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Effectiveness of vancomycin plus cloxacillin compared to vancomycin, cloxacillin, and daptomycin single therapies in the treatment of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in a Rabbit Model of Experimental Endocarditis.

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Running Title: *In vitro* and i*n vivo* vancomycin plus cloxacillin synergy against MRSA, GISA and MSSA strains.

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Synopsis

Objectives: To investigate if the addition of cloxacillin to vancomycin enhances the activity of both monotherapies for treating methicillin-susceptible *Staphylococcus aureus* and methicillin-resistant *S. aureus* experimental endocarditis (EE) in rabbits.

Methods: Vancomycin plus cloxacillin was compared with the respective monotherapies and daptomycin. *In vitro* time-kill studies were performed using standard (10^5) and high (10^8) inocula of five MRSA, one glycopeptide-intermediate (GISA) and five MSSA strains. One MSSA (MSSA-678) and one MRSA (MRSA-277) strains were selected to be used in the *in vivo* model. A human-like pharmacokinetics model was applied and the equivalents of cloxacillin 2g/4h iv and daptomycin 6mg/kg/d iv were administered. To optimize vancomycin activity, dosage was adjusted to achieve an AUC/MIC ≥400.

Results: Daptomycin sterilized significantly more vegetations than cloxacillin (13/13, 100% versus 9/15, 60%; *P*=0.02) and showed a trend of better activity than vancomycin (10/14, 71%; *P*=0.09) and vancomycin plus cloxacillin (10/14, 71%; *P*=0.09) against MSSA-678. Addition of cloxacillin to vancomycin (13/15, 87%) was significantly more effective than vancomycin (8/16, 50%; *P*=0.05) and showed similar activity to daptomycin (13/18, 72%; *P*=0.6) against MRSA-277. In all treatment's arms, the bacterial isolates recovered from vegetations were re-tested and showed the same daptomycin susceptibility as the original strains.

Conclusions: Vancomycin plus cloxacillin proved synergistic and bactericidal activity against MRSA. Daptomycin was the most efficacious option against MSSA and similar to vancomycin plus cloxacillin against MRSA. In settings with high MRSA prevalence, vancomycin plus cloxacillin might be a good alternative for empirical therapy of *S. aureus* IE.

Introduction

Staphylococcus aureus is the leading cause of infective endocarditis (IE) worldwide.[1] Current IE guidelines by the European Society of Cardiology (ESC) and the American Heart Association (AHA) recommend an anti-staphylococcal beta-lactam (nafcillin, cloxacillin or cefazolin) as the treatment of choice for MSSA native valve IE while vancomycin is the treatment of choice for MRSA IE.[2,3]

Vancomycin has been shown to be less effective than anti-staphylococcal beta-lactam therapies in the treatment of MSSA bacteremia[4-6]and IE.[7] Furthermore, albeit vancomycin being the treatment of choice for MRSA bacteremia and IE for more than forty years, several studies have demonstrated increasing failure rates over this period.[8-10]A large meta-analysis examining the results of the studies addressing the impact of vancomycin MIC on MRSA bacteremia and IE concluded that alternatives to vancomycin should be considered in cases of vancomycin MIC ≥2.0 mg/L by Etest.[11] Targeting at achieving an area under the concentration-time curve from 0 to 24h (AUC_{24})/MIC ratio of ≥400 and vancomycin trough >15 mg/L has been associated to improved clinical outcomes.[12-16]Unfortunately, achieving these targets requires high doses of vancomycin, which are not free of side-effects. Notably, higher doses are associated with greater risk of nephrotoxicity.[17,18]

The combination of vancomycin and beta-lactams has been reported to be synergistic against MRSA,[19,20]vancomycin-intermediate *S. aureus*,[21,22]and vancomycin-resistant *S. aureus*[23,24]in experimental models of IE, and initial clinical studies showed encouraging results.[25-27]Regrettably, the CAMERA2 trial recently showed no differences between vancomycin and daptomycin monotherapies compared to their respective combinations with anti-staphylococcal beta-lactams in the treatment of

MRSA bacteremia.[28] There is no evidence, either experimental or clinical, of the activity of vancomycin plus beta-lactams for treating MSSA IE.

The aim of this study was to evaluate if the addition of cloxacillin to vancomycin enhanced its activity against MSSA or MRSA in an animal model of experimental aortic IE. In addition, we compared the activity of this combination with daptomycin monotherapy.

Material and Methods

Bacterial isolates

For *in vitro* studies, five MSSA (MSSA-143, MSSA-175, MSSA-678, MSSA-679 and MSSA-706), five MRSA (MRSA-196, MRSA-277, MRSA-513, MRSA-726 and MRSA-835) and one GISA strain (ATCC700788) isolates were selected. Except for the ATCC collection strain, the rest of them had been isolated from blood cultures of patients diagnosed with IE at our institution. MSSA-678 and MRSA-277 were selected for the *in vivo* studies. The isolates were stored at -80º C in skim milk.

Antimicrobial agents

Daptomycin powder was supplied by Cubist Pharmaceuticals (Lexington, MA, USA) and by MSD (Spain), vancomycin and cloxacillin were purchased from Sigma (St Louis, MO). Drugs were prepared according to the manufacturers' recommendations.

Susceptibility Testing

MICs and MBCs were determined using the broth microdilution method according to standard recommendations.[29] For daptomycin, broth was supplemented with Ca^{2+} to 50 mg/L according to the manufacturer's recommendations. *S. aureus* ATCC 29213 was used as the test control strain. All assays were performed in duplicate. The *in vitro* MRSA MICs/MBCs results have been described elsewhere.[30]

Synergy studies

Time-kill methodology was used to test the activity of combined antibiotics according to previously described criteria.[31] Two different initial inocula were tested: an initial standard inoculum (ISI) of 10^5colony forming units (cfu)/mL and an initial higher inoculum (IHI), to mimic the density of cfu in mature infected vegetation, equal to 10^8cfu/mL.[31,32] For synergy testing, concentration equal to 1 x MIC was chosen for vancomycin and cloxacillin. For the MRSA strains, due to its resistance to cloxacillin, concentrations equal to 64 mg/L or 16 mg/l (equivalent to 0.125 x MIC and 1 x MIC respectively) were used. Synergy was defined as a 2-$\log_{10}$ decrease in the number of cfu/mL between the test tube with the combination and the test tube with the most active agent alone after 24 hours: the number of surviving organisms in the presence of the combination had to be 2 $\log_{10}$ cfu/mL below the starting inoculum. Bactericidal activity was defined as at least a 3-log reduction in cfu at 24h in comparison with the initial inoculum. All experiments were performed in duplicate.

Study animals

Female New Zealand white rabbits (body weight, 2.5 kg) provided by San Bernardo farm (Pamplona, Spain) were used. Housing took place in the animal facilities of the University of Barcelona, School of Medicine, which is equipped with high-efficiency particulate air filter in an automatic air exchange system, as well as circadian light cycle. They were nourished *ad libitum.* The Committee of Animals Ethics of the University of Barcelona approved all animal experimentation in this study.

Human pharmacokinetics (PK) simulation studies

The *in vivo* experimental pharmacokinetics of cloxacillin, vancomycin and daptomycin were described elsewhere.[30,32] Antibiotics were administered using a computer-controlled infusion pump system designed to reproduce human serum pharmacokinetics

in rabbits after an iv infusion. Animal infusion rates were chosen to simulate the human pharmacokinetic profile of vancomycin at two different doses (adjusted to an AUC_{24}/MIC ratio of ≥ 400).[15]Cloxacillin (2g/4h iv) and daptomycin (6 mg/kg iv once daily) regimens were administered, following the recommendations of the AHA[5] guidelines.

Endocarditis model

The experimental aortic valve IE model was induced according to the method described by Garrison and Freedman.[33]Briefly, a catheter was inserted through the right carotid artery into the left ventricle of anaesthetized rabbits; the catheter used for antibiotic administration was placed into the inferior vena cava through the jugular vein.[30]The infusion pump delivered 2 mL/h of 0.9% saline solution until the beginning of antimicrobial administration. Twenty-four hours later, each animal was inoculated via the marginal ear vein with either the MSSA-678 or MRSA-277 strain (1 mL of 5.5 x 10^5cfu/mL). Before initiation of the antimicrobial therapy, one milliliter of blood was obtained to confirm bacteremia. Antibiotic treatments were started and animals were treated for 48 hours using a computer controlled pump. After completion of the treatment, six additional half-lives of the antibiotics were left to elapse, allowing for the growth of residual viable bacteria in the endocardial vegetations. After this, the animals were sacrificed (anesthetized and euthanized using an intravenous bolus of pentobarbital). Aortic valve vegetations were obtained, weighed, homogenized in 2 mL of saline solution, and quantitative and qualitative cultures were performed.

Treatment group

The infected rabbits were separated into the different treatment arms simulating human pharmacokinetics. Monotherapies: vancomycin high dose (HD), 1.25g/8h and 1 g/6h for the MSSA-678 and MRSA-277 strains, respectively; cloxacillin 2g/4h; daptomycin 6

mg/kg/d. Combined therapy: vancomycin 1 g/8h plus cloxacillin 2 g/4h. Each group included 13 to 18 animals.

Analysis of endocardial vegetations

The cfu counts recovered from vegetations were expressed as the number of $\log_{10}$cfu per gram of vegetation ($\log_{10}$cfu/g veg.). The result was assigned a value of zero and the vegetation was considered sterile if there was no growth from the initial quantitative and qualitative cultures and from the homogenates cultured for a week. The result was assigned a value of 2 $\log_{10}$cfu/g veg. if there was no growth on the quantitative plates and growth in the qualitative culture and from the homogenates cultured for a week. All the isolates recovered from vegetations were stored, and their MICs re-tested to detect *in vivo* emerging resistance.

Statistical analysis

The results were expressed as the median and the interquartile range (IQR) of the number of $\log_{10}$cfu/g veg. The Mann Whitney non-parametric test was used to compare the $\log_{10}$cfutissue values among the different treatment groups. The Fisher exact test was used to compare the rate of sterilized vegetations and analyze whether there were differences between treatment groups.

Results

Susceptibility testing

For all MSSA strains, the MICs/MBCs for cloxacillin, vancomycin and daptomycin results are summarized in **Table 1**. All strains were susceptible to daptomycin and vancomycin (apart from the GISA strain).

In vitro synergy study.

After 24 h of incubation with vancomycin plus cloxacillin, synergistic and bactericidal activity was observed in all MRSA strains at ISI including the GISA strain (Figure 1A), When the IHI was used, all the strains lost synergistic activity and bactericidal effect, except for the GISA strain that retained the synergy activity (Figure 1B). For detailed data see supplementary Table S1. Against MSSA strains when ISI were used, the vancomycin plus cloxacillin combination showed synergistic and bactericidal activity against four of them (Figure 2A). None of the strains retained the synergy either the bactericidal activity with the IHI (Figure 2B). For detailed data see supplementary Table S2.

Human PK simulation studies

The mean maximum concentration (C_{max}) and trough concentration (C_{min}) achieved were: cloxacillin 150/1 mg/L for a 2 g/4h simulated dose, vancomycin 96/17 mg/L for a 1.25g/8h simulated dose; in MRSA-277, they were vancomycin 60/20 mg/L for a 1 g/6h simulated dose and daptomycin 86/15 mg/L for a single dose of 6 mg/Kg/d.

Treatment of established endocarditis

The effectiveness of drugs in monotherapy and combined therapy is shown in Figure 3 and Table S3. All control rabbits had infected aortic valve vegetations, with a median

bacterial titer equal to 9 $\log_{10}$ cfu/g veg. for both strains (detailed data [median and interquartile ranges for each group] in Table S3).

For MSSA-678, daptomycin (13/13, 100% sterilization) was significantly more active than cloxacillin (9/15, 60%; *P*=0.02). Compared with vancomycin (10/14, 71%), daptomycin showed a trend of higher activity but without achieving statistical significance (*P* = 0.09). Vancomycin plus cloxacillin displayed the same activity (10/14, 71%) than vancomycin alone (*P*=0.09). No differences were observed between treatment groups regarding the decrease in microorganisms' density in the vegetations.

For MRSA-277, the combined therapy of vancomycin plus cloxacillin significantly improved the efficacy of vancomycin alone (13/85, 87% versus. 8/16, 50%; *P*=0.05) and was as effective as daptomycin (13/85, 87% versus. 13/18, 72%; *P*=0.6).

Daptomycin activity was higher against MSSA-678 than it was against MRSA-277 (100% versus 72% sterilization rate, respectively).

None of the recovered isolates from vegetations exhibited a decrease in daptomycin susceptibility.

Discussion

Our study provides valuable experimental insight with potentially relevant clinical implications. Overall, our findings reveal notable differences in the activity of the combination of vancomycin plus cloxacillin against MSSA and MRSA strains: With the pharmacokinetic/pharmacodynamic (pK/pD) model of MRSA IE, we found that the combination of vancomycin and cloxacillin was more effective than vancomycin in monotherapy. Administered alone, vancomycin activity was poor despite AUC/MIC index and trough levels reaching the target thresholds for a vancomycin MIC of 2 mg/L.

Dilworth *et al.* tested *in vitro* the efficacy of vancomycin in combination with oxacillin against 14 MRSA and four VISA strains, being synergistic and significantly more active than vancomycin alone.[20] Also, Climo *et al.* found synergism *in vivo* between vancomycin and cloxacillin against more than half of 59 MRSA strains, and it correlated directly with vancomycin MICs, showing synergistic activity related to the selective killing of the most vancomycin-resistant subpopulations.[21] In a clinical study comparing microbiological eradication in MRSA bacteremia between patients treated with vancomycin alone or with piperacillin-tazobactam, the combination showed increased efficacy.[25] Contrarily, in the CAMERA1 and CAMERA2 trials comparing clinical outcomes of vancomycin alone or combined with betalactams, no significant differences between the two strategies were found.[28]

In our pK/pD model of MSSA IE, cloxacillin showed similar *in vivo* efficacy than vancomycin adjusted dose to AUC_{24}/MIC ration of ≥400 (1.25g /8h), while the combination of vancomycin plus cloxacillin showed similar activity to that of vancomycin monotherapy. Similarly, the lack of *in vivo* synergism between vancomycin and betalactams against MSSA was also observed by Leonard et al.[19]

The mechanism of synergy between vancomycin and beta-lactams has been broadly studied in MRSA, where the addition of an anti-staphylococcal penicillin not only diminishes cell wall width but also increases neutrophils and cationic host defense peptides, attenuating MRSA virulence.[34,35] Also, it has been hypothesized that the synergism between vancomycin and beta-lactams against MRSA may be related to the substrate specificity of PBP. Thus, the absence of PBP 2A in MSSA could explain the lack of synergy we found against MSSA.

Daptomycin monotherapy showed good activity, especially for MSSA, against which sterilized all the vegetations at 6mg/kg. Notably, when this study in was

performed, the recommended dose of daptomycin in clinical practice was 6 mg/kg/d, accordingly to the first publications.[36] Similarly, Jacqueline *et al.* found high bactericidal *in vivo* activity (reduction of >5 log_{10}cfu/g veg.) with the same dosage against MSSA in the rabbit IE experimental model, but it sterilized only 5/8 animals.[37] Using the rat IE model, Nannini *et al.* recently assessed the activity of nafcillin, cefazolin and daptomycin against a type-A beta-lactamase producing MSSA strain, with daptomycin being the antibiotic that showed the best activity (reduction of 7.1 log_{10}cfu/g veg.).[38] Regarding MRSA, although we found better activity with daptomycin than with vancomycin, it did not significantly sterilize more vegetations than vancomycin and cloxacillin combined. Also noteworthy was that we did not observe the development of daptomycin non-susceptibility in either MSSA or MRSA recovered from vegetations of rabbits treated with daptomycin.

This study has several limitations. First, we studied *in vivo* the activity of the antibiotics against only one strain of MRSA or MSSA. This limits the external reproducibility of our findings. Second, we did not assess changes in cloxacillin or vancomycin MICs after treatment. Third, doses of daptomycin in the assays were lower (6 mg/kg) than those currently recommended (10 mg/kg) in clinical practice. However, this was the most active antibiotic in the MSSA EE model and as active as vancomycin plus cloxacillin against MRSA EE. Notably, equivalent vancomycin dosages required for achieving an AUC_{24}/MIC $\geq$400 or trough levels $\geq$15 mg/L were very high and associated with an unacceptable risk of nephrotoxicity in the clinical setting.[27,28] Finally, we did not assess the efficacy of combining daptomycin plus cloxacillin, which might be efficacious against both MSSA and MRSA. However, when translating our findings into clinical practice, it appears that, whereas vancomycin plus cloxacillin might not be amongst the preferred options for the definite treatment of either MSSA or MRSA IE, it

285 may indeed have a major role as empirical treatment, until antibiotic susceptibilities are
286 available, particularly in settings with high MRSA prevalence where daptomycin or
287 other reliable alternatives are not available. Further studies assessing the efficacy this
288 combination against a larger number of *S. aureus* strains and comparing it with higher
289 doses of daptomycin alone and combined with beta-lactams are warranted.

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467

Table 1. MSSA and MRSA strains tested and corresponding MIC/MBC ratios for vancomycin, cloxacillin and daptomycin.

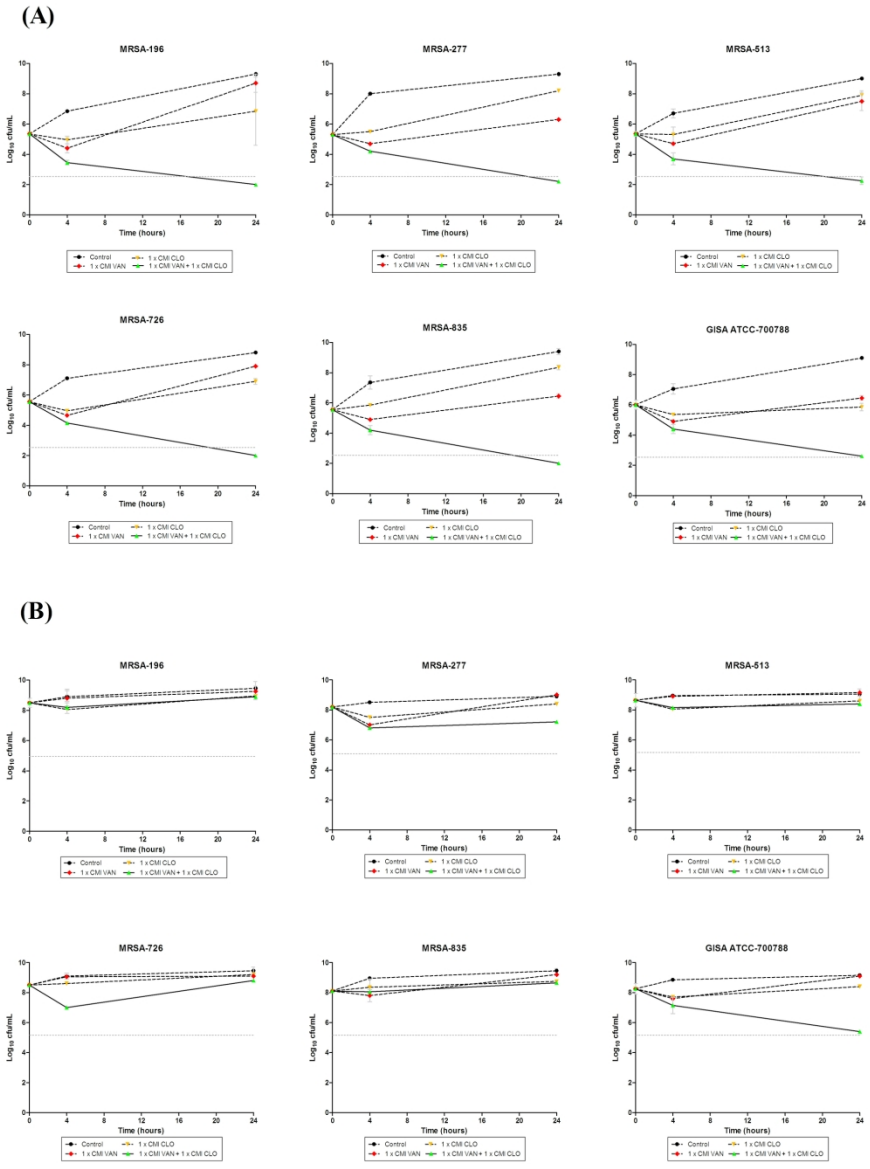
	MIC/MBC (mg/L)		
Strain[a]	**Vancomycin**	**Cloxacillin**	**Daptomycin**
MSSA			
MSSA-143	2/4	0.25/0.5	0.5/1
MSSA-175	1/2	0.5/0.5	0.5/0.5
MSSA-678[a]	1/2	0.25/0.5	0.5/0.5
MSSA-679	1/2	0.25/0.5	0.25/0.5
MSSA-706	0.5/1	0.5/0.5	0.5/0.5
MRSA			
MRSA-196	0.5/8	16/64	0.25/0.5
MRSA-277[a]	2/2	512/512	0.5/0.5
MRSA-513	1/128	512/512	0.5/0.5
MRSA-726	0.5/0.5	16/64	0.5/0.5
MRSA-835	1/16	128/256	0.5/0.5
ATCC700788[b]	8/128	64/512	0.5/1

[a]*In vivo* study strains; [b]GISA strain. Breakpoints susceptibility testing according to the EUCAST standard MIC breakpoints: Vancomycin **S** ≦2 mg/L; Cloxacillin **S** ≤ 2 mg/L and Daptomycin **S** ≤ 1 mg/L.

Figure 1. **Time-kill curve for MRSA strains.** The strains were incubated with vancomycin (VAN) plus cloxacillin (CLO) at a concentration of 0.5 × MIC and 1 × MIC for all antibiotics. **(A)** Standard inoculum equal to 10^5 cfu/mL **(B)** High inoculum equal to 10^8 cfu/mL. Values are means ± standard deviation from two independent experiments. The dashed line indicates the 3 $\log_{10}$ decrease vs. the initial inoculums (bactericidal activity).

Figure 2. **Time-kill curve for MSSA strains.** The strains were incubated with vancomycin (VAN) plus cloxacillin (CLO) at a concentration of 0.5 × MIC and 1 × MIC for all antibiotics. **(A)** Standard inoculum equal to 10^5 cfu/mL **(B)** High inoculum equal to 10^8 cfu/mL. Values are means ± standard deviation from two independent experiments. The dashed line indicates the 3 $\log_{10}$ decrease vs. the initial inoculum (bactericidal activity).

Figure 3. **Treatment of experimental endocarditis caused by strains MSSA 678 and MRSA 277.** Densities of MSSA/MRSA in aortic vegetations in the IE model due to 10^5-CFU/mL challenges of study strains. *nº of rabbits with sterile vegetations / total nº of rabbits (%) are shown for each treatment group under the abscissas bar. Each dot represents one animal. Horizontal black bars indicate mean and interquartile MSSA/MRSA densities.



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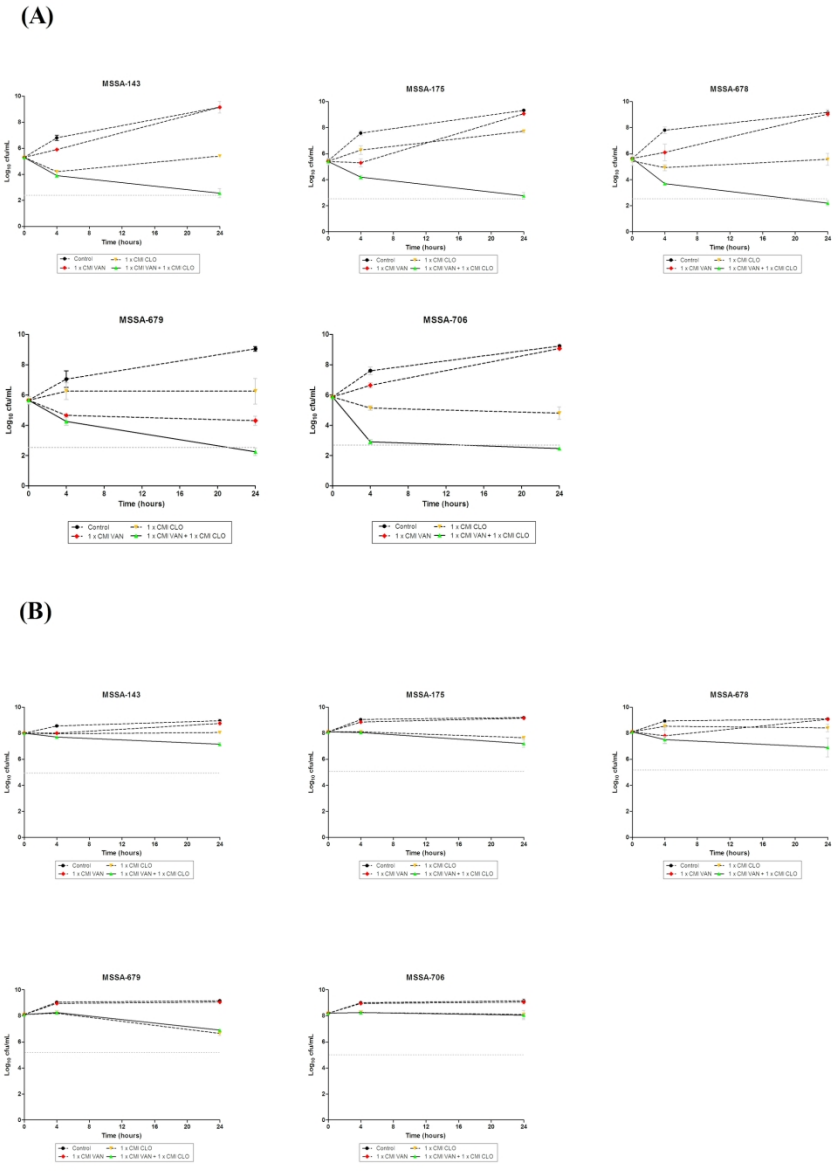

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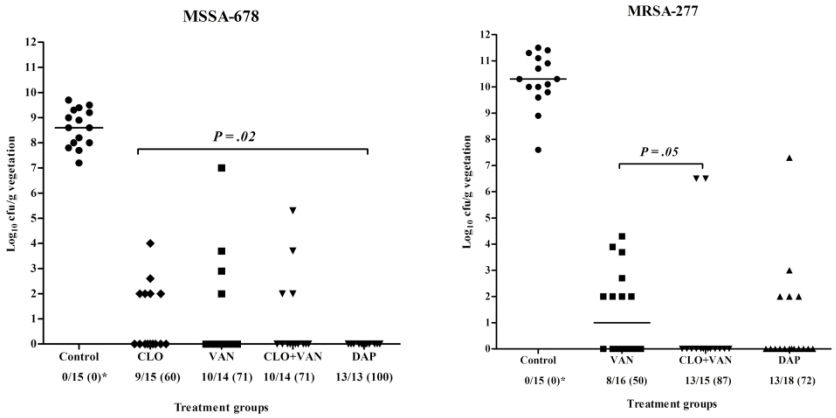

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Supplementary tables

Table S1. *In vitro* time-kill synergy study: MRSA vancomycin plus cloxacillin time-kill curves.

Strains tested		CONTROL Δ Change (x hours) in log_{10} cfu/mL		VAN Δ Change (x hours) in log_{10} cfu/mL		CLO Δ Change (x hours) in log_{10} cfu/mL		VAN + CLO Δ Change (x hours) in log_{10} cfu/mL	
Baseline (0 hours) Log_{10} cfu/mL		4h	24h	4h	24h	4h	24h	4h	24h
Standard inoculum (10^5cfu/mL)									
MRSA-196	5.4	+1.5	+3.9	-0.9	+3.4	-0.4	-0.4	-1.9	-3.4
MRSA-277[a]	5.3	+2.7	+4	-0.6	+1	+0.2	+2.9	-1.1	-3.1
MRSA-513	5.4	+1.4	+3.7	-0.7	+2.2	+0.1	+2.6	-1.7	-3.1
MRSA-726	5.6	+1.6	+3.3	-0.5	+2.5	-0.6	+1.4	-1.4	-3.6
MRSA-835	5.7	+1.8	+3.7	-0.7	+0.9	+0.6	+2.9	-1.4	-3.6
ATCC700788[b]	5.9	+1	+3.1	-0.5	+2.1	-0.7	0	-1.6	-3.1
High inoculum (10^8cfu/mL)									
MRSA-196	8.5	+0.4	+0.9	+0.3	+0.8	-0.4	+0.4	-0.3	+0.4
MRSA-277[a]	8.2	+0.3	+0.7	-1.2	+0.8	-0.7	+0.2	-1.4	-1
MRSA-513	8.2	+0.3	+0.4	+0.3	+0.5	-0.6	-0.4	-0.5	-0.3
MRSA-726	8.5	+0.6	+1	+0.6	+0.6	+0.1	+0.7	-1.5	+0.3
MRSA-835	8.1	+0.9	+1.4	-0.3	+1.1	+0.3	+0.7	-0.1	+0.5
ATCC700788[b]	8.3	+0.6	+0.9	-0.3	+0.9	-0.6	+0.2	-1.1	-2.9

[a]*In vivo* study strain;[b]GISA strain.

Table S2. *In vitro* time-kill synergy study: MSSA vancomycin plus cloxacillin time-kill curves.

Strains tested		CONTROL Δ Change (x hours) in log_{10}cfu/mL		VAN Δ Change (x hours) in log_{10}cfu/mL		CLO Δ Change (x hours) in log_{10} cfu/mL		VAN + CLO Δ Change (x hours) in log_{10} cfu/mL	
Baseline (0 hours) Log_{10} cfu/mL		4h	24h	4h	24h	4h	24h	4h	24h
Standard inoculum (10^5cfu/mL)									
MSSA-143	5.2	+1.6	+3.9	+0.8	+1.8	0.9	+0.3	-1.4	+0.6
MSSA-175	5.5	+2.1	+3.9	-0.2	+3.6	+0.8	+2.5	-1.6	-2.7
MSSA-678[a]	5.6	+2.2	+3.5	+0.5	+3.4	-0.7	-0.1	-1.7	-3.2
MSSA-679	5.7	+1.4	+3.4	-1	-1.4	+0.6	+0.6	-1.4	-3.4
MSSA-706	5.9	+1.7	+3.4	+0.8	+3.2	-0.7	-1.1	-2.9	-3.4
High inoculum (10^8cfu/mL)									
MSSA-143	8	+0.6	+0.9	0	+0.8	0	+0.1	-0.3	-0.9
MSSA-175	8.2	+1	+1.1	+0.8	+1.1	0	-0.4	0	0.9
MSSA-678[b]	8.1	+0.8	+1	-0.3	+1	+0.4	0	-0.6	-1.2
MSSA-679	8.1	+1	+1.1	+0.9	+1	+0.1	-1.5	0.2	-1.2
MSSA-706	8.2	+0.8	+1	+0.8	+0.9	+0.1	-0.1	+0.1	-0.1

[a]I*n vivo* study strain.

Table S3. Treatment of experimental endocarditis caused by strains MSSA-678 and MRSA-277.

Treatment group	No. of rabbit with sterile vegetations / total no. of rabbits (%)	Log_{10} cfu/g vegetation [median (IQR)]
MSSA-678		
Control[a]	0/15 (0)	9 (8-9.2)
Cloxacillin	9/15 (60)[b,c]	0 (0-2)
Vancomycin	10/14 (71)[d]	0 (1-1.5)
Daptomycin	13/13 (100)[b,d,e]	0 (0-0)
Cloxacillin + Vancomycin	10/14 (71)[c,e]	0 (0-1.5)
MRSA-277		
Control[a]	0/15 (0)	9 (8.6-9.3)
Vancomycin	8/16 (50)[f]	1 (0-2.2)[g]
Daptomycin	13/18 (72)[h]	0 (0-1.5)
Cloxacillin + Vancomycin	13/15 (87)[f,h]	0 (0-0)[g]

[a]The control animals were sacrificed 24 h after the infection was started; [b]P=.02; [c]P=.7; [d]P=.09; [e]P=.09; [f]P=.05; [g]P=.09; [h]P=.6. cfu: colony-forming unit; IQR, interquartile range; veg: vegetation