RNAIII-Inhibiting Peptide Significantly Reduces Bacterial Load and Enhances the Effect of Antibiotics in the Treatment of Central Venous Catheter–Associated Staphylococcus aureus Infections

Oscar Cirioni,1 Andrea Giacometti,1 Roberto Ghiselli,2 Giorgio Dell’Acqua,4 Fiorenza Orlando,3 Federico Mocchegiani,2 Carmela Silvestri,1 Alberto Lucchi,1 Vittorio Saba,2 Giorgio Scalise,1 and Naomi Balaban5

1Institute of Infectious Diseases and Public Health, Università Politecnica delle Marche, 2Department of General Surgery, National Institute for Research and Therapy in the Elderly, Università Politecnica delle Marche, and 3Experimental Animal Models for Aging Units, Research Department, National Institute for Research and Therapy in the Elderly, Ancona, Italy; 4BalaPharm International, Grafton, and 5Department of Biomedical Sciences, Cummings School of Veterinary Medicine, Tufts University, North Grafton, Massachusetts

Background. Medical devices used in clinical practice are often associated with biofilm-associated staphylococcal infections.

Methods. An in vitro antibiotic susceptibility assay of Staphylococcus aureus biofilms using 96-well polystyrene tissue-culture plates was performed to test the effects of RNAIII-inhibiting peptide (RIP), ciprofloxacin, imipenem, and vancomycin. Efficacy studies were performed using a rat model of central venous catheter (CVC)–associated infection. Twenty-four hours after implantation, the catheters were filled with RIP (1 mg/mL). Thirty minutes later, rats were challenged, via the CVC, with 1.0 × 107 cfu of S. aureus strain Smith diffuse. The antibiotic-lock technique was begun 24 h later.

Results. Minimum inhibitory concentrations of antibiotics in biofilms were at least 4-fold higher than those against the freely growing planktonic cells. When they were first treated with RIP, the cells in biofilms became as susceptible to antibiotics as did planktonic cells. These data were confirmed by the in vivo studies. In particular, when CVCs were treated with both RIP and antibiotics, the biofilm bacterial load was further reduced to 1 × 103 cfu/mL, and bacteremia was not detected, suggesting that there was 100% elimination of bacteremia and a 6 log10 reduction in biofilm bacterial load.

Conclusion. RIP significantly reduces bacterial load and enhances the effect of antibiotics in the treatment of CVC-associated S. aureus infections.

Medical devices commonly used in clinical practice are often associated with microbial infections. Surgical implantation of the devices leads to tissue damage and inflammation, with increased susceptibility to microbial colonization at sites of injury [1–4]. Multiple-lumen central venous catheters (CVCs) are essential for the clinical management of critically ill patients. More than 2,500,000 CVCs are implanted annually in the United States alone [3–6]. However, use of CVCs is associated with infectious complications (∼5%) that are an important and potentially avoidable source of iatrogenic morbidity and mortality [3–6]. Bacterial colonization of the device is an essential prerequisite in the pathogenesis of catheter-related sepsis. It is common and is affected by many factors, including duration of catheterization, site of insertion, catheter design, and frequency of line manipulation. The CVC may become colonized by 2 main routes: (1) from the skin along the outside of the catheter and (2) via the catheter lumen [3, 7, 8]. Initial colonization is followed by development of a biofilm structure, which usually develops within 3 days of catheterization [3, 9, 10]. In the biofilm, bacteria are protected from both the immune response and antimicrobial therapy, and, thus, such in-
Infections are rarely resolved. Biofilm bacteria can usually survive antibiotics at concentrations 1000–1500 times higher than antibiotic concentrations used to treat planktonic bacteria [7, 10, 11].

Biofilms have a variety of attributes that contribute to their resistance to antibiotics. These attributes include, but are not limited to, a lower growth rate, an exopolysaccharide matrix, and the production of multiple toxins [7, 10, 11]. Resistance to antimicrobial agents by the sessile bacterial communities is at the basis of many persistent and chronic bacterial infections. Thus, the recommended course of treatment is to remove the infected device, treat the patient with rigorous antibiotic therapy, and reinstall a new device [1–3]. Despite this treatment regimen, the recurrence of infection is high.

Staphylococci, especially S. epidermidis and S. aureus, on CVCs are most often found in biofilms [7, 9, 10]. Disease may be associated with detachment of cells or cell aggregates, production of multiple toxins, and provision of foci for the generation of drug-resistant bacteria [1–3]. Antibiotic therapy often fails to eradicate the infection, because of the capacity of the bacteria encased in the biofilm matrix to be resistant to antibacterial treatment, and development of staphylococcal resistance to antibiotics, such as methicillin and vancomycin, further limits available therapeutic approaches [1, 12–18]. The final outcome often is longer hospitalization, need of surgery with removal of the device, and even death due to the toxins that the bacteria produce. Alternative modes of therapy that would be aimed not only at planktonic free-living bacteria but also at biofilms are eagerly sought after. One such approach is to disrupt bacterial cell-to-cell communication, which would interfere with the pathogenic potential of the bacteria (see below).

Bacteria communicate their cell densities by the production of chemical signals in a process known as “quorum sensing.” This type of signalling allows the bacteria to coordinate the expression of particular genes necessary for their survival. In the case of staphylococci and, for example, Pseudomonas aeruginosa, quorum-sensing systems regulate biofilm architecture and detoxication, as well as the production of toxins. In the case of staphylococci, numerous toxins are produced and are involved in disease progression [19–28].

The quorum-sensing system we are focusing on is the RNAIII-activating inhibitor (RAP)/target of RAP (TRAP) system and its peptide inhibitor RNAIII-inhibiting peptide (RIP). RAP is a 33-kDa protein that is secreted by the bacteria and activates the production of numerous toxins through the phosphorylation of its target protein, TRAP. TRAP is a 21-kDa protein that is highly conserved among staphylococci [24, 25, 27, 29]. RIP is a heptapeptide that inhibits toxin production by interfering with the function of RAP, leading to inhibition of TRAP phosphorylation [27, 29, 30].

TRAP has been shown to be a master regulator of virulence, and, when its expression or phosphorylation is disrupted (e.g., by RIP), the expression of the gene regulator agr and the multiple toxins it regulates are suppressed. In addition, most of the adhesion molecules are down-regulated, as are the genes necessary for biofilm formation (such as arc, pyr, and ure). TRAP has, thus, been demonstrated to be a master regulator of staphylococcal pathogenesis [31].

RIP synthesized in its amide form as YSPWTYNF-NH2 has been shown to have strong activity against biofilm formation in vitro and has been shown, in a rat Dacron graft model, to prevent biofilm formation and in vivo infections with both S. aureus and S. epidermidis [27, 32–39]. The mechanism of action of RIP is different from those of common antibiotics, since, instead of killing the bacteria, it inhibits cell-cell communication, leading to prevention of their adhesion and virulence [31, 36, 40]. Genomic studies can easily explain our observation that, in the absence of TRAP expression or phosphorylation, the ability of the bacteria to produce toxins, to attach to host cells or foreign material, to form a biofilm, and to survive within the host is seriously compromised; therefore, in the presence of RIP that inhibits TRAP phosphorylation, biofilm formation is prevented [31, 40]. The aim of the present study was to assess the efficacy of RIP, ciprofloxacin, imipenem, and vancomycin in the treatment of CVC infection, using the antibiotic-lock technique.

**MATERIALS AND METHODS**

**Organisms.** We used S. aureus strain Smith diffuse—a highly encapsulated, slime-producing strain with exopolysaccharides, which are antigenically identical to those of many clinical S. aureus strains tested [27].

**Drugs.** Vancomycin (Sigma-Aldrich), ciprofloxacin (Bayer), and imipenem (Merck) were diluted according to the manufacturers’ recommendations. Solutions were made fresh on the day that the assay was performed or were stored at −80°C in the dark for short periods. The concentration range assayed was 0.25–1024 µg/mL.

**Synthetic peptides.** The amide form of RIP (YSPWTYNF-NH2) was synthesized by Neosystem (Strasbourg, France). RIP was purified, by high-pressure liquid chromatography, to 99% [27]. It was dissolved in distilled H2O at 20 times the required maximal concentration. The solution was made fresh on the day that the assay was performed or was stored at −80°C in the dark for short periods.

**Rats.** Adult male Wistar rats (weight range, 250–300 g) were used (n = 12 rats/experimental group). All rats were housed in individual cages under constant temperature (22°C) and humidity (55%), with a 12-h light/dark cycle, and had access to chow and water throughout the study. The study was approved by the animal research ethics committee of the Na-
In vitro biofilm formation for susceptibility testing. To develop a biofilm in static conditions, $5 \times 10^3$ cfu of *S. aureus* strain Smith diffuse in 50 μL of tryptic soy broth (TSB) (Oxoid) was added, under aseptic conditions, to each well of a tissue culture–treated polystyrene 96-well plate (Becton Dickinson) containing 150 μL of TSB with 2% glucose. After 24 h of incubation at 37°C, the growth medium was discarded, and each well was washed 3 times with PBS, under aseptic conditions, to eliminate unbound bacteria. To evaluate the formation of a biofilm, the remaining attached bacteria were fixed with 0.2 mL of 99% methanol/well, and, after 15 min, plates were emptied and left to dry. Cells were then stained for 5 min with 0.2 mL of 2% crystal violet (which was used for Gram staining)/well. Excess stain was rinsed off by placing the plate under running tap water [41, 42]. The plates were air-dried, and the well. Excess stain was rinsed off by placing the plate under running tap water [41, 42]. The plates were air-dried, and the.

optical density of a well filled with PBS solution. The cutoff (dye that had bound to adherent cells was solubilized with 0.2 mL of 33% (vol/vol) glyacial acid/well. The optical density of each well was determined at 570 nm, using the MR 700 Microplate Reader (Dynatech Laboratories). The blank (negative control) was determined for every plate by measuring the optical density of a well filled with PBS solution. The cutoff optical density for the microtiter-plate test was defined as 3 SDs above the mean optical density of the negative control. The same experiment was performed 2 times: (1) with and (2) without the addition of 10 μg of RIP in a total volume of 10 μL of Mueller-Hinton broth (MHB)/well. Tests were performed in triplicate. Biofilms were also observed by light microscopy.

In vitro susceptibility testing of planktonic cells. The MIC was determined using a microbroth dilution method with MHB (Becton Dickinson Italia) and an initial inoculum of $5 \times 10^3$ cfu of *S. aureus* strain Smith diffuse/mL, in accordance with the procedures outlined by the NCCLS [43]. The MIC was taken as the lowest drug concentration at which observable growth was inhibited. The MBC was taken as the lowest concentration of each drug that resulted in $\geq 99.9\%$ reduction of the initial inoculum. In addition, to investigate the effect of RIP pretreatment on bacterial antibiotic susceptibility, MICs and MBCs were determined after pretreatment of cells for 30 min with 10 μg of RIP in 10 μL of MHB/well. All experiments were performed in triplicate.

In vitro susceptibility testing of biofilms. Biofilms of *S. aureus* strain Smith diffuse (prepared as described above) were washed with PBS to discard unbound bacteria. Sample plates were used to perform the viable count of the remaining adherent bacteria. The plates were washed using saline containing EDTA (0.15%) and Triton X-100 (0.1%). Vigorous mixing and sonication for 3 min was used to remove the adherent cells, and the number of colony-forming units per well was determined by use of a serial dilution method. The mean viable count recovered from 12 different wells of the same line was defined as the adherent inoculum. Subsequently, serial 2-fold dilutions of antibiotics in MHB were added to wells containing adherent organisms. The polystyrene plates were incubated for 18 h at 37°C in an atmosphere of air. The MIC was taken as the lowest drug concentration at which observable growth was inhibited. To determine the MBC, the MHB containing antibiotics was removed from each well and replaced with antibiotic-free MHB; the plates were incubated again for 18 h at 37°C in an atmosphere of air. The MBC was taken as the lowest concentration of each drug that resulted in no bacterial growth following removal of the drug. The same experiments were repeated 30 min after the addition of 10 μg of RIP in a total volume of 10 μL of MHB/well. All experiments were performed in triplicate.

The effect of antibiotics and RIP on CVC–associated infections in vivo. Bacteria (*S. aureus* strain Smith diffuse) were grown in brain-heart infusion broth. When bacteria were in the early log phase of growth, the suspension was centrifuged at 1000 g for 15 min, the supernatant was discarded, and the bacteria were suspended and diluted in sterile saline, to achieve a concentration of $\sim 1 \times 10^6$ cfu/mL.

The rat CVC–associated infection model included the following: 1 control group (no CVC infection); 1 contaminated group that did not receive any antibiotic prophylaxis; 1 contaminated group that received CVCs filled with 0.1 mL of RIP at 1 mg/mL; and 6 contaminated groups that received RIP-filled CVCs or no RIP-filled CVCs plus ciprofloxacin, imipenem, or vancomycin, at concentrations equal to MBCs for adherent cells and 1024 μg/mL, in a volume of 0.1 mL, which filled the CVC. Each experimental group included 12 rats.

All rats were anesthetized by intramuscular injection of ketamine (30 mg/kg of body weight). A silastic catheter (2Bio- logical Instruments) was inserted into the jugular vein and was advanced into the superior vena cava. The proximal portion of the catheter was tunnelled subcutaneously, so that it exited in the midscapular space. The catheters were then flushed with a heparin solution. A rodent restraint jacket was used to protect the catheter and to allow access to it.

Twenty-four hours after CVC placement, blood cultures were obtained from the catheters, to verify sterility [44]. When established (24 h after implantation), the catheters were filled with RIP (1 mg/mL). After 30 min, the rats were challenged, via the CVC, with 1.0 $\times$ 10$^7$ cfu of *S. aureus* strain Smith diffuse in a volume of 0.1 mL of sterile saline.

Six rats (control group 24 h) were killed 24 h after bacterial challenge, to verify, by quantitative cultures, the presence of infection. At the same time, the antibiotic-lock technique was initiated for the other groups: the drugs were allowed to remain for 1 h, and the catheters were then flushed with a heparin solution.
Quantitative peripheral blood cultures and quantitative cultures of the catheters and surrounding venous tissues, for evaluation of treatment, were performed as follows. On day 9, the rats were killed. For quantitative peripheral blood cultures, peripheral blood was obtained by aseptic percutaneous transthoracic cardiac puncture and was cultured on sheep-blood agar plates. Plates were incubated for 48 h at 37°C, and the number of colony-forming units per plate was determined. The isolates were identified by morphological and cultural characteristics, Gram stain, positive catalase reaction, positive coagulase test, and API Staph (bioMérieux). For quantitative cultures of the catheters and surrounding venous tissues, the location of the distal tip of the CVC in the superior vena cava was confirmed visually, and the catheters and surrounding venous tissue were removed aseptically. The explanted catheters/venous tissues were placed in tubes containing 50 mL of PBS solution and were sonicated (Fisher Scientific 300) at 20,000 Hz for 5 min, to remove adherent bacteria. The solution was then cultured by performance of serial dilutions (0.1 mL) of the bacterial suspension in 10 mmol/L sodium HEPES buffer (pH 7.2) (Sigma-Aldrich), to minimize the carryover effect, and by culture of each dilution on blood agar plates. Plates were incubated for 48 h at 37°C, and the number of colony-forming units per plate was determined. The limit of detection for both methods was <10 cfu/mL.

**Statistical analysis.** MICs are presented as the geometric means of 3 separate experiments. Quantitative culture results for all groups are presented as means ± SDs. The bacterial loads obtained from peripheral blood, explanted CVCs, and peripheral tissues were compared using the Kruskal-Wallis test. Significance was accepted when $P ≤ .05$.

## RESULTS

**Increases in in vitro susceptibility of biofilms to antibiotics, by RIP.** *S. aureus* strain Smith diffuse cells in the early log phase of growth were placed in polystyrene plates and grown in static conditions for 24 h. As observed by light microscopy, the growing biofilm covered 5% ± 5% of the surface area after 1 day and increased to 50% ± 35% after 7 days. The mean viable count recovered from 12 different wells of the same line was within the range of $2 \times 10^5$ and $6 \times 10^5$ cfu/mL. Antibiotics were added, and MICs and MBCs were determined and compared to those of cells grown in planktonic conditions. As shown in table 1, the activity of the 3 antibiotics against the biofilm was at least 2-fold lower than that against the freely growing planktonic cells. Ciprofloxacin, imipenem, and vancomycin had MICs of 2.0, 1.0, and 2.0 μg/mL, respectively, against the biofilm, compared with 1.0, 0.5, and 1.0 μg/mL, respectively, against planktonic cells. The MBC, defined as the lowest concentration that resulted in no bacterial growth following removal of the drug, was increased 8-fold for all agents used against the biofilm. Ciprofloxacin, imipenem, and vancomycin had MBCs of 16.0, 8.0, and 16.0 μg/mL, respectively, against the biofilm, compared with 2.0, 1.0, and 2.0 μg/mL, respectively, against planktonic cells.

When the cells were first treated with RIP and then with antibiotics, no difference in MICs and MBCs against planktonic cells was observed, but MICs and MBCs against biofilms were drastically reduced. Specifically, ciprofloxacin, imipenem, and vancomycin had a 2-fold reduction in MICs (1.0, 0.5, and 1.0 μg/mL, respectively) and a 4–8-fold reduction in MBCs (2.0, 2.0, and 2.0 μg/mL, respectively) (table 1). Finally, RIP did not have any microbicidal or microbiostatic activity (MIC, >256 mg/L), as was expected on the basis of its mechanism of action.

**Increases in the efficacy of antibiotics in the treatment of CVC-associated infections in rats, by RIP.** The effects of RIP and antibiotics were tested using the rat CVC-associated infection model. As shown in table 2, all rats included in the infected but untreated control group (killed after 24 h) had evidence of CVC infection, with quantitative culture results showing $6.0 \times 10^6 ± 1.7 \times 10^6$ cfu/mL and no bacteraemia. Quantitative peripheral blood cultures obtained on day 7, by transthoracic cardiac puncture and quantitative catheter/venous tissue culture, showed that control rats had culture results of $9.6 \times 10^7 ± 1.9 \times 10^7$ and $9.1 \times 10^7 ± 1.6 \times 10^7$ cfu/mL, respectively. At MBCs for adherent cells, vancomycin, ciprofloxacin, and imipenem exerted only weak antimicrobial activity, with results not significantly different when compared with those for untreated controls (blood culture results, $4.2 \times 10^5 ± 1.5 \times 10^5$, $6.5 \times 10^5 ± 1.7 \times 10^5$, and $5.8 \times 10^5 ± 1.2 \times 10^5$ cfu/mL, respectively; catheter/venous tissue culture results, $6.3 \times 10^6 ± 2.0 \times 10^6$, $8.7 \times 10^6 ± 2.3 \times 10^6$, and $6.9 \times 10^6 ± 2.1 \times 10^6$ cfu/mL, respectively). In contrast, when the catheters were impregnated with RIP, both blood culture and catheter/venous tissue results were significantly reduced ($3.4 \times 10^5 ± 0.8 \times 10^5$ and $1.4 \times 10^5 ± 0.3 \times 10^5$ cfu/mL, respectively). No additional significant reduction in bacterial load was observed when antibiotics were used at their MBCs. When antibiotics were

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**Table 1. In vitro antimicrobial activity of ciprofloxacin, imipenem, and vancomycin against *Staphylococcus aureus* strain Smith diffuse planktonic cells and biofilm pretreated or not with RNAIII-inhibiting peptide (RIP).**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Planktonic cells</th>
<th>Biofilm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC, μg/mL</td>
<td>MBC, μg/mL</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Ciprofloxacin + RIP$^a$</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Imipenem + RIP$^a$</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Vancomycin + RIP$^a$</td>
<td>1.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

$^a$ Pretreatment with RIP (10 μg of RIP in 10 μL of Mueller-Hinton broth/well).
administered at higher concentrations (1024 µg/mL), the bacterial load was significantly reduced (blood culture results, $5.3 \times 10^5 \pm 1.1 \times 10^5$, $6.6 \times 10^5 \pm 1.4 \times 10^5$, and $5.7 \times 10^5 \pm 1.2 \times 10^5$ cfu/mL, respectively; catheter/venous tissue culture results, $5.4 \times 10^5 \pm 1.6 \times 10^5$, $7.4 \times 10^5 \pm 2.1 \times 10^5$, and $6.0 \times 10^5 \pm 1.9 \times 10^5$ cfu/mL, respectively, for vancomycin, ciprofloxacin, and imipenem). A further significant reduction in bacterial load was observed when catheters were impregnated with RIP and then treated with the higher doses of antibiotics. Specifically, when CVCs were filled with RIP (1 mg/mL) and later injected with 1024 µg/mL vancomycin, ciprofloxacin, or imipenem, only $1.9 \times 10^5 \pm 0.2 \times 10^5$, $3.1 \times 10^5 \pm 0.7 \times 10^5$, and $2.2 \times 10^5 \pm 0.5 \times 10^5$ cfu/mL, respectively, were found for the catheter/venous tissue, and there were no blood culture results at all, suggesting that there was 100% elimination of bacteremia and a 6 log₁₀ reduction in biofilm load.

**DISCUSSION**

One of the methods by which bacterial colonization can be reduced is the use of CVCs precoated with antimicrobials and antiseptics [1, 11, 12, 14, 45]. Catheters that, for example, are coated with chlorhexidine and silver sulfadiazine were shown to be significantly less likely to be associated with catheter-related bloodstream infections than uncoated catheters [17]. However, these catheters were coated only on the external surface, and they were associated with short antimicrobial durability. In addition, some anaphylactic reactions have been documented [17]. Vascular catheters impregnated with minocycline and rifampin have been found to be highly efficacious in preventing catheter-related infections [11]. However, the management of CVCs that become associated with biofilm-based infection remains problematic. Systemic antibiotic therapy is usually administered, but, although it is effective in eliminating circulating bacteria, it usually fails to protect the surfaces of the catheters from colonization, leaving the patient at a continuing risk of complications or recurrence [1, 15, 16, 18]. For these reasons, attention has turned to the in situ treatment of colonized catheters, which is named the “antibiotic-lock technique” and is also known as “intraluminal therapy.” This technique involves the instillation of a concentrated antibiotic solution into a colonized catheter, in a volume chosen to fill the lumen but not spill out into the circulation [46]. Vancomycin, teicoplanin, gentamicin, amikacin, minocycline, clindamycin, and several other drugs have been used as antibiotic locks [46]. However, resistance of biofilms to antibiotic treatment and the emergence of organisms that are resistant to many conventional antibiotics have stimulated the search for antimicrobial agents that have alternative mechanisms of action and that could be used in combination with or instead of the antibiotic-lock technique.

### Table 2. Efficacy of RNAIII-inhibiting peptide (RIP), ciprofloxacin, imipenem, and vancomycin in a rat model of central venous catheter infection induced by *Staphylococcus aureus* strain Smith diffuse.

<table>
<thead>
<tr>
<th>Treatment, dose</th>
<th>Quantitative blood culture, cfu/mL</th>
<th>Quantitative catheter/venous tissue culture, cfu/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment (control group, killed after 24 h)</td>
<td>...</td>
<td>$6.0 \times 10^5 \pm 1.7 \times 10^5$</td>
</tr>
<tr>
<td>Isotonic sodium chloride solution</td>
<td></td>
<td>$9.1 \times 10^5 \pm 1.6 \times 10^5$</td>
</tr>
<tr>
<td>RIP, 1 mg/mL</td>
<td>$9.6 \times 10^2 \pm 1.9 \times 10^2$</td>
<td>$1.4 \times 10^2 \pm 0.3 \times 10^2$</td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 µg/mL</td>
<td>$4.2 \times 10^3 \pm 1.5 \times 10^3$</td>
<td>$6.3 \times 10^3 \pm 2.0 \times 10^3$</td>
</tr>
<tr>
<td>1024 µg/mL</td>
<td>$5.3 \times 10^3 \pm 1.1 \times 10^3$</td>
<td>$5.4 \times 10^3 \pm 1.6 \times 10^3$</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 µg/mL</td>
<td>$6.5 \times 10^3 \pm 1.7 \times 10^3$</td>
<td>$8.7 \times 10^3 \pm 2.3 \times 10^3$</td>
</tr>
<tr>
<td>1024 µg/mL</td>
<td>$6.6 \times 10^3 \pm 1.4 \times 10^3$</td>
<td>$7.4 \times 10^3 \pm 2.1 \times 10^3$</td>
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<tr>
<td>Imipenem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 µg/mL</td>
<td>$5.8 \times 10^3 \pm 1.2 \times 10^3$</td>
<td>$6.9 \times 10^3 \pm 2.1 \times 10^3$</td>
</tr>
<tr>
<td>1024 µg/mL</td>
<td>$5.7 \times 10^3 \pm 1.2 \times 10^3$</td>
<td>$6.0 \times 10^3 \pm 1.9 \times 10^3$</td>
</tr>
<tr>
<td>RIP + vancomycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/mL + 16 µg/mL</td>
<td>$2.6 \times 10^3 \pm 0.2 \times 10^2$</td>
<td>$1.2 \times 10^3 \pm 0.8 \times 10^2$</td>
</tr>
<tr>
<td>1 mg/mL + 1024 µg/mL</td>
<td>$10^0$</td>
<td>$1.9 \times 10^1 \pm 0.2 \times 10^1$</td>
</tr>
<tr>
<td>RIP + ciprofloxacin</td>
<td></td>
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<tr>
<td>1 mg/mL + 16 µg/mL</td>
<td>$4.9 \times 10^3 \pm 1.2 \times 10^3$</td>
<td>$3.4 \times 10^3 \pm 0.3 \times 10^3$</td>
</tr>
<tr>
<td>1 mg/mL + 1024 µg/mL</td>
<td>$10^0$</td>
<td>$3.1 \times 10^1 \pm 0.7 \times 10^1$</td>
</tr>
<tr>
<td>RIP + imipenem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/mL + 8 µg/mL</td>
<td>$3.2 \times 10^3 \pm 0.8 \times 10^2$</td>
<td>$2.5 \times 10^3 \pm 1.0 \times 10^2$</td>
</tr>
<tr>
<td>1 mg/mL + 1024 µg/mL</td>
<td>$10^0$</td>
<td>$2.2 \times 10^1 \pm 0.5 \times 10^1$</td>
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</tbody>
</table>

**NOTE.** Data are means ± SDs.

- P < 0.05, vs. the group treated with isotonic sodium chloride solution.
- P < 0.05, vs. groups singly treated at MBCs.
The aim of the present study was to assess the efficacy of the quorum-sensing inhibitor RIP and the more commonly used antibiotics vancomycin, ciprofloxacin, and imipenem in the treatment of CVC-associated S. aureus infection, using the antibiotic-lock technique. We first established an in vitro system to elucidate the bactericidal activities of 3 conventional antibiotics, with or without RIP, in a planktonic model and in an adherent-cell biofilm model. Subsequently, to assess their efficacies in the absence or the presence of RIP, we used a rat model of CVC infection.

Data presented here indicate that the cells in suspension were susceptible to the antibiotics, as determined by NCCLS guidelines [43]. In contrast, the antimicrobial agents were scarcely active against biofilm bacteria. Biofilms were strongly affected by the presence of RIP, and, in its presence, all antibiotics had MICs and MBCs that were much lower than those obtained in the absence of RIP. The same picture emerged in the in vivo experiments, where the use of RIP-impregnated CVCs with or without combination with conventional antibiotics caused significantly lower bacterial loads. In fact, RIP with ciprofloxacin, imipenem, and vancomycin caused a significant reduction in bacterial load, with a 6 log10 decrease of the bacterial load in the catheter/venous tissue, compared with that in untreated controls, and there was no evidence of bacterial load in the blood. In summary, not only did RIP by itself reduce bacterial load, it also enhanced the effect of commonly used antibiotics. These results are of major importance in view of the fact that biofilm-related infections are notoriously for being so difficult to treat. RIP has been shown to be effective against any staphylococcal strain so far tested, including methicillin- and vancomycin-intermediate–resistant S. aureus and S. epidermidis [37,40], suggesting that RIP-impregnated CVCs may lead to lower rates of infection.

References


