 / Accepted: 13 September 2024

ABSTRACT: New antibacterial strategies are needed in response to increasing antibiotic resistance. Methicillin-resistant *Staphylococcus aureus* infections are serious problem in many countries. Morbidity and mortality due to MRSA have increased over time worldwide. Natural products continue to be a notable source for development of new antibacterial agents. *Curcuma longa* L. products and active constituents have been shown to exhibit remarkable bioactivity. While most studies have focused on curcumin alone, a comparative study on *Curcuma longa* extracts with solvents of differing polarity is needed to determine its potential against MRSA isolates and strains. In this study, antibacterial, antifungal and antibiofilm activity of hexane, dichloromethane, ethanol and water extracts of turmeric rhizomes were assessed against clinical isolates and standard strains. The hexane (MIC: 32 µg/ml, inhibition zone: 10 mm) and dichloromethane extracts (MIC: 32 µg/ml, inhibition zone: 10 mm) exhibited highest activity against clinical isolates. For standard strains of *S. aureus*, MIC values were determined to be 64 µg/ml and 128 µg/ml with an inhibition zone of 11 mm for hexane and dichloromethane extracts, respectively. Both extracts reduced total protein levels in clinical isolates. At MIC values, hexane extract was able to inhibit biofilm formation, whereas dichloromethane extract was not. Against standard strains of *E. coli* and *C. albicans*, highest activity was also determined with hexane and dichloromethane extracts with a MIC value of 64 µg/ml. Although dichloromethane or ethanol extracts have been the main interest to date, hexane extract from turmeric rhizomes may also be evaluated for further research against MRSA.

KEYWORDS: Antimicrobial; biofilm; *Curcuma longa*; MRSA; turmeric.

1. INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) became critical epidemiological issue of hospitals across the world since 1980s. Hospitals of all sizes have been dealing with deadly MRSA infections. Aside from nosocomial infections, MRSA is a deadly agent that has begun to infect healthy children and adults in the community. The risk of infection is increased by *S. aureus* and MRSA's spread and existence in the nose. [1]. *S. aureus* strains are microorganisms that cause serious illnesses as life-threatening infections (bacteraemia, pneumonia, endocarditis) [2]. The infection site and host immunity differences lead to various outcomes ranging from localized infections to serious and invasive infections [3].

High rates of resistance to several antimicrobial drugs are observed in *S. aureus* strains. Distinct virulence factors were determined. These virulence factors have key role in bacterial pathogenesis and enable them to evade host's defense mechanisms [3]. While virulence factors are assumed to be the only cause in certain situations where *S. aureus* is the causal agent, in many cases several virulence factors are thought to impact the course of infection in combination [4]. Biofilms are communities of microorganisms in the "slime" layer formed by bacteria and other microorganisms on foreign surfaces and include monosaccharides, proteins and small peptides. It is also seen in infections caused by catheters, grafts, shunts, prosthetic valves and joints [1]. *S. aureus* increases drug resistance and complicates treatment by forming

How to cite this article: Koyu H, Ozturk I, Tekintas Y, Yurtsever SG. Investigation of antimicrobial and antibiofilm activity of Curcuma longa L. extracts against methicillin-resistant Staphylococcus aureus (MRSA) isolates. J Res Pharm. 2025; 29(3): 1122-1133.

ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1694295

matrix-coated biofilm in tissues and medical devices [5]. Another *S. aureus* virulence factor is staphylococcal protein A. Protein A is bound to the peptidoglycan or cytoplasmic membrane and has specific affinity for the Fc portions of different immunoglobulins (IgG1, IgG2 and IgG4). The binding of protein A to immunoglobulins protects *S. aureus* against antibody-dependent immune clearance [1]. Regulatory genes such as *agr* (accessory gene regulator), and *sar* (staphylococcal accessory regulator) control virulence factors. Global regulatory genes coordinate expression of various staphylococcal genes. Expression ratios of different genes can be clinically important, where varying virulence determinants are determined at different stages of *Staphylococci* infections [6].

Novel antibiotics are increasingly needed to treat infections caused by multidrug-resistant bacteria, such as vancomycin-resistant Staphylococcus aureus (VRSA), MRSA, and Enterobacteriaceae members, that shows resistance to practically all antibacterial medications currently on the market. The COVID-19 pandemic has resulted in a greater clinical usage of antibiotics overall, which has accelerated the evolution of bacterial resistance. This underscores the unfulfilled medical need for novel medicines. Natural products have been a key component in the creation of most antibiotic medications now in clinical use since the mid-1900s, which was described as the golden era of antibiotic discovery. Antibiotics of natural origin work by directly preventing the development of bacteria or eliminating them; they can also function as immunomodulators to protect host cells from pathogenicity, or as potentiators that enhance or change other agents [7].

Curcuma longa L. (turmeric) belongs to the Zingiberaceae family. Turmeric has been grown in tropical and subtropical climates. Turmeric is a native of India and Southeast Asia, which is commonly benefited for cuisine, flavoring, and medicine. Numerous activity investigations addressing antioxidant, anti-inflammatory, antiproliferative, anticancer, and antiaging properties have examined the medicinal potential of turmeric. It has traditionally been employed to treat skin conditions, colds, ulcers, and parasite infections [7, 8]. Turmeric poultices are used in traditional Indian medicine against skin conditions, bites, burns, acne, and common eye infections. Additionally, the poultice is used to help healing of birth canal lacerations. Turmeric powder is eaten with boiled milk to treat respiratory conditions including coughs, while roasted turmeric is used ~~as~~ against dysentery in children. In addition, it is used to treat ulcers, indigestion, gas, dyspepsia, and acidity in the stomach [9]. Besides India, turmeric has been used with various purposes in many other countries. It has been used in drinks in Japan and Korea; utilized for the manufacturing of cosmetics in Thailand and dyes in China; benefited as an antiseptic in Malaysia and as an antiinflammatory agent in Pakistan[10]. Turmeric rhizomes are composed of carbohydrates (69%), proteins (6%), fats (5%), minerals (4%), and moisture (13%). The main constituents are curcuminoids as curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Although the studies have focused mainly on curcumin, other curcuminoids have also been shown to possess biological activities. Curcumin is soluble in acetone, methanol, ethanol, but is not soluble in water [11].

The need for novel antibacterial approaches has arisen from the fast development of antibiotic resistance and a lack of medications in treating drug-resistant bacterial illnesses. Natural products continue to be one of the major fields for developing new antibacterials and have proven to be effective treatments against bacterial infections [12].

A comparative investigation for antimicrobial and antibiofilm activity of turmeric extracts against standard bacterial and fungal strains, and clinical isolates of MRSA were aimed in this study.

2. RESULT

2.1. Resistance profiles of the isolates

The resistance profiles of 81 out of 100 isolates were determined using the BD Phoenix device. The resistance profiles were shown with Table 1 and 2.

81 isolates were found resistant to penicillin and studies were continued with these MRSA isolates. A total of 12 antibiotic drugs were used in antibiotic susceptibility tests. In addition to penicillin resistance, 81 isolates (100%) were found resistant to cefoxitin, 48 (59%) to erythromycin, 40 (49%) to tetracycline, 37 (46%) to levofloxacin, 33 (41%) to rifampicin, 32 (39%) to clindamycin, 30 (37%) to gentamicin and 24 (30%) to trimethoprim/sulfamethoxazole. No resistance to linezolid, vancomycin and teicoplanin was observed. The antibiotic susceptibility test results were presented with Table 2, where among 81 MRSA isolates, 45 were determined with resistance against 5 or more antibiotics. As a representative of the each antibiotic resistant group presented with Table 2, 8 isolates were selected for antimicrobial and antibiofilm activity studies. All

strains were categorised into 8 subgroups based on their antibiotic resistance/sensitivity profiles, as determined by the results of the antibiotic susceptibility testing.

Table 1. Resistance profile of 81 bacterial isolates

Antibiotics	Susceptible	Intermediate	Resistant	Not Determined	Resistance Ratio
Penicillin	-	-	81	-	100%
Cefoxitin	-	-	81	-	100%
Clindamycin	49	-	32	-	39.5%
Erythromycin	32	1	48	-	59.3%
Tetracycline	41	-	40	-	49.4%
Trimethoprim/ Sulfamethoxazole	51	-	24	6	29.6%
Levofloxacin	42	1	37	1	45.7%
Rifampicin	40	1	33	7	40.7%
Gentamicin	49	2	30	-	37.0%
Linezolid	81	-	-	-	0.0%
Vancomycin	81	-	-	-	0.0%
Teicoplanin	81	-	-	-	0.0%

Table 2. Number of isolates determined with antibiotic resistance

Resistance Profile	Number of isolates
9 antibiotics	5 isolates
8 antibiotics	5 isolates
7 antibiotics	13 isolates
6 antibiotics	8 isolates
5 antibiotics	14 isolates
4 antibiotics	10 isolates
3 antibiotics	8 isolates
2 antibiotics	18 isolates
1 antibiotic	-

* Isolates with intermediate susceptibility were considered resistant.
* The resistance profiles of 81 isolates were presented with Supplementary.

2.2. Antimicrobial activity studies

2.2.1. Disk diffusion test

The mean inhibition zone diameters provided by *Curcuma longa* extracts against MRSA isolates and standard strains were determined with disc diffusion test (Table 3 and 4). The inhibition zone diameters were found as ranging 9.00mm to 10.00mm for hexane extract, 9.00mm to 11.00mm for dichloromethane extract, 10.00mm to 11.00mm for ethanol extract and 7.67mm to 8.00mm for water extract. While hexane, dichloromethane and ethanol extracts were determined with inhibition zones against all 8 MRSA isolates, water extract was not observed to produce inhibition zone against 3 isolates.

In disc diffusion tests, different levels of antimicrobial effect against standard strains of bacteria and fungi were also determined with *Curcuma longa* extracts. Inhibition zone diameters were determined as 11.00mm, 12.00mm and 13.00mm with hexane extract, 11.00mm, 11.00mm and 12.00mm with dichloromethane extract, 10.00mm, 11.00mm and 10.00mm with ethanol extract, and 8.00mm, 8.00mm and 8.00mm with water extract against standard strains of *S. aureus*, *E. coli* and *C. albicans*, respectively (Table 4).

2.2.2. Microdilution method

The minimum inhibitory concentration (MIC) values exhibited by *Curcuma longa* extracts against MRSA isolates and standard strains were determined with microdilution method (Table 3 and 4). MIC values were found as ranging between 32 to 1024 µg/ml for hexane extract, 32 to 128 µg/ml for dichloromethane and ethanol extracts, and 512 to >1024 µg/ml for water extract.

Regarding the standard strains, MIC values were determined as 64-64-64 µg/ml for hexane extract, 128-64-64 µg/ml for dichloromethane extract, 128-64-128 µg/ml for ethanol extract, >1024->1024->1024 µg/ml for water extract for *S. aureus*, *E. coli*, *C. albicans*, respectively. To determine if the antimicrobial activity was specific to Gram-positive bacteria, *E. coli* and *C. albicans* were included as a representative of Gram-negative bacteria and yeast.

Table 3. Inhibition zone diameter (mm) and minimum inhibitory concentrations (µg/ml) obtained with *Curcuma longa* extracts against clinical MRSA isolates

Extracts	MRSA-No1	MRSA-No2	MRSA-No3	MRSA-No4	MRSA-No5	MRSA-No6	MRSA-No7	MRSA-No8
Hexane	9.00±0.00 1024 µg/ml	10.00±0.00 1024 µg/ml	10.00±0.00 1024 µg/ml	9.00±0.00 512 µg/ml	9.00±0.00 512 µg/ml	9.00±0.00 1024 µg/ml	10.00±0.00 32 µg/ml	10.00±0.00 1024 µg/ml
Dichloromethane	10.00±0.00 128 µg/ml	11.00±0.00 64 µg/ml	11.00±0.00 32 µg/ml	11.00±0.00 32 µg/ml	10.00±0.00 32 µg/ml	10.00±0.00 64 µg/ml	10.00±0.00 32 µg/ml	9.00±0.00 64 µg/ml
Ethanol	10.00±0.00 128 µg/ml	11.00±0.00 64 µg/ml	11.00±0.00 64 µg/ml	10.00±0.00 64 µg/ml	11.00±0.00 32 µg/ml	11.00±0.00 128 µg/ml	11.00±0.00 32 µg/ml	11.00±0.00 128 µg/ml
Water	8.00±0.00 >1024 µg/ml	7.67±0.58 512 µg/ml	8.00±0.00 512 µg/ml	ND 1024 µg/ml	8.00±0.00 1024 µg/ml	ND 512 µg/ml	ND 1024 µg/ml	8.00±0.00 1024 µg/ml

ND: Inhibition zone diameter not determined

Table 4. Inhibition zone diameter (mm) and minimum inhibitory concentrations (µg/ml) obtained with *Curcuma longa* extracts against standard strains

Extracts	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
Hexane	11.00±0.00 mm 64 µg/ml	12.00±0.00 mm 64 µg/ml	13.00±0.00 mm 64 µg/ml
Dichloromethane	11.00±0.00 mm 128 µg/ml	11.00±0.00 mm 64 µg/ml	12.00±0.00 mm 64 µg/ml
Ethanol	10.00±0.00 mm 128 µg/ml	11.00±0.00 mm 64 µg/ml	10.00±0.00 mm 128 µg/ml
Water	8.00±0.00 mm >1024 µg/ml	8.00±0.00 mm >1024 µg/ml	8.00±0.00 mm >1024 µg/ml

2.3. Antibiofilm activity studies

2.3.1. Biofilm production capacity of the microorganisms

The biofilm production capacities of MRSA isolates were investigated, where 5 MRSA isolates (MRSA-1, 2, 3, 4, 5) were found to produce strong biofilm and 3 MRSA isolates produced moderate biofilm (MRSA-6, 7, 8). Among the standard strains, Gram-positive bacteria *S. aureus* produced moderate biofilm, while *E. coli* and *C. albicans* produced low biofilm.

2.3.2. Antibiofilm activity tests

In experiments with *Curcuma longa* extracts, biofilm formation by *S. aureus*, *E. coli* and *C. albicans* were inhibited as 30.6%, 22.5% and 30.8%, respectively, in the presence of hexane extract. The water extract also inhibited biofilm formation by standard strains (Figure 1 and 2).

Similarly, hexane extract inhibited the biofilm production capacity of all 8 MRSA isolates, whereas water extract suppressed biofilm production levels of 5 isolates. In addition to inhibiting biofilm production levels in MRSA isolates, some extracts were shown to induce biofilm production at MIC levels; dichloromethane and ethanol extracts induced biofilm production in 7 isolates. Dichloromethane and ethanol extracts had no effect on biofilm production in one of the isolates, while water extracts exhibited no effect on 3 isolates.

Extracts	MRSA-No1	MRSA-No2	MRSA-No3	MRSA-No4	MRSA-No5	MRSA-No6	MRSA-No7	MRSA-No8
Hexane	↓	↓	↓	↓	↓	↓	↓	↓
Dichloromethane	↑	↑	-	↑	↑	↑	↑	↑
Ethanol	↑	↑	-	↑	↑	↑	↑	↑
Water	↓	-	↓	↓	↓	↓	-	-

(↓): Biofilm inhibition
(↑): Biofilm induction
(-): No effect

Figure 1. Activity of *Curcuma longa* extracts on biofilm formation in MRSA isolates

Extracts	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
Hexane	↓	↓	↓
Dichloromethane	-	-	-
Ethanol	-	-	-
Water	↓	↓	↓

(↓): Biofilm inhibition
(↑): Biofilm induction
(-): No effect

Figure 2. Activity of *Curcuma longa* extracts on biofilm formation in standard strains

2.4. Polymerase chain reaction studies

As a result of PCR findings, the isolates carrying the investigated gene regions belonging to different virulence factors were shown in Figure 3. Six gene regions (emp-clfA-clfB-fnbpA-fnbpB-cna) investigated in MRSA isolates were found positive in all 8 isolates. Three gene regions (*eap*, *icaD* and *fib*) of different virulence factors were not detected in MRSA isolates.

Gene region	MRSA-No1	MRSA-No2	MRSA-No3	MRSA-No4	MRSA-No5	MRSA-No6	MRSA-No7	MRSA-No8
eap	-	-	-	-	-	-	-	-
emp	+	+	+	+	+	+	+	+
icaD	-	-	-	-	-	-	-	-
clfA	+	+	+	+	+	+	+	+
clfB	+	+	+	+	+	+	+	+
fnbpA	+	+	+	+	+	+	+	+
fnbpB	+	+	+	+	+	+	+	+
cna	+	+	+	+	+	+	+	+
fib	-	-	-	-	-	-	-	-

eap/ map: extracellular bindingprotein gene, emp: extracellular matrix bindingprotein, icaD/pia: polysaccharide intercellular adhesin, clfA: clamp factors A, clfB: clamp factors B, fnbpA: fibronectin bindingproteinsA, fnbpB: fibronectin bindingproteinsB, cna: collagen bindingprotein, fib: fibrinogen bindingprotein
(+): Gene region determined, (-): Gene region not determined

Figure 3. Polymerase chain reaction results

2.5. Protein content analysis of MRSA isolates

The findings presenting the changes in protein amounts in MRSA isolates after exposure to *Curcuma longa* extracts were shown with Figure 4. As a result of the comparison of the groups exposed (experimental group) and not exposed (untreated group) to extracts, it was determined that dichloromethane extract decreased the total protein amount in all MRSA isolates studied. Hexane extract suppressed the total protein amount in 7 isolates.

Extracts	MRSA-No1	MRSA-No2	MRSA-No3	MRSA-No4	MRSA-No5	MRSA-No6	MRSA-No7	MRSA-No8
Hexane	-	↓	↓	↓	↓	↓	↓	↓
Dichloromethane	↓	↓	↓	↓	↓	↓	↓	↓

(↓): Total protein inhibition

(-): No effect

Figure 4. Effect of *Curcuma longa* extracts on total protein content

3. DISCUSSION

As a result of this study, the antimicrobial potential of turmeric extracts on MRSA isolates and some bacterial and fungal standard strains were compared. The activity of extracts against biofilm formation in MRSA isolates and standard strains were also investigated. In addition to phenotypic studies, genotypic

methods as PCR and protein assays were applied to examine gene regions of different virulence factors in MRSA isolates, where the effects of extracts on the total protein amount in isolates were investigated.

With studies on crude extracts, aqueous *Curcuma longa* rhizome extract was shown to provide no inhibition zone and 14.3 mm zone at 250 mg/ml, while MIC values were found as 6 and 4 mg/ml against *S.aureus* and *E.coli* [13]. Ethanol extract (23.42 %w/w curcumin content) was determined with a MIC of 128 and 256µg/ml for *S. aureus* and *C. albicans* [14]. When hexane, ethanol extracts were compared with curcuminoid-rich ethyl acetate fraction (86.5% curcumin content) obtained from *Curcuma longa* rhizomes, the ethanol extract was reported with stronger activity compared to hexane extract and curcuminoid-rich fraction. Inhibition zone diameters were determined as 16.55 mm, 9.87 mm and 11.12 mm against *S. aureus* with ethanol, hexane extracts and curcuminoid fraction, respectively, while inhibition zone was not determined against *E. coli* [15].

Previously, chloroform, methanol, water extracts from turmeric rhizomes were found to possess inhibition zone diameters of 7, 9, 0 mm against *C. albicans*; 8, 10, 7 mm against *E. coli*; 9, 13.5, 0 mm against *S. aureus*. at 12 µg dose, respectively [16]. Benzene, chloroform, methanol and water fractions of turmeric rhizomes were found to provide inhibition zone of 17, 13, 20, 12 mm against standard strains and of 11, 14, 19, 11 mm against clinical isolates of S. aureus at 100 mg/ml dose, respectively. Inhibition zones of 20, 13, 23, 18 mm were determined with benzene, chloroform, methanol and water fractions against standard strains of S. aureus at 250 µg/disc dose [17]. Similar to our findings, water extracts had been determined with lower activity against *S. aureus* compared to moderate/low polarity extracts of ethanol and hexane. The cause of differences for MIC values was credited to solubility of the samples tested and the sample solvent used, the methods used for MIC evaluation, and the purity of the test samples in reviews covering various studies [7, 12].

The antibiofilm activity of the extracts was investigated in MRSA isolates and standard strains. At the MIC values, hexane and water extracts inhibited biofilm formation in MRSA isolates, whereas dichloromethane and ethanol extracts had inductive and no effect on 7 and 1 MRSA isolates, respectively. Similarly, on the standard strains, hexane and water extracts both inhibited biofilm formation, whereas dichloromethane and ethanol extracts had no effect. The use of higher doses than MIC values for dichloromethane and ethanol extracts can be considered for the inhibition of biofilm formation in MRSA isolates, while no effect was also observed in standard strains. One of the primary virulence characteristics of bacteria is their ability to form biofilms, making them extremely difficult to treat. The eps matrix that the biofilm cells embed in themselves reduces their sensitivity to antimicrobial compounds and immune system cells [18]. Therefore, there can often be a discrepancy between the doses that show antibacterial activity and those that are reported to inhibit biofilm [19]. Environmental stress can activate systems associated with biofilm formation, which in turn can cause bacteria to respond and initiate the process of biofilm formation, especially if therapeutic agent's dose is insufficient [20]. As we also noted, although antibacterial activity was obtained, the cause of biofilm induction by dichloromethane and ethanol extracts might be related to this. Membrane permeability might have effect on the efficacy of the extracts. However, it was likely that in the biofilm environment, where MRSA isolates were embedded in the eps matrix, the interaction of the active content with the membrane is reduced, resulting in lower efficacy. There are many variables that can affect the activity of plant extracts with apolar solvents (i.e. hexane) on biofilm. Hexane is generally good at dissolving the fat soluble (lipophilic) components of plant extracts. These components typically consist of lipophilic phytochemicals and sterols [21]. It can be concluded that the hexane extract, which is rich in fat-soluble compounds, may diffuse more efficiently into the lipid-rich biofilm structure and therefore produces stronger antibiofilm activity.

Regarding the content of *Curcuma longa* extracts investigated with our previous study [22] the dichloromethane extract which was determined to have the highest antimicrobial activity against MRSA isolates, had also been quantified as having highest total phenol (204mg GAE/g) and flavonoid (484mg QE/g) contents . Considering the literature data, where curcumin was pointed out with the antimicrobial potency [7, 12], consistent results were observed as the highest curcumin (101mg/g) and total curcuminoid (178mg/g) content had been quantified with LC-MS/MS analysis in dichloromethane extract among *Curcuma longa* extracts [22]. Following the dichloromethane extract, the ethanol extract was secondarily quantified for highest total phenol (188mg GAE/g) and flavonoid (398mg QE/g) contents by spectrophotometry, and curcumin (55mg/g) and total curcuminoid (102mg/g) contents by LC-MS/MS. The highest levels of demethoxycurcumin and bisdemethoxycurcumin were found in the dichloromethane extract with 39mg CE/g and 38mg CE/g. Previous studies had demonstrated the antimicrobial activity of curcumin derivatives as demethoxycurcumin and bisdemethoxycurcumin against MRSA in addition to

curcumin. The MIC of demethoxycurcumin against MRSA was found as 62.5µg/ml [23], while bisdemethoxycurcumin's was found as 7.8µg/ml [24]. Against 10 strains of MRSA (including clinical isolates and standard strains), curcumin was shown to have MIC values of 125-250 µg/ml [25]. In accordance with the results of bioactive content analysis and consistent with the literature data [7, 12], strongest antimicrobial activity was determined with phenolic and curcumin rich extracts. Although the hexane extract was quantified as yielding remarkably lower total phenol (147mg GAE/g) and flavonoid (32mg QE/g) contents, yielding equivalent amounts of curcumin (56mg/g) with the ethanol extract could be interpreted as the reason for its antimicrobial potential against standard strains (Table 4). Both the literature studies and the data obtained from this research indicate that *Curcuma longa* extracts have varying degrees of antibacterial activity, depending on the extraction solvent and the type of bacteria [17]. In this study, dichloromethane and ethanol extracts were found as most effective ones in antibacterial activity experiments against MRSA isolates. As the primary antibacterial effect is thought to be associated with curcumin, it is an expected result that extracts with the highest curcumin content exhibit higher activity [12]. Interestingly, in the antimicrobial tests performed on standard strains, hexane extracts were found to have higher activity. The difference in effect between standard strains and clinical isolates could be related to lower sensitivity of clinical isolates to the antibacterial effects of different groups of compounds. In addition, the possible capsule structure of clinical isolates may allow less penetration of the hexane extract into the cells compared to standard strains. In extracts with higher levels of curcumin, it is likely that the curcumin damages the cell membrane, allowing a higher amount of extract content to penetrate the bacteria [26]. Synergistic antibacterial activity of curcumin with traditional antibacterials was shown [7]. Due to the synergistic effect, MIC of 25 µg/ml was obtained when curcumin's antibacterial activity was assessed against both standard and clinical strains of *S. aureus* using eight different antibiotics. [27]. Curcumin-rich dichloromethane extract could also be benefited with an important potential for combination therapies against MRSA.

The effect on *Curcuma longa* extracts on total protein content of MRSA cells were investigated. The dichloromethane extract was found to decrease total protein amounts in all MRSA isolates, while hexane extract reduced total protein amounts in 7 isolates. *S. aureus* has about 20 proteins covalently linked to peptidoglycan on its surface. These proteins adhere to host cells and produce biofilms, among other functions [28]. As peptidoglycan makes up a large portion of the structure of the Gram-positive cell wall, the presence of molecules such as curcumin, which act by interacting with the cell membrane, results in a remarkable reduction in total protein amounts [29]. Likewise, the results of the BCA assay showed the decrease in total protein levels in the presence of the hexane and dichloromethane extracts.Numerous distinct virulence factors and various combinations of these virulence factors are present in *S. aureus* [30). In terms of the course and severity of the infection, it has been recognised that they are responsible for the virulence factors either individually or in combination. For instance, the fibronectin binding proteins (FnBPs) and collagen binding protein (Cna), which are members of microbial surface components recognising adhesive matrix molecules (MSCRAMMs), are crucial structures that facilitate the adhesion and invasion of microorganisms [31]. As a result, the presence of pertinent genes in all of the isolates included in the study indicates that the strains have the potential to cause systemic complications in addition to superficial infections. Furthermore, it is well-established that fibronectin binding proteins are essential, particularly during the initial phases of biofilm formation, and they are responsible for the production of biofilm [32]. The 100% occurrence of this gene can be attributed to the participation of 8 biofilm producing strains in the study.

The clumping factors, clfA and clfB, share a high degree of sequence similarity and a comparable molecular architecture. However, the fibrinogen binding domains (A domain) exhibit less similarity, and there are also significant variations in their expression. clfA is expressed continuously during bacterial growth, whereas clfB is only expressed during the initial logarithmic phase [33, 34]. This is believed to occur because the presence of clfA and clfb enables them to work jointly in a way that enhances the ability of cells to adhere more strongly to surfaces coated with fibrinogen during the bacterial growth cycle [35]. The identification of all 8 representative strains in the study with 6 different virulence factors is remarkable for assessing the pathogenicity of the strains and determining the potential effects of the extract on highly virulent strains that may be more resistant to treatment.

The antimicrobial and antibiofilm effects of turmeric extracts were investigated in this study. The extracts were found to have antimicrobial and anti-biofilm activity in bacteria, thus suppressing production of virulence factors and reducing infection potential by *Staphylococci*. Resistance rates to antimicrobials in MRSA isolates among staphylococci are increasing day by day and antimicrobial options in the treatment of

infection are becoming limited. Therefore, research on treatment options against staphylococcal infections is still required.

4. CONCLUSION

According to the results, 81 MRSA isolates were found resistant to penicillin. 5 isolates were resistant to 9 antibiotics and 5 isolates were resistant to 8 antibiotics. Among 81 MRSA isolates, 45 isolates were resistant to 5 or more antibiotics. *Curcuma longa* extracts were found to have different levels of antimicrobial activity against MRSA isolates and standard strains, where highest activity was determined with curcumin-rich dichloromethane extract with a MIC of 32µg/ml against MRSA isolates. Six gene regions (emp-clfA-clfB-fnbpA-fnbpB-cna) investigated in MRSA isolates were found positive in 8 isolates. It was found that dichloromethane extract decreased the total protein amount in all isolates. New antimicrobials and alternative treatment strategies like combination of natural products with existing antibiotics are required to control infections caused by drug-resistant pathogenic microorganisms. Further studies involving different virulence factors and mechanism of action, including biofilm structures, are required. The hexane and dichloromethane extracts of *Curcuma longa* rhizomes can be potential sources with remarkable antimicrobial activity for research against MRSA.

5. MATERIALS AND METHODS

5.1. Plant material and extractions

Turmeric (*Curcuma longa* L.) dried rhizomes were obtained from Bagdat Baharat Ltd. A voucher specimen (IZEF-6713) containing the studied plant material was also stored in the IZEF Herbarium of Ege University, Izmir. Plant material was kept in a dark, cold area during the study, and powdered before the extractions.

Prior to extraction, dried rhizomes were powdered using a blender (Isolab). Powdered material (10g) was combined with extraction solvents (200ml) in glass flasks. The extraction solvents (hexane, dichloromethane, ethanol, and water) were selected to cover a wide range of polarity. The extractions were performed in the dark at 30°C for 1 h with ultrasonic bath (Isolab 13lt). After three cycles, collected filtrates were combined and evaporated at 40°C (Buchi R100). The extracts were completely dried via vacuum concentrator (Thermo Scientific-Speedvac) and lyophiliser (Labconco-Freezone). Extracts were stored at -24°C throughout the study [22].

5.2. Chemicals

Hexane, dichloromethane, ethanol, Mueller-Hinton agar, Mueller-Hinton broth, tryptic soy broth, Luria-Bertani broth were obtained from Merck (Darmstadt, Germany); Sabouraud dextrose agar, Sabouraud dextrose broth, phosphate buffered saline, from Oxoid (Wesel, Germany); Taq DNA polimerase, dNTP from Fermentas (Waltham, USA); DNA Ladder from Thermo Scientific (Waltham, USA). All chemicals were of analytical grade. Water was obtained using Stakpure Omnia Type-I UltrapureWater (Niederahr, Germany).

5.3. Microbial strains and isolates

Studies were conducted on standard strains (*Staphylococcus aureus* ATCC29213, *Escherichia coli* ATCC25922 and *Candida albicans* ATCC90028) and 100 of *S. aureus* isolates obtained for a previous study by Department of Medical Microbiology, Izmir Katip Celebi University, Ataturk Education and Research Hospital [36]. To determine if the antimicrobial activity was specific to Gram-positive bacteria, *E. coli* and *C. albicans* were included as a representative of Gram-negative bacteria and yeast.

5.4. Antibiotic susceptibility test

Antibiotic susceptibility tests on 100 isolates were conducted with BD Phoenix system. All strains were categorised into eight subgroups based on their antibiotic resistance/sensitivity profiles, as determined by the results of the antibiotic susceptibility testing. The selection of bacterial representatives was carried out phenotypically, utilising antibiotic susceptibilities (Antibiogram typing or Resistotyping). This method has been employed in studies to facilitate typing *S. aureus* isolates [37, 38]. Representatives of each cluster were randomly selected from the isolates comprising the group.

5.5. Antimicrobial activity

5.5.1. Disc diffusion test

Bacterial strains were incubated with Mueller-Hinton agar (MHA), and fungal strain was incubated with Sabouraud dextrose agar (SDA) (24 h, 37°C). Suspensions were prepared with saline and adjusted to 0.5 McFarland (Biosan, Den-1). Bacterial suspensions were spread on MHA and fungal suspension was spread on SDA, and samples were added to 6 mm sterile blank discs. Solvents without samples were studied separately as negative control. Inhibition zone diameters around the discs were determined after 16 h incubation (37°C). Ciprofloxacin antibiotic disc and *S. aureus* ATCC29213 standard strains were used as positive control. Limit values for quality control were considered in accordance with EUCAST and CLSI recommendations. Three replicates were performed and mean zone diameter ± standard deviation values were calculated [39].

5.5.2. Microdilution method

Bacterial strains were incubated with MHA and fungal strain was incubated with SDA (24 h, 37°C). Fresh colonies of bacteria and fungi were suspended with saline and were adjusted to 0.5 McFarland. Bacterial suspensions were diluted 1/100. Cation adjusted Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi was taken 50 µL each and distributed into 96 microplate wells. In the first wells, the samples (50µl) were placed and diluted with MHB/SDB in 96 microplate wells and finally bacterial and fungal suspensions (50µl) were added to wells. Ciprofloxacin and *S. aureus* ATCC 29213 standard strain were used as positive control. Quality control limit values were considered in accordance with EUCAST and CLSI recommendations. Minimum inhibitory concentration (MIC) was determined as the lowest concentration at which no visible growth was observed after 16 hours of incubation at 37°C [39].

5.6. Biofilm studies

5.6.1. Biofilm production levels of the strains and MRSA isolates

Biofilm studies on bacteria were performed with a modified method [40]. Firstly, biofilm production capacity of bacteria was investigated. In the following stages, the effects of samples on biofilm formation were investigated. All experiments were performed in three replicates. Bacterial strains were incubated overnight in MHA (37°C). Bacterial suspensions were prepared with saline and adjusted to 0.5 McFarland turbidity. Tryptic soy broth (180 µl) containing 2.5% glucose was added to 96 microplate wells. 20 µl each of bacterial suspensions was transferred to wells. The wells were incubated for 24 h (37°C). After the removal of the medium, the wells were washed with PBS (3 times with 200 µl) and the microplates were inverted and dried. Methanol (200 µl) was added and kept in wells for 15 min. After removing the methanol, the wells were dried by inverting the microplates. 0.1% crystal violet solution (200 µl) was transferred to wells and kept for 5 min. Then, tap water (200 µl) was used for washing the wells (3 times). After drying the microplates, absolute ethanol (200 µl) was kept in wells for 15 min. Then, the solutions in the wells were transferred to clean microplates and spectrophotometric measurements (Clariostar, BMG Labtech) were performed at 570 nm.

Results of triplicate experiments were presented as optical density (OD) ± standard deviation. Statistical analyses (t-test) were performed using Graphpad Prism 5. $p<0.05$ were considered as significant.

Biofilm production capacities were determined as;

negative; OD ≤ negative control
weak biofilm formation; negative control ≤ OD ≤ negative controlx2
intermediate biofilm formation; negative controlx2 ≤ OD ≤ negative controlx4
strong biofilm formation; negative controlx4 ≤ OD

Tryptic soy broth (2.5% glucose) was used as negative control.

5.6.2. Effect of Curcuma longa extracts on biofilm production

Bacterial and fungal suspensions were prepared with physiological saline and adjusted to 0.5 McFarland turbidity. Tryptic soy broth (160 µl, containing 2.5% glucose) was added to 96 well microplates. To the wells, 20 µl each of the samples (at MIC levels) and bacterial suspensions were added. The wells were incubated (37°C) for 24 h. After the removal of the medium, the wells were washed with PBS (200 µl, 3 times). The microplates were inverted for drying. Methanol (200 µl) was kept in wells for 15 min, and then removed. 0.1% crystal violet solution (200 µl) was transferred to wells and kept for 5 min. Then, tap water (200 µl) was used for washing the wells (3 times). After drying the microplates, absolute ethanol (200 µl) was kept in wells for 15 min. Then, the solutions in the wells were transferred to clean microplates and spectrophotometric measurements were performed at 570 nm [41].

Results of triplicate experiments were presented as optical density (OD) ± standard deviation. Statistical analyses (t-test) were performed using Graphpad Prism 5. $p<0.05$ were considered as significant.

5.7. Polymerase chain reaction (PCR) studies

For genomic DNA isolation, the method using lysostaphin and proteinase-K was applied with minor modifications [42]. Bacterial strains were incubated overnight on MHA. Fresh colonies were suspended with 50µl of lysostaphin (100 mg/l distilled water) and incubated at 37°C for 10 minutes. 50 µl proteinase-K (100 mg/L distilled water) and 150 µl TE buffer (1 mM EDTA/10 mM Tris, pH 7.5) were added and incubated (37°C) for 20 min. Samples were kept at 95°C for 5 min for deactivation of proteinase-K. DNA-containing suspensions were stored at -20°C and used for PCR studies. PCR experiments were performed according to company recommendations and the specific properties of the primers.

Within the scope of PCR, *eap/map* (extracellular bindingprotein gene), *emp* (extracellular matrix bindingprotein), *icaD/pia* (polysaccharide intercellular adhesin), *clfA* (clamp factors A) were detected in MRSA isolates, The presence of *clfB* (clamp factors B), *fnbpA* (fibronectin bindingproteinsA), *fnbpB* (fibronectin bindingproteinsB), *cna* (collagen bindingprotein) and *fib* (fibrinogen bindingprotein) genes were investigated. PCR reaction mixture (50 µl) was prepared for each sample as containing sterile water (33.6 µl), Taq buffer (5 µl), 25 mM $MgCl_2$ (3 µl), 5 mM dNTP (2 µl), 50 pmol/µl primer forward (1 µl), 50 pmol/µl primer revers (1 µl), 5 U/µl Taq polymerase (0.2 µl), and 50 ng/µl of extraction product.

Samples were placed in the thermal cycling device and amplification was performed at the following times and temperatures; 30 cycles including denaturation (94°C, 1 min), primer annealing (43°C, 1 min), primer extension (72°C, 1 min).

Agarose (1.5%) was dissolved in 1x TBE. Before solidification, 10 µL/100 ml safeview solution (ABM) was added to gel. Mixture was homogenized and transferred to the mould. After solidification of gel, comb was removed and placed in an electrophoresis tank (Thermo Scientific). PCR products were mixed 1/6 with 6X DNA dye (2 µL dye / 10 µL product) and transferred into wells in the gel and electrophoresis was performed at 100 V for 45 min with a power supply (Bio-Rad). The bands formed were visualised with UV imaging device (Gen-Box).

5.8. Protein content studies

For the preparation of bacterial suspensions for total protein isolation, bacteria taken from colonies in fresh bacterial culture were suspended in LB broth (0.5 McFarland adjusted). Bacterial suspensions were transferred to falcon tubes and kept in a shaking incubator at 200 rpm at 37°C and incubated until 4 McFarland turbidity (~ 4 hours). Bacterial suspensions were aliquoted into 15 ml falcon tubes. Plant extracts were prepared considering the concentrations. 10 ml of bacterial suspension was added and incubated (37°C) in a shaking incubator (200 rpm). At 16 h, bacterial suspensions (1 ml) were taken into sterile tubes and used in total protein isolation experiments [43].

Bacterial suspensions (1 ml, with and without exposure to tested samples) were centrifuged at 5000xg for 3 min at 10°C. Pellets were resuspended with buffer A (100 µl, 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5% glycerol, and 1 mg/ml lysozyme and 100 µg/ml lysostaphin). Bacterial suspensions were incubated (42°C) for 5 min. Centrifuged (10.000xg, 4°C) for 45 min and supernatants were taken into clean tubes. Lysates were sonicated with an ultrasonicator (Bandelin, Sonoplus) (10 s, 30%). Lysate protein concentrations were calculated with BCA Protein Assay Kit (Pierce). Bovine serum albumin was used as standard. The protocols described by the kit manufacturer were applied. Absorbance values of standard albumin solutions prepared at different concentrations were obtained with microplate reader and standard curves were drawn. The absorbance values of the samples for protein determination were calculated by considering the absorbance values of the BSA standard solutions with known concentrations and the equation of the line prepared and the changes in the total protein amount were calculated by comparing with the control (untreated) groups.

All experiments were performed with 3 repetitions. Mean protein change rates and standard deviation values were calculated. Using GraphPad Prism 5 program (t-test), $p<0.05$ were considered as statistically significant.

Acknowledgements: This study was part of a project supported by IKCU Scientific Research Projects [2018-ÖNAP-ECZF-0003]. Access to the facilities of Central Research Laboratories (MERLAB) at Izmir Katip Celebi University are highly appreciated.

Conflict of interest statement: The authors declare no conflict of interest.

REFERENCES

[1] Murray PR, Rosenthal KS, Pfaller MA. Tıbbi Mikrobiyoloji, 6. Baskı, Atlas Kitapçılık, Ankara, Türkiye 2010.
[2] Holden MT, Feil EJ, Lindsay JA, Peacock SJ, Day NP, Enright MC, Foster TJ, Moore CE, Hurst L, Atkin R, Barron A, Bason N, Bentley SD, Chillingworth C, Chillingworth T, Churcher C, Clark L, Corton C, Cronin A, Doggett J, Dowd L, Feltwell T, Hance Z, Harris B, Hauser H, Holroyd S, Jagels K, James KD, Lennard N, Line A, Mayes R, Moule S, Mungall K, Ormond D, Quail MA, Rabbinowitsch E, Rutherford K, Sanders M, Sharp S, Simmonds M, Stevens K, Whitehead S, Barrell BG, Spratt BG, Parkhill J. Complete genomes of two clinical *Staphylococcus aureus* strains: Evidence for the rapid evolution of virulence and drug resistance. Proc Natl Acad Sci. 2004; 101(26): 9786-9791. https://doi.org/10.1073/pnas.0402521101.
[3] Zecconi A, Scali F. *Staphylococcus aureus* virulence factors in evasion from innate immune defenses in human and animal diseases. Immunol Lett. 2013; 150(1-2): 12-22. https://doi.org/10.1016/j.imlet.2013.01.004.
[4] Peacock SJ, Moore CE, Justice A, Kantzanou M, Story L, Mackie K, O'Neill G, Day NP. Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*. Infect Immun. 2002; 70(9): 4987-4996. https://doi.org/10.1128/IAI.70.9.4987-4996.2002.
[5] Kaplan JB, Izano EA, Gopal P, Karwacki MT, Kim S, Bose JL, Bayles KW, Horswill AR. Low levels of beta-lactam antibiotics induce extracellular DNA release and biofilm formation in *Staphylococcus aureus*. mBio. 2012; 3(4): e00198-00112. https://doi.org/10.1128/mBio.00198-12.
[6] Lowy FD. *Staphylococcus aureus* infections. N Engl J Med. 1998; 339(8): 520-532. https://doi.org/10.1056/NEJM199808203390806.
[7] Dai C, Lin J, Li H, Shen Z, Wang Y, Velkov T, Shen J. The natural product curcumin as an antibacterial agent: current achievements and problems. Antioxidants (Basel). 2022; 11(3):459. https://doi.org/10.3390/antiox11030459.
[8] Amalraj A, Pius A, Gopi S, Gopi S. Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives - A review. J Tradit Complement Med. 2017; 7(2): 205-233. https://doi.org/10.1016/j.jtcme.2016.05.005.
[9] Hatcher H, Planalp R, Cho J, Torti FM, Torti SV. Curcumin: From ancient medicine to current clinical trials. Cell Mol Life Sci. 2008; 65(11): 1631-1652. https://doi.org/10.1007/s00018-008-7452-4.
[10] Hewlings SJ, Kalman DS. Curcumin: A review of its effects on human health. Foods (Basel). 2017; 6(10). https://doi.org/10.3390/foods6100092.
[11] Kocaadam B, Sanlier N. Curcumin, an active component of turmeric (*Curcuma longa*), and its effects on health. Crit Rev Food Sci Nutr. 2017; 57(13): 2889-2895. https://doi.org/10.1080/10408398.2015.1077195.
[12] Teow SY, Liew K, Ali SA, Khoo AS, Peh SC. Antibacterial action of curcumin against *Staphylococcus aureus*: A brief review. J Trop Med. 2016; 2016: 2853045. https://doi.org/10.1155/2016/2853045.
[13] Niamsa N, Sittiwet C. Antimicrobial activity of *Curcuma longa* aqueous extract. J Pharm Toxicol. 2009; 4: 173-177. https://doi.org/10.3923/jpt.2009.173.177.
[14] Singchangchai P, Itharat A, Rattanasuwan P, Sungkarak S, Ungphaiboon S, Supavita T. Study on antioxidant and antimicrobial activities of turmeric clear liquid soap for wound treatment of HIV patients. Songklanakarin J Sci Technol. 2005; 27: 569-578.
[15] Lawhavinit O, Kongkathip N, Kongkathip B. Antimicrobial activity of curcuminoids from *Curcuma longa* L. on pathogenic bacteria of shrimp and chicken. Kasetsart J. 2010; 44: 364-371.
[16] Gul P, Bakht J. Antimicrobial activity of turmeric extract and its potential use in food industry. J Food Sci Technol. 2015; 52(4): 2272-2279. https://doi.org/10.1007/s13197-013-1195-4.
[17] Gupta A, Mahajan S, Sharma R. Evaluation of antimicrobial activity of *Curcuma longa* rhizome extract against *Staphylococcus aureus*. Biotech Rep (Amst). 2015; 6: 51-55. https://doi.org/10.1016/j.btre.2015.02.001.
[18] Peng Q, Tang X, Dong W, Sun N, Yuan W. A review of biofilm formation of *Staphylococcus aureus* and its regulation mechanism. Antibiotics. 2023; 12(1): 12. https://doi.org/10.3390/antibiotics12010012.
[19] Archer NK, Mazaitis MJ, Costerton JW, Leid JG, Powers ME, Shirtliff ME. *Staphylococcus aureus* biofilms: Properties, regulation, and roles in human disease. Virulence. 2011; 2(5): 445-459. https://doi.org/10.4161/viru.2.5.17724.
[20] Uzunbayir-Akel N, Tekintas Y, Yilmaz FF, Ozturk I, Okeer M, Aydemir SS, Cilli FF, Hosgor-Limoncu M. Effects of disinfectants and ciprofloxacin on quorum sensing genes and biofilm of clinical *Pseudomonas aeruginosa* isolates. J Infect Public Health. 2020; 13(12): 1932-1938. https://doi.org/10.1016/j.jiph.2020.10.002.
[21] Rapinel V, Claux O, Abert-Vian M, McAlinden C, Bartier M, Patouillard N, Jacques L, Chemat F. 2-Methyloxolane (2-MeOx) as sustainable lipophilic solvent to substitute hexane for green extraction of natural products. Properties, applications, and perspectives. Molecules. 2020; 25(15): 3417. https://doi.org/10.3390/molecules25153417.
[22] Koyu H, Istanbullu H, Turunc Ozoglu SE, Kaya Temiz T. Investigation of in vitro HDAC 1 inhibitory activity of *Curcuma longa* L. extracts, isolated fractions and curcumin. Eur Food Res Technol. 2024; 250(2): 623-631. https://doi.org/10.1007/s00217-023-04424-5.
[23] Li QQ, Kang OH, Kwon DY. Study on demethoxycurcumin as a promising approach to reverse methicillin-resistance of *Staphylococcus aureus*. Int J Mol Sci. 2021; 22(7). https://doi.org/10.3390/ijms22073778.
[24] Wang S, Kim MC, Kang OH, Kwon DY. The mechanism of bisdemethoxycurcumin enhances conventional antibiotics against methicillin-resistant *Staphylococcus aureus*. Int J Mol Sci. 2020; 21(21):7945. https://doi.org/10.3390/ijms21217945.

[25] Mun SH, Joung DK, Kim YS, Kang OH, Kim SB, Seo YS, Kim YC, Lee DS, Shin DW, Kweon KT, Kwon DY. Synergistic antibacterial effect of curcumin against methicillin-resistant *Staphylococcus aureus*. Phytomed. 2013; 20(8-9): 714-718. https://doi.org/10.1016/j.phymed.2013.02.006.

[26] Mun SH, Kim SB, Kong R, Choi JG, Kim YC, Shin DW, Kang OH, Kwon DY. Curcumin reverse methicillin resistance in *Staphylococcus aureus*. Molecules. 2014; 19(11): 18283-18295. https://doi.org/10.3390/molecules191118283.

[27] Teow SY, Ali SA. Synergistic antibacterial activity of Curcumin with antibiotics against *Staphylococcus aureus*. Pak J Pharm Sci. 2015; 28(6): 2109-2114.

[28] Foster TJ. Surface proteins of *Staphylococcus aureus*. Microbiol Spectr. 2019; 7(4): https://doi.org/10.1128/microbiolspec.GPP3-0046-2018.

[29] Irazoki O, Hernandez SB, Cava F. Peptidoglycan muropeptides: Release, perception, and functions as signaling molecules. Front Microbiol. 2019; 10. https://doi.org/10.3389/fmicb.2019.00500.

[30] Zecconi A, Scali F. *Staphylococcus aureus* virulence factors in evasion from innate immune defenses in human and animal diseases. Immunol Lett. 2013; 150(1-2): 12–22. https://doi.org/10.1016/j.imlet.2013.01.004.

[31] Hauck CR, Ohlsen K. Sticky connections: Extracellular matrix protein recognition and integrin-mediated cellular invasion by *Staphylococcus aureus*. Curr Opin Microbiol. 2006; 9(1): 5-11. https://doi.org/10.1016/j.mib.2005.12.002.

[32] Herman-Bausier P, El-Kirat-Chatel S, Foster TJ, Geoghegan JA, Dufrêne YF. *Staphylococcus aureus* fibronectin-binding protein A mediates cell-cell adhesion through low-affinity homophilic bonds. mBio. 2015; 6(3). https://doi.org/10.1128/mBio.00413-15.

[33] McDevitt D, Nanavaty T, House-Pompeo K, Bell E, Turner N, McIntire L, Foster T, Höök M. Characterization of the interaction between the *Staphylococcus aureus* clumping factor (ClfA) and fibrinogen. Eur J Biochem. 1997; 247(1): 416-24. https://doi.org/10.1111/j.1432-1033.1997.00416.x.

[34] Ní Eidhin D, Perkins S, Francois P, Vaudaux P, Höök M, Foster TJ. Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of *Staphylococcus aureus*. Mol Microbiol. 1998; 30(2): 245-57. https://doi.org/10.1046/j.1365-2958.1998.01050.x.

[35] Entenza JM, Foster TJ, Ni Eidhin D, Vaudaux P, Francioli P, Moreillon P. Contribution of clumping factor B to pathogenesis of experimental endocarditis due to *Staphylococcus aureus*. Infect Immun. 2000; 68(9): 5443-5446. https://doi.org/10.1128/IAI.68.9.5443-5446.2000.

[36] Yurtsever SG, Aygul A, Oztürk I, Nemli SA, Kaya S, Ermertcan S. Investigation of various virulence factors and SCCmec types in the healthcare-associated and community-associated methicillin resistant *Staphylococcus aureus* strains. Eur J Ther. 2020; 26(2): 111-116. https://doi.org/10.5152/eurjther.2019.19062.

[37] Bernardo WL, Boriollo MF, Gonçalves RB, Höfling JF. *Staphylococcus aureus* ampicillin-resistant from the odontological clinic environment. Rev Inst Med Trop Sao Paulo. 2005; 47(1): 19-24. https://doi.org/10.1590/s0036-46652005000100004.

[38] Mehndiratta PL, Bhalla P. Typing of methicillin resistant *Staphylococcus aureus*: A technical review. Indian J Med Microbiol. 2012; 30(1) :16-23. https://doi.org/10.4103/0255-0857.93015.

[39] Jonasson E, Matuschek E, Kahlmeter G. The EUCAST rapid disc diffusion method for antimicrobial susceptibility testing directly from positive blood culture bottles. J Antimicrob Chemother. 2020; 75(4): 968-978. https://doi.org/10.1093/jac/dkz548.

[40] Stepanović S, Vuković D, Hola V, Di Bonaventura G, Djukić S, Cirković I, Ruzicka F. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by *Staphylococci*. APMIS. 2007; 115(8): 891-899. https://doi.org/10.1111/j.1600-0463.2007.apm_630.x.

[41] Ozturk I, Tekintas Y, Temel A, Ermertcan S, Kurutepe S, Hosgor Limoncu M. In vitro effects of antibiofilm agents and antibiotics oncoagulase-negative *Staphylococci*. J Res Pharm. 2020; 24(6): 821-832. https://doi.org/10.35333/jrp.2020.241.

[42] Merlino J, Watson J, Rose B, Beard-Pegler M, Gottlieb T, Bradbury R, Harbour C. Detection and expression of methicillin/oxacillin resistance in multidrug-resistant and non-multidrug-resistant *Staphylococcus aureus* in Central Sydney, Australia. J Antimicrob Chemother. 2002; 49(5): 793-801. https://doi.org/10.1093/jac/dkf021.

[43] Ozturk I, Erac Y, Ballar Kirmizibayrak P, Ermertcan S. Nonsteroidal antiinflammatory drugs alter antibiotic susceptibility and expression of virulence-related genes and protein A of *Staphylococcus aureus*. Turk J Med Sci. 2021; 51(2): 835-847. https://doi.org/10.3906/sag-2003-60.