New in vitro and in vivo models to evaluate antibiotic efficacy in Staphylococcus aureus prosthetic vascular graft infection

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Matthieu Revest, C. Jacqueline, R. Boudjemaa, J. Caillon, V. Le Mabecque, et al.. New in vitro and in vivo models to evaluate antibiotic efficacy in Staphylococcus aureus prosthetic vascular graft infection. Journal of Antimicrobial Chemotherapy, 2016, 71 (5), pp.1291-1299. 10.1093/jac/dkv496 . hal-01274236

HAL Id: hal-01274236

https://univ-rennes.hal.science/hal-01274236

Submitted on 22 Mar 2016

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Title page

New *in vitro* and *in vivo* Models to evaluate Antibiotic Efficacy in *Staphylococcus aureus* Prosthetic Vascular Graft Infection

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Running title: evaluation of different antibiotics for vascular prosthesis infections treatment

Key words: Staphylococcus aureus, animal models, biofilm, surgical infection, vascular prosthesis infections

Abstract:

Objective. Prosthetic vascular graft infection (PVGI) is an emerging disease, mostly due to staphylococci, with limited data regarding efficacy of current antistaphylococcal agents. We aimed to assess the efficacy of different antibiotic regimens. **Methods.** Six different strains of methicillin-susceptible (MSSA) and methicillin-resistant *S. aureus* (MRSA) were used. We compared results of minimal biofilm inhibitory and eradicating concentrations (MBICs and MBECs) obtained with a Calgary Biofilm Pin lid Device (CBPD) to those yielded by an original Dacron®-related minimal inhibitory and eradicating concentrations measure model. We then used an original murine model of *Staphylococcus aureus* vascular material infection to evaluate efficacy of different antibiotic regimens: vancomycin and daptomycin combined or not with rifampicin for MRSA and same groups with cloxacillin and cloxacillin associated with rifampicin for MSSA. **Results.** We demonstrated that classical measures of MBICs and MBECs with CPBD could overestimate the decrease of antibiotic susceptibility in material-related infections and that the nature of the support used might influence the measure of biofilm susceptibility since results yielded by our Dacron®-related minimal eradicating assay were lower than those found on a plastic device. In our in vivo model, we showed that daptomycin was significantly more bactericidal than comparators for some strains of MRSA or MSSA but not for all. For the majority of strains, it was as efficient as comparators. The addition of rifampicin to daptomycin did not enhance daptomycin efficacy. **Conclusions.** Despite the heterogeneity of results according to bacterial strains, these innovative models represent an option to better evaluate *in vitro* efficacy of antibiotics on Dacron®-related biofilm *S. aureus* infections, and to screen different antibiotic regimens in a mouse-model of PVGIs.

New *in vitro* and *in vivo* Models to evaluate Antibiotic Efficacy in *Staphylococcus aureus* Prosthetic Vascular Graft Infection

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Introduction

More than 400 000 vascular grafts are inserted annually in the United States[1] and approximately 50 000 in France.[2] Prosthetic vascular graft infections (PVGIs) are among the most serious complications associated with these procedures,[1] with 30 day- and one year-mortality rates of, respectively, 10–25%, and 50%.[3] *Staphylococcus aureus* is the main pathogen, and methicillin resistant *S. aureus* (MRSA) accounts for almost 50% of *S. aureus* PVGIs in North America.[4] Clinical data regarding the optimal antibiotic therapy for these infections are scarce, in the absence of any comparative clinical trial dealing with the treatment of PVGIs.[5] Hence, experimental data are needed to better identify the optimal antibiotic regimens for PVGIs.

Biofilm developed onto the vascular prosthesis plays a significant role in the difficulties encountered for treating PVGIs. Firstly, biofilm acts as a mechanical barrier against antibiotic penetration. Secondly, bacteria embedded in a mature biofilm enter into an altered metabolic state associated with a dramatic decrease of susceptibility to most antibiotics.[6, 7] Many studies showed that minimal biofilm inhibitory concentrations (MBIC) and minimal biofilm eradicating concentrations (MBEC) of antibiotics are much higher than their respective minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC), as measured on planktonic bacteria.[8-10] However, most studies evaluated antibiotic efficacy on artificial materials not used in clinical practice, although the nature of the material may influence these results. In addition, although helpful, *in vitro* data may fail to capture what

happens *in vivo*. Animal models of PVGIs have mainly been used to evaluate techniques to prevent PVGIs,[11-14] but no animal model assessing the efficacy of antibiotics on infection of the Dacron® vascular prosthesis used in clinical daily practice has been described so far.

In this study, we investigated the activities of cloxacillin, vancomycin, daptomycin and rifampicin against different strains of methicillin-susceptible *S. aureus* (MSSA) and MRSA *in vitro*. Those activities were determined against planktonic bacteria (MIC and MBC), and against adherent bacteria (MBIC and MBEC). To evaluate whether the nature of the support influences results for MBIC and MBEC, two different techniques were used: a largely used modified version of the Calgary Biofilm Pin Lid Device (CBPD) and an original model of Dacron®-related biofilm. Then, cloxacillin, vancomycin, daptomycin whether or not combined with rifampicin, were evaluated in a new mouse vascular-material infection model, using the same strains of *S. aureus*.

Materials and methods

Bacterial strains

Six different strains of *S. aureus* were used for all experiments. For MSSA: two different clinical strains, thereafter named 171 and 176, isolated from patients with *S. aureus* bloodstream infections, and one strain from the American Type Culture Collection: ATCC 27217. For MRSA: two different strains, thereafter named BCB8 and 117, also isolated from blood cultures, and one ATCC strain: ATCC 33591. Bacteria were stored in a cryovial bead preservation system at -80°C.

Antimicrobial agents

Clinical forms of the following antibiotics were used: cloxacillin (Astellas Pharma, Levallois-Perret, France) stored in a 100 mg/mL stock-solution, vancomycin (Sandoz, Levallois-Perret,

France) stored in a 50 mg/mL stock-solution, daptomycin (Novartis Pharma SAS, Rueil-Malmaison, France) stored in a 50 mg/mL stock-solution and rifampicin (Sanofi-Aventis, Paris, France) stored in a 60 mg/mL stock-solution. All the stock-solutions were prepared in sterile and pyrogen-free 0.9% saline except for rifampicin, which was prepared in sterile water, and stored at -80°C before utilization.

In vitro experiments

All the following experiments were performed at least in duplicate, with all the staphylococcal strains evaluated. Biofilm formation was compared between polystyrene (CPBD) and Dacron® using confocal microscopy.

MICs and MBCs

The MICs and the MBCs values for cloxacillin, vancomycin, daptomycin and rifampicin were determined by the broth macrodilution method in cation-adjusted Mueller-Hinton broth (CAMHB), according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Media were supplemented with 50 mg/L Ca^{2+} for daptomycin.

MBICs and MBECs

A modified Calgary device was used as previously described.[8, 15] Briefly, biofilm was formed by immersing pegs of a modified microtiter lid into wells of a flat-bottom 96-well microtiter plate. Each well was filled with 150 µL of a 3 McFarland *S. aureus* broth medium solution. After a 24-h incubation at 37°C, pegs lids were rinsed 3 times in sterile water, placed onto flat-bottom microtiter plates containing antibiotic twofold dilutions in 150 µL of CAMHB per well, and incubated for 24 h at 37°C. The MBIC were defined as the minimal concentration of antibiotic inhibiting bacterial growth, as determined by reading turbidity of media at 650 nm. Pegs with no bacterial growth were again rinsed 3 times with sterile water and sonicated (Aquasonic sonicator, 35 kHz for 5 minutes) followed by vortexing for 30 seconds to remove

biofilm from the support. A new cover plate was added and this new device was cultured for 24 h at 37°C in CAMHB. The MBEC was defined as the minimal concentration of antibiotic where no bacterial growth was documented.

Dacron®-related minimal biofilm inhibitory concentrations (dMBICs) and the Dacron®-related minimal biofilm eradicating concentrations (dMBECs)

Commercially available woven Dacron® grafts (Cardial™, Bard, Saint-Etienne, France) were cut into 1 cm x 1 cm squares and sterilized. To cover the biomaterial with proteins and thus to facilitate the bacterial graft, these Dacron® sheets were incubated at 37°C under sterile conditions with horse serum for 24 h. They were rinsed 3 times with sterile water to remove the horse serum and then incubated for 24 h at 37°C with 1 mL per patch of Mueller-Hinton broth (MHB) containing 10^8 colony forming units (CFU)/mL of *S. aureus*. Again, they were rinsed 3 times in sterile water to eliminate the planktonic bacteria, dried with a sterile gauze compress, and plunged into tubes filled with CAMHB containing none (control groups) or serial antibiotic dilutions, and incubated for 24 h at 37°C. dMBIC for each antibiotic and strain couple was defined as the antibiotic concentration of the first tube with no visible bacterial growth. Then, Dacron® patches with no bacterial growth were removed from their tubes, rinsed 3 times in sterile water, dried with a sterile gauze compress and plunged again in MHB. A sonication (35kHz for 5 minutes) was performed followed by vortexing for 30 seconds. These MHB containing the Dacron® sheets were incubated for 24 h at 37°C. dMBECs were defined as the antibiotic concentration of the first tube with no bacterial growth.

Confocal microscopy evaluating biofilm formation depending on the support

Twenty-four h-biofilms prepared as previously described were observed using a Leica TCS SP5 confocal laser-scanning microscope (Leica Microsystems, France). Bacteria were stained with 2.5 µM Syto9® (Invitrogen), which is able to penetrate all bacteria, and 5 µL of 1-

mg/mL propidium iodide (PI, Invitrogen), which can only penetrate dead cells. Syto9® and PI were excited at 488 nm and 543 nm, respectively, and their fluorescence emissions were collected between 500 and 600 nm for Syto9® and between 640 and 750 nm for PI. Images were acquired using a 63x with a 1.4 numerical aperture oil immersion objective. The size of the confocal images was 512 x 512 pixels (82 x 82 µm²), recorded with a z-step of 1 µm and a 3x zoom. For each biofilm, at least four different regions were analysed.

In vivo experiments

All experiments were approved by the French ministry of research and the regional committee of animal ethics and animals were cared for in line with national guidelines.

Animals

Four-weeks old female Swiss mice (RjOrl/SWISS, Janvier laboratory St Berthevin, France) weighing approximately 20 g were maintained on a 12-h light/dark cycle with free access to food and water. Eight to 15 animals per group were used for analysis.

Biomaterial

Sterile 1 cm^2 squares of commercially used Dacron® were incubated with sterile serum of healthy female Swiss mice during 24 h at 37°C. Then, they were implanted into mice.

Surgical procedures

Mice were anesthetized with ketamine (70 mg/kg) and Xylazine (10 mg/kg) through intraperitoneal (IP) injection. Under sterile conditions, a 10-mm horizontal incision in the center of the back was made to create a subcutaneous pocket. A sterile Dacron® patch was implanted into this pocket. Skin was closed with sutures (Vicryl 5/0). Two days after Dacron® implantation, a saline solution (0.2 mL) containing 10^7 CFU of *S. aureus* was transcutaneously inoculated onto the graft surface. During inoculation, mice were anesthetized with isoflurane (0.8 L/min, 3%).

Antimicrobial treatment regimens

All the antibiotics used were administered at dose regimens resulting in serum concentrations similar to those obtained in humans. Mice were randomized into 14 groups. For MRSA: no treatment (controls); vancomycin group (subcutaneous injection (SC), 110 mg/kg/12 h);[16] daptomycin group (50 mg/kg/24 h, SC);[17] Rifampicin group (30 mg/kg/12 h, IP);[18] vancomycin-rifampicin group; daptomycin-rifampicin group; for MSSA: same groups + cloxacillin group (200 mg/kg/12 h, SC)[19] and cloxacillin-rifampicin group. Vancomycin and cloxacillin solutions were prepared in sterile 0.9% saline, daptomycin in sterile Ringer-Lactate and rifampicin in sterile 5% glucose serum. Mice were treated for 48 h and then euthanized following international guidelines.

Bacterial counts

Dacron® patches were removed under aseptic conditions, homogenized in 0.5 mL of saline buffer and vortexed during 30 seconds. Fifty microliters of this solution were used for quantitative bacterial cultures. The Dacron® patches were then sonicated (35kHz for 5 minutes) and 50 µL of the supernatant was inoculated for cultures on Tryptic Soy (TS) and Chapman agar plates, incubated at 37°C. The bacterial count was performed after 48 h of incubation. Spleens were also homogenized in 1 mL of saline buffer for bacterial cultures. Animals for which the spleen bacterial cultures were positive were considered to be bacteriemic. In case of a positive bacterial culture, *in vitro* drug susceptibility testings were performed using the Vitek 2 automated identification and susceptibility testing system with the Advanced Expert System (bioMerieux, Lyon, France), and interpreted according to the EUCAST criteria.

Statistical analysis

GraphPad prism software (version 6.0; GraphPad Software, San Diego, CA, USA) was used. Normally distributed data were analyzed using analysis of variance to compare the effects

between the different groups, followed by a Bonferroni's test to compare the groups two by two. $P < 0.05$ was considered to be statistically significant.

Results

In vitro efficacy of antibiotics in biofilms are influenced by the nature of the support

Confocal microscopy confirmed a biofilm formation for all strains on polystyrene (Figure 1a), or Dacron® (Figure 1b). However, biofilms formed on Dacron® sheets were less dense and thick as compared to those found with the modified CPBD.

MBIC and MBEC measures demonstrated a dramatic reduction of bacterial susceptibilities to all antibiotics tested, for all strains. dMBICs and dMBECs were also higher than MICs and MBCs for all strains tested. However, decreases of susceptibilities were less pronounced on Dacron® than on polystyrene, with the modified Calgary device (Table 1): for all the conditions evaluated, MBECs were much higher than dMBECs.

Daptomycin efficacy on MRSA vascular material infection is superior to comparators for one strain but not for others

There was no difference between bacterial cultures before and after Dacron® sonication. For MRSA BCB8, daptomycin was significantly more bactericidal than vancomycin with a dramatic reduction of bacterial load after a 48 h-treatment (- 5.85 $\log_{10}$ CFU/cm^2 as compared to the control group, $P < 0.001$, and - 3.47 $\log_{10}$ CFU/cm^2 as compared to vancomycin, $P < 0.001$), whether or not combined with rifampicin. The bactericidal activity of vancomycin was significantly improved when combined with rifampicin, although this combination remained less bactericidal than daptomycin alone on this isolate ($P < 0.001$). Rifampicin monotherapy demonstrated good efficacy (- 4.5 $\log_{10}$ CFU/cm^2 vs control group, $P < 0.001$)

(Figure 2). No emergence of antibiotic resistance was observed on bacteria recovered from positive cultures.

For MRSA 117 and 33591, results were strikingly different. For both strains, there were no significant differences between daptomycin, vancomycin and rifampicin monotherapies. Combined therapies demonstrated no significant benefit as compared to monotherapies (Figure 2). Although not statistically significant, there was a trend towards higher efficacy of vancomycin-rifampicin as compared to daptomycin-rifampicin. Once again, no antibiotic resistance was documented after treatment for both strains.

Spleen cultures were more frequently positive for controls than for therapeutic groups. There was no significant difference between therapeutic groups.

Daptomycin efficacy on MSSA vascular material infection depends also on bacterial strains

As for MRSA, bacterial cultures after sonication did not differ from those without sonication. Daptomycin was significantly more bactericidal than cloxacillin for MSSA 176 (– 1.27 $\log_{10}$ CFU/cm^2 (CI 95%: 0.32 to 2.22), $P < 0.05$), but not for MSSA 27217 and MSSA 171 (Figure 3). A trend towards better efficacy of daptomycin vs vancomycin on MSSA 176 (- 1.1 $\log_{10}$ CFU/cm^2; CI 95%: -0.16 to 2.37) was observed. Daptomycin and cloxacillin demonstrated higher efficacy than vancomycin for MSSA 171 (respectively – 2.72 $\log_{10}$ CFU/cm^2 (CI 95%: 0.75 to 3.79) and – 2.1 $\log_{10}$ CFU/cm^2 (CI 95%: 0.55 to 3.65); $P < 0.001$ for both). Combination of cloxacillin, vancomycin, or daptomycin, with rifampicin was more bactericidal than monotherapies for the 27217 strain. This effect was less pronounced for 171 and 176. Surprisingly, the association of daptomycin and rifampin was less efficient than cloxacillin-rifampicin (- 1.76 $\log_{10}$ CFU/cm^2 (CI 95%: 0.61 to 2.9); $P < 0.01$) or vancomycin-

rifampicin (- 2.17 $\log_{10}$ CFU/cm^2 (CI 95%: 1.1 to 3.25); $P < 0.001$), for MSSA 27217. No emergence of antibiotic resistance was documented for any of the conditions tested.

Spleen cultures were less often positive for MSSA than for MRSA, and no significant difference between the different conditions were noticed.

Discussion

The main findings of these experimental studies are: i) the results of the *in vitro* biofilm antibiotic susceptibility assays may vary according to the nature of the support used; ii) our mouse model of *S. aureus* vascular graft infection allowed us to test a large number of bacterial strains and antibiotic regimens and could pave the way toward a better understanding of antibiotic efficacy in PVGIs; iii) although more efficient than comparators for some bacterial strains, daptomycin was most of the time not superior to vancomycin or cloxacillin; and iv) combination of rifampicin and daptomycin did not enhance the bactericidal effect of daptomycin in this model.

Decrease of antibiotic efficacy in material-related infections has been highlighted by many studies using biofilm susceptibility tests, including the Calgary device.[8, 15, 20, 21] Although not recommended in routine,[22] those techniques illustrate that MBICs and MBECs are much higher than MIC and MBC, with limited perspective of clinical cure since these concentrations are not achievable in humans.[9, 21] However, those techniques use polystyrene devices, significantly different from biomaterials used for vascular prosthesis. Therefore, results yielded with those procedures may not be relevant for PVGIs. We developed a model to evaluate specific MBICs and MBECs on Dacron®, referred as dMBICs and dMBECs, to better assess the decrease of antibiotics efficacy on bacteria embedded inside the biofilm on vascular prostheses. In this model, while dMIBCs and MBICs were comparable, dMBECs

were lower than MBECs, although higher than MBCs. For instance, dMBICs and dMBECs of rifampicin were in the range of concentrations achievable in humans for most bacterial strains tested. Biofilm developed onto plastic was more dense, and thicker, than the one found on Dacron® (figure 1), and adhesion of bacteria onto the Dacron® appeared weaker, explaining why antibiotics are less efficient in the polystyrene model. Our results demonstrated that classical techniques used to measure MBICs and MBECs could overestimate the decrease of antibiotic efficacy in the particular context of PVGIs, and that our model, specific to PVGIs, could yield more relevant findings. They do not question the recommendations of removing all infected material whenever possible for PVGI.[23, 24] However, our data suggest that antibiotics alone may be a reasonable therapeutic option in selected cases when surgery would be associated with a high probability of severe adverse outcomes.[25-27]

Animal models constitute a critical step for the evaluation of antibiotics for PVGIs. A rat model of PVGI using a Dacron® patch implanted in a dorsal subcutaneous pouch evaluated different prophylactic procedures.[13, 28-30] Other models mimicked prosthetic-joint infections using a Teflon®-cage implanted in a dorsal subcutaneous pouch in Guinea-pigs,[31-35] or rats.[10] To our knowledge, no specific model evaluated the curative treatment of PVGI so far. We combined two approaches to evaluate different antibiotic regimens in a PVGI mouse model. For technical reasons, it was impossible to implant our Dacron® along the vascular system, and this represents one weakness of our work. Some authors described rabbit,[36] pig[14] or dog[11] models of aortic graft infections, but those models do not allow the use of a large number of animals, and consequently are not appropriate for the screening of antibiotic strategies on different bacterial strains. Moreover, in a clinical setting, most PVGIs occur from the wound or from an adjacent infectious focus, and not through hematogenous route.[37] Therefore, the infection process usually starts along the external part of the vascular prosthesis, not the endoluminal layer. Our model reproduces this pathway.

Although all antibiotics tested in this model demonstrated efficacy when compared to controls, antibiotic efficacy varied according to bacterial strains. For instance, daptomycin was more bactericidal than vancomycin for MRSA BCB8 and MSSA 171, and more bactericidal than cloxacillin for MSSA 176, but this was not superior to other antibiotics for other strains. For MRSA BCB8, dMBIC and dMBEC were lower for daptomycin than for vancomycin and this could partially explain the differences noticed. One other explanation could be the different capabilities of antibiotics to penetrate the biofilm *in vivo*., although this would not explain the striking differences observed between strains.[38] Daptomycin has already demonstrated better efficacy against MRSA than vancomycin *in vitro*[39, 40] and in animal models.[40, 41] This higher efficacy is thought to be linked to a better biofilm penetration for daptomycin than for vancomycin,[42] and a better bactericidal activity against bacteria in stationary phase.[40] However, some authors did not found any difference between daptomycin and vancomycin efficacy.[43, 44] Our results highlight a possible differential activity of daptomycin according to the bacterial strain, that may explain the discrepancies between previous studies.

Rifampicin has demonstrated its efficacy for the treatment of material-related staphylococcal infection[45] and its use in combination is largely recommended. In the present study, we did not found any improvement of therapeutic efficacy when rifampicin was added to daptomycin for MRSA, but rifampicin enhanced the activity of vancomycin. The combination of daptomycin and rifampicin increased only moderately the bactericidal activity of daptomycin for MSSA 27217, while the addition of rifampicin to vancomycin or cloxacillin strongly enhanced their bactericidal effect for this strain. These results are in contrast with those of Sakoulas *et al.*,[46] who demonstrated in a rat model of MRSA endocarditis a synergistic effect of daptomycin plus rifampicin. Saleh-Mghir *et al.* found similar results in a rabbit model of prosthetic joint infection.[47] However, an *in vitro* pharmacodynamic model evaluating

daptomycin and rifampicin against different MRSA strains found variable activity of this association (i.e. increased bactericidal activity with combinations in some, but not all strains tested.[48] Likewise, the addition of rifampicin to daptomycin did not enhance the bactericidal activity of daptomycin in a rabbit model of MRSA endocarditis.[49] Some studies suggested that daptomycin-fosfomycin or daptomycin-cloxacillin may be more synergistic.[10, 50]

Our model could not capture the utility of combinations to prevent the emergence of resistance. Indeed, even when bacterial load was high after treatment, we did not document any bacterial resistance. This was unexpected, since emergence of resistance under treatment is one of the main caveats with rifampicin,[41] or daptomycin monotherapies.[41, 47] An experimental study evaluating the *in vivo* fitness of rifampicin-resistant *S. aureus* mutants in a mouse biofilm infection model found that rifampicin-resistant strains appeared after 3 to 9 days of treatment.[18] This delay may explain why our 48 h-treatment regimens were not associated with emergence of resistance. Thus, although no resistance was documented in our model, clinical data indicate that rifampicin must be used in combination for PVGI, as for any other infection.

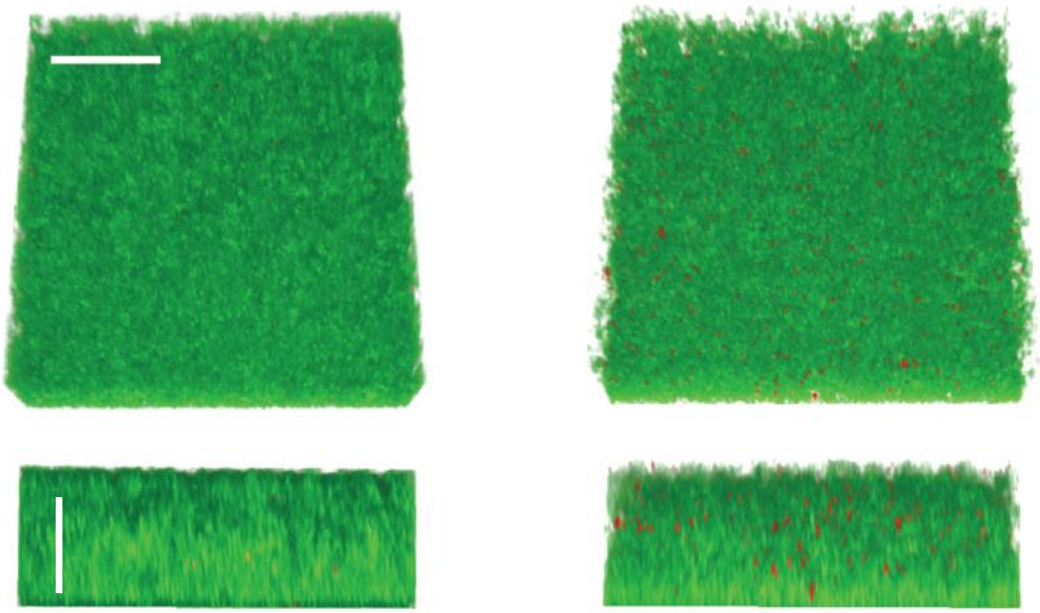
In conclusion, we found that biofilm formation and bacterial adhesion are weaker onto the Dacron® than onto polystyrene devices, resulting in a less pronounced biofilm-related decrease of antibiotic efficacy in the particular setting of PVGIs. We implemented an innovative mouse model of PVGI allowing the evaluation of a large number of antibiotic regimens. In this model, we demonstrated that daptomycin was more efficient than comparators for some strains but not all, and that the addition of rifampicin did not enhance daptomycin efficacy. However, due to the variability of findings according to bacterial strains, we were not able to determine the best antibiotic regimen for PVGIs.

Acknowledgements: The authors want to acknowledge the Collège des enseignants des Maladies Infectieuses et Tropicales (CMIT) and the Société Française de Pathologie Infectieuse de Langue Française (SPILF) for their support. They also want to acknowledge Prof Erwan Flecher who kindly provided the Dacron®.

Funding: This work was supported by a grant from Novartis and a grant from the Collège des enseignants des Maladies Infectieuses et Tropicales (CMIT). Sponsors had no role in the design and the realization of this work, and had no access to results until this work was submitted.

Transparency Declarations: Matthieu Revest has been supported by MSD and Pfizer laboratories to attend international conferences. The other authors have nothing to declare.

a



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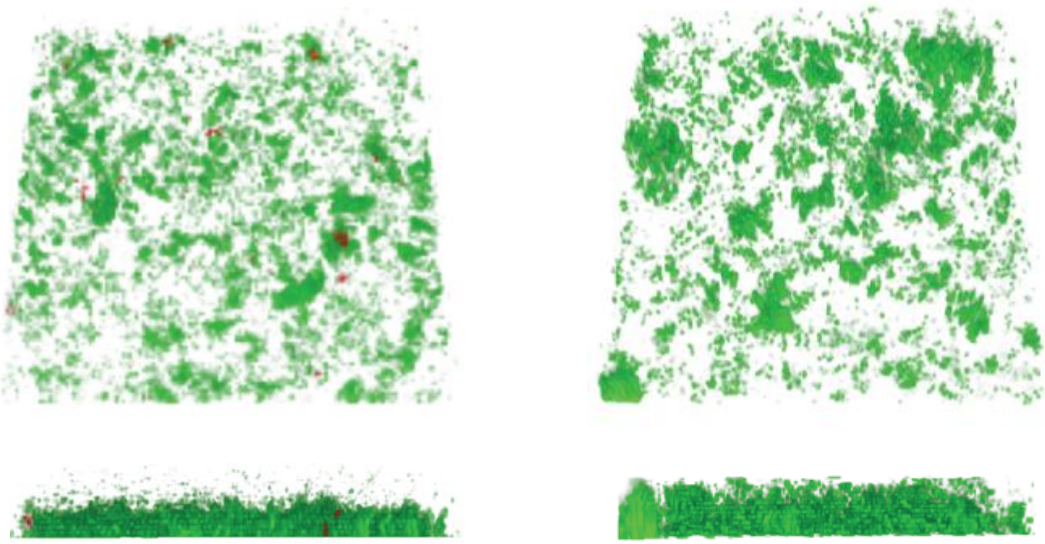

MSSA 27217 **MRSA BCB8**

Figure 1. Visualization of MSSA and MRSA 24h-biofilms on polystyrene (a) and on Dacron (b) using section views to observe biofilm thickness. All bacteria were stained green with Syto9®. The acquisition was performed on the whole biofilm thickness with an axial displacement of 1 µm. Images dimension is 82 x 82 µm². The scale bars correspond to 20 µm. Only biofilm for MSSA 27217 and MRSA BCB8 are represented since they were representative of all the biofilms visualized for other strains.

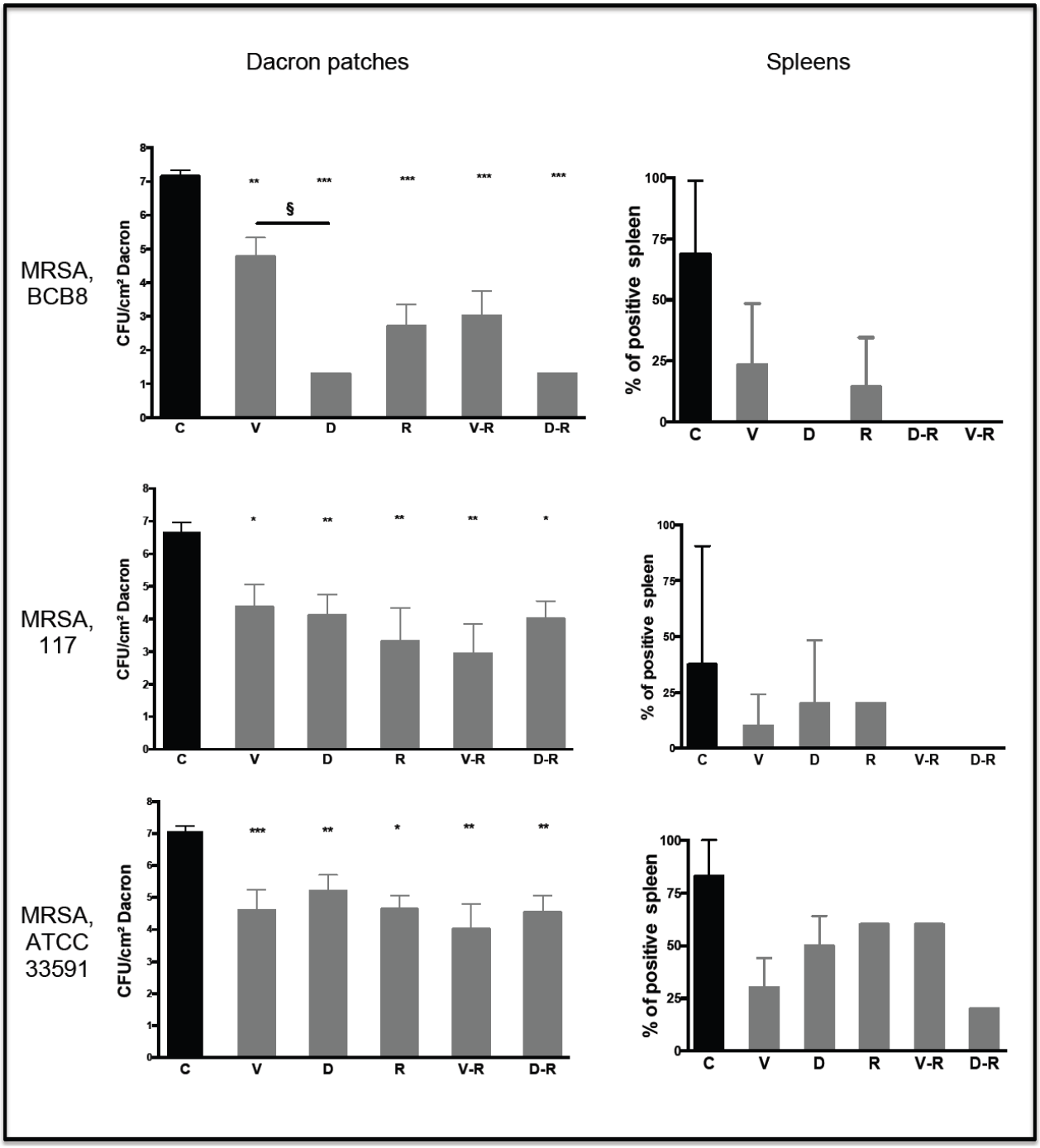

Figure 2. Results of bacterial count on Dacron patches and spleens after 48 h of treatment for MRSA infection.

C: controls; V: vancomycin; D: daptomcyin; R: rifampicin; V-R: vancomycin-rifampicin; D-R: daptomycin-rifampicin. CFU: Colony Forming Unit

Stars represent results of comparison between control groups and each therapeutic group.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. § $P < 0.001$, comparison between vancomycin and daptomycin for MRSA BCB8.

Number of mice per condition:

- BCB8: n= 12 per antibiotic regimen
- 117: n= 10 per antibiotic regimen
- 33591: n= 14 per antibiotic regimen

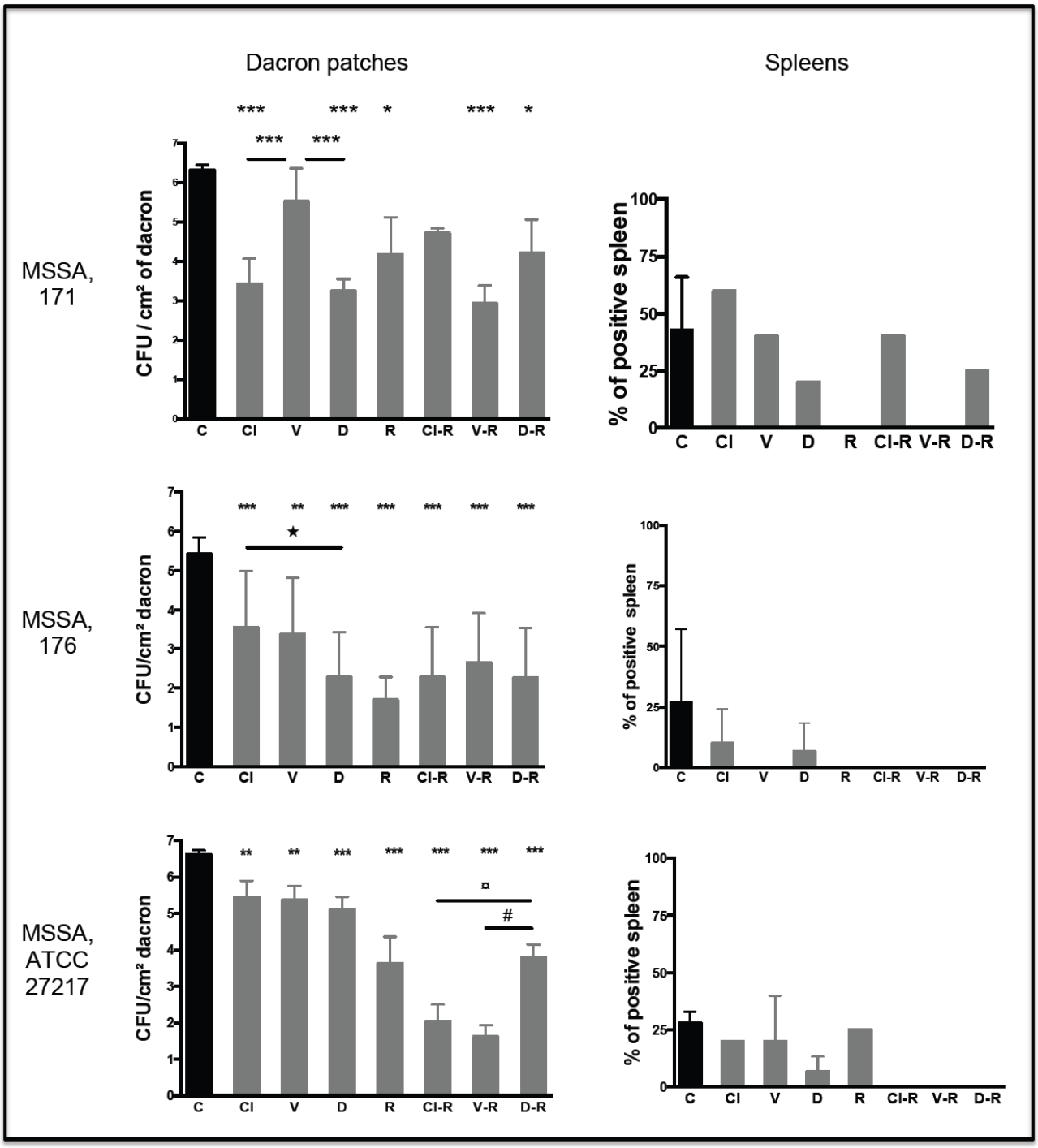

Figure 3. Results of bacterial count on Dacron sheets and spleen after 48 h of treatment for MRSA infection.

C: controls; V: vancomycin; D: daptomcyin; R: rifampicin; V-R: vancomycin-rifampicin; D-R: daptomycin-rifampicin. CFU: Colony Forming Unit

Stars represent results of comparison between control groups and each therapeutic group.

* $P < 0.05$; *** $P < 0.001$. $P < 0.05$, comparison between controls and daptomycin for MSSA 176. ¤ $P < 0.01$, comparison between cloxacillin-rifampicin and daptomycin-rifampicin for

MSSA 27217. # $P < 0.001$, comparison between vancomycin-rifampicin and daptomycin-rifampicin for MSSA 27217

Number of mice per condition:

- 171: n= 8 to 12 per antibiotic regimen
- 176: n= 10 to 15 per antibiotic regimen
- 27217: n= 12 to 15 per antibiotic regimen

Table 1. *In vitro* drug-susceptibility testing for the bacterial strains used in this study

Bacterial strains		Antibiotic	Classical methods		Biofilm on Dacron		Biofilm on polystyrene	
			MIC	MBC	dMBIC	dMBEC	MBIC	MBEC
MRSA	BCB8	Vancomycin	1	1	1	> 32	1	> 256
		Daptomycin	0.125	0.125	0.5	8	1	> 256
		Rifampicin	< 0.06	< 0.06	< 0.125	> 1	0,015	> 8
	117	Vancomycin	1	4	4	16	1	> 256
		Daptomycin	1	2	2	64	1	> 256
		Rifampicin	0.015	0.03	0.015	> 0.5	0,015	> 8
	33591	Vancomycin	1	2	4	16	2	> 256
		Daptomycin	0.25	0.5	2	> 16	1	> 256
		Rifampicin	0.0075	0.015	0.015	0.5	0,0075	> 8
MSSA	171	Cloxacillin	0.25	0.5	2	> 32	0,25	> 256
		Vancomycin	1	1	8	32	4	> 256
		Daptomycin	0.5	0.5	4	> 32	4	> 256
		Rifampicin	0.015	0.03	0.125	> 0.25	0,015	> 8
	176	Cloxacillin	0.5	0.5	2	> 32	0,5	> 256
		Vancomycin	1	1	2	16	1	> 256
		Daptomycin	0.5	0.5	2	> 64	0,5	> 256
		Rifampicin	0.015	0.03	0.125	> 0.5	0,015	> 8
	27217	Cloxacillin	0.5	0.5	1	16	0,25	> 256
		Vancomycin	1	1	8	> 32	8	> 256
		Daptomycin	0.25	0.25	1	> 8	2	> 256
		Rifampicin	< 0.06	0.125	2	2	0,03	> 8

MIC: Minimum inhibitory concentration; MBC: minimum bactericidal concentration; dMBIC: Dacron-related minimal biofilm inhibitory concentration; dMBEC: Dacron-related minimal biofilm eradicating concentration; MBIC: minimal biofilm inhibitory concentration; MBEC: minimal biofilm eradicating concentration

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