Clinical and Experimental Health Sciences

In Vitro Activity of Fosfomycin on Biofilm in Community-Acquired *Staphylococcus aureus* Isolates

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ABSTRACT

Objective: *Staphylococcus aureus (S. aureus)* is a significant pathogen microorganism that can lead to serious infections. In this study, we researched the activity of biofilm formation and fosfomycin on biofilm in community-acquired *S. aureus* isolates that were drawn from human noses.
Methods: Microtitration plate method was used to determine biofilm formation. The effect of fosfomycin on sessile cells was studied on biofilm matrix composed around plastic beads. The icaA, icaD, icaB, icaC, bap, eno, fnbA, fnbB, clfA, clfB, fib, ebpS, cna and mecA genes were screened by Polymerase Chain Reactions (PCR).
Results: *S. aureus* was isolated from 87 samples (13.2%) out of a total 658 nasal samples. We found that 10 of these isolates (11.4%) were methicillin-resistant *S. aureus* (MRSA). A total of 86 isolates had the ability to form biofilm. The biofilm inhibitor concentration (BIC) and minimum biofilm eradication concentration (MBEC) of fosfomycin were determined as 8 µg/ml and 32 µg/ml, respectively. In the molecular detection results of biofilm-related genes of these isolates, ica-dependent genes were determined to be quite high. However, no bap gene was observed to be positive in any of the isolates. Among the other genes, the most frequent genes to be declared positive were eno (97.6%) and fnbA (94.1%).
Conclusion: This study indicates that prevalence of biofilm genes in *S. aureus* isolates in nasal flora is high and fosfomycin is an effective anti-biofilm agent alone. However, to increase fosfomycin's efficiency, there is a need for more combination studies to make it more effective.
Keywords: *Staphylococcus aureus*, biofilm, fosfomycin, biofilm-related genes, nasal colonisation

1. INTRODUCTION

Staphylococcus aureus emerges as a pathogenic microorganism in many community-based and hospital-acquired infections (1). It leads to serious morbidity and mortality by causing various infections such as bacteraemia, infective endocarditis, septic arthritis, osteomyelitis and prosthetic joint and artificial graft infections (2, 3). In most staphylococcal infections, the agent is endogenous. Its colonisation in healthy humans' nasal mucosa is a risk factor for later infections (4). Nasal carriage of about 10%-40% has been reported for *S. aureus* in the human population (5).

The most pressing concern regarding *S. aureus* isolates today is their growing resistance to antibiotics (6, 7). Methicillin-resistant *S. aureus* (MRSA) isolates are common pathogens all over the world. However, community-acquired MRSA infections have increased the severity of the problem (1). With limited treatment options, decreased sensitivity and reports of resistance to vancomycin have become a problem (8). One of the reasons for antibiotic resistance in *S. aureus* isolates is their formation of biofilm (9). Biofilm is an important virulence factor because of survive in hospitals for a long time and antibiotic resistance (10).

Biofilm is a community formed by microorganisms residing in a living or inanimate surface that are embedded in an organic exopolysaccharide matrix of their own production and adhered to one another on a solid surface or interface (11, 12). Bacteria in biofilm are known to be 100-10,000 times more resistant to antibiotics, than their planktonic forms (13). Bacteria that do not die in the presence of antibiotics in the biofilm cause persistent infections that are difficult to treat (3). High morbidity and mortality rates associated with these infections are critical burdens that lead to high cost (9). Therefore, preventing these infections effectively and treating the infections are vital.

Fosfomycin trometamol, first obtained in Streptomyces cultures in Spain in 1969 and originally named fosfomycin, has been used for many years in the treatment of various infections, mainly urinary tract infections (14). Fosfomycin prevents the formation of UDP-NAMA by inhibiting the enzyme MurA and demonstrates its antibacterial activity by preventing the synthesis of the peptidoglycan layer (15). Recently, in addition to low resistance, its pharmacokinetic and pharmacodynamic advantages, in vivo activity, clinical efficiency, high level of tolerability and reliability and existence as a treatment option for infections other than

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urinary system infections caused by resistant bacteria are some of these remarkable features (16).

In this study, we examined the biofilm formation by community-acquired *S. aureus* nasal culture isolates and the effect of fosfomycin against adhered bacteria in the biofilm.

2. METHODS

2.1. Bacterial isolates

Samples of nose swabs were taken from 658 patients who were admitted to the Otorhinolaryngology polyclinic and had no history of hospitalisation prior to being involved in the 6-month study at the Kirsehir Ahi Evran University Education and Research Hospital. *S. aureus* isolation and identification of nose swab samples were done by using conventional methods (using mannitol salt agar (BD, USA), coagulase tube test) and the Vitek-2 system (bioMérieux, France). Methicillin resistance was tested with a cefoxitin (30µg) disk diffusion method in line with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and was confirmed with the positive presence of the mecA gene in the isolates (17). The isolates were stored in 20% glycerol at - 80°C until run time.

Written informed consent was obtained from all participants. The study was performed in accordance with the Declaration of Helsinki's Good Clinical Practice guidelines and approved by the Turgut Ozal University Faculty of Medicine Ethical Committee (Ethical approval number-date: 99950669/32-9.01.2015).

2.2. Determination of biofilm production by microtiter plate assay

Biofilm formation was determined semi-quantitatively as defined in previous studies (18, 19). All isolates were incubated overnight at 37°C using Trypticase soy broth (TSB) supplemented with 2% glucose. TSB cultures of *S. aureus* isolates were diluted 1:100 with fresh TSB and 150 µl aliquots of each dilution were placed in 96-well plate. Three wells were used for each isolate. Plates were incubated for 48 h at 37°C. After incubation, the plates were washed 3 times with phosphate-buffered saline and 2% of crystal violet was used for staining. After washing the plates again with PBS, 150 µl of ethanol-acetone mixture (80:20) was put into each well and optical densities (OD) were determined by scanning at 540 nm. Using *S. aureus* ATCC 25923 (which forms strong biofilm) as a positive control and *E. coli* ATCC 25922 (which does not form biofilm) as a negative control, biofilm formation was determined in accordance with OD values (20). The isolates that gave absorbance values equal to or below the absorbance value of *E. coli* 25922 strains that did not form biofilms were evaluated as negative. The isolates that gave absorbance values equal to or above the absorbance value of *S. aureus* 25923 strains known to produce strong biofilms were identified as strong biofilm-producing isolates. The isolates with absorbance values between both controls were evaluated as moderate biofilm-producing isolates. Experiments were repeated 3 times.

2.3. Antibiotic study on biofilms

In order to investigate the efficacy of fosfomycin on biofilm, three MRSA isolates and three MSSA isolates were selected for the basis that produced a strong biofilm.

2.3.1. Antimicrobial agent and Minimum inhibitor concentration (MIC) determination

Fosfomycin was supplied as a dry powder for laboratory use by Sigma-Aldrich (St. Louis, MO, USA). A 0.20 µm filter-sterilised stock solution was prepared with fosfomycin at 5120 µg/mL. MIC values for fosfomycin of isolates were tested (0.5-64 µg/mL range) with an agar dilution method in accordance with the recommendation of EUCAST. An agar medium supplemented with glucose-6-phosphate (25 mg/L) was used for the antibiotic susceptibility testing of fosfomycin. The inoculum of each isolate contained 10^4 cfu/mL. The inoculated plates were incubated at 37°C for 24 h. The MIC was defined as the lowest antibiotic concentration that did not yield visible growth after overnight incubation. *S. aureus* ATCC 29213 was included in each assay as the control strain. Experiments were repeated 3 times.

2.3.2. Inhibition of biofilm formation

The effect of fosfomycin on the biofilm formed at this stage was examined. The isolates, which were incubated at 37°C for 24 h on TSB medium containing 2% glucose, were diluted to 1/100 and distributed to each well in the amount of 100 µl. After incubation for 48 h at 37°C, the microtiter plate was washed 3 times with PBS by aspirating the supernatant. Fosfomycin was added to wells in amounts of 100 µl by dilution in TSB as twofold increasing concentrations (0.5-128 µg/mL). Microtiter plates were incubated at 37°C for 20 h. Plates were washed after incubation and stained in crystal violet; then OD values were determined in the ELISA reader (BMG LABTECH, Germany) at 540 nm. The well in which the fosfomycin was not added was taken as the positive control well, and the well without the isolate was taken as the negative control well. All the isolates were run 3 times. Biofilm ODs at different concentrations of fosfomycin were compared and interpreted statistically.

2.3.3. Minimum biofilm eradication concentration (MBEC)

After all isolates were incubated overnight at 37°C in Glucose-TSB medium for biofilm formation, they were distributed in 200 µl, 96-well microplates with 1/20 dilution. Sterile plastic beads were placed in each well on the plates and incubated for 48 h at 37°C. Serial dilutions (0.5-128 µg/mL) of fosfomycin were made in freshly-prepared Mueller Hinton Broth (MHB) in another microplate. After biofilm-formed plastic beads were placed into each well of microplates with antibiotic dilution, they were incubated for 1 night at 37°C. The next

day, the beads in the wells were transferred to eppendorf tubes containing 200 µl MHB medium and vortexed for 5 min in the fast cycle to separate the biofilm layer. After this step, 100 µl of supernatant from the tubes was taken and added to wells containing 100 µl of MHB in a new microplate. The lowest concentration at which the growth was not found after overnight incubation at 37°C was determined as the biofilm eradication concentration (20). Experiments were repeated 3 times.

2.4. Detection of the icaA, icaD, icaB, icaC, bap, eno, fnbA, fnbB, clfA, clfB, fib, ebpS, cna and mecA genes by Polymerase Chain Reactions (PCR)

The genomic DNAs of *S. aureus* isolates were purified using the Genomic DNA Purification Kit (Thermo Fisher Scientific, USA). All primers used in this study are given in Table 1.

Table 1. *Primers were used in this study*

Genes	Sequences (5'-3')	T_m (°C)	Amplicon size (bp)	Reference
***mec*A**	F: GTAGAAATGACTGAACGTCCGATAA R: CCAATTCCACATTGTTTCGGTCTAA	50	310	Geha et al., 1994
***ica*A**	F: GAGGTAAAGCCAACGCACTC R: CCTGTAACCGCAAGTTT	58	151	Atshan et al., 2013
***ica*B**	F: ATACCGGCGACTGGGTTTAT R: TTGCAAATCGTGGGTATGTGT	57	140	Atshan et al., 2013
***ica*C**	F: CTTGGGTATTTGCACGCATT R: GCAATATCATGCCGACACCT	56	209	Atshan et al., 2013
***ica*D**	F: ACCCAACGCTAAAATCATCG R: GCGAAAATGCCCATAGTTTC	56	211	Atshan et al., 2013
bap	F: CCCTATATCGAAGGTGTAGAATTG R: GCTGTTGAAGTTAATACTGTACCTGC	57	971	Cucarella et al., 2004
eno	F: TGCCGTAGGTGACGAAGGTGGTT R: GCACCGTGTTCGCCTTCGAACT	58	195	Atshan et al., 2013
***fnb*A**	F: AAATTGGGAGCAGCATCAGT R: GCAGCTGAATTCCCATTTTC	56	121	Atshan et al., 2013
***fnb*B**	F: ACGCTCAAGGCGACGGCAAAG R: ACCTTCTGCATGACCTTCTGCACCT	58	197	Atshan et al., 2013
***clf*A**	F: ACCCAGGTTCAGATTCTGGCAGCG R: TCGCTGAGTCGGAATCGCTTGCT	58	165	Atshan et al., 2013
***clf*B**	F: AACTCCAGGGCCGCCGGTTG R: CCTGAGTCGCTGTCTGAGCCTGAG	58	159	Atshan et al., 2013
fib	F: CGTCAACAGCAGATGCGAGCG R: TGCATCAGTTTTCGCTGCTGGTTT	58	239	Atshan et al., 2013
***ebp*S**	F: GGTGCAGCTGGTGCAATGGGTGT R: GCTGCGCCTCCAGCCAAACCT	58	191	Atshan et al., 2013
cna	F: AATAGAGGCGCCACGACCGT R: GTGCCTTCCCAAACCTTTTGAGCA	58	156	Atshan et al., 2013

Methicillin resistance was confirmed by the presence of the mecA gene. Thirteen genes related to biofilm formation and microbial surface components recognising adhesive matrix molecules (MSCRAMMs) were analysed by PCR. The genes of icaA, icaB, icaC and icaD (intercellular adhesion genes A through D); bap (encoding biofilm-associated protein), eno (encoding laminin-binding protein); fnbA and fnbB (fibronectin-binding proteins A and B); clfA and clfB (clumping factors A and B); fib (fibrinogen-binding protein); ebpS (elastin-binding protein) and cna (collagen-binding protein) were determined in all *S. aureus* isolates.

The reaction mixtures of PCR were 25 µL in total volume, containing 1X Taq DNA Polymerase Buffer, 200 µM each dNTPs, 1.5 mM MgCl2, 0.2 pmol/µl forward and reverse primers, 200 ng genomic DNA and 2 U Taq DNA Polymerase (Thermo Fisher Scientific, USA).

PCRs were carried out with an initial denaturation step of 3 min at 95°C, followed by 35 cycles of denaturation (1 min at 94°C), annealing (1 min at the primer binding temperature calculated for each primer set) and extension (1 min at 72°C). The reactions were finalised by polymerisation for 5 min at 72°C. The PCR products were loaded in 1% agarose gel electrophoresis including ethidium bromide and were visualised under UV light.

2.5. Statistical analysis

In the statistical analysis, since the number of cases in the group did not satisfy the normal distribution conditions, the Wilcoxon sign test followed by a Freudian Variance Analysis was used for intragroup evaluation of the results obtained at different times. Chi-square test was used for the relationship between biofilm levels and gene presence. The Statistical Package for the Social Sciences (SPSS) version 20.00 (SPSS Inc., Chicago, IL, USA) statistical package program was used to analyse the data set. A value of $p<0.05$ was considered significant in the evaluation of the data.

3. RESULTS

S. aureus reproduced in 87 (13.2%) of the 658 nasal swab samples. Methicillin resistance was detected in 10 (11.4%) of the reproduced 87 isolates, and the presence of the mecA gene was confirmed. When biofilm formation was examined, it was determined that 86 of the 87 isolates produced biofilm. Biofilm formation was evaluated by taking control strains into account. When the results were evaluated in accordance with OD values of the positive controls and negative controls, strong biofilm formation was observed in (57.5%) 46 isolates and moderate biofilm formation was observed in (42.5%) 40 isolates.

Six of the positive *S. aureus* isolates identified as producing strong biofilms were selected (based on the amount of biofilm formed by *S. aureus* ATCC 25923, they had an equal and higher absorbance rate). Selected isolates were sensitive to fosfomycin, and their MIC values were between 1 and 2 µg/ml (MIC90, 2µg/ml). The effect of fosfomycin on the biofilms of the same isolates was also studied. The lowest BIC value of isolates for fosfomycin was 8 µg/ml. When OD averages according to fosfomycin concentrations were examined, a difference was detected, between 8 µg/ml of fosfomycin and 4 µg/ml of fosfomycin. This is shown in Figure 1.

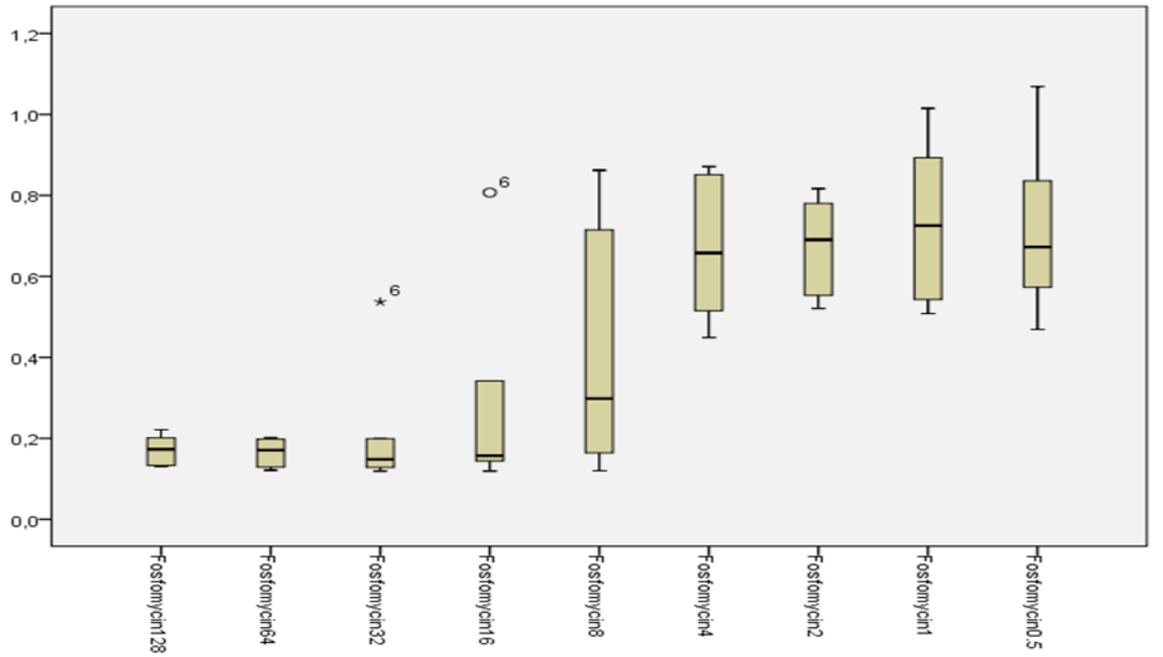

Figure 1. *Box graphical representation of average biofilm OD values of six clinical isolates of Staphylococcus aureus at different fosfomycin concentrations*

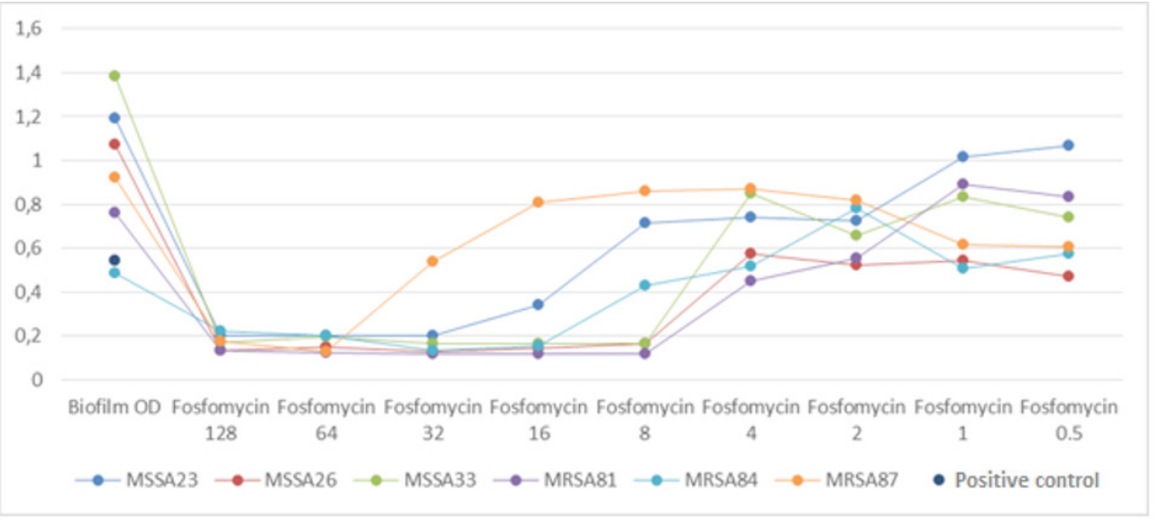

Figure 2. *Change of biofilm ODs according to fosfomycin concentrations of each isolate*

When the effect of fosfomycin concentrations on biofilm ODs are compared, it can be said that the difference between them is due to a statistically significant increase (Table 2).

Table 2. *Effect of Fosfomycin concentrations on biofilm ODs.*

	Fosfomycin16-Fosfomycin32	Fosfomycin8 - Fosfomycin16	Fosfomycin4 - Fosfomycin8	Fosfomycin2 - Fosfomycin4	Fosfomycin1 - Fosfomycin2
Z	-1,753[a]	-2,201[a]	-2,201[a]	-,105[b]	-,734[a]
p	0,08	0,028	0,028	0,916	0,463

Biofilm ODs changes according to fosfomycin concentrations are seen in Figures 1 and 2. ODs of the biofilm formation in accordance with methicillin resistance are shown in Table 3. No significant difference was detected between them in statistical analysis (p>0.05).

Table 3. *ODs of biofilm formation at different fosfomycin concentrations according to methicillin resistance*

	MRSA	MSSA
Biofilm OD	0,722±0,12	1,215±0,15
Fosfomycin128	0,170±0,02	0,160±0,20
Fosfomycin64	0,149±0,24	0,180±0,29
Fosfomycin32	0,262±0,13	0,164±0,20
Fosfomycin16	0,359±0,22	0,216±0,63
Fosfomycin8	0,471±0,21	0,348±0,18
Fosfomycin4	0,611±0,13	0,722±0,80
Fosfomycin2	0,711±0,82	0,634±0,59
Fosfomycin1	0,672±0,11	0,797±0,13
Fosfomycin0,5	0,671±0,82	0,759±0,17

The lowest MBEC value for fosfomycin of these isolates was found to be 32 µg/ml, which is 16 times the MIC90 value (Table 4).

Table 4. *Minimum inhibitory concentration (MIC) and minimum biofilm eradication concentration (MBEC) of the fosfomycin against six clinical isolates of Staphylococcus aureus.*

Isolates	Fosfomycin	
	MIC (µg/ml)	MBEC (µg/ml)
MSSA23	1	32
MSSA26	1	128
MSSA33	1	64
MRSA81	1	64
MRSA84	2	64
MRSA87	2	32

Prevalence of adhesion and regulation of biofilm-related genes in the 86 biofilm-positive *S. aureus* isolates were as follows: eno (97.6%), fnbA (94.1%), icaB (93%), icaD (91.8%), icaA (90.6%), icaC (84.9%), fib (75.5%), ebpS (58.1%), clfB (36%), clfA (15.1%), cna (4.7%). None of the isolates were bap or fnbB positive. However, no statistically significant difference was found between moderate and strong biofilm formation and presence of genes (p>0.05) (Table 5).

Table 5. *Relationship between biofilm level of isolates and gene presence*

	Moderate biofilm-forming isolates total=40 n (%)		Strong biofilm-forming isolates total=46 n (%)		*p*	X^2
Genes	**Negative**	**Positive**	**Negative**	**Positive**		
BAP	40 (100)	0	46 (100)	0	-	-
ICA-A	5 (12.5)	35 (87.5)	4 (8.6)	42 (91.4)	0,565	0,330
ICA-D	5 (12.5)	35 (87.5)	4 (8.6)	42 (91.4)	0,565	0,330
ICA-B	3 (7.5)	37 (92.5)	4 (8.6)	42 (91.4)	0,84	0,410
ICA-C	6 (15)	34 (85)	8 (17.4)	38 (82.6)	0,764	0,900
Fnb-A	4 (10)	36 (90)	2 (4.3)	44 (95.7)	0,305	1,053
Fnb-B	40 (100)	0	46 (100)	0	-	-
CLF-A	33 (82.5)	7 (17.5)	40 (86.9)	6 (13.1)	0,565	0,331
CLF-B	29 (72.5)	11 (27.5)	29 (63)	17 (37)	0,351	0,871
Fib	10 (25)	30 (75)	10 (21.7)	36 (78.3)	0,721	0,127
Ebps	16 (40)	24 (60)	22 (47.8)	24 (52.2)	0,466	0,531
Eno	2 (5)	38 (95)	3 (6.5)	43 (93.5)	0,764	0,090
Cna	39 (97.5)	1 (2.5)	43 (93.4)	3 (6.6)	0,377	0,780

4. DISCUSSION

S. aureus is the most adaptable and common human pathogen. Nasal carriage increases *S. aureus* infection risk by creating endogenous and exogenous sources (5). Biofilms that result in antibiotic resistance are heterogeneous microorganism populations. Biofilms lead to treatment failure since they are sources of infection. Determining the right antibiotic for biofilm formation and choosing a highly effective antibiotic for biofilm layers are significant steps in preventing the infections. In this study, we examined the effectiveness of fosfomycin on *S. aureus* isolates colonised in the nose and we also analysed biofilm-related genes. The effectiveness of fosfomycin on the biofilm formed by 3 MRSA and 3 MSSA isolates created a significant difference between concentrations. There was considerable biofilm inhibition at concentrations of 64, 32, 16 and 8 µg/ml according to the concentration of 4 µg/ml of fosfomycin, and the ODs were decreased. However, it was determined that for the total eradication of biofilm via fosfomycin, the effective dose should be as high as 16 times the value of MIC90 (32 µg/ml).

There are few studies in literature about the effectiveness of fosfomycin in biofilm medium formed by *S. aureus* isolates. In the studies, it was found that a combination of fosfomycin with other antibiotics showed strong in vitro activity against *S. aureus* isolates in biofilm; however, researchers are still trying to determine in vivo efficacy. Tang et al. (2) conducted a new study using a model of the methicillin-resistant *S. aureus* biofilm, and they specified that the antibacterial effectiveness of vancomycin combined with fosfomycin is better than vancomycin alone. Shi et al. (22) detected that fosfomycin and vancomycin are effective in-vivo synergistic bactericides to bacteria in biofilm of MRSA infections. Chai et al. (23), parallel with our study, observed that fosfomycin alone showed an activity of 8-32 µg/ml, depending on the concentration; however, linezolid and fosfomycin together is a stronger combination against MRSA biofilm both in vitro and in vivo. However, in a study conducted with biofilms on polystyrene and metal surfaces, the biofilm inhibitor concentration (BIC) value of fosfomycin was found to be quite high (>256) (24). It has also been reported that fosfomycin is effective in biofilm studies with *P. aeruginosa* and *E. coli* (25, 26).

Another important point that we want to emphasise is that these isolates are not hospital-originated. Nasal colonisation with MRSA is increasing in healthy societies. Nasal carriage is a major risk factor for community-aquired *S. aureus* infections (5, 27). Of the 658 outpatients without a previous hospital history, 13.2% were nasal *S. aureus* carriers and 11.4% of these isolates were MRSA. This rate was lower in our study compared with other studies conducted in our country. In these studies, *S. aureus* carriage was found to exist between 19.1% and 38%; however, MRSA carriage was observed to be much lower than our study (0%-5%) (28). In another study conducted in 2015, nasal carriage was noted to be 17.3% and 0.5% for *S. aureus* and MRSA respectively in healthy university students (29). MRSA carriage around the world has been increasingly reported (30-33). Especially in communities, the frequency of infections caused by MRSA has increased in the last decade (5). It is believed that the prevalence of MRSA carriage increases in a healthy population, and for this reason, the follow-up of the nasal carriage of MSSA and MRSA in healthy individuals is important (29).

Adhesive matrix proteins play a role in the first step of biofilm formation, which is attaching to the surface. Polysaccharide intercellular adhesin (PIA) production is controlled by the icaADBC gene cluster, and it is known that *S. aureus* isolates

harbouring this gene cluster are potential biofilm producers. Our results are compatible with all other studies that show ica-operon is present in almost all *S. aureus* isolates and that it is expression (34, 35). In addition to this, *S. aureus* may excrete a variety of adhesive matrix molecules that interact with the extracellular ligands of the host. Elastin-binding protein (ebpS), laminin-binding protein (eno), collagen-binding protein (cna), fibronectin-binding proteins A and B (fnbA, fnbB), fibrinogen-binding protein (fib) and clumping factors A and B (clfA, clfB) can be named as examples for these molecules (30). Eno (97.6%), fnbA (94.1%), fib (75.5%), ebpS (58.1%), clfB (36%), clfA (15.1%) and cna (4.7%) genes have been detected in our study respectively. fnbB has not been noted in any of the isolates. Athans et al. (35) found that these genes are highly positive in different clonal *S. aureus* isolates producing biofilms. Barbieri et al. (34) emphasised that *S. aureus* isolates that cause breast peri-implant infections in oncologic patients, expression these genes at high rates and the cna gene has an important role in these infections. When compared with other studies, gene prevalence in our study is quite high (36, 37). Also, no statistically significant difference was found between moderate and strong biofilm formation and presence of genes.

Biofilm-associated protein (bap) has been reported as one of the necessary structures for biofilm formation. Studies have shown that bap acts both in adhesion to the abiotic surfaces and in the intercellular adhesion steps (38). Studies on bap in *S. aureus* strains are restricted since the frequency of the bap gene presence is low. The bap gene was predominantly found in chronic bovine mastitis (39, 40). Vautor et al. (41) suggest that the prevalence of the bap gene is low because it is not yet as common among human and animal origin *S. aureus* isolates. No bap gene was detected in any of the isolates in our study as well. We believe that it is important to genotypically characterise the biofilm genes in order to better understand the complex biofilm process that leads to infections. One restriction of our study is that clonal typing was not performed in the *S. aureus* we isolated.

5. CONCLUSION

In conclusion, antibiotics are often ineffective eradicating *S. aureus* and resistance to topical antibiotics such as mupirocin has been reported (42, 43). We detected that methicillin resistance is significant in community-aquired *S. aureus* isolates obtained from the nose and fosfomycin is an effective antibiotic on biofilm; however, it can even become more effective when it is combined with other antibiotics. We also detected that the prevalence of biofilm-related genes is high. Especially in the infections caused by the species forming the biofilm layer, their treatment process is harder and takes longer, since the biofilm layer provides some advantages for bacteria in the protection phase from antimicrobial agents. For this reason, it is important to understand the biofilm mechanism formed by *S. aureus* bacteria. By understanding the processes of the genes and antibiotics involved in the biofilm mechanism, it will be possible to shed light on the production of a new generation of medicines for the treatment of *S. aureus* infections.

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