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

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Objective: Prosthetic vascular graft infection (PVGI) is an emerging disease, mostly caused by 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 MSSA and 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) with those yielded by an original Dacron®-related minimal inhibitory and eradicating concentration measure model. We then used a murine model of *Staphylococcus aureus* vascular prosthetic material infection to evaluate efficacy of different antibiotic regimens: vancomycin and daptomycin combined or not with rifampicin for MRSA and the same groups with cloxacillin and cloxacillin combined with rifampicin for MSSA.

Results: We demonstrated that classical measures of MBICs and MBECs obtained with a CPBD could overestimate the decrease in 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 with 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 the *in vitro* efficacy of antibiotics on Dacron®-related biofilm *S. aureus* infections, and to screen different antibiotic regimens in a mouse model of PVGIs.

Introduction

More than 400 000 vascular grafts are inserted annually in the USA[1] and ~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 1 year mortality rates of 10%–25% and 50%, respectively.[3] *Staphylococcus aureus* is the main pathogen, and 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 development on the vascular prosthesis plays a significant role in the difficulties encountered when 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 in susceptibility to most antibiotics.[6,7] Many studies showed that minimal biofilm inhibitory concentrations (MBICs) and minimal biofilm eradicating concentrations (MBECs) of antibiotics are much higher than their respective MICs, and their MBCs as measured in 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 against 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 MSSA and MRSA *in vitro*. Those activities were determined against planktonic bacteria (MIC and MBC) and adherent bacteria (MBIC and MBEC). To evaluate whether the nature of the support influences MBIC and MBEC results, two different techniques were used: a widely used modified version of the Calgary Biofilm Pin Lid Device (CBPD) and an original model of Dacron®-related biofilm. Then, cloxacillin, vancomycin and daptomycin, combined or not with rifampicin, were evaluated in a new mouse model of vascular prosthetic material infection, 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 we used two clinical strains, hereafter 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 we used two strains, hereafter 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 stock solutions were prepared in sterile, 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 (CBPD) and Dacron® using confocal microscopy.

MICs and MBCs

The MIC and the MBC values for cloxacillin, vancomycin, daptomycin and rifampicin were determined by the broth macrodilution method in CAMHB, according to 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 microtitre lid into wells of a flat-bottom 96-well microtitre plate. Each well was filled with 150 μL of a 3 McFarland *S. aureus* broth medium solution. After 24 h of incubation at 37°C, peg lids were rinsed three times in sterile water, placed onto flat-bottom microtitre plates containing 2-fold dilutions of antibiotic in 150 μL of CAMHB per well, and incubated for 24 h at 37°C. The MBIC was defined as the minimal concentration of antibiotic inhibiting bacterial growth, as determined by reading the turbidity of the medium at 650 nm. Pegs with no bacterial growth were again rinsed three times with sterile water and sonicated (Aquasonic sonicator, 35 kHz for 5 min) followed by vortexing for 30 s 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 Dacron®-related minimal biofilm eradicating concentrations (dMBECs)

Commercially available woven Dacron® grafts (Cardial™, Bard, Saint-Etienne, France) were cut into 1 cm×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 three 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 cfu/mL of *S. aureus*. Again, they were rinsed three times in sterile water to eliminate planktonic bacteria, dried with a sterile gauze compress and plunged into tubes filled with CAMHB containing no antibiotic (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 three times in sterile water, dried with a sterile gauze compress and plunged again into MHB. Sonication (35 kHz for 5 min) was performed followed by vortexing for 30 s. MHB containing the Dacron® sheets was incubated for 24 h at 37°C. dMBEC was defined as the antibiotic concentration of the first tube with no bacterial growth.

Confocal microscopy evaluating biofilm formation depending on the support

Twenty-four hour 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 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 ×63 oil immersion objective with a numerical aperture of 1.4. The size of the confocal images was 512×512 pixels (82×82 μm^2), recorded with a z-step of 1 μm and a ×3 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 animal ethics committee and animals were cared for in line with national guidelines.

Animals

Four-week-old female Swiss mice (RjOrl/SWISS, Janvier Laboratory, St Berthevin, France) weighing ~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 from healthy female Swiss mice for 24 h at 37°C. Then, they were implanted into mice.

Surgical procedures

Mice were anaesthetized with ketamine (70 mg/kg) and xylazine (10 mg/kg) through intraperitoneal (ip) injection. Under sterile conditions, a 10 mm horizontal incision in the centre 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 anaesthetized 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 the groups were as follows: no treatment (controls); vancomycin group [subcutaneous (sc) injection, 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; and daptomycin/rifampicin group. For MSSA we used the same groups plus a cloxacillin group (200 mg/kg/12 h, sc)[19] and a cloxacillin/rifampicin group. Vancomycin and cloxacillin solutions were prepared in sterile 0.9% saline, daptomycin in sterile Ringer–lactate solution 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 for 30 s. Fifty microlitres of this solution was used for quantitative bacterial cultures. The Dacron® patches were then sonicated (35 kHz for 5 min) and 50 μL of the supernatant
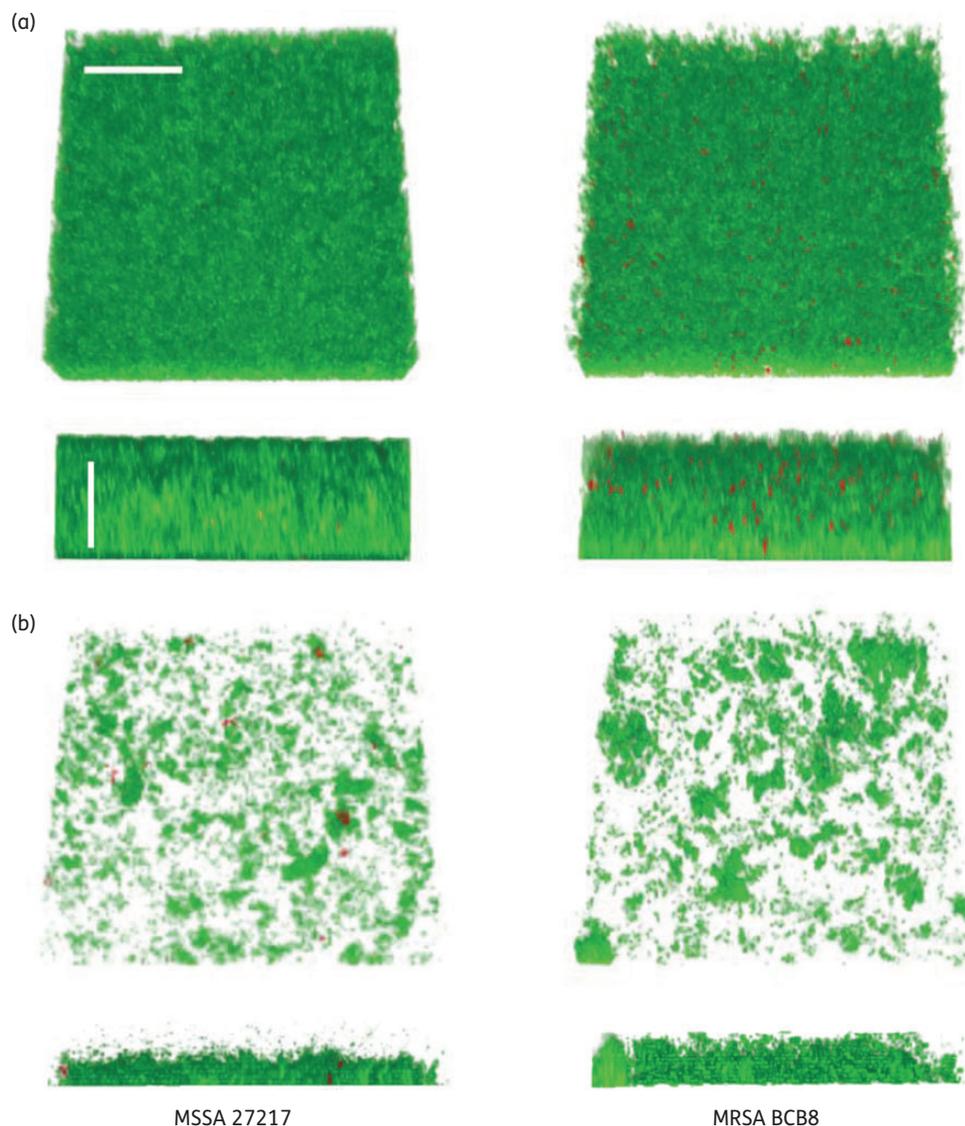

(a)

(b)

MSSA 27217   MRSA BCB8

Figure 1. Visualization of MSSA and MRSA 24 h biofilms on polystyrene (a) and on Dacron (b) using section views to observe biofilm thickness. All bacteria were stained green with Syto9®. Acquisition was performed on the whole biofilm thickness with an axial displacement of 1 μm. Image dimension is 82×82 μm. Scale bars correspond to 20 μm. Only biofilms for MSSA 27217 and MRSA BCB8 are represented since they were representative of all biofilms visualized for other strains. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

was inoculated for cultures on tryptic soy 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 bacteraemic. When there was a positive bacterial culture, *in vitro* drug susceptibility testing was performed using the Vitek 2 automated identification and susceptibility testing system with the Advanced Expert System (bioMérieux, Lyon, France), and results were 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 analysed using analysis of variance to compare effects between the different groups, followed by 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 is influenced by the nature of the support

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

MBIC and MBEC measures demonstrated a dramatic reduction in 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 in susceptibilities were less

pronounced on Dacron® than on polystyrene, with the modified Calgary device (Table 1); for all conditions evaluated, MBECs were much higher than dMBECs.

Daptomycin efficacy on MRSA vascular prosthetic 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 48 h of treatment (-5.85 log$_{10}$ cfu/cm^2 compared with the control group, $P < 0.001$; -3.47 log$_{10}$ cfu/cm^2 compared with vancomycin, $P < 0.001$), whether or not it was combined with rifampicin. The bactericidal activity of vancomycin was significantly improved when combined with rifampicin, although this combination remained less bactericidal than daptomycin alone against this isolate ($P < 0.001$). Rifampicin monotherapy demonstrated good efficacy (-4.5 log$_{10}$ cfu/cm^2 versus 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 compared with monotherapies (Figure 2). Although not statistically significant, there was a trend towards higher efficacy of vancomycin/rifampicin compared with daptomycin/rifampicin. Once again, no antibiotic resistance was documented after treatment for both strains.

Table 1. *In vitro* drug susceptibility testing for the bacterial strains used in this study (mg/L)

| 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
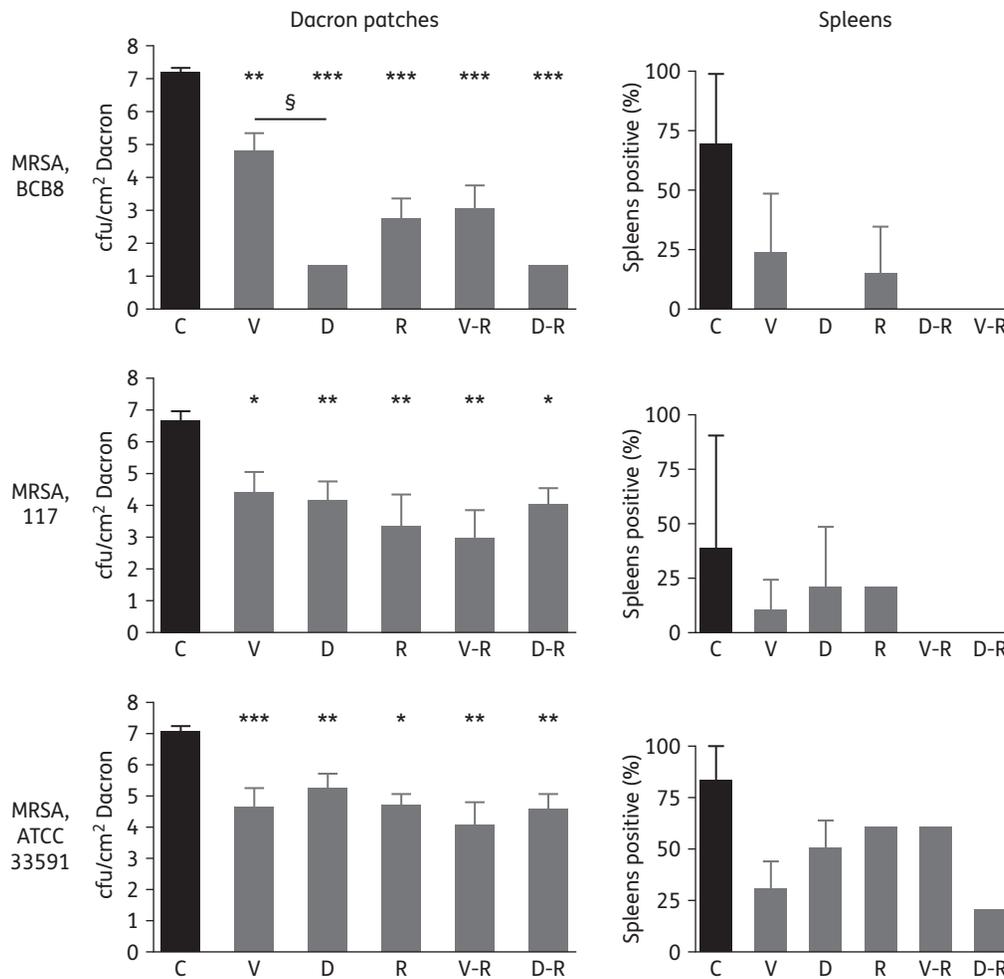
Figure 2. Bacterial counts on Dacron patches and spleens after 48 h of treatment for MRSA infection. C, controls; V, vancomycin; D, daptomycin; R, rifampicin; V-R: vancomycin/rifampicin; D-R, daptomycin/rifampicin. Asterisks represent results of comparisons between control groups and each therapeutic 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 antibiotic regimen: strain BCB8, 12; strain 117, 10; and strain 33591, 14.

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

Daptomycin efficacy against MSSA vascular prosthetic material infection also depends 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, 95% CI 0.32–2.22, $P<0.05$), but not for MSSA 27217 and MSSA 171 (Figure 3). A trend towards better efficacy of daptomycin versus vancomycin against MSSA 176 (-1.1 $\log_{10}$ cfu/cm^2, 95% CI -0.16 to 2.37) was observed. Daptomycin and cloxacillin demonstrated higher efficacy than vancomycin against MSSA 171 [-2.72 $\log_{10}$ cfu/cm^2 (95% CI 0.75–3.79) and -2.1 $\log_{10}$ cfu/cm^2 (95% CI 0.55–3.65), respectively; $P<0.001$ 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 strains 171 and 176. Surprisingly,

the combination of daptomycin with rifampicin was less efficient than cloxacillin/rifampicin (-1.76 $\log_{10}$ cfu/cm^2, 95% CI 0.61–2.9, $P<0.01$) or vancomycin/rifampicin (-2.17 $\log_{10}$ cfu/cm^2, 95% CI 1.1–3.25, $P<0.001$) 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 was noticed.

Discussion

The main findings of these experimental studies are: (i) the results of *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 towards 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
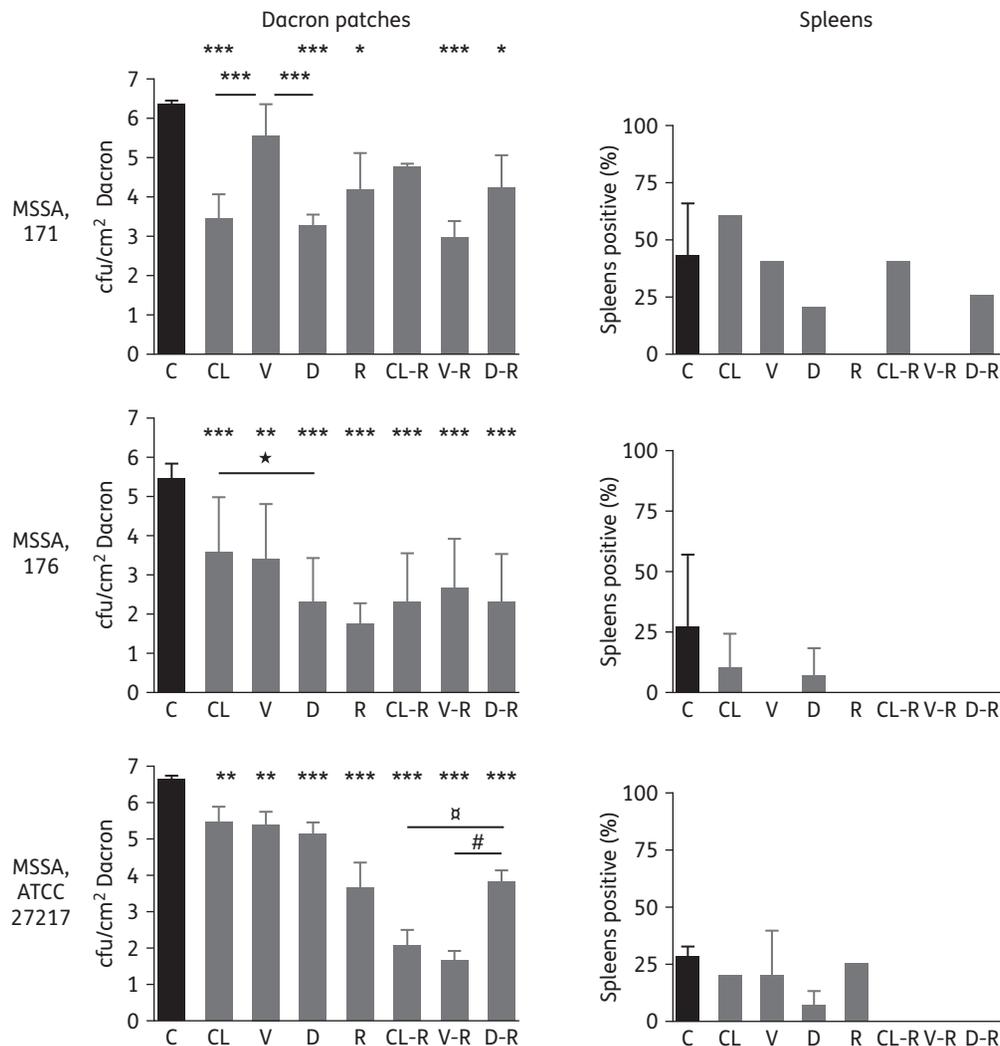
Figure 3. Bacterial count on Dacron sheets and spleen after 48 h of treatment for MRSA infection. CL, cloxacillin; C, controls; V, vancomycin; D, daptomycin; R, rifampicin; V-R, vancomycin/rifampicin; D-R, daptomycin/rifampicin. Asterisks represent results of comparisons between control groups and each 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 antibiotic regimen: strain 171, 8–12; strain 176, 10–15; strain 27217, 12–15.

to vancomycin or cloxacillin; and (iv) combination with rifampicin did not enhance the bactericidal effect of daptomycin in this model.

Decreased 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 routinely,[22] these techniques illustrate that MBICs and MBECs are much higher than MICs and MBCs, with limited prospects for clinical cure since these concentrations are not achievable in humans.[9,21] However, these techniques use polystyrene devices, which are significantly different from the biomaterials used for vascular prostheses. Therefore, results obtained with these 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 in antibiotic efficacy on bacteria embedded inside the biofilm on vascular prostheses. In this model, while dMBICs 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 on plastic was more dense, and thicker, than that found on Dacron® (Figure 1), and adhesion of bacteria to 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 in 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 recommendation to remove 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 in 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 has evaluated the curative treatment of PVGI so far. We combined these 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 have described rabbit,[36] pig[14] or dog[11] models of aortic graft infections, but these models do not allow the use of a large number of animals, and consequently are not appropriate for the screening of antibiotic strategies in different bacterial strains. Moreover, in a clinical setting, most PVGIs arise from the wound or from an adjacent infectious focus, and not through a haematogenous 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 with 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 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 better biofilm penetration for daptomycin than for vancomycin[42] and better bactericidal activity against bacteria in stationary phase.[40] However, some authors did not find any difference between daptomycin and vancomycin efficacy.[43,44] Our results highlight a possible differential activity of daptomycin according to bacterial strain, which 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 widely recommended. In the present study, we did not find 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 the bactericidal activity of daptomycin against MSSA 27217 only moderately, while the addition of rifampicin to vancomycin or cloxacillin strongly enhanced their bactericidal effect on 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.[47] found similar results in a rabbit model of prosthetic joint infection. 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 3–9 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 on Dacron® than on polystyrene devices, resulting in a less pronounced biofilm-related decrease in 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.

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