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Farnesol Decreases Biofilms of *Staphylococcus epidermidis* and Exhibits Synergy with Nafcillin and Vancomycin

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Abstract

Biofilm infections are frequently caused by *S. epidermidis*, are resistant to antimicrobial agents and adversely affect patient outcomes. We evaluated farnesol, the Candida quorum sensing molecule, on *S. epidermidis* biofilms, *in vitro* and *in vivo*. We evaluated ED_{50}, ED_{75} and ED_{90} – (drug concentrations causing 50%, 75% and 90% inhibition respectively) of farnesol and evaluated synergy with nafcillin and vancomycin. Farnesol's effects on morphology of *S. epidermidis* biofilms were analyzed using confocal microscopy and real-time changes using a bioluminescent strain of *S. epidermidis*, Xen 43. In mice, effects of farnesol treatment on subcutaneous catheter biofilms, cultures of blood, kidney, catheter and peri-catheter tissues and bioluminescence in strain Xen 43 were evaluated. Farnesol inhibited biofilms (ED_{50} ranged from 0.625 to 2.5 mM) and was synergistic with nafcillin and vancomycin at most combination ratios. Farnesol significantly decreased biovolume, substratum coverage and mean thickness of *S. epidermidis* biofilms. In mice, farnesol significantly decreased viable colony counts of *S. epidermidis* from blood, kidney, catheter and peri-catheter tissues and decreased Xen 43 bioluminescence. We confirmed the anti-biofilm effects of farnesol both *in vitro* and *in vivo*, in a bioluminescent strain and its synergy with antibiotics. Farnesol may be effective against clinical *S. epidermidis* biofilm infections.

Introduction

Biofilms are three dimensional communities of microorganisms that are surface-associated and encased in an extracellular matrix, composed of polysaccharides, proteins and extracellular DNA (1). Biofilms contribute significantly to medical infections that include catheter and other device-associated infections(2). Catheter-related blood stream infections (CRBSI) significantly increase mortality, morbidity and healthcare costs (3). Approximately 250,000 cases of CRBSIs occur in the USA and it costs more than $50,000 to treat an episode of CRBSI (3).

S. epidermidis is the most common etiological agent isolated in device-associated biofilm-related infections (4). *S. epidermidis* is a normal skin commensal but as an opportunistic pathogen leads the list for healthcare-associated infections. While *S. epidermidis* lacks secreted virulence factors like the exotoxins produced by *S. aureus*, it is uniquely adapted to

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cause chronic biofilm infections. In addition, *S. epidermidis* is also the major cause for late-onset neonatal sepsis in infants born at < 1500 g (5). Neonatal sepsis is associated with significant mortality, morbidity and adverse neurodevelopmental outcomes (5, 6).

Microbial biofilms including those of *S. epidermidis* are inherently resistant to host defense and antimicrobial agents and hence difficult to eradicate. Clinical treatment strategies involve treatment with multiple antibiotics and removal of the life-saving catheters and devices. Novel strategies are necessary to combat biofilm-related infections to improve clinical outcomes. Farnesol, the Candida quorum sensing molecule has anti-biofilm activity. Farnesol is a sesquiterpene alcohol and is the first quorum sensing molecule described in eukaryotes (7) and produced by most candida species (8). Exogenous farnesol inhibits the development of Candida biofilms *via* inhibition of filamentation (9). Farnesol has also been reported to have efficacy against *S. aureus* biofilms and increases biofilm susceptibility to antibiotics (10). We evaluated the anti-microbial susceptibilities of *S. epidermidis* biofilms to farnesol and synergy with antistaphylococcal antibiotics *in vitro*. Quorum sensing mutants of *S. epidermidis* form larger biofilms and are implicated in chronic biofilm infections in humans and hence we evaluated mutants of the *agr* and *luxS* quorum sensing systems in addition to clinical isolates (11). We confirmed our findings in a clinically relevant mouse model of catheter infection *in vivo*.

METHODS

Strains and culture conditions

Organisms: *S. epidermidis* strains ATCC 55133, 1457, 1457 *agr* mutant, 1457 *luxS* mutant and Xen 43 were used. ATCC 55133 and 1457 are biofilm forming clinical isolates. Xen 43 (Caliper Life Sciences Inc., USA) is a bioluminescent strain derived from 1457 and was used to monitor infection in real-time (12). **Growth media:** Trypticose soy agar with 5% sheep blood was used for plating organisms and trypticose soy broth (TSB) for subcultures and antimicrobial susceptibility testing.

Farnesol preparation: A stock solution of trans-trans-farnesol (Sigma, USA) in DMSO was freshly prepared at 500 mM before use and diluted to required concentrations. Farnesol suspensions were prepared fresh to preserve the antioxidant effect. Farnesol was prepared in concentration ranges of 80 to 0.16 mM for biofilm susceptibility testing. Nafcillin and vancomycin were prepared in concentration ranges of 80 to 0.6 μg/ml for biofilm antimicrobial susceptibility testing.

Antimicrobial susceptibility testing of *S. epidermidis* biofilms

Biofilms were formed in 96-well microtiter plates by adding 100 μl of *S. epidermidis* at approximately 10^7 colony forming units/ml (CFU ml^{-1}) in RPMI 1640 (pH of 7) and incubated for 24 h at 35° C. The supernatant was discarded and the biofilms washed with PBS to remove unadhered cells. Biofilm formation was confirmed by light microscopy. Antimicrobial susceptibility of biofilms to farnesol was performed in duplicate by adapting Clinical and Laboratory Standards Institute (CLSI) guidelines for planktonic antimicrobial testing (13). The *S. epidermidis* biofilms in the microtiter plates were exposed to serial dilutions of antimicrobial agent (farnesol from 0.2 mM to 80 mM, nafcillin and vancomycin from 80 to 0.6 μg/ml) for 24 h. The endpoint of inhibition of the biofilms by the antimicrobial agent was estimated by the colorimetric method using 2, 3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyl-amino) carbonyl]-2H-tetrazolium hydroxide (XTT) (14). A solution of XTT (0.5 g L^{-1}) was prepared in phosphate buffered saline, filter sterilized (0.22 μm size) and stored at −80 °C. Menadione was prepared as 10 mM solution in acetone (99.9% HPLC grade) and stored at −80 °C and 100 μl of which added to 100 μl of XTT

gives 1 μM concentration. Hundred μl of this combination is added to the wells of 96 well micro-titer plates with the biofilms with the antimicrobial and then incubated in the dark for 2 h. Microtiter plates were centrifuged for 5 min at 3000 rpm, 100 μl supernatant was transferred to a new microtiter plate and the color read at OD 490 nm (15). Growth and sterility controls on the same microtiter plate were used for comparison. The experiments were done in duplicate, repeated on 3 different days and the mean of the readings of XTT reduction were used to determine the biofilm inhibitory concentrations. We evaluated ED_{50}, ED_{75} and ED_{90} (drug concentrations causing 50%, 75% and 90% inhibition respectively) of farnesol, nafcillin and vancomycin.

Confocal imaging to assess biofilm morphology: Biofilms of *S. epidermidis* were formed on optical bottom microwell Petri dishes (Mattek Corp, USA) for 24 h and exposed to 0.5 mM of farnesol or DMSO control for another 24 hrs. The biofilms were washed with PBS, stained with LIVE/DEAD stain (Molecular probes, USA) and examined using Zeiss Meta confocal microscope. Serial sections of the biofilm were obtained at 1 μm intervals along the z-axis and the z-stack images were analyzed with the software PHLIP, for MATLAB toolbox, for biovolume (in μm^3), substratum coverage (in %) and mean thickness (in μm) (16). At least 2 representative fields in the biofilm on any particular day were analyzed and measurements were averaged. Biofilm experiments were repeated on 3 different days. Biofilms exposed to farnesol were compared with the controls by t test and statistical significance was assumed at $p < 0.05$.

Synergy evaluation of farnesol antibiotic combinations

Biofilm inhibition by combinations of farnesol with nafcillin or vancomycin was evaluated in an 8 by 8 checkerboard format, in 96-well micro-titer plates, in 2-fold serial dilutions across rows and columns, and inhibitory endpoints were assessed by the XTT reduction assay. Inhibitory effects at equipotent drug-dose ratios (1:1 ratios of ED_{50}, ED_{75} and ED_{90}) and non-equipotent ratios (1:2, 1:4 or 2:1) of the combinations were determined. The median effects method described by Chou *et al.* was used to study interactions in drug combinations by the calculation of combination indices (CI) (17). A CI <1 indicates synergy, CI >1 antagonism and CI=1, an additive effect. Multiple drug dose-effect calculations were performed using Calcusyn software (Biosoft, Cambridge, U.K.) with constant ratios of drug combinations.

Mouse model of subcutaneous catheter infection

In 3 week old FVB albino mice (Charles River Laboratories Inc., USA) mice, 1.5 inch segment of 18 gauge Teflon catheter was inserted subcutaneously on the back of each animal and each animal receiving one catheter segment. Prior to insertion, catheters were immersed in suspensions of *S. epidermidis* (10^7 CFU/ml) for 2 h (catheter CFU/ml pre-insertion was 3 to 5 10^5 CFU/ml), to facilitate biofilm formation. Farnesol (100 μg/g in 0.1 ml DMSO, 6.7 mM, n=7) or DMSO (control, n=5) was injected for 6 consecutive days from day 2 of infection, once per day, s.c. near the catheter. The dose of farnesol was extrapolated from other animal studies and was higher than our estimated ED_{50} *in vitro* (18). The animals were euthanized on day 8 and cultures of blood, kidney homogenates, catheter (by sonication) and peri-catheter (skin and subcutaneous) tissues were plated in serial dilutions. Catheter biofilms were confirmed by confocal and electron microscopy of explanted catheter segments. Sample size calculations revealed that a sample size of 5 in each group gave a power of 93% in detecting a log difference in CFU/ml of catheter cultures. The differences in CFU/ml between farnesol and DMSO treatment were assessed for significance by the Kruskal Wallis test. The protocol for animal experiments was approved by The Institutional Animal Care and Use Committee at Baylor College of Medicine.

Scanning electron microscopy: A 5 mm sample was cut from each of the explanted catheter segments from mice with subcutaneous catheter infection. The catheter samples were cut in cross sections and fixed with 2% glutaraldehyde, followed by fixing with osmium tetroxide, tannic acid and uranyl acetate. Fixation was followed by a series of ethanol dehydration steps and samples were sputter-coated with gold palladium. The samples were then scanned by pathologists who were blinded for farnesol treatment.

Real-time imaging using the bioluminescent strain Xen 43

***In vitro*:** Biofilms of Xen 43 were developed in 30 wells of an opaque 96-well microtiter plate (Corning, USA) and washed with PBS. At 48 h, 3 groups of ten wells each were exposed to DMSO, farnesol (FSL) (0.5 mM), or TSB for 24 h. Bioluminescence was monitored at 24, 48, 72 and 96 h and compared among the three exposures and the experiments were performed in duplicate. ***In vivo*:** Subcutaneous catheter infection was also established with the bioluminescent strain Xen 43, as described earlier. Subcutaneous injections of farnesol (100 μg/g in 0.1 ml DMSO, 6.7 mM, n=5) or DMSO (n=5) were administered only from days 2 to 5. Live animal imaging for bioluminescence was performed daily for 5 days.

Results

Antimicrobial susceptibilities of *S. epidermidis* biofilms

The antimicrobial susceptibilities of *S. epidermidis* biofilms at ED_{50}, ED_{75} and ED_{90} for strains ATCC 55133, 1457, 1457 *agr* mutant and 1457 *luxS* mutant are shown in Table. 1. We were unable to establish ED_{90} of farnesol, nafcillin or vancomycin for strains 1457, 1457 *agr* mutant or the 1457 *luxS* mutant indicating the inherent resistance of the biofilm state. The *agr* and *luxS* quorum sensing mutants of 1457 were similar in susceptibilities to the parent strain 1457 (within two, 2-fold dilutions of the antimicrobial) except the *luxS* mutant, whose ED_{75} for nafcillin was more than 2 dilutions than the parent 1457 strain (5 μg/ml vs. 0.625 μg/ml).

In other experiments, 24 h biofilms of *S. epidermidis* strains were exposed to farnesol at 0.5 mM or DMSO (control). We chose 0.5 mM of farnesol, which was lower than the ED_{50} of all the strains to evaluate biofilm morphology. Confocal images of farnesol exposed biofilms and DMSO (control) obtained at 40 X magnification were examined at z intervals of 1 μm (Fig. 1) and analyzed. Farnesol significantly decreased biovolume, substratum coverage and mean thickness of *S. epidermidis* biofilms of all the four strains studied ($p < 0.05$) (Fig. 2).

Evaluation of synergy of the antimicrobial combinations by combination indices

We evaluated farnesol synergy with nafcillin and vancomycin by calculating combination Indices (CI) against four strains of *S. epidermidis* (Table. 2). We estimated CIs at equipotency ratios (1:1 ratios of ED of farnesol and the antibiotic) of ED_{50}, ED_{90} concentrations (ATCC 55133) and ED_{75} ratios for 1457, 1457 *agr* mutant and 1457 *luxS* mutant (where ED_{90} measurements were not available). We also evaluated non-equipotency ratios at 1:2, 1:4 and 2:1, where possible, from our checkerboard matrix of combinations. For farnesol and vancomycin combinations, ED_{50} 1:1 combination ratios were the same as ED_{90} 1:1 for strain 55133, and same as ED_{75} 1:1 for strains 1457, 1457 *agr* mutant and 1457 *luxS* mutant. Farnesol was synergistic with nafcillin and vancomycin (CI <1) at most equipotency and non-equipotency drug-dose ratios at 75% and 90% combination inhibitory effects of the combination (CED_{75} and CED_{90}) with few exceptions. The combinations that were not synergistic are bolded and italicized in the table (Table. 2). Strains 55133 and 1457 *agr* mutant were synergistic at all combinations tested and strains 1457 and 1457 *luxS* mutant had 2 and 1 exceptions respectively.

Farnesol decreased viable colony counts of *S. epidermidis* in the mouse model of subcutaneous catheter infection

In mice after subcutaneous catheter infection, mice were euthanized on day 8 of infection, catheters explanted and cultures performed. Catheter biofilms were confirmed by confocal and electron microscopy (Figs 3A & 3B). Farnesol decreased catheter biofilms (Fig. 3C) compared to controls (Fig. 3B). Farnesol treatment decreased catheter and pericatheter infection of *S. epidermidis* as shown by significantly decreased viable colony counts (CFU/ml) from catheter (Fig 4A) and peri-catheter tissues (Fig. 4B). Farnesol treatment also decreased systemic dissemination of *S. epidermidis* as shown by decreased blood and kidney CFU/ml (Fig. 4C & 4D). There were not significant differences in weight gain, activity or local reactions in farnesol treated mice compared to controls (data not shown).

Farnesol inhibits biofilms of bioluminescent *S. epidermidis* Xen 43 *in vitro* and *in vivo*

As an alternative approach to assess the effects of farnesol on *S. epidermidis* biofilms *in vivo*, a bioluminescent strain was used. The utility of this strain was validated *in vitro*. After 48 h, Xen 43 biofilms were divided into three groups of 10 wells and treated with farnesol, DMSO or fresh medium. *In vitro*, Xen 43 biofilms, bioluminescence was not significantly different among the 3 groups of 10 wells at 48 h, before exposure to farnesol (Fig. 5A). After exposure to DMSO, farnesol (FSL) (0.5 mM), or Trypticose soy broth (TSB) for 24 h, farnesol significantly reduced bioluminescence compared to DMSO or TSB (* and ** $p < 0.05$) (Fig. 5B and 5C). Bioluminescence did not differ significantly between DMSO and TSB exposed biofilms.

In vivo, subcutaneous catheter biofilm infection of Xen 43, bioluminescence over the infected catheters was monitored daily for 5 days in live animals (Fig 6A & 6B). Average radiance was similar on day 1 and day 2, before exposure to farnesol. After farnesol treatment, a significant decrease in bioluminescence was observed on day 3, 4 and 5 of infection. Farnesol treatment significantly decreased biofilm infection *in vivo*.

Discussion

Farnesol inhibited biofilms of *S. epidermidis* biofilms both *in vitro* and *in vivo* and was synergistic with nafcillin and vancomycin at most combination ratios. In our model of subcutaneous catheter infection in mice that is clinically relevant, farnesol treatment decreased catheter infection and systemic dissemination. We also confirmed the biofilm inhibiting effects of farnesol in real-time, using a bioluminescent strain of *S. epidermidis*.

We report ED_{50}, ED_{75} and ED_{90} of farnesol, nafcillin and vancomycin against biofilms of 2 clinical isolates and 3 laboratory strains of *S. epidermidis*, all of which were sensitive to nafcillin (minimum inhibitory $concentration_{50}$ (MIC_{50}) < 0.5 μg/ml) and vancomycin (MIC_{50} < 2 μg/ml) in the planktonic state (data not shown). We evaluated quorum sensing mutants (*agr* and *luxS* mutants) of *S. epidermidis* 1457, as these mutant strains form thicker biofilms than WT strains and spontaneous *agr* mutants predominate in chronic biofilm infections (11). Quorum sensing mechanisms determine antibiotic and biocide susceptibility in *Pseudomonas aeruginosa* and we sought to clarify farnesol susceptibility of these quorum sensing mutants in *S. epidermidis* (19). The *agr* and *luxS* quorum sensing mutants were similar in susceptibilities to the parent strain 1457 (within two, 2-fold dilutions of the antimicrobial) except the *luxS* mutant, whose ED_{75} for nafcillin was more than 2 dilutions than the parent strain (5 μg/ml vs. 0.625 μg/ml).

Gomes *et al* reported the antibacterial effects of farnesol on planktonic cells of *S. epidermidis* at concentrations up to 300 μM and reported *S. epidermidis* susceptibility to farnesol at 100 μM (20). However, biofilms were tolerant to farnesol *in vitro*. Gomes *et al*

did not report MICs or EDs performed according to standardized guidelines or the effects of farnesol on biofilms *in vivo*. Antibacterial effects of farnesol on *S. aureus* at 150 μM concentration and synergy with gentamicin at 100 μM on *S. aureus* biofilms have been reported (10). Possible mechanisms for the antibacterial effects of farnesol have been explored. Farnesol at 100 μg/ml inhibits the pro-coagulant effect, production of *S. aureus* exotoxins and potentiated the effects of cell wall acting ampicillin (21). Inoue *et al* demonstrated that K^+ ion leakage from *S. aureus* induced by the terpene alcohols such as farnesol correlated with the antibacterial effects (22). Thus, the antibacterial effects of farnesol may be due to its effects on membrane integrity.

We evaluated the morphology of *S. epidermidis* biofilms exposed to farnesol at 0.5 mM and observed a significant inhibiting effect. Detailed evaluation on confocal imaging revealed significant decrease in biovolume, mean thickness and substratum coverage of farnesol exposed biofilms, of both WT and quorum sensing mutants. The efficacy of farnesol at concentrations much lower than the estimated ED_{50} (0.625 to 2.5 mM) against *S. epidermidis* biofilms may have clinical implications in the treatment of biofilm-related catheter and device-associated healthcare infections.

Biofilms are inherently resistant to antibiotics, and antimicrobial combinations may be an important strategy against biofilm infections. Antimicrobial combinations against biofilms may enhance efficacy, reduce drug dosages and minimize the development of drug resistance. Therefore, we evaluated combinations of farnesol with the commonly used antistaphylococcal antibiotics; nafcillin and vancomycin, by discerning inhibitory endpoints by the XTT assay. We used the median-effects principle expounded by Chou *et al*, to evaluate synergy for the antimicrobial combinations (17). Evaluation of antimicrobial combinations by the median-effects method is widely used in cancer and infectious diseases research (17). Advantages of this method include surmounting the assumption that drug interactions are linear across drug dosages and effects. No general equation fits all the dose-response curves because mechanisms of drug actions differ. Dose-response curves evaluated at various dose effects may overcome this problem. Therefore, we evaluated drug combinations in a systematic manner at 2 different dose-effects, CED_{75} and CED_{90} at constant drug ratios, including equipotency ratios of the drug combinations. We observed synergy at most combination ratios with few exceptions.

We also evaluated the effects of farnesol *in vivo* in a mouse model of subcutaneous catheter infection that is a clinically relevant model of device-associated infection. We confirmed the formation of biofilms on the subcutaneously implanted catheters in mice, by electron and confocal laser microscopy. Farnesol treatment significantly decreased catheter infection and systemic dissemination. The dose of farnesol was extrapolated from animal studies and that was higher than our estimated ED_{50} *in vitro* (18). Other investigators have evaluated higher doses of farnesol (1 ml of 20 mM i.p.) in systemic candidiasis in mice (23, 24). Farnesol treatment by injections once a day at 100 μg/g did not cause any local or systemic adverse effects in mice. We are not aware of other studies evaluating the anti-biofilm effects of farnesol on *S. epidermidis* biofilms *in vivo*.

Real-time photonic imaging of animals offers advantages over conventional animal infection models. Real-time monitoring has the advantage of serial monitoring of infection and may decrease the sample size of animals (25). Growth curves of Xen 43 are similar to the parent strain 1457 and the bioluminescence is directly proportional to the CFU/ml (data not shown). The feasibility of monitoring catheter infection in mice using a bioluminescent strain of *S. epidermidis* Xen 43 has been reported (12). We evaluated the effects of farnesol on biofilms *in vitro* and on subcutaneous catheter infection using the bioluminescent strain

of *S. epidermidis* Xen43, *in vivo*. Farnesol significantly decreased Xen 43 biofilm bioluminescence *in vitro* and in our catheter infection model *in vivo*.

Standard guidelines for biofilm antimicrobial susceptibility do not exist and we adapted CLSI guidelines for planktonic cells, to evaluate EDs against biofilms. We evaluated farnesol (0.5 mM) on biofilms formed *in vitro* and a daily dose of 100 μg/g subcutaneously, for 6 days in the animal model. It is possible that several other concentrations or doses are effective and the optimum effective dose that has clinical applicability needs to be established. The mouse model of subcutaneous catheter infection mimics a foreign device infection and to evaluate the effects on intravascular catheter biofilm infection, our future experiments will focus on a vascular indwelling catheter infection in mice.

We have evaluated and confirmed the efficacy of farnesol against biofilms of *S. epidermidis* by multiple approaches, both *in vitro* and in a clinically relevant subcutaneous catheter biofilm infection in mice *in vivo*. No adverse effects of farnesol treatment were observed in this animal model. We also observed synergy of farnesol with nafcillin and vancomycin. We conclude that farnesol alone or in combination with nafcillin or vancomycin, may be effective against device-associated biofilm infections and improve clinical outcomes.

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Abbreviations

CRBSI	Catheter-related Bloodstream Infection
CFU/ml	Colony Forming Units/ml
CI	Combination index
CED_{50} and CED_{90}	Combination effective dose causing 50% and 90% inhibition respectively
ED_{50}, ED_{75} and ED_{90}	Drug concentrations causing 50%, 75% and 90% inhibition respectively
FSL	Farnesol
TSB	Trypticose Soy Broth
XTT	2, 3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyl-amino) carbonyl]-2H-tetrazolium hydroxide

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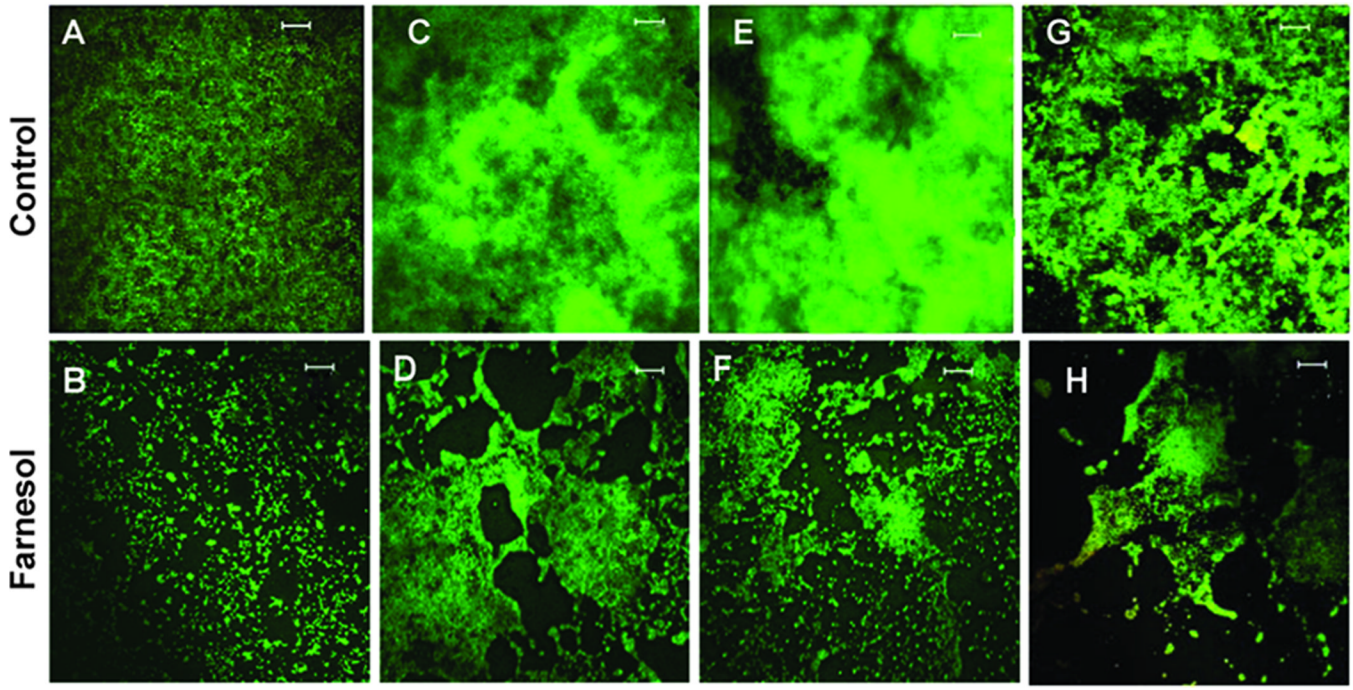

Fig. 1. Biofilm evaluation by confocal microscopy

Representative images of biofilms of 4 strains of *S. epidermidis* exposed to DMSO (control) or farnesol (0.5 mM) for 24 h: ATCC 55133 (A and B), 1457 (C and D), 1457 *agr* mutant (E and F), and 1457 *luxS* mutant (G and H). Scale bars measure 20 μm. Biofilms were stained and examined by the Zeiss confocal microscope. Farnesol exposed biofilms are thinner and more sparse than controls.

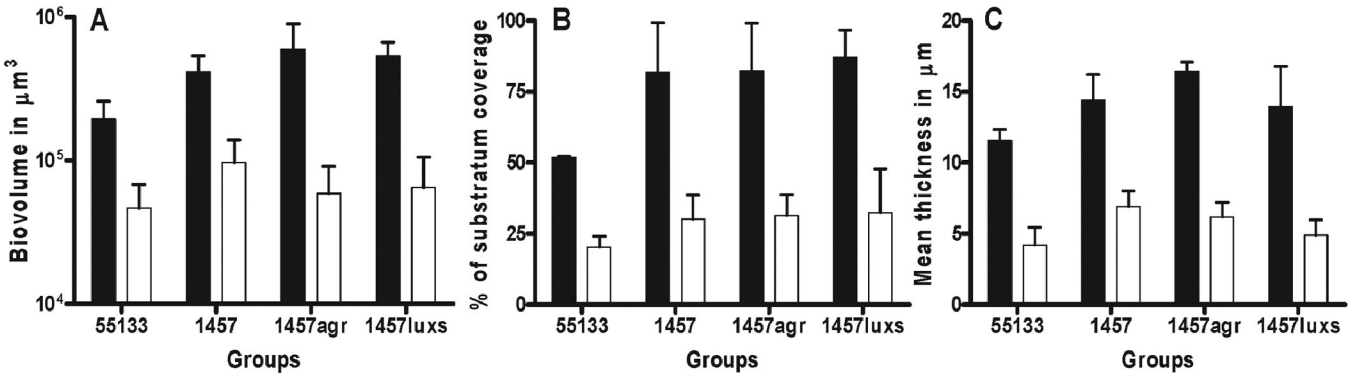

Fig. 2. Farnesol decreases biovolume, substratum coverage and mean thickness in biofilms in vitro

The confocal images of the 24 h biofilms exposed to DMSO or farnesol were analyzed by PHLIP software, using MATLAB toolbox. The z stack images were analyzed for biovolume (μm^3) (**A**) substratum coverage (%) (**B**) and mean thickness (µm) (**C**). Farnesol (□) decreased biovolume, substratum coverage and mean thickness significantly ($p < 0.05$), when compared to DMSO control (■) for all four *S. epidermidis* strains.

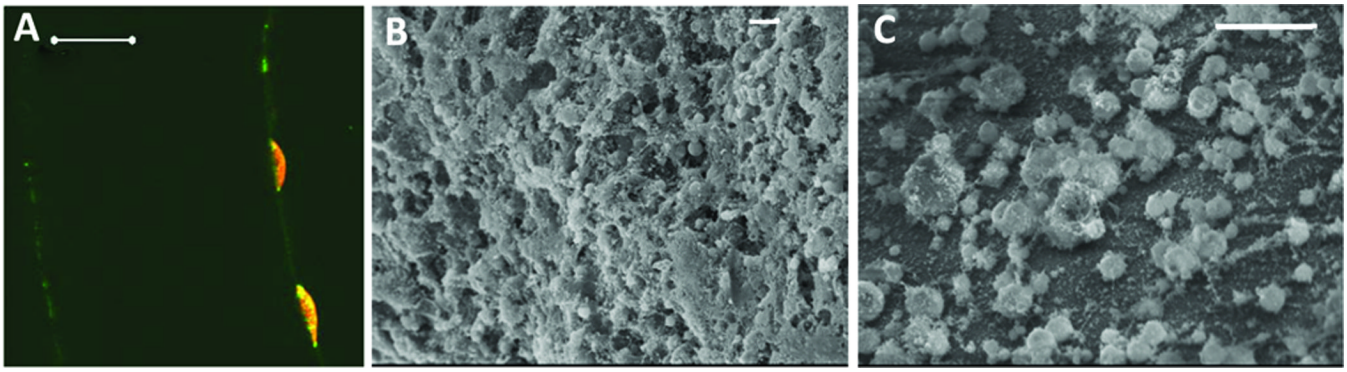

Fig. 3. Farnesol decreases catheter biofilms

In 3 week old FVB mice, 1.5 inch Teflon catheter segments were inserted subcutaneously. Catheters were preincubated in *S. epidermidis* suspension for 2 h before insertion, to aid biofilm development. The catheter segments were explanted on day 8 of implantation, sectioned into thin slices, stained with LIVE/DEAD stain and examined under the Zeiss confocal microscope (**A)**. Scale bar measures 50 μm in **A**. Some catheter segments were examined by scanning electron microscopy (**B & C**). Confocal imaging and electron microscopy confirmed *S. epidermidis* biofilm formation (**A & B**). Farnesol treatment (100 μg/g, s.c. for 6 days) decreased *S. epidermidis* catheter biofilms (**C**). Scale bars measure 10 μm in **B** and **C**.

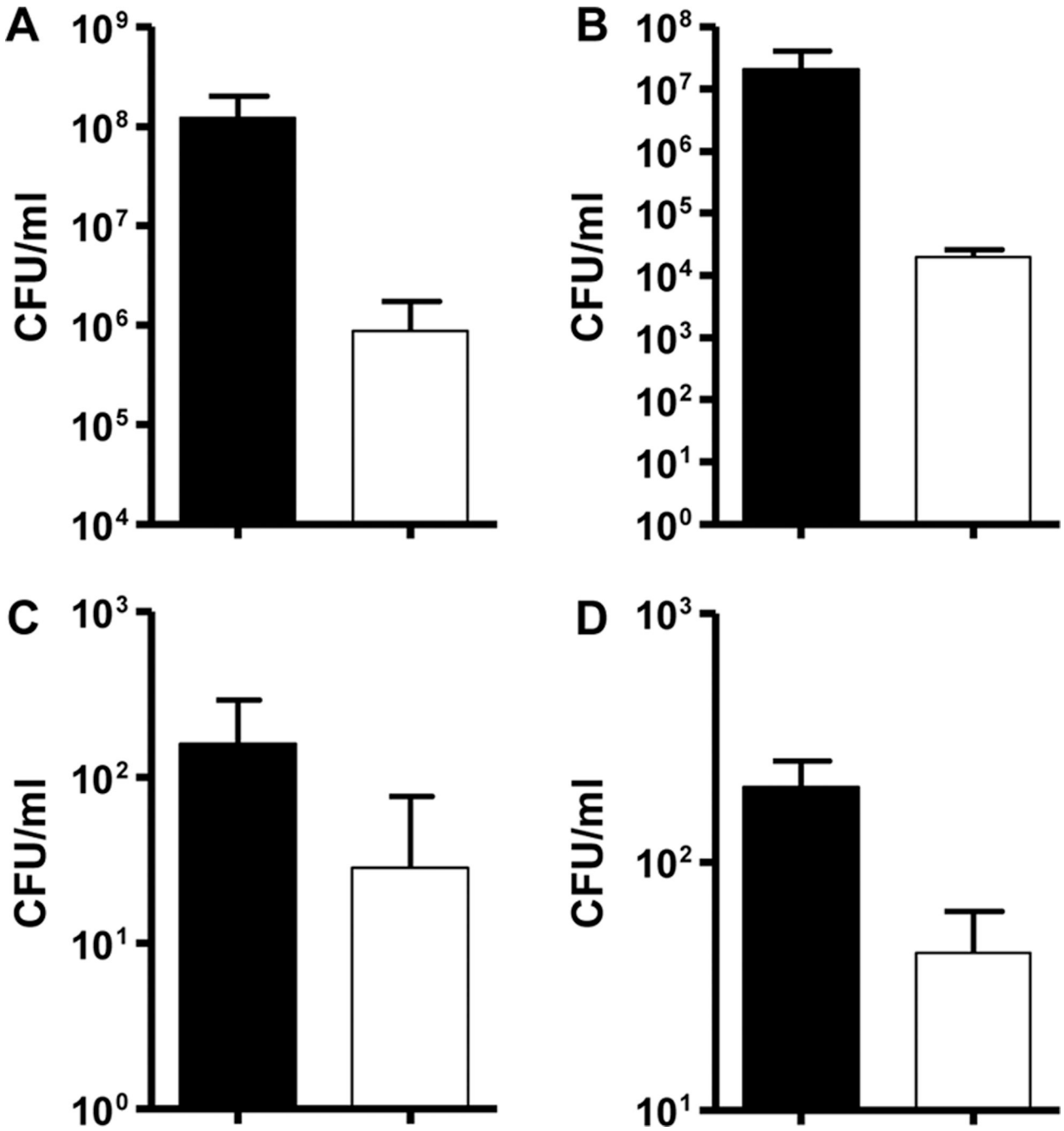

Fig. 4. Farnesol decreases viable cell counts of *S. epidermidis* during catheter infection in mice

Mice were euthanized on day 8 of catheter implantation and cultures of the catheters (**A**) and skin and tissue around the catheter (peri-catheter) (**B**) were performed. Systemic dissemination was assessed by cultures of blood (**C**) and kidney homogenate (kidneys of one animal homogenized in 1 ml of PBS) (**C**). Farnesol treatment (n=7) was compared to DMSO control (n=5). Farnesol treatment significantly decreased catheter CFU/ml, peri-catheter infection and systemic dissemination ($p < 0.05$). All panels: DMSO control (■);Farnesol treatment (□).

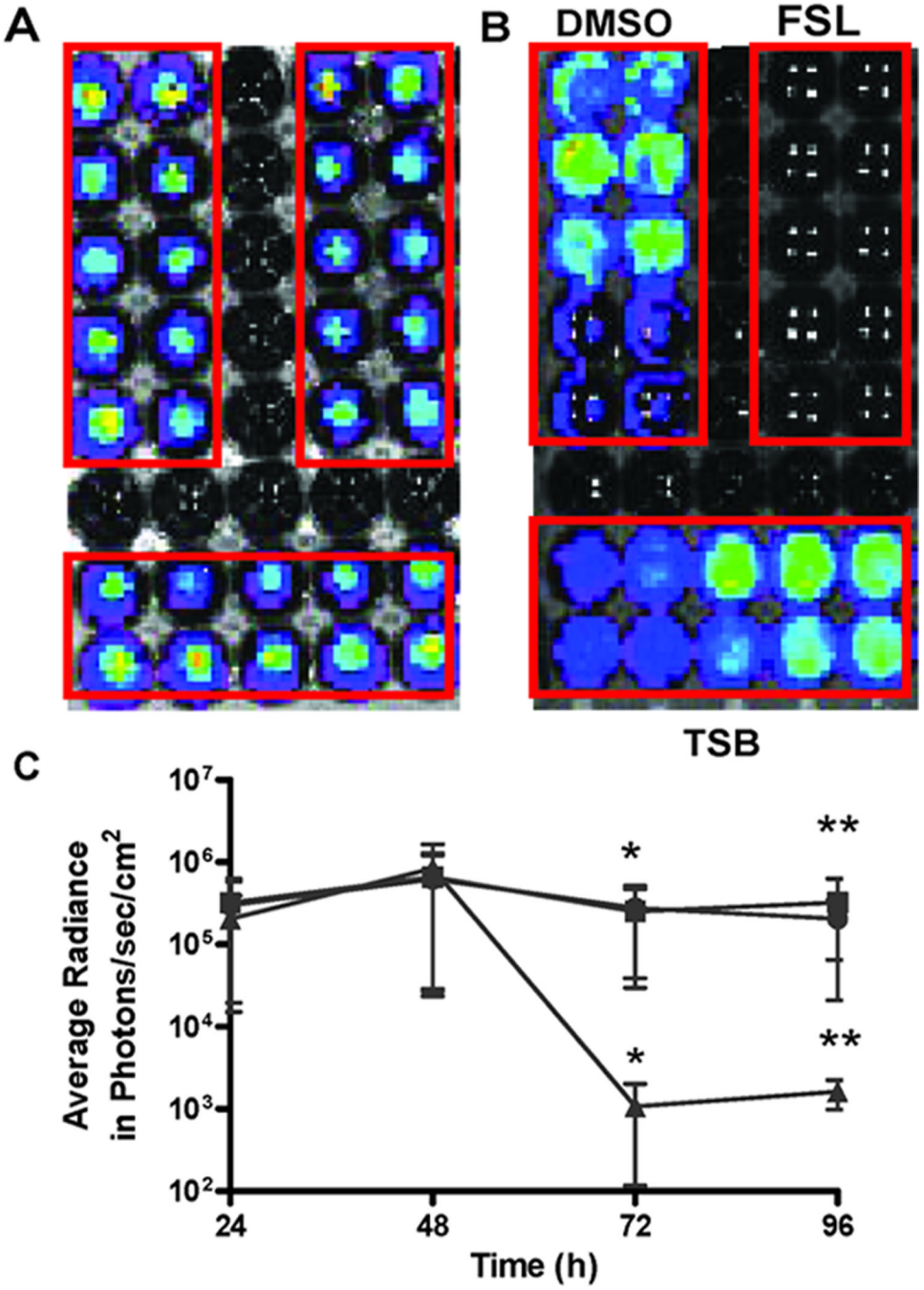

Fig. 5. Biofilm bioluminescence of Xen 43 decreased by farnesol

Xen 43 biofilms were developed in 30 wells of an opaque 96-well microtiter plate (**A**). At 48 h, supernatants were removed and unadhered cells were washed with PBS. Three groups of ten wells each were exposed to DMSO, farnesol (FSL) (0.5 mM), or Trypticose soy broth (TSB) media for 24 h (**B**). Bioluminescence was monitored at 24, 48, 72 and 96 h and compared among the three exposures. Farnesol exposure significantly reduced average radiance compared with DMSO or TSB exposed wells (* and ** $p < 0.05$) (**C**). ■: DMSO; ●: TSB; ▲: Farnesol.

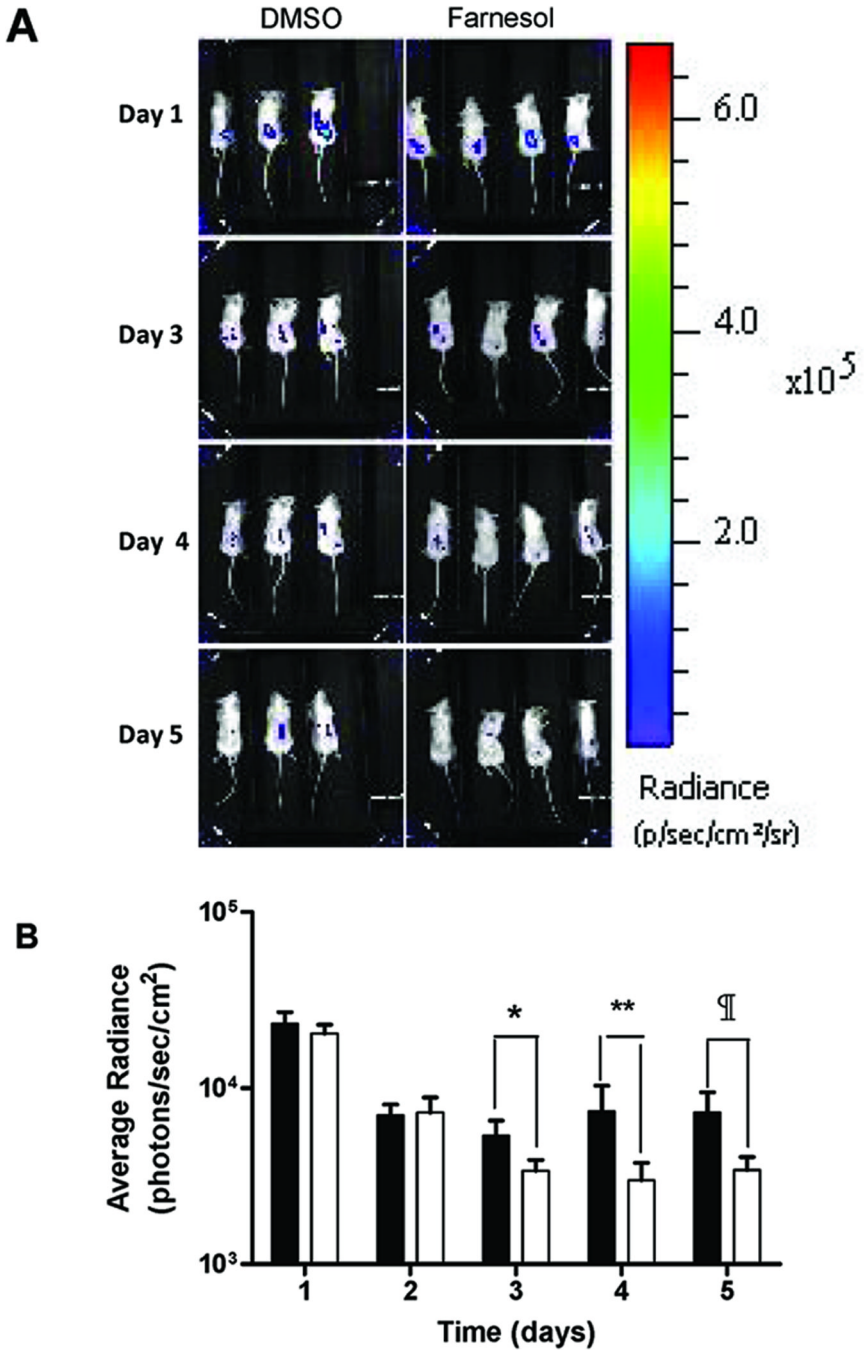

Fig. 6. Farnesol decreases bioluminescence in *S. epidermidis* catheter infection *in vivo*

A bioluminescent strain of *S. epidermidis* 1457 Xen 43 was used in the subcutaneous catheter infection model in mice and bioluminescence followed serially for 5 days (**A**). Mice were treated with farnesol at 100 μg/g/day or DMSO (control) from day 2 of infection for 4 days. Bioluminescence was quantified and expressed as average radiance in photons/sec/cm2 (**B**). Farnesol treatment (□) decreased bioluminescence significantly (*, ** and #, $p < 0.05$) on days 3, 4 and 5 compared to DMSO control (■).

Table 1

Antimicrobial Susceptibility of *S. epidermidis* Biofilms

Strains	**ATCC 55133**	**1457**	**1457 *agr-***	**1457 *luxS-***
Farnesol (mM)				
ED_{50}	1.25	2.5	1.25	0.625
ED_{75}	10	10	10	10
ED_{90}	20	>80	>80	>80
Nafcillin (μg/ml)				
ED_{50}	0.32	0.08	0.08	0.08
ED_{75}	0.625	0.625	1.25	5
ED_{90}	80	>80	>80	>80
Vancomycin (μg/ml)				
ED_{50}	5	2.5	2.5	2.5
ED_{75}	10	10	10	10
ED_{90}	80	>80	>80	>80

Biofilm inhibitory concentrations of the antimicrobial agent at 50%, 75% and 90% inhibition are reported as effective dose$_{50}$ (ED_{50}), ED_{75} and ED_{90} respectively.

TABLE 2

Combination Indices for Farnesol-Antibiotic Combinations

Ratios	**CI values in mean (SD)**		**Ratios**	**CI values in mean (SD)**	
FSL: NAF	**CED_{75}**	**CED_{90}**	**FSL:VAN**	**CED_{75}**	**CED_{90}**
ATCC 55133					
ED_{50} 1:1	0 (0)	0.26 (0.45)	ED_{50} 1:1 = ED_{90} 1:1	0.13 (0.11)	0.1 (0.02)
ED_{90} 1:1	0.5 (0.5)	0.04 (0.01)	ED_{90} 1:2	0.10 (0.07)	0.07 (0.02)
ED_{90} 1:2	0.13 (0.11)	0.06 (0.01)	ED_{90} 2:1	0.18 (0.17)	0.1 (0.04)
ED_{90} 2:1	0.22 (0.19)	0.07 (0.03)			
1457					
ED_{75} 1:1	*3.82 (0.53)*	0.05 (0)	ED_{50} 1:1 = ED_{75} 1:1	0.24 (0.05)	0.08 (0.02)
ED_{75} 1:2	*2.41 (1.80)*	0.21 (0.13)	ED_{75} 1:2	0.29 (0.09)	0.15 (0.03)
ED_{75} 1:4	0.46 (0.53)	0.34 (0.13)	ED_{75} 2:1	0.21 (0.03)	0.07 (0.04)
1457 *agr*-					
ED_{75} 1:1	0.69 (0.26)	0.41 (0.35)	ED_{50} 1:1 = ED_{75} 1:1	0.30 (0.29)	0.2 (0.11)
ED_{75} 1:2	0.55 (0.51)	0.59 (0.13)	ED_{75} 1:2	0.33 (0.28)	0.35 (0.16)
ED_{75} 1:4	0.48 (0.42)	0.9 (0.79)	ED_{75} 2:1	0.26 (0.25)	0.14 (0.06)
1457*luxS*-					
ED_{75} 1:1	0.21 (0.30)	0.03 (0.02)	ED_{50} 1:1 = ED_{75} 1:1	0.27 (0.06)	0.05 (0.02)
ED_{75} 1:2	0.01 (0.02)	0.09 (0.07)	ED_{75} 1:2	0.28 (0.06)	0.06 (0.024)
ED_{75} 1:4	0.08 (0.07)	0.12 (0.09)	ED_{75} 2:1	0.25 (0.1)	0.05 (0.02)
ED_{50} 1:1	0.29 (0.27)	*10.57* (182)	ED_{50} 1:1	0.10 (0.03)	0.03 (0.014)

FSL- farnesol, NAF-nafcillin, VAN-vancomycin. ED_{50}, ED_{75}, and ED_{90} represent 50%, 75% and 90% inhibitory effects of the individual antimicrobial agent respectively. CED_{75} and CED_{90} represent 75% and 90% inhibitory effects of farnesol-antibiotic combination respectively. Combination indices (CI) derived by the median effects principle, indicate synergy if CI < 1, additive effect if CI=1 and antagonism if CI > 1. Non synergistic combinations are italicized and bolded.