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ORIGINAL ARTICLE

Erythromycin resistance features and biofilm formation affected by subinhibitory erythromycin in clinical isolates of *Staphylococcus epidermidis*

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Background/Purpose: Subminimal inhibitory concentration (sub-MIC) of antibiotics can modify the phenotype of biofilm formation in bacteria. However, the relationship between resistance phenotypes, genotypes, and the biofilm formation phenotype in response to sub-MIC antibiotics remains unclear.
Methods: Here, we collected 96 clinical isolates of *Staphylococcus epidermidis* (*S. epidermidis*) and investigated the erythromycin (ERY) susceptibility, the biofilm formation in respond to sub-MIC ERY, the presence and transcription expression of *erm* genes. Serial passage of induction resistance was used against ERY-susceptible isolates and biofilm formation in response to their new sub-MIC ERY was determined.
Results: The incidence of biofilm phenotype modification in ERY-resistant isolates was significantly higher than that of ERY-susceptible isolates [27/85 (31.8%) vs. 0/11 (0%), $p = 0.031$]. Yet, ERY-susceptible isolates displayed the phenomenon of biofilm phenotype modification (7/11), after induction of resistance to ERY. The *ermC* gene was absolutely dominant among the three macrolide resistant genes including *erm* (*A*, *B*, *C*) [6/96 (6.2%), 6/96 (6.2%), and 91/96 (94.8%), respectively]. With statistic stratification analysis, a linear and positive correlation was identified between the two factors in the biofilm-enhanced strains, a linear and negative correlation in biofilm-inhibited strains, and a weakly positive correlation in biofilm-unaffected strains ($R^2 = 0.4992$, 0.3686, and 0.0512, respectively).

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Conclusion: The results suggest that the ERY resistance phenotype and the transcription expression of *ermC* gene could be considered as important signs to estimate whether the biofilm formation phenotype in *S. epidermidis* clinical isolates can be easily affected by sub-MIC ERY.

Introduction

Antibiotic therapy is a very important means of treating bacterial infections.[1,2] Once inside the body, a subminimal inhibitory concentration (sub-MIC) of antibiotic always follows supra-inhibitory concentration *in vivo*[3] and it is an inevitable process during antibiotic treatment. Effects of sub-MIC antibiotics on bacteria are interesting; current studies mainly focus on the biofilm formation *in vitro*. Some antibiotics, when present at concentrations below the MIC, can significantly modify the phenotype of biofilm formation in a variety of bacterial species *in vitro*. Schadow et al[4] first demonstrated that sub-MIC rifampin can induce biofilm formation in *Staphylococcus epidermidis* (*S. epidermidis*) *in vitro*. The most classic paper on this subject was published in 2005, which showed that sub-MIC tobramycin could induce biofilm formation in *Pseudomonas aeruginosa* (*P. aeruginosa*).[5] However, sub-MIC macrolide antibiotics such as azithromycin (AZM) and clarithromycin (CLR) can inhibit biofilm formation in *P. aeruginosa*.[6,7]

S. epidermidis, a Gram-positive bacterium with low virulence and weak pathogenicity, is the leading pathogen causing biofilm-associated infections of surgical implants, central venous catheters, artificial pacemakers and so on.[8–10] Many materials, such as some antibiotics, phenolic triterpenoids,[11] thiophenones,[12] farnesol,[13] and some synthetic cationic peptides[14] can act against *S. epidermidis* or inhibit biofilm formation of *S. epidermidis*. Macrolide antibiotics are mainly used for a variety of infections caused by Gram-positive bacteria. Recently, we reported that sub-MIC levels of erythromycin (ERY), AZM, and CLR markedly enhanced biofilm formation of 20% macrolide-resistant clinical isolates of *S. epidermidis in vitro*.[15] The aim of this study was to determine in detail the features in 96 clinical isolates of *S. epidermidis*, including the profile of biofilm formation treated with or without sub-MIC ERY, ERY-resistant phenotype and genotype, and the transcription expression levels of *ermC* gene treated with sub-MIC ERY. These data will reveal new insights into associations between biofilm formation in response to sub-MIC ERY and these resistant characteristics of the clinical isolates of *S. epidermidis*.

Materials and methods

Stains and medium

Ninety-six clinical isolates of *S. epidermidis* with a variety of ERY susceptibility patterns were recovered from various samples in the Southwest Hospital of the Third Military Medical University from January 2010 to April 2011. Duplicate isolates from the same patient were not included in the study. Distributions of *S. epidermidis* strains by origin of recovery were 25 strains from blood (26%), 21 strains from wound (21.9%), 18 strains from sputum (18.8%), 12 strains from catheter (12.5%), seven strains from urine (7.3%), five strains from eye secretions (5.2%), and eight strains from other samples (8.3%). Species identification was based on BBL Crystal TM Mind (BBL Crystal Autoreader, Sparks, Maryland, USA). *S. epidermidis* ATCC 35984 and *S. epidermidis* ATCC 12228 were included as the reference of biofilm-positive and -negative strains, respectively, in the biofilm formation experiments.

The liquid growth medium for *S. epidermidis* was tryptic soy broth (TSB, Fluka, Saint Louis, Missouri, USA). The solid growth medium was prepared from TSB by addition of agar to 1.5%. Mueller-Hinton agar (Luqiao Technique Co. Ltd, Beijing, China) plates with different concentrations of antibiotics were used to determine MIC.

Susceptibility testing

The determination of the MICs of ERY (Fluka) was performed by the agar dilution method according to the suggestions of the Clinical and Laboratory Standards Institute (CLSI).[16] The quality control was performed with *S. aureus* ATCC 29213 and values of MICs were in the ranges stipulated by the CLSI.[16]

Biofilm assay

Biofilm formation was tested by the growth of isolates in 96-well Corning flat-bottom plates, essentially as described previously.[6,17] An overnight culture of cells grown in TSB (37°C, 180 rpm) was diluted 1:100, and 100 μL aliquots and 100 μL TSB in the presence and absence of 0.5 MIC ERY (final concentration: 0.25 MIC) were added to the 96-well plate with four replicates. Every test was repeated three times. After 24 hours of incubation at 37°C without shaking, the wells of the microtiter plate were rinsed with water and the biofilms were stained with 1% (w/v) crystal violet (Sigma, Saint Louis, Missouri, USA) and absorbance measurements were taken with a Sunrise Tecan ELISA (Sunrise, Tecan, Melbourne, Australia) at 590 nm. If the absorbance value of the stained sample exceeded 3.0, we would correctly dilute it to assay.

The criteria of biofilm formation were the optical density (OD) value of biofilm formation was equal to or greater than twice that of the measured OD values of biofilm formation of *S. epidermidis* ATCC12228. The conditions of biofilm formation, including the concentration of ERY and the incubated time (0.25 MIC and 24 hours) were

determined according to the results of the dose-effect and time-effect tests (data not shown).

The definition for the biofilm formation to be categorized as enhanced, inhibited, or unaffected with the addition of 1/4 MIC ERY was the following: firstly, to compare whether the biofilm formations per strain with or without 1/4 MIC ERY were similar ($p > 0.05$) or significantly different ($p < 0.05$) with the *t* test. If the arithmetical mean of the OD value with 1/4 MIC ERY was greater than that of the OD value without 1/4 MIC ERY and $p < 0.05$, then the biofilm formation of the strain can be considered as enhancement. If the arithmetical mean of the OD value with 1/4 MIC ERY was less than that of the OD value without 1/4 MIC ERY and $p < 0.05$, then the biofilm formation of the strain can be considered as inhibition. If $p > 0.05$, the biofilm formation of the strain would be considered as not affected, even if the arithmetical mean of OD value with 1/4 MIC ERY was less or greater than that of the OD value without 1/4 MIC ERY.

Serial passage of induction resistance in vitro

This was performed as previously described with modifications.[18–20] A 24-hour culture of each ERY-susceptible *S. epidermidis* isolate was first transferred with a swab onto Mueller-Hinton agar containing 0.25 MIC of ERY. Surface growth after 24—72 hours of incubation was then transferred with a swab onto ERY-free agar for isolation and onto Mueller-Hinton agar containing a doubled amount of ERY. These plates were then incubated for 24—72 hours. This process was repeated serially until no growth occurred. All experiments for induction of resistance were performed on the same media, and at the same incubation temperature and atmosphere. The MIC of ERY was determined for isolates that were grown with the highest concentration of ERY. Isolates were also transferred three times on ERY-free agar, followed by a re-determination of the MIC of ERY to assess the stability of the selected resistance.

PCR analysis

The genomic DNA was isolated from the planktonic cells of *S. epidermidis* isolates using a Wizard Genomic DNA Purification Kit (Promega, Shanghai, China). Then the genomic DNA was used as a template for PCR using Go Taq Green Master Mix (Promega). The primers used were *gryB*-F-TTC GCA TAC GTT AAT AAG TTG G and *gryB*-R-TGA CGA GGC ATT AGC AGG T (ref. 15, amplification: 675 bp); *ermA*-F-AAA ACC CTA AAG ACA CGC and *ermA*-R-CAC TTG ACA TAA GCC TCC (GENEBANK: NC_002976, amplification: 191 bp); *ermB*-F-CAC TAG GGT TGC TCT TGC and *ermB*-R-ACT TTG GCG TGT TTC ATT (GENEBANK: AB300568, amplification: 241 bp); *ermC*-F-ATC GGC TCA GGA AAA GGG CAT and *ermC*-R-CGT CAA TTC CTG CAT GTT TTA AGG (GENEBANK: AFEF01000006, amplification: 559 bp) and synthesized by Sangon Biotech Co., Ltd., Shanghai, China. The *gyrB* gene was the positive control. All amplification reactions were prepared in a 25 μL volume. All strains were detected the presence of such genes associated with macrolides resistance in *Staphylococci* as *ermA*, *ermB* and *ermC*. PCR products were analyzed by agarose gel electrophoresis and further sequenced in Sangon Biotech.

Real time reverse transcription polymerase chain reaction

Total RNA was isolated from intact *S. epidermidis* cells grown in liquid TSB medium with or without 0.25 MIC ERY for 2 hours using ultrasonication and a rapid bacterial RNA extraction kit (ABigen, Beijing, China). The incubated time was determined according to the results of the time-effect tests of transcriptional expression of *ermC* gene (data not shown). A total of eight ERY-resistant, *ermC* gene-positive and biofilm-inhibited isolates, SH04, SH66, SH87, SH92, SH40, SH46, SH52, and SH77 were all selected for transcription expression of the *ermC* gene. Isolates SH40, SH46, SH52, and SH77 were induced resistant isolates and their biofilm formations were inhibited by the new sub-MIC ERY. The selection of the other strains for transcription expression of the ermC gene were randomly selected. Reverse transcription (RT) was performed using the PrimeScript RT Reagent Kit following the manufacturer's protocol (TaKaRa, Dalian, China). Then the cDNA was used as a template for real-time polymerase chain reaction (PCR) using SYBR Premix Ex Taq II (TaKaRa) in an iCycler Real Time PCR instrument TY0678 (Bio-Rad, California, USA). The primers used were *gyrB*-F-AAG GGT ATT ATG GCT TCA CG and *gyrB*-R-TTT CAC TTT CTT CAG GGT TC (ref. 15, amplification: 139 bp); *ermC*-F-GAA ATC GGC TCA GGA AAA GGG C and *ermC*-R-TGG TCT ATT TCA ATG GCA GTT ACG A (GENEBANK: AFEF01000006, amplification: 80 bp). All reactions were normalized to the *gyrB* gene, which encodes the protein DNA gyrase β-subunit.

Statistical analysis

Continuous variables (for example, the optical density (OD) value of biofilm formation) were compared using the independent samples *t* test ($p < 0.05$ was considered statistically significant), and categorical variables were compared using the Chi-square test (Fisher's exact test). Correlation analysis was performed using the Crosstabs test. Statistical analyses were performed using SPSS v.11.5.

Results

Identification of ERY resistance phenotype

Among the 96 clinical isolates of *S. epidermidis*, we found 85 resistant to ERY. The ERY MICs of these resistant isolates ranged from 8 to 256 μg/mL, most of which were highly resistant to ERY, with MIC ≥128 μg/mL.

Biofilm formation

We examined the biofilm phenotypes in response to 0.25 MIC of ERY for all of the 96 *S. epidermidis* clinical strains. Twenty-three of the 85 (27%) ERY-resistant isolates exhibited biofilm induction (Table 1). In 18 of these 23 strains, biofilm induction intensity ranged from 1.11-fold to 2.0-fold, and in five strains, biofilm induction intensity was more than 2-fold. At the same time, we found that in 4/85 ERY-resistant isolates of *S. epidermidis*, biofilm formation

Table 1 Identification of erythromycin (ERY) resistance genotype and biofilm phenotype in response to subminimal inhibitory concentration (sub-MIC) ERY of 96 *Staphylococcus epidermidis* (*S. epidermidis*) strains tested

PCR amplification of *ermA*, *B*, *C* genes	Biofilm phenotype in response to sub-MIC ERY		
	Inhibited[a]	Enhanced[b]	Unaffected[c]
− − +	SH04, SH66, SH87, SH92	SH10, SH14, SH15, SH17, SH21, SH23, SH27, SH28, SH39, SH41, SH42, SH43, SH45, SH56, SH58, SH60, SH68, SH78, SH86, SH89	SH01, SH02, SH03, SH09, SH11, SH12, SH24, SH26, SH29, SH30, SH32, SH33, SH34, SH36, SH38, SH44, SH47, SH48, SH49, SH53, SH55, SH59, SH61, SH62, SH64, SH65, SH69, SH70, SH71, SH72, SH73, SH74, SH75, SH76, SH79, SH80, SH81, SH82, SH83, SH85, SH88, SH91, SH93, SH95, SH96, SH97, SH98, SH22, SH25, SH35, SH40, SH46, SH51, SH52, SH67, SH77, SH94
+ − +			SH05, SH07, SH13, SH16, SH06
− + +		SH08, SH63,	SH31, SH57, SH84
+ − −			SH50
− + −		SH18,	
− − −			SH19, SH20, SH37

[a] The arithmetical mean of OD value per strain with 1/4 MIC erythromycin was less than that of OD value without 1/4 MIC erythromycin and p value <0.05.
[b] The arithmetical mean of OD value per strain with 1/4 MIC erythromycin was greater than that of OD value without 1/4 MIC erythromycin and p value <0.05.
[c] The p value >0.05, in spite of the arithmetical mean of OD value per strain with 1/4 MIC erythromycin was less or greater than that of OD value without 1/4 MIC erythromycin.

was inhibited by sub-MIC ERY (Table 1). However, the biofilm phenotype modification in response to sub-MIC ERY was observed in none of the 11 ERY-susceptible isolates. There was a statistically significant difference of the incidence of biofilm phenotype modification in response to sub-MIC of ERY between ERY-resistant isolates and ERY-susceptible isolates [27/85 (31.8%) vs. 0/11 (0.0%), $p = 0.031$].

Induction resistance in vitro and biofilm formation in response to the new sub-MIC ERY in the inducible resistance isolates

Induction of resistance to ERY was tested against the 11 ERY-susceptible *S. epidermidis* clinical isolates. All of the 11 ERY-susceptible isolates became resistant to ERY after serial passage technique of induction resistance. The new ERY MIC of these 11 induction resistant *S. epidermidis* clinical isolates ranged from 16 μg/mL to 256 μg/mL (Table 2).

We assayed the biofilm formation in response to sub-MIC ERY again in all of the 11 isolates. Parallel experiments were performed in which biofilm formation of induced and uninduced cells in the presence and absence of their respective sub-MIC ERY was determined. There were three isolates, SH22, SH51 and SH67, in which biofilm formation of induced cells in response to 0.25 MIC (32, 16, and 32 μg/mL) of ERY was significantly enhanced compared with that of induced cells without ERY and that of uninduced cells in the presence and absence of the original sub-MIC ERY. There were four isolates, SH40, SH46, SH52, and SH77, in which biofilm formation of induced cells in response to 0.25 MIC (32, 64, 4, and 64 μg/mL) of ERY was significantly inhibited compared with that of induced cells without ERY and that of uninduced cells in the presence and absence of the original sub-MIC ERY (Table 2).

Presence of macrolide resistance genes

Positive PCR amplifications of *ermC* gene were obtained for 80/85 (94.1%) ERY-resistant *S. epidermidis* isolates tested and all of the 11 ERY-susceptible isolates. The positive amplifications for another two genes *ermA* and *ermB* were 6/96 (6.2%) and 6/96 (6.2%), respectively. The data of identification of ERY resistance genotype and biofilm phenotype in response to 0.25 MIC of ERY for all 96 *S. epidermidis* clinical isolates are given in Table 1. Eighty-one strains were *ermC* type, five were *ermA* and *ermC* type, five were *ermB* and *ermC* type, one was *ermA* type, one was *ermB* type, and three were *ermA*-, *ermB*- and *ermC*-negative strains.

Real time RT-PCR analysis of transcriptional expression of ermC gene

We investigated the transcription expression of *ermC* gene incubated with sub-MIC ERY for 2 hours in 25 ERY-resistant isolates (including 7 induced resistant isolates) with

Table 2 Susceptibility and biofilm formation to erythromycin (ERY) before and after induction of resistance *in vitro* in 11 ERY-susceptible *Staphylococcus epidermidis* (*S. epidermidis*) (μg/mL)

Strains	MIC_0	OD value with ERY before induced	OD value without ERY before induced	The actual fold changes of OD values	MIC_1	OD value with ERY after induced	OD value without ERY after induced	The actual fold changes of OD values
SH06	0.25	1.04 ± 0.15	0.96 ± 0.21	1.08	32	0.84 ± 0.12	0.86 ± 0.13	0.98
SH22	0.0625	1.49 ± 0.25	1.50 ± 0.26	0.99	128	1.83 ± 0.33**	1.32 ± 0.17	1.39
SH25	0.0625	0.44 ± 0.09	0.46 ± 0.10	0.96	16	0.60 ± 0.17	0.49 ± 0.08	1.22
SH35	1	1.36 ± 0.18	1.24 ± 0.12	1.10	128	0.84 ± 0.11	0.93 ± 0.17	0.90
SH40	0.0625	1.29 ± 0.15	1.32 ± 0.13	0.98	128	0.65 ± 0.15**	0.96 ± 0.18	0.68
SH46	0.0625	2.05 ± 0.15	2.01 ± 0.09	1.02	256	1.62 ± 0.09**	1.83 ± 0.12	0.88
SH51	0.0625	0.91 ± 0.13	0.85 ± 0.08	1.07	64	1.10 ± 0.18**	0.58 ± 0.88	1.90
SH52	0.25	1.82 ± 0.43	1.76 ± 0.39	1.03	16	1.80 ± 0.31**	2.30 ± 0.51	0.78
SH67	0.25	1.35 ± 0.11	1.37 ± 0.17	0.99	128	0.89 ± 0.09*	0.79 ± 0.10	1.13
SH77	0.25	1.24 ± 0.17	1.12 ± 0.15	1.11	256	1.01 ± 0.11**	1.56 ± 0.24	0.65
SH94	0.125	3.35 ± 0.06	3.38 ± 0.06	0.99	16	3.29 ± 0.04	3.30 ± 0.09	0.99

MIC_0 = minimal inhibitory concentration of ERY before induction of resistance *in vitro* in ERY-susceptible *S. epidermidis* strains; MIC_1 = minimal inhibitory concentration of ERY after induction of resistance *in vitro* in ERY-susceptible *S. epidermidis* strains.
*$p < 0.05$.
**$p < 0.01$ (*t* test, comparing with the optical density (OD) value without ERY after induced).

different biofilm formation phenotypes (9 enhanced isolates, 8 inhibited ones and 8 unaffected ones) and found that there was no correlation between the transcription expression of *ermC* gene and the ratio of biofilm OD value of ERY-treated and -untreated groups, $R^2 = 0.0044$. However, with statistic stratification analysis, a linear and positive correlation was identified between the two factors in the biofilm-enhanced strains, a linear and negative correlation in biofilm-inhibited strains, and a weakly positive correlation in biofilm-unaffected strains ($R^2 = 0.4992$, 0.3686, and 0.0512, respectively, as shown in Fig. 1).

Discussion

Oliveira et al[21] tested 34 enterococci isolates and found that resistance to gentamicin, streptomycin, and enrofloxacin was related to biofilm production at some time points. Elisotetta et al[22] showed that the β-lactamase positive *Proteus mirabilis* strains were statistically better significant in biofilm formation than negative ones, regardless of growth medium. These studies suggested that there was probably some correlation between the phenotype of antibiotic resistance and bacteria biofilm formation. In this study, we collected 96 *S. epidermidis* clinical isolates and found that all strains in which biofilm formation was enhanced or inhibited by sub-MIC ERY were ERY-resistant isolates. However, such modification of biofilm phenotype by sub-MIC ERY was not found in any of the ERY-susceptible strains. At the same time, a significant difference was found between the phenotype of antibiotic resistance and modification of biofilm phenotype in response to sub-MIC ERY ($p < 0.05$). Afterwards, by serial passage technique of induction resistance, ERY-susceptible isolates tested in this study, which were *ermC* gene-positive, became resistant to ERY, and the biofilm formation in some of them was also enhanced or inhibited by their new sub-MIC ERY. This phenomenon was similar with that in the ERY-resistant clinical isolates and further suggested that there may be some relation between the phenotype of antibiotic resistance and modification of biofilm phenotype in response to sub-MIC ERY. Numerous studies have investigated the effects of sub-MIC of antibiotics on biofilm formation in a number of bacterial species, with most of them focused on resistant strains.[5,7,15] In contrast, Dunne[23] tested 23 clinical isolates of *S. epidermidis* which were susceptible to cefamandole and vancomycin, and found that sub-MIC of these two antimicrobial agents could, respectively, enhance and repress the biofilm formation in the isolates *in vitro*. In another study, Boehm et al[24] demonstrated that sub-MIC streptomycin could induce biofilm formation in a streptomycin-sensitive *E. coli* strain, but not in a streptomycin-resistant mutant. However, Kaplan[25] showed that in two strains of *MRSA* and one strain of *MSSA*, sub-MIC of methicillin could induce biofilm formation. Elliott et al[26] found that sub-MIC of tobramycin induced biofilm formation in both tobramycin-resistant and tobramycin-susceptible *P. aeruginosa*, regardless of the MIC of tobramycin for the strains. Taking together the results mentioned above, there is still no clear relationship between antibiotic susceptibility and the modification of biofilm phenotype in response to sub-MIC antibiotics. Although our results alone cannot demonstrate that the modification of biofilm phenotype by sub-MIC ERY only happens in ERY-resistant *S. epidermidis* clinical isolates, it suggests that the biofilm formations of ERY-resistant *S. epidermidis* clinical isolates are more likely to be modified by sub-MIC ERY than that of ERY-susceptible ones.

Resistance to macrolides is conferred mostly by methylases, encoded by *erm* genes that modify the ribosomal target of macrolides.[27] Thus far, several groups of investigators[28–30] have examined the distribution of macrolide resistance genes in coagulase-negative staphylococci or *S. epidermidis* strains. The predominant genetic determinants about methylases for resistance to macrolides mostly include *erm* (*A*, *B*, *C*) genes. Therefore we

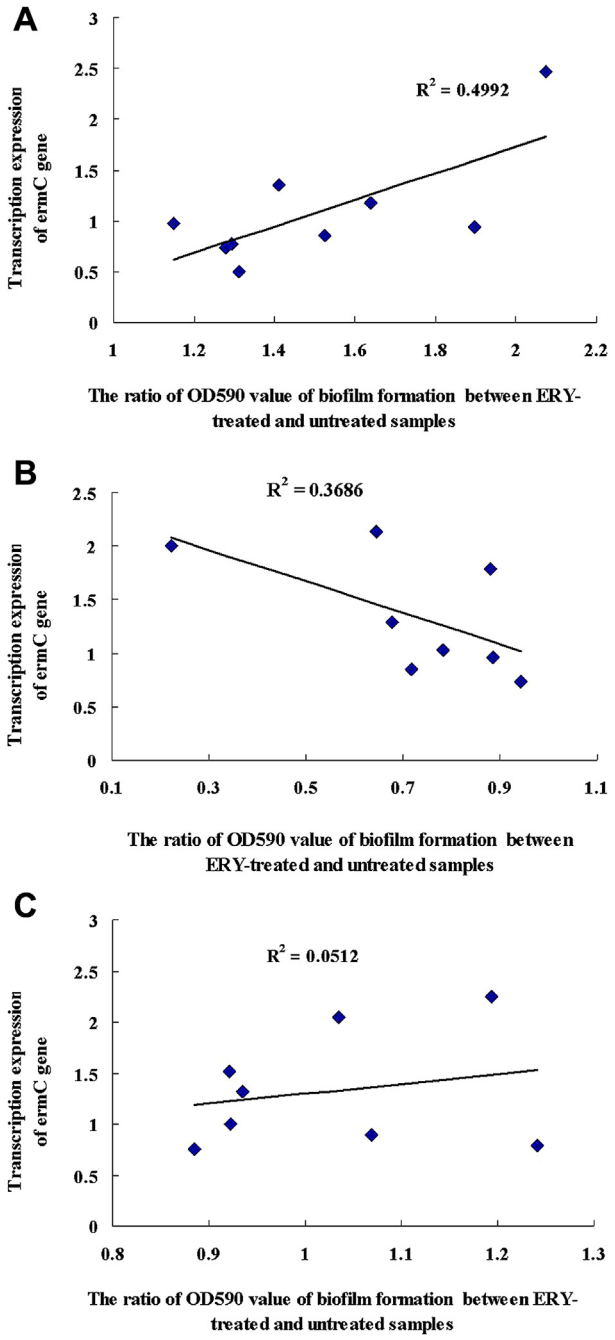

Figure 1. Biofilm formation phenotype in response to sub-minimal inhibitory concentration (sub-MIC) erythromycin (ERY) depending on the transcription expression of the *ermC* gene. (A) Isolates in which biofilm formation was enhanced by sub-MIC ERY ($n = 9$); (B) isolates in which biofilm formation was inhibited by sub-MIC ERY ($n = 8$); and (C) isolates in which biofilm formation was not affected by sub-MIC ERY ($n = 8$).

identified the presence or absence of these genes by PCR approach in all isolates tested and found that the *ermC* gene was absolutely dominant among the three *erm* genes tested. The finding of the predominant *ermC* gene among the isolates is similar to those reported by the others. Abbassi et al[28] collected 30 methicillin-resistant *S. epidermidis* strains and found that ERY resistance was encoded by the *ermC* (11 strains) and *ermA* (6 strains). Campoccia et al[31] investigated the prevalence of *ermA* and *ermC* genes in 70 clinical isolates of *S. epidermidis* and found that the *ermC* gene was observed in 33% of the isolates, while *ermA* was detected in a single isolate. Gatermann et al[32] identified the distribution of *erm* (*A*, *B*, *C*) genes in 494 isolates of coagulase-negative staphylococci and found that most (63%) ERY-resistant isolates carried constitutively expressed *ermC* as the sole resistance determinant; however, the *ermA* and *ermB* determinants were comparatively rare.

We further compared the transcription expression of the *ermC* gene in sub-MIC ERY-treated cells with that of the *ermC* gene in sub-MIC ERY-untreated cells, and found that there was a significant correlation between the transcription expression of the *ermC* gene and the ratio of biofilm OD value of ERY-treated and -untreated groups in biofilm enhanced and inhibited strains. However, the relationship was only weakly positive between the two factors in biofilm unaffected strains. These results suggest that the modification of biofilm phenotype in response to sub-MIC ERY was probably related with the transcription expression of the *ermC* gene in *S. epidermidis* clinical isolates, yet how the transcription expression variation of *ermC* gene launched the modification of biofilm formation needs further investigation to validate. There are other genes, which confer resistance to macrolides by encoding macrolides efflux, for example *msrA*, *mefA*. It is necessary to further investigate if there is a relationship between the *msrA* or *mefA* gene with the modification of biofilm phenotype in response to sub-MIC ERY in *S. epidermidis* clinical isolates.

In staphylococci, the most important molecule in biofilm formation is the polysaccharide intercellular adhesion (PIA),[33,34] which is also called poly-N-acetylglucosamine, with its biosynthesis accomplished by the products of the *ica* gene locus. Several studies revealed that some global regulators, including the DNA-binding protein SarA, the alternative sigma factor SigB, and the quorum-sensing system LuxS regulated *ica* transcription or PIA expression.[35–37] More studies are needed to clarify whether and how sub-MIC ERY triggered the global regulators mentioned above to modify the biofilm formation phenotype by changing the transcription expression of *ermC* gene in *S. epidermidis* clinical isolates.

In conclusion, we found that approximately 30% of the *S. epidermidis* clinical isolates exhibited biofilm enhancement or inhibition in response to sub-MIC ERY, and this phenomenon is closely associated with the ERY-resistant phenotype of *S. epidermidis* and the transcription expression of the *ermC* gene. These results suggest that the treatment of biofilm-associated infections of *S. epidermidis* will become more complex when using macrolide antibiotics. The difference of biofilm formation in response to sub-MIC ERY in ERY-resistant and -susceptible isolates warrants further investigations of the mechanism.

Conflicts of interest

All authors declare no conflicts of interest.

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