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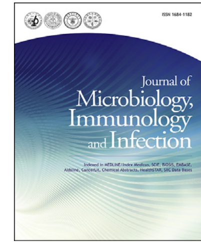

ORIGINAL ARTICLE

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.

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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

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