



Evaluation of Antibacterial Activity of *Zataria multiflora* Against the Expression of *icaADB* and *aap* Gene and Biofilm Formation in *Staphylococcus epidermidis*

Zahra Mottaghiyan¹, Mohammad Aghazadeh^{1,*}, Hamideh Mahmoodzadeh Hosseini² and Abbas Ali Imani Fooladi^{2,**}

¹Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

²Applied Microbiology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

*Corresponding author: Immunology Research Center, Tabriz University of Medical Sciences, Tehran, Iran. Email: aghazadeh@tbzmed.ac.ir

**Corresponding author: Applied Microbiology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Vanak Sq. Molasadra St., P.O. Box: 19395-5487, Tehran, Iran. Tel: +98-2182482568, Fax: +98-2188068924, Email: imanifooladi.a@gmail.com

Received 2017 December 19; Revised 2018 December 29; Accepted 2019 January 20.

Abstract

Background: This study aimed at evaluating the effect of the extract and essential oil of *Zataria multiflora* on reducing phenotypes and genotypes of biofilm formation in clinical isolates of *Staphylococcus epidermidis*.

Methods: The ability to produce biofilm was evaluated by microtiter plate (MtP) for phenotype and through the presence of *icaADB* and *aap* by PCR for genotype among the 153 clinical isolates of *Staphylococcus epidermidis*. The impact of ethanol extract and essential oil of *Zataria multiflora* were measured on biofilm formation in phenotypic evaluation based on MtP and on the expression of *ica* operon and *aap* by real time-PCR.

Results: The results showed that 71.2% of isolates were able to produce biofilms. The PCR results showed that 52.2% and 88.9% of the isolates had *icaABD* and *aap*, respectively. In addition, the relative mRNA expression of *icaA*, *icaD*, and *aap* genes were significantly reduced compared to the negative control after treating the *Staphylococcus epidermidis* RP62A with subMIC concentration of essential oil and extract ($P < 0.001$).

Conclusions: Given the significant inhibitory effect of the extract and essential oil of *Zataria multiflora* on biofilm formation, it seems that these substances are good options for studies related to controlling biofilm formation in *Staphylococcus epidermidis*

Keywords: Biofilm, *Staphylococcus epidermidis*, *Zataria multiflora*

1. Background

Staphylococcus epidermis is the most frequent cause of implanted medical device or catheter-related infections and it has caused an increase in resistance to different types of antibiotics in hospitals through its most significant virulence factor, i.e. biofilm production. Polysaccharide intercellular adhesive (PIA) is a product of the *ica* operon and is strongly associated with staphylococcal cell surface and the main composition, which mediates cell to cell adhesion. The *ica* operon contains *ica* ADDB genes, which are required for PIA synthesis (1). Other pathways also contribute to biofilm formation in *Staphylococcus epidermidis* independent of the *ica* operon, such as the biofilm accumulation associated protein (Aap) forming fibrillary structures on the cell surface (2).

Zataria multiflora is a plant from the Labiatae fam-

ily that grows in Iran, Pakistan, and Afghanistan. The traditional uses of *Zataria multiflora* include antiseptics, anesthetics, and antispasmodic (3). In this plant, the most antibacterial activity is related to essential oil with compounds including thymol and/or carvacrol, phenolic derivatives. The anti-biofilm activity of *Zataria multiflora* was reported against *Pseudomonas aeruginosa* and *Listeria monocytogenes*, and antibacterial activity of *Zataria multiflora* was shown against *Staphylococcus epidermidis* strains, yet there is no study about the role of *Zataria multiflora* on inhibition of biofilm formation in *Staphylococcus epidermidis* isolates (4).

Many studies have investigated the effect of different substances on biofilm production in *Staphylococcus epidermidis*, including silver colloidal nanoparticles and *Cassia alata* leaves (5). The effect of *Zataria multiflora* Boiss on biofilm production in *Staphylococcus epidermidis* has not

been addressed in the literature, although its inhibitory effects on growth and biofilm production has been studied in other bacteria (6).

2. Objectives

The present study was conducted to examine the in-vitro effect of *Zataria multiflora* Boiss extract and essence on biofilm production in *Staphylococcus epidermidis*.

3. Methods

3.1. Sample Collection

A total of 153 *S. epidermidis* clinical isolates were obtained between April 2014 and January 2015 from Baqiyatallah Hospital, Tehran, Iran. The isolates were from blood, wound, bronchoalveolar lavage, urine, cerebral spinal fluid, tracheal, sputum, throat, and eye samples. Identification included biochemical tests, such as catalase, coagulase, PYR, and urea tests and susceptibility to novobiocin (7). After identification, samples were inoculated in BHI-broth at 37°C for 24 hours. Each sample was stored at -80°C.

3.2. Antimicrobial Susceptibility Test

Antimicrobial susceptibility was performed for clindamycin (2 µg), cefoxitin (30 µg), erythromycin (15 µg), tetracycline (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), rifampicin (5 µg), linezolid (30 µg), theicoplanin (30 µg), amikacin (30 µg), and meropenem (10 µg) by the disk-diffusion method using antimicrobial disks (MAST) and Muller-Hinton agar medium (Merk, Germany) (8) and interpreted according to CLSI procedures.

3.3. Analysis of Biofilm Forming Isolates

To identify the biofilm forming isolate, the microtiter plate test (mpt) was used, with a modification of the method described by Merritt et al. (9) The BHI broth cultures were used to produce a bacterial suspension in Muller-Hinton Broth with a density of 0.5 McFarland, then 200 µL from each suspension was added to 96-well microtiter plates. The plates were incubated overnight at 37°C. After incubation, the free floating cells were removed and submerged in a tray full of distilled water. Following the washing, the plates were stained with 125 µL of 0.2% (w/v) crystal violet, and then incubated at room temperature for 15 minutes. The dye was rinsed with distilled water three times, and the plates were allowed to air-dry. Then, 200 µL of absolute ethanol was added and incubated for a further 15 minutes at room temperature. A total of 200

µL from each well was transferred to a fresh microtiter plate well. Finally, the optical density of each suspension was measured at 570 nm. *Staphylococcus epidermidis* RP62A was used as a positive control for the biofilm assay while the negative control consisted of all added reagents with the exception of the bacterial suspension. The test was repeated in triplicates. Mean absorbance at 570 nm ± SD was calculated. According to Merritt et al., if the optical density is higher than 0.2, the strain is considered a biofilm producer (10).

3.4. Detection of *icaABD*, *IS256* and *aap* Genes

In order to study the frequency of *icaABD*, *IS256*, and *aap* genes among the tested isolates, the PCR method was used. Total genomic DNA extraction of *S. epidermidis* isolates was performed using a DNA extraction kit (GenAll Biotechnology, Korea). DNA content was determined by measuring absorbance at 260 nm. Overall, 1 µL of each DNA was added to 25 µL of mixture reaction including 10X reaction buffer, 2 mM MgCl₂, 0.1 mM of the deoxynucleoside triphosphates (dNTPs), 10 pmol of designed primers, according to Table 1 and 1.5 U of Taq DNA Polymerase (Cinnagen, Tehran, Iran) and was amplified based on PCR conditions in a DNA thermal cycler, including pre-denaturation at 95°C for five minutes, 35 cycles of denaturation at 95°C for one minute, annealing at 59°C for one minute, extension at 72°C for one minute, and final elongation at 72°C for five minutes for *icaABD* and *IS256* genes and pre-denaturation at 94°C for five minutes, 35 cycles of denaturation at 94°C for 45 seconds, annealing at 54°C for 45 seconds, extension at 72°C for one minute, and final elongation at 72°C for five minutes for *aap* gene. In this study, the researchers used the *IS256* gene to determine the pathogenicity of *S. epidermidis* strains and detected *ica* and *aap* genes to analyze the potency of biofilm formation. The PCR products were detected in 1.5% agarose gel with the use of ethidium bromide and visualized by UV trans-illumination.

3.5. Plant Material

During year 2014, fresh plant leaves and seeds of *Zataria multiflora* were purchased from a local herbal medicinal shop in Tehran, Iran.

3.6. Preparation of the Essential Oil of *Zataria multiflora*

Overall 20 g of ground parts of leaves and seeds of *Zataria multiflora* were soaked in 500 mL of sterile distilled water and distillate by Clevenger apparatus for two hours. The yield of essential oil was stored at 4°C.

Table 1. Specific Primers Used for PCR

Primer Name	Sequence (5'-3')	Amplicon Size (bp)	Ref
icaBD			
F	TTATCAAT GCCGCAGTTGTC	516	(11)
R	GTTTAACGCG AGTGCGCTAT		
IS256			
F	TGAAAAGCGAAGAGATCAAAGC	1013	(12)
R	ATGTAGGTCCATAAGAACGGC		
Aap			
F	ATGGGCAACCGTAGACAAG	1100	(13)
R	ACCGTAAAAATCGTAATTATCTC		
Gyr			
F	CTTATATGAGAATCCATCTGTAGG	154	(14)
R	AGAACAATCTGCCAATTTACC		
IcaD			
F	CCGGAGTATTTGGATGTATTG	197	(15)
R	TGAAACGCGAGACTAAATGTA		
IcaA			
F	TCTCTTCAGGAGCAATCAA	186	(16)
R	AGGCCACTAACATCCAGCA		
Aap			
F	AGAAACAAGCTGGTCAAG	117	(17)
R	CTGCGTAGTTAAGAAAATC		

3.7. Determination of MIC and MBC Concentration by the Broth Microdilution Method

The MIC of *Zataria multiflora* essential oil was determined in LB broth by broth microdilution method to yield concentrations ranging from 0.25 to 10 (% v/v). Microbial suspension of *Staphylococcus epidermidis* RP62A strain was justified to 0.5 MacFarland. Overall, 40 µL of microbial suspension with LB broth medium and essential oil concentration were added to each tube, and tubes were incubated at 37°C for 18 hours. The lowest concentration of essential oil with no visible bacterial growth was determined as the MIC. The MBC of essential oil was reported using the first tube showing no growth on LB agar. Finally, the sub-MIC of essential oil was determined.

3.8. Preparation the Ethanol Extract of *Zataria multiflora*

Leaves and seeds of *Zataria multiflora* were dried, ground in a grinder, and about 100 g of dry powder was extracted with 1500 mL of 70% ethanol for 48 hours. Then the extract was filtered through Wathman no 1 filter paper. The solvent of the extract was evaporated by a vacuum evaporator, and finally residues were dried and used

for the experiment. The major components of *Zataria multiflora* extract were analyzed using a gas chromatography-mass spectroscopy (GC-MS).

3.9. Determination of MIC and MBC Concentration by Broth Microdilution Method

The MIC of *Zataria multiflora* extract were determined in LB broth by the microdilution method. The extract was diluted in 80% DMSO to yield concentrations ranging from 0.25 to 10 (% w/v). Microbial suspension of *Staphylococcus epidermidis* RP62A strain was adjusted to 0.5 MacFarland. Overall, 40 µL of suspension bacterial with LB broth medium and extract concentrations were added to each tube and tubes were incubated at 37°C for 18 hours. The lowest concentration of extract with no visible bacterial growth was determined as the MIC. The MBC of the extract was reported using the first tube showing no growth on LB agar. Finally, the subMIC of the extract was determined.

3.10. Effect of Sub-Inhibitory Concentration of Essential Oil and Extract on Biofilm Formation

Microbial suspensions of 109 Biofilm positive strains in Muller Hinton Broth medium were adjusted to 0.5 MacFarland and 100 µL were added to each well of 96-well microtiter plate. Then, 100 µL of sub-inhibitory concentration (sub-MIC) of essential oil (9%) and extract (4 mg/mL) were dispensed in each well. Microbial suspensions and Muller Hinton Broth medium without the essential oil and extract were used as the negative control. Microplates were incubated at 37°C for 18 hours. Each assay was repeated in duplicates. Following incubation, biofilm formation was performed as described above (9).

3.11. Effect of Sub-Inhibitory Concentration of Essential Oil and Extract on the Expression of *icaAD* and *aap* Genes by Use of Real Time-PCR

The microbial suspension of *Staphylococcus epidermidis* RP62A in LB broth medium was adjusted to 0.5 MacFarland. Overall, 20 µL of microbial suspension with LB broth medium were separately added to tubes containing sub-MIC concentration of essential oil (9%) and extract (4 mg/mL). The microbial suspension without plant was evaluated as a negative control. After overnight incubation at 37°C, the bacterial pellet was collected using centrifuge at 5000 Xg for 20 minutes and RNA extraction was performed using the Genall kit (GenAll Biotechnology, Korea). The RNA concentration was determined by nano-drop. Complementary DNA (cDNA) was synthesized using the Thermo scientific kit. Quantitative real time PCR was performed for appointment of scale of *icaAD* and *aap* genes expression. The primers that were used in this study are shown

in Table 1. One microliter of each cDNA was amplified in 20 μ L of mixture reaction containing 2X cyber green mixture reaction and 10 μ mol of designed primers (Viragene, USA). The expression level of *gyr* gene was surveyed as an internal control. The PCR protocol was exerted by Corbet 6000 with an initial denaturation step during three minutes at 94°C, 38 cycles of 30 seconds at 94°C, 30 seconds at the desired annealing temperature, and 30 seconds at 72°C. All tests were done in triplicates. The relative fold change alterations in the expression were analyzed by the Rest 2009 software.

3.12. Statistical Analysis

The Pearson chi-squared test was performed in order to explore the relationship between biofilm production and the presence of *icaABD* and *aap* genes, the correlation between biofilm formation and drug resistance, and the relationship between the presence of *icaABD*, *aap* genes, and drug resistance. P value was considered significant at < 0.05. Data analysis was performed using SPSS 16.0 (SPSS Inc., IBM, USA).

4. Results

4.1. Antibiotic Resistance

Antimicrobial susceptibility was determined using the disk diffusion method. *Staphylococcus epidermidis* strains showed various susceptibilities to tested chemotherapeutics (Figure 1). The strains had high resistance to erythromycin (86.2%) and lower resistance to linezolid (6.5%) (Figure 1).

4.2. Biofilm Formation

The MtP method was used to determine biofilm forming ability of isolates. Overall, 109 (71.2%) of the isolates were biofilm positive and they were used for evaluating the effect of extract and essential oil. Table 2 summarizes the antibiotic resistance pattern among biofilm producer and non-producer isolates. There was no significant discrepancy in antibiotic resistance between isolates with the ability of biofilm production and those without these properties ($P > 0.05$).

4.3. Presence of *IS256*, *icaABD* and *aap* Genes

Genetic analysis of 153 *S. epidermidis* strains showed the presence of *icaABD* in 80 (52.2%) strains (Figure 2A), and the presence of *aap* gene in 136 (88.8%) isolates (Figure 2B), and the presence of *IS256* gene in 139 (90%) isolates (Figure 2C). The pattern of antibiotic susceptibility was not different between the strains carrying the *icaABD* gene and those possessing the *aap* gene ($P > 0.05$). Additionally, among the strains with *icaABD* genes, only 53 (66.2%) exhibited the

ability to form biofilms, and among the isolates with *aap* gene, 86 (63.2%) were biofilm positive yet there is no significant discrepancy in biofilm formation with the presence of *icaABD* and *aap* genes ($P > 0.05$). In addition, 27 (17.6%) and 50 (32.7%) of non-biofilm producer strains carried the *icaABD* and *aap* genes, respectively.

4.4. Major Components of *Zataria multiflora* Extract

The compositional analysis of *Zataria multiflora* extract by GC/MS is shown in Table 3. The major components were phenol, 2-methyl-5-1-methylethyl (26.9%), Formamide, N-methoxy (21.7%), and thymol (16.4%).

4.5. MIC and MBC of *Zataria multiflora* Essential Oil and Extract

To determine the MIC and MBC of *Zataria multiflora*, the biofilm forming isolates were selected and examined. The MBC and MIC of *Zataria multiflora* essential oil were 10% and sub-MIC concentration was 9%. Furthermore, the MBC and MIC of *Zataria multiflora* extract was 6 mg/mL and 5 mg/mL, respectively.

4.6. Phenotypic and Genotypic Impacts of Sub-Inhibitory Concentration (subMIC) of Essential Oil and Extract on Biofilm Formation

In order to determine the impact of *Zataria multiflora* extract and essential oil on biofilm forming ability and expression of genes involved in biofilm production in selected isolates, the sub-inhibitory concentration (subMIC) of essential oil and extract was tested. Among 109 biofilm positive isolates, the sub-MIC concentration of essential oil and extract reduced biofilm formation ability in 93 (85.3%) and 106 (97.2%), respectively.

In order to determine the effect of *Zataria multiflora* essential oil and extract on the expression of key genes involved in biofilm formation, the real time-PCR was used. The expression efficiency was between 97% and 103%. The results of sub-inhibitory concentration of essential oil and extract on the expression of *icaA*, *icaD*, and *aap* genes are shown in Figure 3. Based on the findings of Rest2009, the relative mRNA expression of *icaA*, *icaD*, and *aap* genes were reduced by 0.15, 0.1, and 0.07 folds compared to the negative control after treating the *Staphylococcus epidermidis* RP62A with subMIC concentration of essential oil ($P < 0.001$). In addition, the relative expression of *icaA*, *icaD*, and *aap* genes after exposure to the subMIC concentration of extract was lower (0.37, 0.42 and 0.41, respectively) than the negative control ($P < 0.001$).

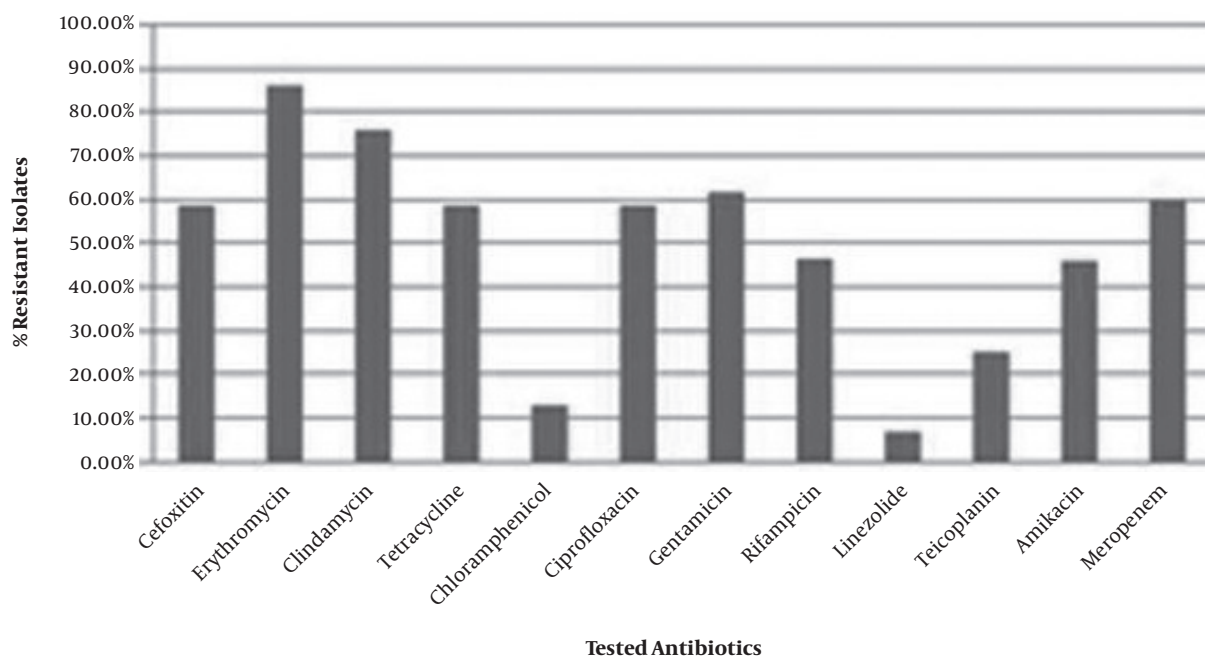


Figure 1. The number/percentage of *S. epidermidis* strains resistant to selected antibiotics

Table 2. Frequency of Antibiotic Resistance Among Biofilm Producer and Non-Producer *S. epidermidis* Isolates^a

Antibiotics	Total Isolates	Resistant	
		Biofilm Forming Isolates	Non Biofilm Forming Isolates
FOX	90 (58.8)	50 (52.6)	40 (69)
E	132 (86.2)	77 (81.1)	55 (94.8)
DA	116 (75.8)	65 (68.4)	51 (87.9)
T	90 (58.8)	52 (54.7)	38 (65.5)
C	20 (13)	8 (8.4)	12 (20.7)
CIP	90 (58.8)	54 (56.8)	36 (62.1)
CN	94 (61.4)	51 (53.7)	43 (74.1)
RIF	71 (46.4)	35 (36.8)	36 (62.1)
LZD	10 (6.5)	4 (4.2)	6 (10.3)
TEC	38 (24.8)	20 (21.1)	18 (31)
AK	70 (45.7)	32 (33.7)	36 (62.1)
MEM	91 (59.4)	51 (53.7)	40 (69)
Total	153 (100)	63 (100)	90 (100)

Abbreviations: AK, amikacin; C, chloramphenicol; CN, gentamicin; DA, clindamycin; E, erythromycin; FOX, cefoxitin; LZD, linezolid; MEM, meropenem; RIF, rifampicin; TEC, teicoplanin.

^a Values are expressed as No. (%).

5. Discussion

The present study investigated biofilm-forming strains of *S. epidermidis* by phenotypic and genotypic methods, and the association between biofilm production and antimicrobial resistance. The study used two methods including MTP for the evaluation of biofilm formation, and PCR for the detection of *icaABD*, *IS256*, and *aap* genes.

The findings were in agreement with Sahal and Bilkay (11) and Farajzadeh Sheikh and Mehdinejad studies (18) that showed the isolates of blood and wound were the most common clinical materials. The researchers demonstrated that in some isolates of *S. epidermidis*, sub-inhibitory concentrations of azithromycin, clarithromycin, and erythromycin enhanced biofilm formation in a dose-dependent manner (19). Based on Sahal and Bilkay study,

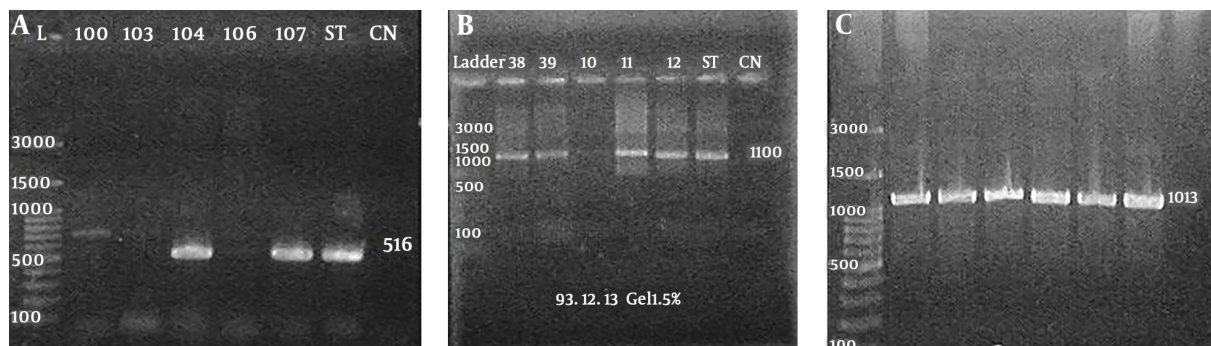


Figure 2. Detection of the operon *icaBD*, and *Aap* and *IS256* genes in *S. epidermidis* strains. (A) PCR results with primer for *icaABD* gene, (B) PCR results with primer for *aap* gene, (C) PCR results with primer for *IS256* gene

Table 3. Compositional Analysis of *Zataria multiflora* Extract

Compounds	Percentage (%)	Retention Time
Propanoic acid,3-(acetylthio)-2-methyl	4.51	5.569
Formamide, N-methoxy	21.7	5.923
Dodecane	4.13	16.546
Thymol	16.4	19.595
Phenol,2-methyl-5-(1-methylethyl)	26.9	19.87
Tetradecane	16	21.873
Phenol,3,5-bis(1,1-dimethylethyl)	0.72	24.954
7-methyl-Z-tetradecane-1-ol acetat	7.62	26.643
t-Buthyl(1)-dimethyl-silane	1.69	28.002

S. epidermidis strains from blood, wound, and cardiothoracic surgery samples showed sensitivity to vancomycin, while 65% were resistant to all β -lactams and 60% were multi-drug resistant (11); researchers have suggested that vancomycin is an efficient antibiotic for inhibition of *S. epidermidis* (11, 20-22). The current results are in agreement with other studies that showed resistance to erythromycin (8, 19). Erythromycin is thought to be ineffective for the eradication of biofilms. In the current study on 153 strains, 109 (71.2%) isolates produced biofilm, and the current results showed no significant association between antibiotic resistance and biofilm formation.

Wojtyczka demonstrated that *S. epidermidis* showed high susceptibility to rifampicin, ciprofloxacin, and chloramphenicol yet less susceptibility to erythromycin and SXT trimethoprim/sulfamethoxazole. Wojtyczka evaluated 32 *S. epidermidis* strains and 12 (37.5%) were biofilm positive. There was no significant relationship between biofilm formation and antimicrobial resistance (8). In another study, out of 80 strains of *S. epidermidis*, 53 (66%) were biofilm

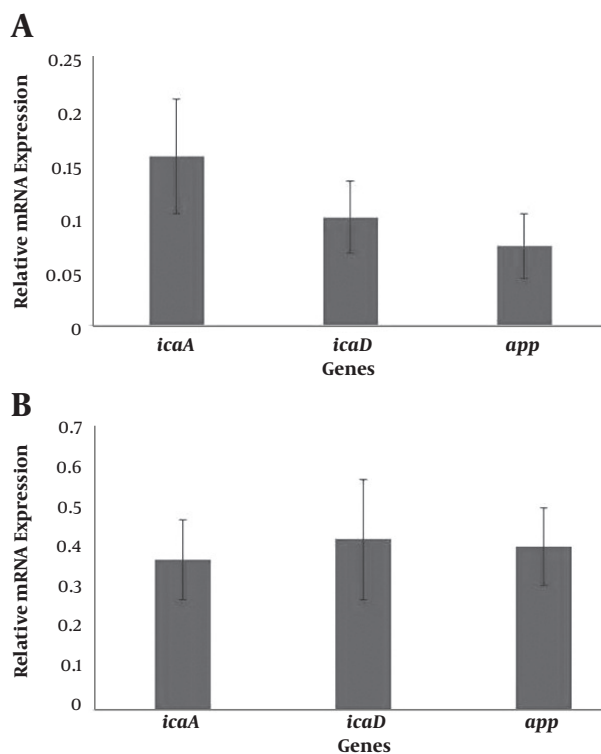


Figure 3. The relative expression of *icaA*, *icaD* and *aap* genes after treating with sub-MIC concentration of essential oil (A) and extract (B)

positive and 12 (15%) were weak producers (23). It is believed that *ica* operon genes are associated with biofilm production (24). In this study, out of 135 strains, 53 (55.8%) possessed the *icaBD* gene yet did not exhibit biofilm formation. This result may be due to high frequency of biofilm producer isolates in the population. Eftekhari studied biofilm phenotype and *ica* operon genes carriage in

two groups of isolates, clinical strains from symptomatic patients, and skin isolates from healthy patients. In total, 52% of the clinical strains and 56% of the skin isolates were biofilm-forming strains with *ica* operon genes carriage being 30% and 8%, respectively. Eftekhar indicated that *aap* is the most likely factor for biofilm formation via a PIA non-dependent pathway. In the current findings, the presence of the *aap* gene was 88.8% among 153 *S. epidermidis* isolates and showed no association with phenotype. Dadashi et al. reported that the chloroformic extract of *Zataria multiflora* had significant inhibitory impact on the ESBL-producing *Klebsiella pneumoniae* strains with MIC₅₀ = 1.56 mg/mL and MIC₉₀ = 3.12 mg/mL. Furthermore, methanolic and acetic extracts showed lower impact with MIC₅₀ of 3.12 and 6.25 mg/mL, respectively (6).

Of note, the presence of the *aap* gene was greater than *IS256* and *icaABD* genes among blood samples. The current study revealed no association between the presences of *IS256* as an invasiveness factor with clinical samples. Regarding MtP results, there was a greater number of isolates from wound and blood samples yet there was no significant association with *icaABD* and *aap* genes, therefore, the results demonstrate no particular pattern among the phenotypic with genotypic method.

Other studies have found no relationship between biofilm formation and the presence of the *ica* operon (25, 26). Analysis of data showed 27 (46.6%) strains with the *icaABD* gene and 50 (86.2%) strains with the *aap* gene among non-biofilm forming strains; these observations suggested there were other factors regulating biofilm formation (27, 28).

Comparing other studies and the current findings, it could be suggested that clinical and commensal isolates have similar virulence factors. In the current research, some strains were biofilm negative/*ica* positive and biofilm negative/*Aap* positive. In commensal strains, it may not be necessary to express *ica* operon genes and form biofilms, yet in clinical strains, in order to avoid the host immune system, expression of the *ica* operon or other factors is necessary. In conclusion, the expression of virulence factors depends on external environmental conditions, such as the presence of patient's immune system. In the current findings, there were both biofilm positive/*ica* negative and biofilm positive/*Aap* negative strains, and that the environmental conditions determine which virulence factors *S. epidermidis* expresses to ensure survival.

The phenolic components of extracts derived from *Zataria multiflora* are predominant; however, geographic differences, age, and preparation protocol could alter the content of extracts. Carvacrol (61.29%) and thymol (25.18%) were the dominant components in *Zataria multiflora* extract from Yazd (3) yet in Fars plant, only carvacrol (71.12%)

was seen in the extract content (4). The major content of the extract contains thymol and phenol compounds, however, there was no carvacrol compound in the extract. The presence of thymol and phenolic compound results in antimicrobial impacts. Based on the outcomes, the extracts of *Zataria multiflora* had anti-bacterial and anti-biofilm effects against *S. epidermidis*. There are a few findings concerning the impacts of *Zataria multiflora* on *S. epidermidis*. In the work by Mahboubi et al., the MIC of *Zataria multiflora* was 1.56 mg/mL (29), which is lower than the current findings (5 mg/mL). However, according to the researcher's knowledge, there is no document to assess the effects of *Zataria multiflora* on biofilm forming phenotype and the expression of genes involved.

The current study is the first to report the anti-biofilm property of *Zataria multiflora*. This effect is both phenotypic and genotypic and leads to significant attenuation of mRNA expression of momentous genes involved in biofilm formation, such as *icaA*, *icaD*, and *aap* genes. Given the significant inhibitory effect of the extract and essential oil of *Zataria multiflora* on biofilm formation, it seems that these substances are good options for studies related to controlling biofilm formation in *Staphylococcus epidermidis*.

Acknowledgments

This article was extracted from a thesis.

Footnotes

Authors' Contribution: Acquisition of data and drafting of the manuscript was done by Zahra Mottaghiyan, administrative, technical, and material support was done by Mohammad Aghazadeh, analysis and interpretation of data and statistical analysis were performed by Hamideh Mahmoodzadeh Hosseini. Study concept and design were done by Abbas Ali Imani Fooladi.

Conflict of Interests: None.

Funding/Support: None declared.

References

1. Bryers JD. Medical biofilms. *Biotechnol Bioeng*. 2008;**100**(1):1-18. doi: [10.1002/bit.21838](https://doi.org/10.1002/bit.21838). [PubMed: [18366134](https://pubmed.ncbi.nlm.nih.gov/18366134/)]. [PubMed Central: [PMC2706312](https://pubmed.ncbi.nlm.nih.gov/PMC2706312/)].
2. Kampf G, Löffler H, Gastmeier P. Hand hygiene for the prevention of nosocomial infections. *Dtsch Arztebl Int*. 2009;**106**(40):649-55. doi: [10.3238/arztebl.2009.0649](https://doi.org/10.3238/arztebl.2009.0649). [PubMed: [19890431](https://pubmed.ncbi.nlm.nih.gov/19890431/)]. [PubMed Central: [PMC2770229](https://pubmed.ncbi.nlm.nih.gov/PMC2770229/)].
3. Shafiee A, Javidnia K. Composition of essential oil of *Zataria multiflora*. *Planta Med*. 1997;**63**(4):371-2. doi: [10.1055/s-2006-957707](https://doi.org/10.1055/s-2006-957707). [PubMed: [17252397](https://pubmed.ncbi.nlm.nih.gov/17252397/)].

4. Azizkhani M, Misaghi A, Basti AA, Gandomi H, Hosseini H. Effects of *Zataria multiflora* Boiss. essential oil on growth and gene expression of enterotoxins A, C and E in *Staphylococcus aureus* ATCC 29213. *Int J Food Microbiol.* 2013;**163**(2-3):159-65. doi: [10.1016/j.ijfoodmicro.2013.02.020](https://doi.org/10.1016/j.ijfoodmicro.2013.02.020). [PubMed: 23558199].
5. Saito ST, Trentin Dda S, Macedo AJ, Pungartnik C, Gosmann G, Silveira Jde D, et al. Bioguided fractionation shows *Cassia alata* extract to inhibit *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* growth and biofilm formation. *Evid Based Complement Alternat Med.* 2012;**2012**:867103. doi: [10.1155/2012/867103](https://doi.org/10.1155/2012/867103). [PubMed: 22548121]. [PubMed Central: PMC3323858].
6. Dadashi M, Hashemi A, Eslami G, Fallah F, Goudarzi H, Erfanimanesh S, et al. Evaluation of antibacterial effects of *Zataria multiflora* Boiss extracts against ESBL-producing *Klebsiella pneumoniae* strains. *Avicenna J Phytomed.* 2016;**6**(3):336-43. [PubMed: 27462557]. [PubMed Central: PMC4930541].
7. Bannerman TL, Peacock S, Murray P, Baron E, Jorgensen J, Landry M, et al. *Staphylococcus*, *Micrococcus* and other catalase-positive cocci. *Manual Clin Microbiol.* 2006:390-411.
8. Wojtyczka RD, Orlewska K, Kepa M, Idzik D, Dziedzic A, Mularz T, et al. Biofilm formation and antimicrobial susceptibility of *Staphylococcus epidermidis* strains from a hospital environment. *Int J Environ Res Public Health.* 2014;**11**(5):4619-33. doi: [10.3390/ijerph110504619](https://doi.org/10.3390/ijerph110504619). [PubMed: 24776724]. [PubMed Central: PMC4053877].
9. Merritt JH, Kadouri DE, O'Toole GA. Growing and analyzing static biofilms. *Curr Protoc Microbiol.* 2005;Chapter 1:Unit 1B 1. doi: [10.1002/9780471729259.mc01b01s00](https://doi.org/10.1002/9780471729259.mc01b01s00). [PubMed: 18770545]. [PubMed Central: PMC4568995].
10. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, et al. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: A quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol.* 1985;**22**(6):996-1006. [PubMed: 3905855]. [PubMed Central: PMC271866].
11. Sahal G, Bilkay IS. Multi drug resistance in strong biofilm forming clinical isolates of *Staphylococcus epidermidis*. *Braz J Microbiol.* 2014;**45**(2):539-44. [PubMed: 25242939]. [PubMed Central: PMC4166280].
12. Kozitskaya S, Cho SH, Dietrich K, Marre R, Naber K, Ziebuhr W. The bacterial insertion sequence element IS256 occurs preferentially in nosocomial *Staphylococcus epidermidis* isolates: Association with biofilm formation and resistance to aminoglycosides. *Infect Immun.* 2004;**72**(2):1210-5. [PubMed: 14742578]. [PubMed Central: PMC321601].
13. Djalalinia S, Owlia P, Forouzan AS, Habibi E, Dejman M, Eftekhari MB, et al. Health research evaluation and its role on knowledge production. *Iran J Public Health.* 2012;**41**(2):39-46. [PubMed: 23113133]. [PubMed Central: PMC3481679].
14. Lou Q, Qi Y, Ma Y, Qu D. Two-component signal transduction system SaeRS positively regulates *Staphylococcus epidermidis* glucose metabolism. *ScientificWorldJournal.* 2014;**2014**:908121. doi: [10.1155/2014/908121](https://doi.org/10.1155/2014/908121). [PubMed: 24592198]. [PubMed Central: PMC3921950].
15. Tormo MA, Marti M, Valle J, Manna AC, Cheung AL, Lasa I, et al. SarA is an essential positive regulator of *Staphylococcus epidermidis* biofilm development. *J Bacteriol.* 2005;**187**(7):2348-56. doi: [10.1128/JB.187.7.2348-2356.2005](https://doi.org/10.1128/JB.187.7.2348-2356.2005). [PubMed: 15774878]. [PubMed Central: PMC1065223].
16. Arciola CR, Baldassarri L, Montanaro L. Presence of *icaA* and *icaD* genes and slime production in a collection of staphylococcal strains from catheter-associated infections. *J Clin Microbiol.* 2001;**39**(6):2151-6. doi: [10.1128/JCM.39.6.2151-2156.2001](https://doi.org/10.1128/JCM.39.6.2151-2156.2001). [PubMed: 11376050]. [PubMed Central: PMC88104].
17. Juarez-Verdayes MA, Ramon-Perez ML, Flores-Paez LA, Camarillo-Marquez O, Zenteno JC, Jan-Roblero J, et al. *Staphylococcus epidermidis* with the *icaA(-)/icaD(-)/IS256(-)* genotype and protein or protein/extracellular-DNA biofilm is frequent in ocular infections. *J Med Microbiol.* 2013;**62**(Pt 10):1579-87. doi: [10.1099/jmm.0.055210.0](https://doi.org/10.1099/jmm.0.055210.0). [PubMed: 23861297].
18. Farajzadeh Sheikh A, Mehdinejad M. Identification and determination of coagulase-negative *Staphylococcus* species and antimicrobial susceptibility pattern of isolates from clinical specimens. *Afr J Microbiol Res.* 2012;**6**(8). doi: [10.5897/ajmr11.076](https://doi.org/10.5897/ajmr11.076).
19. Wang Q, Sun FJ, Liu Y, Xiong LR, Xie LL, Xia PY. Enhancement of biofilm formation by subinhibitory concentrations of macrolides in *icaADBC*-positive and -negative clinical isolates of *Staphylococcus epidermidis*. *Antimicrob Agents Chemother.* 2010;**54**(6):2707-11. doi: [10.1128/AAC.01565-09](https://doi.org/10.1128/AAC.01565-09). [PubMed: 20231401]. [PubMed Central: PMC2876384].
20. Doğruman Al FAG, Sipahi B, Sultan N. Kan örneklerinden soyutlanan stafilokok suşlarının antibiyotiklere direnç durumları. *Ankem Derg.* 2005;**19**(1):14-6.
21. Chaieb K, Abbassi MS, Touati A, Hassen AB, Mahdouani K, Bakhrour A. Molecular characterization of *Staphylococcus epidermidis* isolated from biomaterials in a dialysis service. *Annal Microbiol.* 2005;**55**(4):307.
22. McCann MT, Gilmore BF, Gorman SP. *Staphylococcus epidermidis* device-related infections: Pathogenesis and clinical management. *J Pharm Pharmacol.* 2008;**60**(12):1551-71. doi: [10.1211/jpp/60.12.0001](https://doi.org/10.1211/jpp/60.12.0001). [PubMed: 19000360].
23. Arciola CR, Campoccia D, Gamberini S, Donati ME, Pirini V, Visai L, et al. Antibiotic resistance in exopolysaccharide-forming *Staphylococcus epidermidis* clinical isolates from orthopaedic implant infections. *Biomaterials.* 2005;**26**(33):6530-5. doi: [10.1016/j.biomaterials.2005.04.031](https://doi.org/10.1016/j.biomaterials.2005.04.031). [PubMed: 15949842].
24. Fredheim EG, Klingenberg C, Rohde H, Frankenberg S, Gaustad P, Flaegstad T, et al. Biofilm formation by *Staphylococcus haemolyticus*. *J Clin Microbiol.* 2009;**47**(4):1172-80. doi: [10.1128/JCM.01891-08](https://doi.org/10.1128/JCM.01891-08). [PubMed: 19144798]. [PubMed Central: PMC2668337].
25. Ninin E, Caroff N, Espaze E, Marailac J, Lepelletier D, Milpied N, et al. Assessment of *ica* operon carriage and biofilm production in *Staphylococcus epidermidis* isolates causing bacteraemia in bone marrow transplant recipients. *Clin Microbiol Infect.* 2006;**12**(5):446-52. doi: [10.1111/j.1469-0691.2006.01382.x](https://doi.org/10.1111/j.1469-0691.2006.01382.x). [PubMed: 16643521].
26. de Silva GD, Kantzanou M, Justice A, Massey RC, Wilkinson AR, Day NP, et al. The *ica* operon and biofilm production in coagulase-negative *Staphylococcus* associated with carriage and disease in a neonatal intensive care unit. *J Clin Microbiol.* 2002;**40**(2):382-8. [PubMed: 11825946]. [PubMed Central: PMC153361].
27. Eftekhari F, Mirmohamadi Z. Evaluation of biofilm production by *Staphylococcus epidermidis* isolates from nosocomial infections and skin of healthy volunteers. *Int J Med Sci.* 2009;**1**(10):438-41.
28. Dobinsky S, Kiel K, Rohde H, Bartscht K, Knobloch JK, Horstkotte MA, et al. Glucose-related dissociation between *icaADBC* transcription and biofilm expression by *Staphylococcus epidermidis*: Evidence for an additional factor required for polysaccharide intercellular adhesion synthesis. *J Bacteriol.* 2003;**185**(9):2879-86. [PubMed: 12700267]. [PubMed Central: PMC154395].
29. Mahboubi A, Kamalinejad M, Ayatollahi AM, Babaeian M. Total phenolic content and antibacterial activity of five plants of labiatae against four foodborne and some other bacteria. *Iran J Pharm Res.* 2014;**13**(2):559-66. [PubMed: 25237351]. [PubMed Central: PMC4157031].