Antimicrobial peptide LL-37 is bactericidal against Staphylococcus aureus biofilms

Jason Kang, Matthew J. Dietz *, Bingyun Li*

Department of Orthopaedics, School of Medicine, West Virginia University, Morgantown, WV, United States of America

* mdietz@hsc.wvu.edu (MJD); bili@hsc.wvu.edu (BL)

Abstract

Our current challenge in the management of prosthetic joint infection is the eradication of biofilms which has driven the need for improved antimicrobial agents and regimens. In this study, the antimicrobial peptide, LL-37, and silver nanoparticles (AgNPs) were investigated for their antimicrobial efficacies against Staphylococcus aureus (S. aureus), a microorganism commonly implicated in biofilm-related infections. These antimicrobials were compared to conventional antibiotics and combination treatments with rifampin. Using a Centers for Disease Control reactor, 24 h S. aureus biofilms were formed on cobalt-chromium discs and the anti-biofilm activity was determined by quantifying the amount of colony forming units following treatments. We found that LL-37 was the most efficacious antimicrobial agent with a more than 4 log reduction in colony counts. In comparison, silver nanoparticles and conventional antibiotics were not as efficacious, with a less than 1 log reduction in colony counts. Antimicrobial combination treatments with rifampin significantly increased the log reduction for AgNPs and gentamicin, although still significantly less than LL-37 in isolation. Furthermore, kinetic studies revealed the rapid elimination of S. aureus biofilm with LL-37. Collectively, the results of this study demonstrated that LL-37 was an effective agent against S. aureus biofilms and may have potential clinical applications in the eradication of biofilms and treatment of prosthetic joint infection.

Introduction

Primary arthroplasty, such as total knee arthroplasty (TKA), is one of the most commonly performed orthopedic procedures. The number of TKAs performed in the US is expected to increase by 143% from 2012 to 2050 with 1.5 million cases per year by 2050 [1]. This procedure provides symptomatic pain relief and helps improve mobility. However, a small proportion of these patients will become infected, as between 0.5 to 2% of these procedures may result in prosthetic joint infection (PJI), an infection of the prosthesis, joint, and adjacent tissue [2–6]. This type of implant failure causes considerable morbidity and is associated with significant financial costs to the healthcare system. In 2009, the total estimated cost for treating PJI was about $566 million [2]. Thus, with an increasing number of primary arthroplasties being performed each year, we can expect the incidence of PJI and its associated burdens to rise as well.
Staphylococcus aureus (S. aureus) is the most frequently isolated microorganism in PJI [7]. The persistence of S. aureus in PJI is attributed to many factors, which include increasing antibiotic resistance [8], intracellular survival of bacteria [9–11], and formation of biofilms [12]. In particular, biofilms are believed to have a large role in the pathogenesis of PJI, as foreign medical devices, such as prostheses, are prone to biofilm formation, and biofilms have many properties that make treatment difficult, especially with antibiotics [13].

The goals of PJI treatment are aimed at eradicating the infection. This is best accomplished through a combination of surgical and antimicrobial therapies. Depending on the severity and timing of the infection, surgical options may include resection arthroplasty with re-implantation, in a one- or two-stage exchange, or debridement with retention of prosthesis. These surgical procedures are typically accompanied by four to six weeks of parenteral antibiotics, followed by three to six months of oral antibiotic therapy in some cases [14]. However, there are many challenges in treating these infections with antibiotics. Long-term, systemic administration of antibiotics may cause adverse effects [15]. Furthermore, biofilms have many properties that may limit the efficacy of antibiotics and can generate resistance, allowing infection to recur [13]. As a result, current regimens of treatment have relatively high failure rates. Overall re-infection rates after first-line treatment [1-stage, 2-stage, or irrigation and debridement (I&D)] are 26% after one year and 35.8% after six years [16]. The five-year infection free survival rate following I&D with oral antibiotic therapy has been reported as 68.5% [17]. Thus, it is important to continue to identify antimicrobial agents that could be more efficacious (compared to conventional antibiotics) in treating infections thereby reducing the incidence of re-infection.

One promising antimicrobial agent is the antimicrobial peptide (AMP) LL-37. Rapid and efficient methods have been developed over the years to yield recombinant forms of LL-37, allowing for increased clinical and functional characterization, on what is an otherwise prohibitively expensive peptide [18,19]. Previous studies in the literature regarding the efficacy of LL-37 against pre-formed biofilms vary. Some studies suggest that LL-37 does not disrupt pre-formed biofilms, inhibit bacterial attachment, or prevent early biofilm formation [20,21]. In contrast, other studies have demonstrated that LL-37 can disrupt 24 and 48 h mature S. aureus biofilms [22]. In our evaluation, biofilms are formed using the Centers for Disease Control (CDC) biofilm reactor (CBR), which better replicates biofilms that are found clinically. There remains a need to further elucidate the antimicrobial properties of LL-37 and its potential use in combination with conventional antibiotics against CBR-formed biofilms.

The objectives of this study were to determine the in vitro treatment of S. aureus biofilms with two non-antibiotic antimicrobial agents (i.e. LL-37 and silver nanoparticles (AgNPs)) and to compare them with conventional antibiotics (e.g. gentamicin, vancomycin, and rifampin). S. aureus biofilms were grown on cobalt chrome (Co-Cr) discs and the efficacy of the treatments of various antimicrobial agents were evaluated by quantifying the number of viable bacteria. We hypothesized that LL-37 and AgNPs would be more efficacious than conventional antibiotics (i.e. gentamicin, vancomycin, and rifampin) in eradicating S. aureus biofilms.

Materials and methods
Bacterial strain
A clinical isolate of S. aureus (SA 1004) was previously obtained from a patient’s chronic wound from Ruby Memorial Hospital in Morgantown, WV [23] and was used in this study. Susceptibility tests revealed that the isolate was resistant to ampicillin, cefoxitin, and penicillin, and susceptible to gentamicin, vancomycin, rifampin, cefazolin, clindamycin, ciprofloxacin, levofloxacin, erythromycin, linezolid, oxacillin, moxifloxacin, tigecycline, and tetracycline.
Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of human LL-37 (Sigma Aldrich, St. Louis, MO), AgNPs (40 nm, polyvinylpyrrolidone-capped) (nanoComposix, Inc., San Diego, CA), and conventional antibiotics (i.e. gentamicin, vancomycin, rifampin, clindamycin, cefazolin) (Sigma Aldrich) were conducted by the broth microdilution technique based on the guidelines set by the Clinical and Laboratory Standards Institute (CLSI) [24]. Briefly, in 96-well microtiter plates, increasing concentrations of antimicrobial agents were added to each well, starting at 0.25 μM, and doubling in concentration until reaching a final concentration of 128 μM. Equal volumes of bacterial inoculum grown in Muller-Hinton Broth (MHB) (Becton Dickinson, Sparks, MD) containing 1×10^6 colony forming units (CFUs)/ml were subsequently added. The 96-well plate was incubated at 37˚C for 18 h. After incubation, the MIC was read as the lowest concentration of antimicrobial agent that visibly inhibited bacterial growth.

Biofilm formation

To prepare an inoculum, colonies of *S. aureus* were suspended in brain heart infusion (BHI) (Becton Dickinson) and incubated for 18 h at 37˚C. The inoculum was diluted to a 0.5 McFarland standard with fresh BHI and seeded in a CBR (BioSurfaces Technologies, Bozeman, MT). Biofilms were formed on surgical grade Co-Cr discs (BioSurfaces Technologies) with a total surface area of 1.57 cm^2^. The CBR was run for 24 h at 37˚C with continuous stirring at 120 rpm.

Dose-response and kinetics of antimicrobial agents

Following 24 h biofilm formation, Co-Cr discs were removed from the CBR, placed in round bottom polystyrene tubes, and washed in phosphate buffered saline (PBS) (Corning, Manassas, VA) to remove planktonic bacteria. The dose-response and kinetics of LL-37, AgNPs, conventional antibiotics (gentamicin, vancomycin, and rifampin) and combinations with rifampin were investigated. For the dose response studies, LL-37 (1.75, 2.5, 3.75, 5, 10, and 100 μM), AgNPs (100, 500, and 1000 μM), gentamicin (100, 500, and 1000 μM), vancomycin (100, 500, and 1000 μM), rifampin (100, 500, and 1000 μM), AgNPs + rifampin (100 μM each), gentamicin + rifampin (100 μM each), vancomycin + rifampin (100 μM each), and controls (plain PBS) were applied and incubated at 37˚C for 60 min. For the kinetic studies, LL-37 (10 μM), rifampin (100 μM), gentamicin (100 μM), gentamicin + rifampin (100 μM each), and controls (plain PBS) were applied at incubation intervals of 5, 30, and 60 min at 37˚C. Shorter incubation intervals of 5 and 30 minutes were chosen, as our previous study indicated that LL-37 was fast acting at these time points [23]. For all assays, the total treatment volume was 1 mL. Following treatment, the discs were washed in PBS to remove planktonic bacteria and residual chemicals from the treatment, and were transferred to another tube containing 2 mL of BHI. The discs were then sonicated for 10 min at 50 Hz in a TRU-SWEEP Ultrasonic Cleaner (CREST Ultrasonics, Trenton, NJ) and vortexed for 2 min to break up the remaining biofilm. The samples were serially diluted in PBS to 10^-1, 10^-2, and 10^-3 and plated on 5% sheep blood agar plates (Remel, Lenexa, KS). The plates were incubated for 24 h at 37˚C and the number of CFUs were quantified. All samples were performed in triplicate and the average was reported. The outcomes were reported as log reduction, which was calculated by subtracting the difference between the log CFU/cm^2^ of the control and the log CFU/cm^2^ of the treated samples. Bactericidal activity was defined as a more than 3 log reduction in colony count from the initial inoculum [25].
Statistical analysis

All data were presented as means ± standard deviations. Differences in log reduction of all treatments were assessed using JMP-V14 statistical software (SAS Institute, Cary, NC). Comparisons between groups were conducted with one-way ANOVA followed by Tukey’s honestly significant difference (HSD) test. Combination therapy analysis was performed by taking the logarithm of the percent colonies remaining after treatment and comparing the combined and individual treatments and their interactions for significance with one-way ANOVA. A p-value < 0.05 was considered statistically significant.

Results

Minimum inhibitory concentration

The antimicrobial susceptibility of LL-37, AgNPs, gentamicin, vancomycin, rifampin, clindamycin, and cefazolin was determined against a clinical strain of *S. aureus*. The MIC of LL-37 was determined to be 32 μM (Table 1). In comparison, the MIC of AgNPs, gentamicin, vancomycin, rifampin, clindamycin, and cefazolin was found to be >128, 0.5, 0.5, <0.25, 0.5, and 2 μM, respectively (Table 1).

Dose-response effect on biofilms

Treatment of 24 h *S. aureus* biofilms formed on Co-Cr discs with LL-37, AgNPs, conventional antibiotics, and combination treatments with rifampin were investigated. Treatment with LL-37 at concentrations of 1.75, 2.5, 3.75, 5, 10, and 100 μM resulted in a log reduction of 0.9, 1.2, 2.8, 3.5, 4.3, and 4.3, respectively (Fig 1). In comparison, there was a low eradication of *S. aureus* biofilm for AgNPs, conventional antibiotics, and combinations with rifampin. At concentrations as high as 1000 μM, there was a less than 1 log reduction in CFU for AgNPs, gentamicin, vancomycin, and rifampin (Fig 2). Combination treatments of AgNPs + rifampin, gentamicin + rifampin, and vancomycin + rifampin resulted in log reductions of 0.76, 1.71, and 0.30, respectively (Fig 3). The combination of gentamicin + rifampin demonstrated a significant increase in log reduction over gentamicin and rifampin in isolation. Though gentamicin + rifampin was found to be the only combinatory treatment with statistical significance over its combined individual counterparts, its log reduction was still significantly less than that of LL-37 in isolation (Fig 3).

Kinetics analysis

The log reduction kinetics of LL-37 was compared to gentamicin, rifampin, and gentamicin + rifampin. LL-37 had a 3.2 log reduction in CFU within 5 min of treatment time (Fig 4).

### Table 1. Minimum inhibitory concentration (MIC) of novel and conventional antimicrobial agents against planktonic *S. aureus*.

| Treatment | MIC (μM) |
|-----------|----------|
| LL-37     | 32       |
| AgNPs     | 128      |
| Gentamicin| 0.5      |
| Vancomycin| 0.5      |
| Rifampin  | 0.25     |
| Clindamycin| 0.5    |
| Cefazolin | 2        |

https://doi.org/10.1371/journal.pone.0216676.t001
In contrast, the log reduction at 5 min for gentamicin, rifampin, and gentamicin + rifampin was 0.14, 0.36, and 1.3, respectively (Fig 4). Increasing the treatment time up to 60 min for LL-37, gentamicin + rifampin, and gentamicin demonstrated a significant increase in log reductions and demonstrated the presence of time trend for these antimicrobial agents (Fig 4).

Discussion
There are hundreds of antimicrobial peptides (AMPs) in humans that are part of the innate immune system [26]. Of interest in this study is LL-37, a cathelicidin-derived AMP, which functions as an immunomodulatory agent and has direct antimicrobial activities against many Gram-positive and -negative bacteria, including _S. aureus_ [27]. In this study, we investigated the antimicrobial efficacy of LL-37 against _S. aureus_ biofilms. The biofilm was created in the CBR, which is a rugged system that allows for similar replication of biofilms found _in vivo_,...
with fluid shear dynamics in the growth medium [28]. In addition, more practical applications may be achieved with this method, as biofilms can be formed on metal substrates, such as Co-Cr, which is a common orthopedic material used in prosthetic implants.

This study demonstrated that LL-37 has excellent antimicrobial activity against 24 h *S. aureus* biofilms. Our results showed that, at sub-MIC concentrations, starting at 10 μM, LL-37 was bactericidal against *S. aureus* biofilms with a more than 4 log reduction in colony counts. No change in log reduction was observed up to 100 μM. In addition, LL-37 was also fast acting against *S. aureus* biofilms, requiring low incubation times (e.g. 5 min) to achieve a more than 3 log reduction in colony counts. Though the mechanism that LL-37 employs against biofilms is still largely unknown, it is likely that LL-37 penetrates the biofilm and has antimicrobial activity against the embedded bacteria [29]. Other mechanisms may include the decreased attachment of planktonic bacteria to prevent biofilm formation and down-regulation of quorum-sensing systems related to biofilm development and maintenance [30].

This study also investigated the effect of AgNPs, conventional antibiotics, and their combinations against *S. aureus* biofilms. Gentamicin, vancomycin, and rifampin were chosen as conventional antibiotics to study due to their widespread use in the treatment of orthopedic-related infections. Vancomycin is commonly chosen among physicians as a first-line empiric antibiotic due to its excellent Gram-positive coverage and action against methicillin-resistant *S. aureus* (MRSA) [14,31]. Gentamicin is also a common empiric agent due to its broad spectrum of action, particularly against Gram-negative suspected infections, and can also be found as an additive in cement spacers and polymethylmethacrylate (PMMA) beads [32,33].
Rifampin is a frequently recommended combinatorial antibiotic in PJI due to its ability to penetrate biofilms and activity against biofilm microorganisms [14,34]. Finally, as a novel antimicrobial agent, AgNPs are gaining popularity due to their broad spectrum of action and anti-biofilm efficacy [35,36], and are even being used in orthopedic applications such as coatings for prosthetic implants and external fixation pins [37].

In our study, LL-37 was found to have much greater anti-biofilm activity in comparison to AgNPs and conventional antibiotics. At concentrations of 1000 μM, which is 2000 times to
4000 times the MIC of conventional antibiotics, there was a less than 1 log reduction in colony counts after treatment with AgNPs, gentamicin, vancomycin, and rifampin. Combination treatments with rifampin demonstrated a significant increase in log reduction for AgNPs and gentamicin. Interestingly, there was a negative log reduction observed with vancomycin. The limited response to vancomycin is concerning, as it is frequently used in the management of infections suspected to be caused by MRSA. A few other studies have reported an increase in biofilm density after vancomycin treatment, which may be due to non-responsive bacterial strains to vancomycin [38] or sub-inhibitory concentrations during treatment [39]. Vancomycin has been shown to have a time-dependent nature against *S. aureus* biofilms [40], as the relatively brief treatment duration in this study may not be adequate and could be the reason for the negative log reduction.

Our findings demonstrate some of the challenges in eradicating biofilms with AgNPs and conventional antibiotics. Although AgNPs and antibiotics are frequently being used in the treatment and prevention of PJL, our study suggests that these antimicrobials result in incomplete eradication of the *S. aureus* biofilm, which may ultimately lead to chronic and recurrent infection. In comparison, LL-37 was shown to be fast-acting and have a superior eradication of biofilms, with a greater log reduction in colony counts at sub-MIC concentrations. The higher efficacy against biofilms of LL-37 was consistent with the findings that some antimicrobial peptides are more effective against biofilms compared to other antimicrobial agents including conventional antibiotics, and LL-37 was found to be more potent against intracellular *S. aureus*, similar to biofilm persister cells, compared to conventional antibiotics [23]. As a result,
LL-37 may be a better choice as a therapeutic agent against biofilm-related infections and may find increased applications toward infection treatment and prevention.

Some concerns with the use of AMPs as therapeutic agents include its inactivation in environments of elevated salt concentration, which may limit its use in vivo, as studies have demonstrated reduced activity of LL-37 under physiological and high salt conditions [41,42]. Our study demonstrated that LL-37 had a MIC of 32 μM, whose relative increase in comparison to antibiotics could be attributed to inactivation under media conditions. Comparable studies report a similar MIC for LL-37. Depending on the strain of bacteria, reported MICs for LL-37 have ranged from 6.25 to >128 μM [21,22]. Although LL-37 may lose its antimicrobial activity under elevated salt conditions, it may still retain its anti-biofilm properties. Dean et al. reported that, under conditions of high salt, LL-37 has significant inhibition of *S. aureus* biofilm at sub-antimicrobial concentrations [43]. Furthermore, Chennupati et al. demonstrated the eradication of *Pseudomonas aeruginosa* (*P. aeruginosa*) biofilm with LL-37 in an animal model of sinusitis [44].

Another concern with the use of AMPs is its potential for bacterial resistance. Some studies have reported that the use of LL-37 leads to a selection of AMP-resistant pathogens. Leszczyńska et al. reported that clinical *S. aureus* strains developed increased resistance after three passages with sub-MICs of LL-37 [45]. Lofton et al. found that AMP-resistant strains of *Salmonella typhimurium* were generated after prolonged exposure to LL-37, with an increase in MIC after 490–553 generations [46]. Furthermore, Strempel et al. showed that physiologic concentrations of LL-37 upregulated resistance factors in *P. aeruginosa*, such as increased production of quorum-sensing molecules, secreted virulence factors, lipopolysaccharide modification, and genes encoding multidrug efflux pumps [47]. In general, mechanisms of resistance may include membrane modifications to reduce the binding of AMPs, efflux pumps, and proteolytic degradation [48]. Though the generation of AMP-resistant bacteria is concerning and warrants continued evaluation, AMPs are less susceptible to bacterial resistance when compared to antibiotics, due to several mechanisms of inhibiting bacteria including membrane disruption and inhibition of cellular processes [49].

One limitation of this study is the need to determine the potential cytotoxicity of LL-37 against eukaryotic cell lines. Previous studies have investigated this issue, and Johansson et al. reported that LL-37 displayed toxicity toward T-lymphocyte cell lines at concentrations of 13 to 25 μM [50]. Furthermore, Säll et al. reported that LL-37 reduced the number and viability of human MG63 osteoblasts at an IC₅₀ value of about 5 μM [51]. There are many strategies that exist in reducing its cytotoxicity. It is possible to reduce the cytotoxicity of LL-37 through truncation of its N-terminal amino acid residues [52]. In addition, LL-37 selectively permeabilizes apoptotic cells over viable cells [53]. There are also eukaryotic host cell defenses, such as the globular C1q receptor, p33, which antagonizes and binds to LL-37 [54]. The determination of cytotoxicity is important in delineating potential therapeutic windows. Considering previous cytotoxic reports, a narrow window may exist for LL-37 in the low micromolar range and is especially likely with the body of literature detailing its reduction in cytotoxicity.

In summary, our study demonstrates the excellent antimicrobial activity and kinetics of LL-37 against *S. aureus* biofilms. In comparison, conventional antibiotics and AgNPs were not as efficacious in eradicating *S. aureus* biofilms. Though the log reduction with the addition of rifampin was significantly increased for AgNPs and gentamicin, it was still significantly less than LL-37. The treatment model utilized in this study allows for insight toward the treatment of clinical applications, such as the treatment of PJI. This study identified current challenges in treating PJI with conventional antibiotics and suggests that LL-37 may be an alternative therapeutic agent in eradicating infection, particularly those related to biofilm formation.
Supporting information

S1 Datasets. Data sets for Table 1 and Figs 1–4 are available.

(DOCX)

Acknowledgments

We acknowledge Gerald R. Hobbs at West Virginia University for help with data analysis and Suzanne Danley for proofreading.

Author Contributions

Conceptualization: Matthew J. Dietz, Bingyun Li.

Formal analysis: Jason Kang, Matthew J. Dietz, Bingyun Li.

Funding acquisition: Bingyun Li.

Investigation: Jason Kang, Bingyun Li.

Methodology: Jason Kang, Matthew J. Dietz, Bingyun Li.

Project administration: Bingyun Li.

Resources: Bingyun Li.

Supervision: Matthew J. Dietz, Bingyun Li.

Validation: Jason Kang, Matthew J. Dietz, Bingyun Li.

Writing – original draft: Jason Kang.

Writing – review & editing: Jason Kang, Matthew J. Dietz, Bingyun Li.

References

1. Inacio MCS, Paxton EW, Graves SE, Namba RS, Nemes S. Projected increase in total knee arthroplasty in the United States—an alternative projection model. Osteoarthr Cartil. 2017; 25: 1797–1803. https://doi.org/10.1016/j.joca.2017.07.022 PMID: 28801208

2. Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J. Economic burden of periprosthetic joint infection in the United States. J Arthroplasty. 2012; 27: 61–65.e1. https://doi.org/10.1016/j.arth.2012.02.022 PMID: 22554729

3. Pulido L, Ghanem E, Joshi A, Purtill JJ, Parvizi J. Periprosthetic joint infection: The incidence, timing, and predisposing factors. Clin Orthop Relat Res. 2008; 466: 1710–1715. https://doi.org/10.1007/s11999-008-0209-4 PMID: 18421542

4. Ong KL, Kurtz SM, Lau E, Bozic KJ, Berry DJ, Parvizi J. Prosthetic joint infection risk after total hip arthroplasty in the medicare population. J Arthroplasty. 2009; 24: 105–109. https://doi.org/10.1016/j.arth.2009.04.027 PMID: 19493644

5. Kurtz SM, Ong KL, Lau E, Bozic KJ, Berry D, Parvizi J. Prosthetic joint infection risk after TKA in the medicare population. Clin Orthop Relat Res. 2010; 468: 52–56. https://doi.org/10.1007/s11999-009-1013-5 PMID: 19669386

6. Huotari K, Peltola M, Jämsen E. The incidence of late prosthetic joint infections. Acta Orthop. 2015; 86: 321–325. https://doi.org/10.3109/17453674.2015.1035173 PMID: 25813645

7. Tande AJ, Patel R. Prosthetic Joint Infection. Clin Microbiol Rev. 2014; 27: 302–345. https://doi.org/10.1128/CMR.00111-13 PMID: 24696437

8. Foster TJ. Antibiotic resistance in Staphylococcus aureus. Current status and future prospects. FEMS Microbiol Rev. 2017; 41: 430–449. https://doi.org/10.1093/femsre/fux007 PMID: 28419231

9. Connolly BP. Staphylococcus aureus chronic and relapsing infections: Evidence of a role for persister cells. BioEssays. 2014; 36: 991–996. https://doi.org/10.1002/bies.201400080 PMID: 25100240
10. Hudson MC, Ramp WK, Nicholson NC, Williams AS, Nousiainen MT. Internalization of Staphylococcus aureus by cultured osteoblasts. Microb Pathog. 1995; 19: 409–419. https://doi.org/10.1006/mpat.1995.0075 PMID: 8852281

11. Hamza T, Li B. Differential responses of osteoblasts and macrophages upon Staphylococcus aureus infection. BMC Microbiol. 2014; 14: 207. https://doi.org/10.1186/s12866-014-0207-5 PMID: 25059520

12. McConoughey SJ, Howlin R, Granger JF, Manring MM, Calhoun JH, Shirtliff M, et al. Biofilms in peri-prosthetic orthopedic infections. Future Microbiol. 2014; 9: 987–1007. https://doi.org/10.2217/fmb.14.64 PMID: 25309955

13. Ciofu O, Rojo-Moliner E, Macià MD, Oliver A. Antibiotic treatment of biofilm infections. APMIS. 2017; 125: 304–319. https://doi.org/10.1111/apm.12673 PMID: 28407419

14. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, et al. Diagnosis and management of prosthetic joint infection: Clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis. 2013; 56: e1–e25. https://doi.org/10.1093/cid/cis803 PMID: 23223583

15. Siqueira MBP, Saleh A, Klika AK, O’Rourke C, Schmitt S, Higuera CA, et al. Chronic suppression of Staphylococcus aureus by cultured osteoblasts. Microb Pathog. 1995; 19: 409–419. https://doi.org/10.1006/mpat.1995.0075 PMID: 8852281

16. Noore J, Noore A, Li B. Cationic antimicrobial peptide LL-37 is effective against both extra- and intracellular Staphylococcus aureus. Antimicrob Agents Chemother. 2013; 57: 1283–1290. https://doi.org/10.1128/AAC.01650-12 PMID: 23274662

17. Mohamed MF, Abdelkhalak A, Seleem MN. Evaluation of short synthetic antimicrobial peptides for treatment of drug-resistant and intracellular Staphylococcus aureus. Sci Rep. 2016; 6: 29707. https://doi.org/10.1038/srep29707 PMID: 27405275

18. Moon J-Y, Hentzer-Wildman KA, Ramamoorthy A. Expression and purification of a recombinant LL-37 from Escherichia coli. Biochim Biophys Acta—Biomerembr. 2006; 1758: 1351–1358. https://doi.org/10.1016/j.bbamem.2006.02.003 PMID: 16542635

19. Li Y. A novel protocol for the production of recombinant LL-37 expressed as a thioredoxin fusion protein. Protein Expr Purif. 2012; 81: 201–210. https://doi.org/10.1016/j.pep.2011.10.011 PMID: 22085721

20. Luo Y, McLean DTF, Linden GJ, McAuley DF, McMullan R, Lundy FT. The naturally occurring host defense peptide, LL-37, and its truncated mimetics KE-18 and KR-12 have selected biocidal and anti-biofilm activities against Candida albicans, Staphylococcus aureus, and Escherichia coli in vitro. Front Microbiol. 2017; 8. https://doi.org/10.3389/fmicb.2017.00544 PMID: 28408902

21. Mishra B, Golla RM, Lau K, Lushnikova T, Wang G. Anti-Staphylococcal biofilm effects of human cathelicidin peptides. ACS Med Chem Lett. 2016; 7: 117–121. https://doi.org/10.1021/acsmedchemlett.5b00433 PMID: 26819677

22. Mohseni M, Momen-Sharghi M, Salehi A, Shaker S. Evaluation of cationic antimicrobial peptides against Escherichia coli: A novel laboratory method for growing biofilms. Microbiol. 2005; 151: 757–762. https://doi.org/10.1099/mic.0.27709-0 PMID: 15758222

23. Moon J-Y, Hentzer-Wildman KA, Ramamoorthy A. Expression and purification of a recombinant LL-37 from Escherichia coli. Biochim Biophys Acta—Biomerembr. 2006; 1758: 1351–1358. https://doi.org/10.1016/j.bbamem.2006.02.003 PMID: 16542635

24. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

25. Hall Snyder AD, Vidaillic C, Rose W, McRoberts JP, Rybak MJ. Evaluation of high-dose daptomycin versus vancomycin alone or combined with clarithromycin or rifampin against Staphylococcus aureus and S. epidermidis in a novel in vitro PK/PD model of bacterial biofilm. Infect Dis Ther. 2015; 4: 51–65. https://doi.org/10.1007/s40121-015-0080-z

26. Ganz T. The role of antimicrobial peptides in innate immunity. Integr Comp Biol. 2003; 43: 300–304. https://doi.org/10.1093/icb/43.2.300 PMID: 12680437

27. Vandamme D, Landuyt B, Luyten W, Schoofs L. A comprehensive summary of LL-37, the factotum peptide. Cell Immunol. 2012; 280: 22–35. https://doi.org/10.1016/j.cellimm.2012.11.009 PMID: 23246832

28. Goeres DM, Loetterle LR, Hamilton MA, Murga R, Kirby DW, Donlan RM. Statistical assessment of a laboratory method for growing biofilms. Microbiology. 2005; 151: 757–762. https://doi.org/10.1099/mic.0.27709-0 PMID: 15758222

29. Segev-Zarko L, Saar-Dover R, Brumfeld V, Mangoni ML, Shai Y. Mechanisms of biofilm inhibition and degradation by antimicrobial peptides. Biochem J. 2015; 468: 259–270. https://doi.org/10.1042/BJ20141251 PMID: 25761937

30. Overhage J, Campisano A, Bains M, Torfs ECW, Rehm BHA, Hancock REW. Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun. 2008; 76: 4176–4182. https://doi.org/10.1128/IAI.00318-08 PMID: 18591225
31. Marschall J, Lane MA, Beekmann SE, Polgreen PM, Babcock HM. Current management of prosthetic joint infections in adults: results of an Emerging Infections Network survey. Int J Antimicrob Agents. 2013; 41: 272–277. https://doi.org/10.1016/j.ijantimicag.2012.10.023 PMID: 23312602
32. Iarikov D, Demian H, Rubin D, Alexander J, Nambiar S. Choice and doses of antibacterial agents for cement spacers in treatment of prosthetic joint infections: Review of published studies. Clin Infect Dis. 2012; 55: 1474–1480. https://doi.org/10.1093/cid/cis735 PMID: 22918993
33. Klemm K. The use of antibiotic-containing bead chains in the treatment of chronic bone infections. Clin Microbiol Infect. Elsevier; 2001; 7: 28–31. https://doi.org/10.1016/S1476-1448(00)00186-X PMID: 11284941
34. Saginur R, StDenis M, Ferris W, Aaron SD, Chan F, Lee C, et al. Multiple combination bactericidal testing of Staphylococcal biofilms from implant-associated infections. Antimicrob Agents Chemother. 2006; 50: 55–61. https://doi.org/10.1128/AAC.50.1.55-61.2006 PMID: 16377667
35. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv. 2009; 27: 76–83. https://doi.org/10.1016/j.biotechadv.2008.09.002 PMID: 18854209
36. Martinez-Gutierrez F, Boegli L, Agostinho A, Sanchez EM, Bach H, Ruiz F, et al. Anti-biofilm activity of silver nanoparticles against different microorganisms. Biofouling. 2013; 29: 651–660. https://doi.org/10.1080/08927014.2013.794225 PMID: 23731460
37. Kang J, Hughes K, Xing M, Li B. Orthopedic Applications of silver and silver nanoparticles. Orthopedic Biomaterials. Springer International Publishing; 2017. pp. 63–83. https://doi.org/10.1007/978-3-319-73664-8_3
38. Abdelhady W, Bayer AS, Seidl K, Nast CC, Kiedrowski MR, Horswill AR, et al. Reduced vancomycin susceptibility in an in vitro catheter-related biofilm model correlates with poor therapeutic outcomes in experimental endocarditis due to methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2013; 57: 1447–1454. https://doi.org/10.1128/AAC.02073-12 PMID: 23295925
39. Cargill JS, Upton M. Low concentrations of vancomycin stimulate biofilm formation in some clinical isolates of Staphylococcus epidermidis. J Clin Pathol. 2009; 62: 1112–1116. https://doi.org/10.1136/jcp.2009.069021 PMID: 19640858
40. Post V, Wahl P, Richards RG, Moriarty TF. Vancomycin displays time-dependent eradication of mature Staphylococcus aureus biofilms. J Orthop Res. 2017; 35: 381–388. https://doi.org/10.1002/jor.23291 PMID: 2715462
41. Travis SM, Anderson NN, Forsyth WR, Espiritu C, Conway BD, Greenberg EP, et al. Bactericidal activity of mammalian cathelicidin-derived peptides. Infect Immun. 2000; 68: 2748–2755. https://doi.org/10.1128/IAI.68.5.2748-2755.2000 PMID: 10768969
42. Cox DL, Sun Y, Liu H, Lehrer RI, Shafer WM. Susceptibility of Treponema pallidum to host-derived antimicrobial peptides. Peptides. 2003; 24: 1741–1746. https://doi.org/10.1016/j.peptides.2003.07.026 PMID: 15019205
43. Dean SN, Bishop BM, van Hoek ML. Natural and synthetic cathelicidin peptides with anti-microbial and anti-biofilm activity against Staphylococcus aureus. BMC Microbiol. 2011; 11: 114. https://doi.org/10.1186/1471-2180-11-114 PMID: 21605457
44. Chennupati SK, Chiu AG, Tamashiro E, Banks CA, Cohen MB, Bleier BS, et al. Effects of an LL-37-derived antimicrobial peptide in an animal model of biofilm Pseudomonas sinensis. Am J Rhinol Allergy. 2009; 23: 46–51. https://doi.org/10.2500/ajra.2009.23.3261 PMID: 19379612
45. Leszczynska K, Namiot D, Byfield FJ, Cruz K, Zendzian-Piotrowska M, Fein DE, et al. Antibacterial activity of the human host defence peptide LL-37 and selected synthetic cationic lipids against bacteria associated with oral and upper respiratory tract infections. J Antimicrob Chemother. 2013; 68: 610–618. https://doi.org/10.1093/jac/dks434 PMID: 23134677
46. Loften H, Pränting M, Thulin E, Andersson DI. Mechanisms and fitness costs of resistance to antimicrobial peptides LL-37, CNY100HL and wheat germ histones. Marinus MG, editor. PLoS One. 2013; 8: e68875. https://doi.org/10.1371/journal.pone.0068875 PMID: 23894360
47. Strempel N, Neidig A, Nusser M, Geffers R, Villard J, Lesouhaitier O, et al. Human host defense peptide LL-37 stimulates virulence factor production and adaptive resistance in Pseudomonas aeruginosa. Fleischig S, editor. PLoS One. 2013; 8: e82240. https://doi.org/10.1371/journal.pone.0082240 PMID: 24349231
48. Andersson DI, Hughes D, Kubicek-Sutherland JZ. Mechanisms and consequences of bacterial resistance to antimicrobial peptides. Drug Resist Updat. 2016; 26: 43–57. https://doi.org/10.1016/j.drup.2016.04.002 PMID: 27198309
49. Tavares LS, Silva CSF, de Souza VC, da Silva VL, Diniz CG, Santos MO. Strategies and molecular tools to fight antimicrobial resistance: resistome, transcriptome, and antimicrobial peptides. Front Microbiol. 2013; 4: 412. https://doi.org/10.3389/fmicb.2013.00412 PMID: 24427156
50. Johansson J, Gudmundsson GH, Rottenberg ME, Berndt KD, Agerberth B. Conformation-dependent antibacterial activity of the naturally occurring human peptide LL-37. J Biol Chem. 1998; 273: 3718–3724. https://doi.org/10.1074/jbc.273.6.3718 PMID: 9452503

51. Säll J, Carlsson M, Giqlöf O, Holm A, Humlén J, Öhman J, et al. The Antimicrobial peptide LL-37 alters human osteoblast Ca²⁺ handling and induces Ca²⁺-independent apoptosis. J Innate Immun. 2013; 5: 290–300. https://doi.org/10.1159/000346587 PMID: 23406612

52. Ciornei CD, Sigurdardottir T, Schmidtchen A, Bodelsson M. Antimicrobial and chemoattractant activity, lipopolysaccharide neutralization, cytotoxicity, and inhibition by serum of analogs of human cathelicidin LL-37. Antimicrob Agents Chemother. 2005; 49: 2845–2850. https://doi.org/10.1128/AAC.49.7.2845-2850.2005 PMID: 15980359

53. Björstad A, Askarieh G, Brown KL, Christenson K, Forsman H, Onnheim K, et al. The host defense peptide LL-37 selectively permeabilizes apoptotic leukocytes. Antimicrob Agents Chemother. 2009; 53: 1027–1038. https://doi.org/10.1128/AAC.01310-08 PMID: 19075071

54. Svensson D, Wilk L, Morgelin M, Herwald H, Nilsson B-O. LL-37-induced host cell cytotoxicity depends on cellular expression of the globular C1q receptor (p33). Biochem J. 2016; 473: 87–98. https://doi.org/10.1042/BJ20150798 PMID: 26508735