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ORIGINAL ARTICLE

Antibacterial fatty acids destabilize hydrophobic and multicellular aggregates of biofilm in *S. aureus*

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Present study is based on 20 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from different food items. These isolates were identified on the basis of colony morphology, Gram staining and growth on different selective and differential media. Studies on 16S RNA and positive reactions on DNase agar and Prolex Latex Agglutination system confirm it as *Staphylococcus aureus*. Oxacillin susceptibility testing and PCR with *mecA* gene-specific primer results showed that these isolates are MRSA-carrying *mecA* gene that belongs to *SCCmecA* type IV and also harbor *agr* type II. Phenotypic study revealed that these isolates adopt biofilm mode of growth after exposure to subinhibitory doses of oxacillin. The biofilm and cell surface hydrophobicity have a strong correlation. It was noticed that affinity to hexadecane (apolar-solvent) of planktonic cells was low, suggesting its hydrophilic character. However, as the cells are exposed to oxacillin, they adopt biofilm mode of life and the affinity to apolar solvent increases, indicating a hydrophobic character. In biofilm consortia, the cells with more hydrophobic surfaces show incomplete septation and produce multicellular aggregates. This is due to reduced expression of *atl* gene. This was confirmed by real-time PCR studies. Moreover, the planktonic or wild-type phenotypes of these isolates were more tolerant to antibacterial effect of the fatty acids used; that is, *cis*-2-decanoic acid and *cis*-9-*octadectanoic acid*. These fatty acids were more effective against biofilms. After exposure to these fatty acids, established biofilms were dispersed and surviving cells were unable to readopt biofilm mode of life. The planktonic or wild-type phenotypes produce fatty acid-modifying enzyme (FAME) to inactivate the bactericidal activity of fatty acids by esterification to cholesterol. The biofilm indwellers are metabolically inactive and unable to produce FAME; hence, they are vulnerable to antibiofilm effect of *cis*-2-decanoic acid and *cis*-9-octadectanoic acid.
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INTRODUCTION

Staphylococcus aureus is a Gram-positive opportunistic pathogen that lives on the skin or in the nose of humans and animals and causes significant morbidity and mortality worldwide.[1] This is one of the hardiest bacteria and survives in high concentrations of salt and sugar where other bacteria would die.[2] Because of this character, *S. aureus* is a widely distributed bacterial pathogen in hospitals as well as communities and is well known for its adaptive evolution in the antibiotic era.[3] Strong adaptive response to antibiotics has resulted in the emergence of multidrug-resistant *S. aureus* that poses a great challenge to the prevention and treatment of *S. aureus* infections.[3] The prevalence of community-acquired multidrug-resistant *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA), is a cause of concern nowadays.[4] The widespread and rapid growth in community-acquired MRSA has raised the question as to whether MRSA is indeed a food-borne pathogen.[4] These pathogens may enter food through food handlers or poor hygiene practices in food-processing plants that may result in the contamination of the finished food products. Once *S. aureus* contaminate the food product, it is very difficult to remove because these pathogens are resistant to commonly used food preservatives (that is, salt, glucose) and also survive under refrigeration conditions (albeit grow slowly).[5,6] These isolates are even more difficult to eradicate when they adopt biofilm mode of growth.[7] Biofilms are aggregates of microbial cells surrounded by a matrix of exopolymers, which confers resistance to these microorganisms. *S. aureus* is well known for its character to attach on food contact surfaces to resist hostile environment, which is a serious problem in food industry.[7,8] This enhances the chances of the recurrence of *S. aureus* food contamination, because biofilm communities continuously release planktonic cells from the outer layer.[7,8] At present, there is no standard treatment option for biofilm control. *In vitro* studies indicated that bacterial strains growing in biofilms may become 10–1000 times more resistant to the effects of antibacterial agents than the same strain in planktonic form.[9] The biofilm formation in Staphylococci and in other bacteria depend on multiple factors, including the age of the cell, attachment surface, bacterial cell surface properties, presence of other bacteria, temperature, availability of nutrients and pH.[10] Biofilm formation often results in changes in

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cellular morphology and growth rates. Additionally, diverse genes are expressed in bacteria that are attached to surfaces as compared with their planktonic counterparts. As a result of these changes, biofilm indwellers display increased resistance to antibiotics, toxic chemicals and biocides.[11] Food-borne infections, most frequently caused by Staphylococci, are of increasing importance in modern era. The most important factor in the pathogenesis of staphylococcal infections is the formation of biofilms. Therefore, this study was designed to explore the biofilm-formation mechanisms in a group of food-borne isolates of MRSA. Moreover, the effect of selected fatty acids has also been studied in biofilm indwellers and planktonic cells of MRSA.

MATERIALS AND METHODS

Identification of *S. aureus*

During the study, a total of 20 biofilm-producing isolates of MRSA, recovered from different food commodities, have been studied. For isolation and identification of *S. aureus*, growth was monitored on differential and selective media; for example, Manitol Salt Agar (BioM, Durham, NC, USA), Staph-chromo agar (Merck, Darmstadt, Germany), *Staphylococcus* 110 Agar (BioM), Baird–Parker Agar (Oxoid, Basingstoke, UK), DNase Agar (Merck) and Blood Agar (Oxoid). Staph Latex Kit (Prolix Latex Agglutination System, Pro Lab Diagnostics, South Wirral, UK) was used for confirmation.

Oxacillin susceptibility testing and molecular analysis

The oxacillin susceptibility of subject isolates was determined by agar-dilution method as recommended by the Clinical and Laboratory Standards Institute.[12] Antimicrobial powders were purchased from Sigma-Aldrich (St Louis, MO, USA). The SCC*mec* elements (I–V) and *mec*A gene were identified as previously described.[13] Expression of *atl* gene was studied by using total RNA, recovered from exponentially growing cells in Tryptic Soy Broth (OD at 578 nm) using a dedicated kit (Qiagen Rneasy Mini, Qiagen, Hilden, Germeny) and stored at −20 °C. DNA was removed from RNA extractions using DNase according to the manufacturer's instructions[14] and RNA concentration was quantified by spectrophotometer (Evolution 300BB, Thermoelectro Corporation, Madison, WI, USA). One microgram of RNA was used per reverse transcriptase-PCR (Qiagen One-step RT-PCR Kit, Qiagen) together with gene-specific primers (*atl*-F: 5′-CAGTTAGCAAGATTGCTCAAG-3′, *atl*-R: 5′-CCGTTACCTGT-TTC TAATAGG-3′, *atl*-promoter F: 5′-GGAAGGCATCGAGCAT-3′, *atl*-promoter R: 5′-GCGTTAATGCAACCAT-3′).

Determination of fatty acid MIC values

Anti-*S. aureus* activity of selected fatty acids was determined by broth micro-dilution method according to protocol M07-A8 published by the Clinical and Laboratory Standards Institute.[12] Briefly, subject fatty acids (*cis*-2-octadecanoic acid and *cis*-9-octadecanoic acids) were dissolved in 5% DMSO (dimethyl sulfoxide) to obtain 5 mg ml^{-1} stock solution. Susceptibility testing was carried out in Muller–Hinton Broth (Oxoid). A control tube, containing the micro-organisms without the fatty acid, was also prepared and incubated at 35 °C for 24 h.

Collection of culture filtrate and fatty acid-modifying enzyme (FAME) assay

One milliliter from overnight culture of selected isolates of *S. aureus* were added to 100 ml of Tryptic Soya Broth (Oxoid) and incubated at 37 °C in shaking incubator (at 100 r.p.m.) for 48 h. Cell-free broth was collected by centrifugation at 15 000 r.p.m. and passed through 0.45-μm membrane (Sartorius Stedium-Biotech, Goettingen, Germeny) and stored at −20 °C. Fatty acid-modifying activities were performed according to the protocol of Lu *et al.*[15] Culture was taken after 24 and 48 h, and all experiments were performed in triplicate.

Bacterial hydrophobicity assay

The hydrophobicity of strains was evaluated by the microbial adhesion to solvent test as described by Kouidhi *et al.*[16] It consisted of evaluating the affinity of the cells towards apolar solvents (hexadecane). For the experiment, bacterial cells were harvested by centrifugation at 8500 *g* for 5 min and resuspended to Abs 578 nm in 0.01 M potassium phosphate buffer (pH 7.0). This bacterial suspension was mixed with a solvent in a ratio of 1:6 (0.4/2.4 v/v) by vortexing for 3 min to make an emulsion. This mixture was then left for 30 min until the separation of two phases. Aqueous phase absorbance was measured (Abs2) and the percentage of adhesion was expressed as: % adhesion= $(1-\text{Abs2}/\text{Abs1})\times 100$.

Phenotypic characterization of slime-producing bacteria

Biofilm formation was initially confirmed by Congo-Red Agar method as described earlier.[17] Briefly, BHI agar (Oxoid) plates containing 50 g l^{-1} sucrose and 0.8 g l^{-1} Congo-Red were prepared and streaked with strains and incubated aerobically for 24–48 h at 37 °C. Positive results were indicated by black colonies with dry crystalline appearance. Weak slime producers usually remained pink, though occasional darkening at the center of colonies was observed.

Biofilm assay

A qualitative assessment of biofilm formation on glass slides was evaluated as described earlier by Mirani and Jamil.[18]

Scanning electron microscopy

Scanning electron microcopy was carried out to analyze the production of extracellular matrix material after exposure to oxacillin. Biofilm slides were divided into 4-mm sections and washed with distilled water to remove the debris and were then negatively stained with 0.02% Uranyl acetate for 30 s. These 4-mm slide sections showed the presence of biofilm material when examined directly in a GOEL-JEM-1200 EX II Electron Microscope (JEOL, Peabody, MA, USA).[18]

RESULTS

Present study is based on 20 MRSA isolates recovered from different food items. These isolates were identified as *S. aureus* on the basis of colony morphology, Gram staining, growth on different selective and differential media; that is, black colonies with opaque zone on Baird–Parker agar supplemented with egg yolk tellurite and mannitol fermentation on Mannitol Salt agar. Positive reactions on 16S RNA and DNase agar and on Prolex Latex Agglutination System further confirmed it as *S. aureus*. Oxacillin susceptibility testing results showed that these isolates are MRSA. Out of the 20 isolates, 6 exhibited high-level oxacillin resistance (MIC 64 μg ml^{-1}), 7 isolates exhibited intermediate oxacillin resistance (MIC 32 μg ml^{-1}) and 7 isolates showed low-level oxacillin resistance (MIC 8–16 μg ml^{-1}). Moreover, PCR studies showed that these subject isolates of MRSA carry *mecA* gene, belong to *SCCmecA* type IV and harbor *agr* type II. Phenotypic study revealed that subject isolates of MRSA adopt biofilm mode of growth after exposure to subinhibitory doses of oxacillin. Further studies showed that cell surface hydrophobicity and biofilm formation have a strong correlation. The results of microbial adhesion to apolar solvent (hexadecane) are summarized in Table 1. It was noticed that planktonic cells' affinity to hexadecane was low, suggesting its hydrophilic character. However, as the cells are exposed to oxacillin they adopt biofilm mode of life and the affinity to apolar solvent increases, indicating a hydrophobic character. The surface hydrophobicity is associated with oxacillin resistance (that is, highly oxacillin-resistant isolates) and were more hydrophobic. Moreover, cells with more hydrophobic surfaces produce strong biofilm consortia in which cells show multicellular aggregates (Figure 1a). In these biofilm structures, most of the cells remain dormant and non-septated (Figures 1b and c). Because of incomplete septation, cells appear multicellular in biofilm consortia (Figure 1a). This is due to reduced activity of muriene hydrolase, the enzyme involved in daughter cell

Table 1 Biofilm formation, fatty acid-modifying enzymes activities, response to antibacterial fatty acids and cell-surface hydrophobicity of subject isolates of MRSA

Isolate no.	MIC in $\mu g\ ml^{-1}$	SCCmecA type	Agr type	Biofilm OD		[a]Relative range of FAME activity				MICs to cis-2-DCA, $\mu g\ ml^{-1}$		MIC of cis-9-ODCA, $\mu g\ ml^{-1}$		Cell-surface hydrophobicity			
				24 h	48 h	6 h	12 h	24 h	48 h	24 h	48 h	24 h	48 h	Plan at 18 h	At 24 h Prebiofilm	At 48 h biofilm stage	Replank
1	64	IV	II	0.09	0.96	150	170	73	ND	192	96	192	96	0.43	0.51	0.75	0.37
2	64	IV	II	0.08	0.92	150	175	70	ND	192	96	128	96	0.39	0.55	0.79	0.32
3	64	IV	II	0.23	0.89	55	78	35	33	192	192	128	192	0.33	0.57	0.75	0.31
4	64	IV	II	0.27	0.73	141	101	47	ND	192	96	128	92	0.46	0.5	0.81	0.43
5	32	IV	II	0.17	0.86	120	110	80	21	320	320	128	320	0.42	0.59	0.71	0.51
6	32	IV	II	0.21	0.80	ND	ND	ND	ND	384	196	192	96	0.34	0.53	0.69	0.44
7	32	IV	II	0.22	0.79	ND	ND	ND	ND	384	192	128	96	0.36	0.61	0.71	0.41
8	32	IV	II	0.35	0.76	ND	ND	ND	ND	384	192	128	96	0.34	0.5	0.72	0.33
9	32	IV	II	0.33	0.75	ND	ND	ND	ND	384	192	192	96	0.35	0.51	0.71	0.44
10	16	IV	II	0.55	0.87	46	80	ND	ND	384	192	96	56	0.36	0.47	0.61	0.41
11	16	IV	II	0.54	0.81	42	80	ND	ND	384	768	96	192	0.31	0.45	0.59	0.41
12	08	IV	II	0.55	0.86	26	88	ND	ND	384	768	192	384	0.28	0.35	0.55	0.35
13	08	IV	II	0.25	0.81	ND	ND	ND	ND	384	192	192	96	0.26	0.45	0.56	0.46
14	64	IV	II	0.09	0.85	ND	90	ND	ND	384	192	96	58	0.35	0.59	0.87	0.31
15	64	IV	II	0.08	0.97	50	47	ND	ND	384	192	64	32	0.37	0.51	0.77	0.32
16	32	IV	II	0.23	0.82	30	67	ND	ND	384	192	192	96	0.37	0.44	0.59	0.41
17	32	IV	II	0.27	0.79	93	105	09	ND	384	192	192	64	0.29	0.47	0.62	0.37
18	16	IV	II	0.17	0.81	99	99	21	ND	384	192	64	32	0.28	0.33	0.42	0.31
19	08	IV	II	0.21	0.83	110	145	45	25	384	768	192	384	0.31	0.32	0.37	0.28
20	08	IV	II	0.22	0.76	123	149	ND	ND	384	192	192	96	0.25	0.28	0.31	0.26

Abbreviations: cis-2-DCA, *cis*-2-decaqnoic acid; cis-9-ODCA, *cis*-9-octadecanoic acid; FAME, fatty acid-modifying enzyme; MRSA, methicillin-resistant *Staphylococcus aureus*; ND, not detected.
[a]Percent (%) esterification compared with a standard with significant activity $P<0.05$ from negative control.

separation during cell division. Muriene hydrolase activities are regulated by *atl* gene in *S. aureus* isolates. Real-time PCR results showed that it is gradually reduced in biofilm-forming isolates of MRSA. At prebiofilm stage, the *atl* gene expression was high, but with the passage of incubation period, when cells enter the biofilm phase of life, a gradual reduction was noticed in *atl* gene expression (Figure 2). At log phase (12 h of incubation at 35 °C), the *atl* gene expression was high along with proper daughter cell separation and complete cell septation. At this stage, there is no evidence of biofilm formation. As stationary phase started (after 18 h of incubation at 35 °C), a reduction in *atl* gene expression was noticed along with biofilm formation. As biofilm became stronger, cells showed multicellular aggregates along with incomplete septation and daughter cell separation. These multicellular aggregates were rooted in extracellular matrix material and adhered strongly to the surface (Table 1). Interestingly, these biofilm indwellers dominated by metabolically inactive population were sensitive to *cis*-2-decanoic acid and *cis*-9-octadecanoic acid. After exposure to lethal dose of *cis*-2-decanoic acid or *cis*-9-octadecanoic acid, established biofilms were mostly eradicated and surviving cells were unable to readopt biofilm mode of life. The planktonic or prebiofilm populations of these isolates were more tolerant to the toxic effect of these fatty acids (Table 1). Although they resist high level of subject fatty acids, they are unable to form biofilm. Comparative analysis showed that at planktonic stage these isolates produce FAMEs, which is not produced by biofilm indwellers. These FAMEs inactivate the bactericidal activity of fatty acids by their esterification to cholesterol. Detectable levels of FAME activity were not obtained until 6 h of growth and reached at the highest detectable level after 12 h of incubation at 35 °C. As cells enter the biofilm stage, the FAME activity is reduced and not detected after maturation of biofilms at 48 h. Fourteen out of the 20 isolates showed detectable FAME activity after 6 h of incubation. At 12 h of incubation, 15 isolates showed the FAME production with all increased activity. After that, a reduction in FAME was observed and at 24 h of incubation only eight isolates showed FAME production (Table 1). This number was reduced to three after 48 h of incubation. Although biofilm formation was induced by subinhibitory doses of oxacillin, the FAME activity was not affected by oxacillin.

DISCUSSION

In the present study, 20 biofilm-positive food isolates of MRSA that belong to *SCCmecA* type IV were used. All of these isolates adopted biofilm mode of life after exposure to sublethal doses of oxacillin. These oxacillin-induced biofilms reached maximum thickness after 48 h of incubation at 35 °C. In a previous study, we described that oxacillin has regulatory effect on *icaA* gene and it induces the *icaA*-dependent polysaccharide intercellular adhesion (PIA) production and biofilm formation.[19] These biofilms harbor heterogeneous populations comprised of wild type as well as small colony variants (SCVs).[20] These phenotypes have a major role in persistence and stability of biofilm consortia.[20] In the present study, it has been observed that wild-type phenotype of biofilm consortia also undergo some changes; after exposure to oxacillin and before initiation of biofilm formation, the surface hydrophobicity of these isolates increases. This change from hydrophilic to hydrophobic surface seems to be a prerequisite for biofilm formation. This was confirmed by growing the isolates in the absence of oxacillin where these isolates were found to be more hydrophilic and unable to adopt biofilm mode of growth. This might be a defensive response of *S. aureus* to antibiotics, because isolates with hydrophilic surface are unable to adopt biofilm mode of growth, hence they are sensitive to antibiotic. This is also supported by Norouzi *et al.*,[21] who described that cell surface hydrophobicity has a

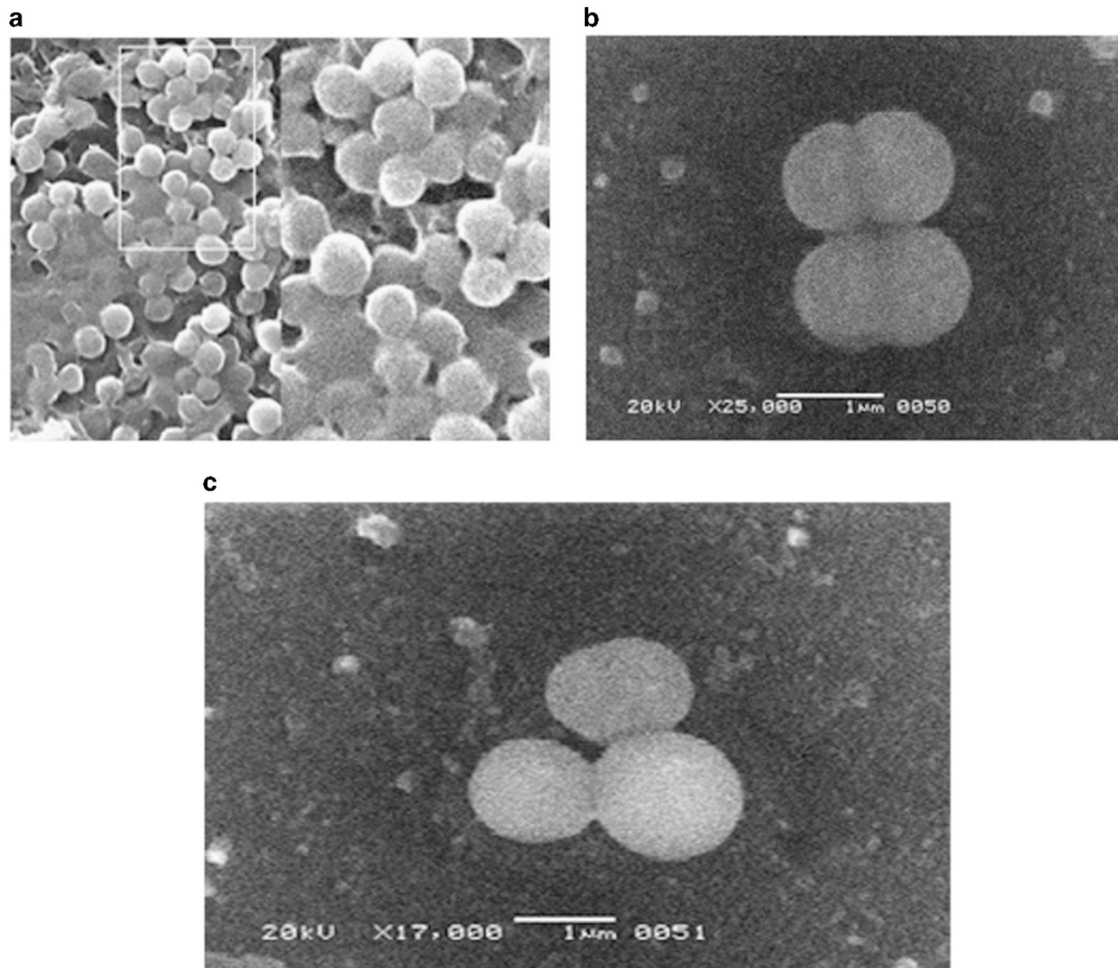

Figure 1 Biofilm consortia showing (**a**) multicellular aggregates and (**b**, **c**) non-septated cells.

critical role in bacterial adhesion and biofilm formation. According to Ambalam *et al.*,[22] biofilm formation is associated with an increase in hydrophobic cell surface proteins; for many pathogens, cell surface hydrophobicity is the first step for biofilm formation. In the present study, this was confirmed that exposure to oxacillin enhances cell surface hydrophobicity, which results in biofilm formation. Accordingly, Goulter *et al.*[23] also reported a significant correlation between hydrophobicity, attachment and biofilm-formation ability in bacteria. Gomes *et al.*[24] reported that sub-MICs of penicillin and erythromycin enhance cell-surface hydrophobicity and biofilm formation. This particular feature of resistant bacteria has a major role in bacterial survival inside the host. Bacterial cells with hydrophobic surfaces strongly adhere to host cell through hydrophobic interactions with the cell membrane and are not easily attacked by lysosomal enzymes.[25] Because of strong adhesion and aggregation, these cells also become resistant to phagocytosis, which likely contributes to biofilm persistence and chronic infection.[25] The other characteristic change in the MRSA isolates is the inhibition of cell separation. After maturation of biofilm structure at 48 h of incubation, a major population of biofilm indwellers produce multicellular aggregates without daughter cell separation. At this stage, biofilm hydrophobicity was found to be comparatively high and these phenotypes seem to stabilize biofilm structure and render it more resistant to oxacillin. In our previous study, it was described that biofilm consortia harbor heterogeneous population: group one is SCVs and the other group consists of wild-type isolates.[20] This latter group seems to protect itself from killing effect of oxacillin and starvation by forming multicellular aggregates. These aggregates are more hydrophobic and very difficult to disperse. Although cell surface hydrophobicity and cell aggregation seems to be two different phenomena, both are influenced by oxacillin. Therefore, we hypothesize that exposure to oxacillin results in increased cell-surface hydrophobicity, which induces biofilm formation and multicellular aggregation embedding in extracellular matrix material that protect bacteria from toxic effects of oxacillin. Interestingly, in biofilm consortia the aggregate builders were non-septated daughter cells that remained attached to mother cells. This appears to be a more important step in biofilm structure where mother cells retain their daughter cells in biofilm consortia. This might be due to arrested metabolism or inhibition of enzymatic activities. It is a well known fact that cell separation or cell division in *S. aureus* is catalyzed by murein hydrolases, which is regulated by *atl* gene.[26,27] Several studies suggested that *atl* also has an important role in biofilm formation.[26,28,29] *S. aureus* use bacterial DNA as extracellular matrix that is released via *atl*, a multifunctional murein hydrolase.[30] Keeping this in view, we studied the role of *atl* gene in subject isolates of *S. aureus*. It was noticed that *atl* gene expression gradually increases. At prebiofilm stage (that is, at 12 h of incubation), highest *atl* gene expression was observed when most of the cells are metabolically active. As biofilm formation reaches its maximum level (that is, at 48 h of incubation), a reduction or inhibition in *atl* gene expression was

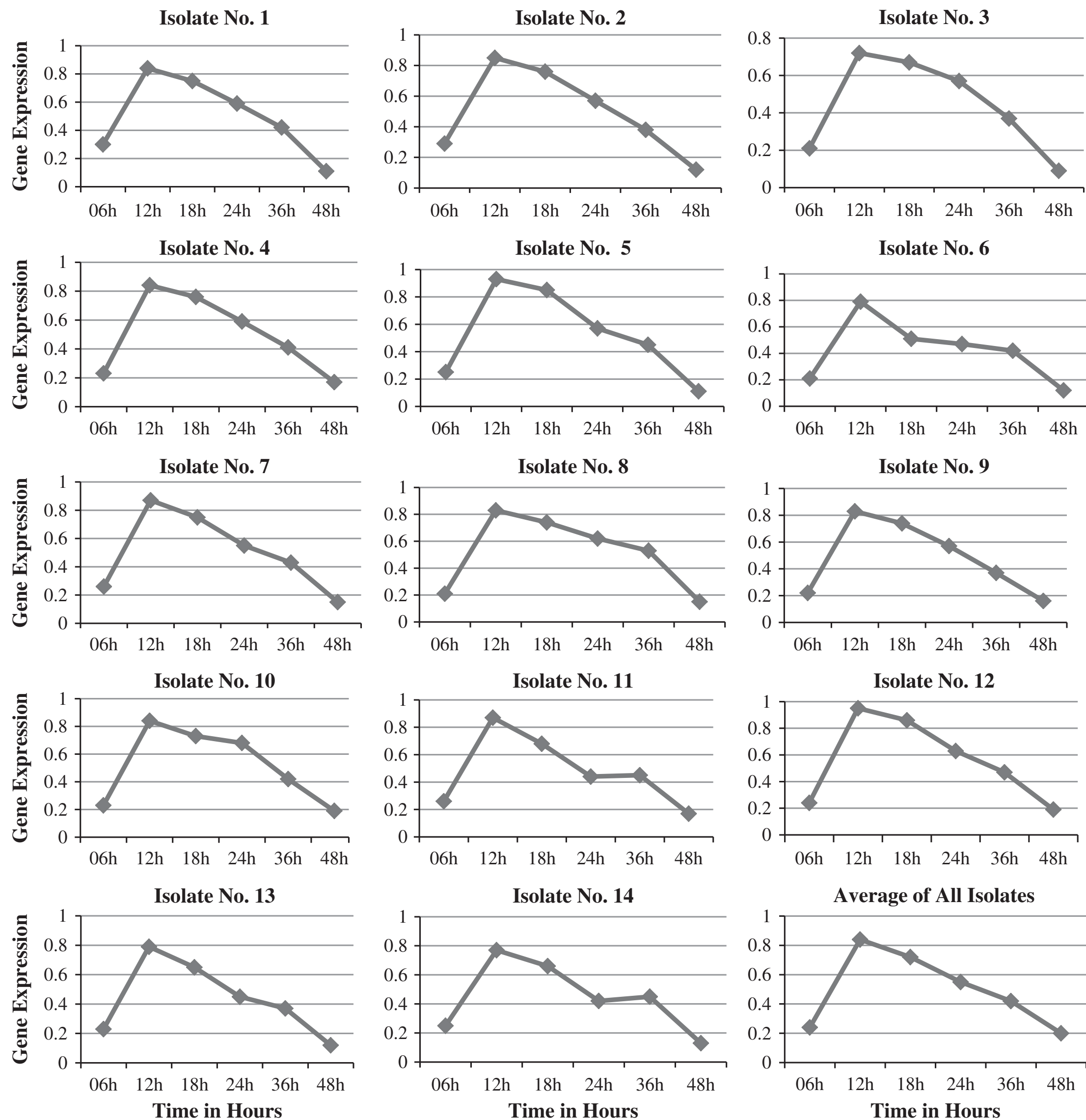

Figure 2 Expression of *atl* gene in biofilm-positive MRSA isolates recorded at prebiofilm, biofilm and postbiofilm stages.

noticed. This is due to arrest of metabolism. In our previous studies, it was reported that in biofilm consortia most of the cells are in dormant state owing to starvation or reduced oxygen level.[20] Initially, bacteria require extracellular matrix material for microcolony formation and adhesion to surface. Therefore, at prebiofilm stages, *atl* gene expression was at the highest level. This results in release of extracellular DNA and extracellular adhesion proteins to mediate or proceed with biofilm-formation process. In biofilm consortia, *atl* gene expression is reduced owing to inhibition of metabolism, which results in incomplete septation and separation of daughter cells. This is also a factor for strengthening biofilm by the formation of multicellular aggregates. These phenotypic variations (for example, cell-surface hydrophobicity and multicellular aggregates formation) appeared owing to application of subinhibitory dose of oxacillin. This suggests that improper use of antibiotics may result in induction of resistant bacteria. Bruhn *et al.*[31] suggested that, after administration, antibiotic must reach the bloodstream and tissues in sufficient concentrations required for bacterial killing. Otherwise, low doses of antibiotic may permit induction of resistance mechanisms (for example, subinhibitory concentrations of clarithromycin) and induces clarithromycin resistance in *Mycobacterium tuberculosis in vivo*.[31] The other major characteristic was noticed in terms of FAME production by planktonic cells and biofilm indwellers. FAMEs are extracellular enzymes produced by *S. aureus* and *S. epidermidis*, responsible for inactivation of bactericidal fatty acids by esterifying them to cholesterol.[32] In the present study, a negative correlation was noticed in FAME production and biofilm formation. The planktonic cells showed highest FAME production at 6–12 h of incubation, when there were no signs of biofilm formation. However, after adaptation of biofilm mode of growth a reduction in FAME production was noticed. At 12 h of incubation, a majority of the subject isolates were FAME positive, which is confirmed by esterification of *cis*-9-octadecanoic acid to butyl oleate. However, as they adopt biofilm mode of growth (at 48 h), only 20% of the isolates showed FAME production although it is very weak. Because of reduction in FAME production, antibacterial fatty acids studied (that is, *cis*-2-octadecanoic acid and *cis*-9-octadecanoic acid) was more active against biofilms. The MIC values against planktonic cells were found to be 192–384 $\mu g\ ml^{-1}$ while against biofilm this was reduced

to 96 μg ml^{-1}. It is well known that in biofilm consortium most of the cells are slow growing or in dormant state with reduced or arrested metabolism.[20] The presence of dormant or metabolically inactive population is a hallmark of biofilms, which makes them resistant to antimicrobials. However, in the present study, this dormancy resulted in reduction of FAME production, which is a weapon to save bacteria from the effect of antibacterial fatty acids. So our finding suggests that bacterial dormancy is disadvantageous to bacteria and provides a way to dislodge the biofilm by the help of antibacterial fatty acids. Although, it is well known that fatty acids; for example, *cis*-2-decenoic acid and oleic acid, are working effectively as biofilm inhibitors,[33] but the mechanism of antibiofilm activity is not defined until now. In the present study, the bacterial defenses (that is, FAMEs) active at planktonic stage and weakness of highly protected biofilm population (that is, increase hydrophobicity) has been elucidated. The unsaturated fatty acids (for example, *cis*-2-decenoic acid and *cis*-9-octadecanoic acid) utilizes this property of biofilm indwellers for hydrophobic interaction to bind and penetrate hydrophobic surface. This is also supported by Kohler *et al.*[34] and Clarke *et al.*[35] It is suggested that surface hydrophobicity of bacteria render them sensitive to the bactericidal effect of fatty acids and peptides.[35] Biofilms are a major cause of catheter-related infections because most of the silicone catheters are hydrophobic. It is mentioned that higher hydrophobicity is probably responsible for strong biofilm formation on these surfaces. Fatty acids may also make strong attachment with catheters by hydrophobic interaction. Therefore, it is suggested that surface coating of catheters with these fatty acids may restrain bacterial attachment and biofilm formation. By this application, shelf life of catheters may be increased with reduced treatment cost and duration. Moreover, in a recently published work, it has been mentioned that accessory gene regulator (*Agr*) has a major contribution in biofilm dispersion. Hence, mutation of the *agr* locus drastically affects FAME production.[36] It has also been reported that *agr* locus is controlled by *sar*. Therefore, a mutation in *sar* also affects the expression of *agr*[36] In this regard, our recent study showed that biofilm consortia is dominated by dormant cells and a drastic reduction in *sarA* expression was noticed in this metabolically inactive population.[20] The subject isolates used in the present study belong to *agr* type II. Therefore, transcriptional activity of the *agr* locus may also repress, resulting in inhibition of FAME production. There is no clear evidence about how these fatty acids inhibit or disperse biofilm. However, it is reported that *cis*-2-decenoic acid and *cis*-11-methyl-2-dodecenoic acid act as a positive signal that is sensed by bacteria, thereby inducing a cascade of events that result in degradation of the biofilm matrix and biofilm dispersal.[37,38] In a recent work, Marques *et al.*[39] described that *cis*-2-decenoic acid, a fatty acid signaling molecule, is able to change the status of *Pseudomonas aeruginosa* and *Escherichia coli* persister cells from a dormant to a metabolically active state without an increase in cell number. In our previous study, it was described that biofilm consortia is dominated by persister cells. These cells are metabolically inactive with arrested *agr* and *sar* expression.[20] Possibly, *cis*-2-decanoic acid and *cis*-9-octadecanoic acid also activate metabolic machinery of *S. aureus* persister cells that enable them to revert to an active state. This results in induction of *agr* and dispersion of biofilm or readaptation of planktonic state.

CONCLUSION

This study suggests that subinhibitory dose of oxacillin changes cell-surface charges from hydrophilic to hydrophobic and induces multicellular aggregates in biofilm consortia. These oxacillin-induced biofilms were metabolically inactive and unable to produce FAMEs. Hence, these biofilm indwellers were found sensitive to fatty acids (that is, *cis*-2-decanoic acid and *cis*-9-octadecanoic acid) because FAMEs deactivate fatty acids and protect bacteria. The highly protected and resistant populations in biofilms show a weakness (that is, increased hydrophobicity) that attract and allow antibacterial fatty acids to penetrate inside the protective covering and disperse the biofilm. This penetration capability of fatty acids may also enhance the efficacy of other antimicrobials as most of the available antibiotics are unable to penetrate and disperse bacteria in biofilm consortia. This finding may open new avenues for research in treatment against biofilm-associated infections.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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