Cloxacillin or Fosfomycin plus Daptomycin Combinations are More Active Than Cloxacillin monotherapy or Combined with Gentamicin against Methicillin-susceptible Staphylococcus aureus (MSSA) in Rabbit Model of Experimental Endocarditis (EE).

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Title: Cloxacillin or Fosfomycin plus Daptomycin Combinations are More Active Than Cloxacillin monotherapy or Combined with Gentamicin against Methicillin-susceptible Staphylococcus aureus (MSSA) in Rabbit Model of Experimental Endocarditis (EE).

Running Title: Daptomycin combinations for Methicillin-susceptible Staphylococcus aureus (MSSA) Endocarditis

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Synopsis

Background: *In vitro* and *in vivo* activity of daptomycin alone or plus either cloxacillin or fosfomycin compared with cloxacillin alone and cloxacillin plus gentamicin were evaluated in a rabbit model of methicillin-susceptible *Staphylococcus aureus* (MSSA) experimental endocarditis (EE).

Methods: Five MSSA strains were used in time-kill studies at standard ($10^5$) and high ($10^8$) inocula. The following antibiotic combinations were evaluated in the EE model: cloxacillin (2 g/4h) alone or combined with gentamicin (1 mg/kg/8h) or daptomycin (6 mg/kg), daptomycin (6 mg/kg/d) alone or combined with fosfomycin (2 g/6h).

Results: At standard and high inocula, daptomycin plus fosfomycin or cloxacillin were bactericidal against 4/5 strains, while cloxacillin plus gentamycin was bactericidal against 3/5 strains at standard inocula but against none at high inocula. Fosfomycin, cloxacillin, gentamicin, and daptomycin MIC/MBCs of the strain used in the EE model were: 2/2, 0.25/0.5, 0.25/0.5, and 0.25/0.25 mg/L, respectively. Adding gentamicin to cloxacillin significantly reduced bacterial density in vegetations compared with cloxacillin monotherapy ($P=0.026$). Adding fosfomycin or cloxacillin significantly improved the efficacy of daptomycin in sterilizing vegetations (10/11 [93%] vs. 8/11 [73%] vs. 0/11 [0%], $P<0.001$ for both combinations) and showed better activity than cloxacillin alone (0/10 [0%], $P<0.001$ for both combinations) and cloxacillin plus gentamicin (3/10 [30%], $P=0.086$ for cloxacillin plus daptomycin and $P=0.008$ for fosfomycin plus daptomycin). No recovered isolates showed increased daptomycin MIC.

Conclusions: The addition of cloxacillin or fosfomycin to daptomycin is synergistic and rapidly bactericidal, showing better activity than cloxacillin plus gentamicin for treating MSSA EE, supporting their clinical use.
Introduction

Methicillin-susceptible *Staphylococcus aureus* (MSSA) is the most common cause of infective endocarditis (IE) (1), with associated mortality rates of around 20% (2, 3). Therefore, there is a need to find more effective antibiotic combinations to face this infection, and also alternative therapies for beta-lactam allergic patients. Cloxacillin, with the addition of gentamicin during the first 3-5 days, has been the treatment of choice for decades (4, 5), based on its synergistic activity, leading to a reduction of the length of bacteremia (6). However, recent studies showed that the addition of gentamicin did not reduce mortality while increasing the risk of renal toxicity (7). Thus, the 2015 American Heart Association (AHA) and European Society of Cardiology (ESC) Infective Endocarditis Guidelines no longer recommended the use of gentamicin for MSSA IE (8, 9).

Daptomycin plus oxacillin (or nafcillin) is synergistic and effective for treating patients with refractory MRSA bacteremia (10). However, despite synergy has also been proven in vivo against MSSA isolates in a foreign body infection model (11), there is a lack of information regarding the activity of this combination against MSSA IE. Other cell-wall agents also showed synergistic activity with daptomycin against MRSA, due to the reduction of MRSA cell-wall charge (12). Our group has shown that the combination of daptomycin plus fosfomycin was synergistic in vitro and in vivo against several MSSA strains, and this combination was successfully used to treat two patients with native valve IE due to MSSA (13).

Our hypotheses were that the combinations of daptomycin plus cloxacillin or fosfomycin are more active against MSSA than daptomycin in monotherapy and at least as synergistic and bactericidal as cloxacillin plus gentamicin. The aim of study was to evaluate the activity of these combinations with daptomycin in comparison with
cloxacillin alone or combined with gentamicin \textit{in vitro} and in a MSSA experimental endocarditis model.

\textbf{MATERIALS AND METHODS}

\textbf{Bacterial isolates}

For \textit{in vitro} studies, five MSSA isolates were selected: MSSA-143, MSSA-175, MSSA-678, MSSA-679 and MSSA-706; all them had been isolated from blood cultures of patients diagnosed with infective endocarditis at our institution. MSSA-678 was also selected for the \textit{in vivo} studies. The isolates were stored at -80\degree C in skim milk.

\textbf{Antimicrobial agents}

Daptomycin powder was supplied by MSD, Spain; cloxacillin, gentamicin, fosfomycin and vancomycin were purchased from Sigma (St Louis, MO). The drugs were prepared according to the manufacturer’s recommendations.

\textbf{Susceptibility Testing}

Minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) were determined using the broth microdilution method according to standard recommendations (14). Fosfomycin susceptibility testing was done in Mueller-Hinton broth supplemented with D-glucose 6-phosphate (Sigma, St Louis, MO, USA) to a final concentration of 25 mg/L. For daptomycin, broth was supplemented with Ca\textsuperscript{2+} to 50 mg/L according to the manufacturer’s recommendations. \textit{S. aureus} ATCC 29213 was used as the test control strain. All the results were double-checked.
**Time-kill curves** were performed with cloxacillin plus gentamicin and cloxacillin or fosfomycin plus daptomycin at concentrations of ¼xMIC and 1xMIC, using two different initial inocula as already described (15). Before inoculation, each tube of fresh cation-adjusted Mueller-Hinton broth was supplemented with Ca²⁺ to 50 mg/L and D-glucose 6-phosphate to test fosfomycin, as described previously (15). All experiments were performed in duplicate. Bactericidal activity was defined as a ≥3-log₁₀ decrease in cfu/ml of the initial inoculum at 48 h. At 24 h, the results of the combination were compared with those of the most active single drug; synergy, indifference, and antagonism were then defined as a ≥2-log increase in killing, a <2-log change (increase or decrease) in killing, and a ≥2-log decrease in killing, respectively.

**Study animals**

Female, New Zealand white rabbits (body weight, 2.5 Kg) provided by San Bernardo farm (Pamplona, Spain) were housed in the animal facilities of the University of Barcelona’s School of Medicine, which is equipped with high-efficiency particulate air filter in an automatic air exchange system, as well as a circadian light cycle. The animals 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**

Antibiotics were administered using a computer-controlled infusion pump system designed to reproduce human serum pharmacokinetics in rabbits. The following antibiotic regimens and doses were chosen to be simulated in the rabbits: cloxacillin (2 g/4h iv)(16), gentamicin (1 mg/kg/8h iv) (17), daptomycin (6 mg/kg iv once daily) (18) and fosfomycin (2 g/6h iv)(15). Simulated doses of cloxacillin and fosfomycin were
those usually used in clinical practice against staphylococcal infective endocarditis. Doses of gentamycin were selected based on the former recommended doses (4). Daptomycin simulated dose was established at 6 mg/kg instead of 10 mg/kg because lower doses of daptomycin help us to figure out the synergism and bactericidal activity of the antimicrobial combination.

**Experimental Endocarditis model**

The experimental aortic valve IE model was induced according to the method described by Garrison and Freedman (19). 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 (18). 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 the MSSA-678 strain (1 ml of $5.5 \times 10^5$ cfu/ml). Before the initiation of antimicrobial therapy, one milliliter of blood was obtained to confirm bacteremia. Antibiotic treatments were started and animals were treated for 24 h using a computer-controlled pump. After completion of the treatment, an additional six half-lives were allowed to elapse before the animals were anesthetized and afterward 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 assigned into the following treatment arms using the previously stated antibiotic dosage: A) Monotherapies: cloxacillin or daptomycin; and,
B) Combined therapies: cloxacillin plus gentamicin or daptomycin plus cloxacillin or fosfomycin. Each group included from 10 to 11 animals.

Analysis of endocardial vegetations

Results were expressed as the number of \( \log_{10} \) cfu per gram of vegetation (\( \log_{10} \) cfu/g veg). Vegetations were assigned a value of \( 2 \log_{10} \) CFU/g when growth was detected in the culture of the remaining homogenate in tryptic soy broth (qualitative culture) but not detected in the quantitative cultures on plates containing Columbia agar with 5% sheep blood. Vegetations, in which no growth was detected from the initial quantitative and qualitative culture (incubated for one week), were considered sterile and a value of zero was assigned.

All the isolates recovered from vegetations were stored, and their MICs re-tested to detect in vivo emerging resistance to daptomycin.

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 \( \log_{10} \) cfu tissue values among the different treatment groups. Fisher’s exact test was used to compare the rate of sterilized vegetations and to analyze whether there were differences among treatment groups.

Results

Susceptibility testing

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of cloxacillin, fosfomycin, gentamicin, daptomycin and
vancomycin of the five strains used in the *in vitro* studies are summarized in Table 1. All strains were susceptible to all antibiotics tested according to the Clinical and Laboratory Standards Institute (CLSI standard) (14) and EUCAST (20) standard MIC breakpoints.

**In vitro time-kill studies**

The results of the time-kill synergy studies for cloxacillin plus gentamicin and cloxacillin or fosfomycin plus daptomycin combinations are displayed in Table 2 (A-C). Two different initial inocula were tested: an initial standard inoculum (ISI) of $10^5$-$10^6$ colony-forming units (cfu)/ml and an initial higher inoculum (IHI), to mimic the density of cfu in mature infected vegetation, equal to $10^8$ cfu/ml.

After 24 hours of incubation with cloxacillin plus gentamicin (Table 2A), synergistic and bactericidal activity was observed in three of five strains at ISI, and synergistic activity in one strain at IHI; the combination was indifferent against the other four strains.

Daptomycin plus cloxacillin (Table 2B) showed synergistic activity at 24 hours against all five strains at ISI, while bactericidal effect was observed against four of them. At IHI, the combination was bactericidal for four strains, and synergistic in all four.

Daptomycin plus fosfomycin (Table 2C) was synergistic against all five strains at ISI, while bactericidal effect was observed in four of them. At IHI, the combination was synergistic against four strains and bactericidal effects were observed against those four strains too.

**Treatment of experimental endocarditis**
In vivo studies to compare the efficacy of drugs in monotherapy or in combination in the experimental model, are shown in Table 3. All control rabbits had infected aortic valve vegetations, with a median bacterial titer of 9 log_{10} cfu/g veg. In monotherapy, neither cloxacillin nor daptomycin were able to sterilize any vegetation. The activity improved with combined therapies: the addition of cloxacillin or fosfomycin to daptomycin significantly improved efficacy in sterilizing vegetations ($P=.001$ and $P<.001$, respectively). Cloxacillin plus gentamicin showed a significant decrease in the density of cfu/gr ($P=.026$), but showed no significant difference compared with cloxacillin monotherapy in sterilizing vegetations ($P=.211$).

Although not achieving statistical significance, daptomycin plus cloxacillin decreased pronouncedly vegetation density ($P=.080$) and sterilized more vegetations ($P=.086$) compared to daptomycin. Daptomycin plus fosfomycin was the most active combination, which sterilized more vegetations ($P=.008$) and decreased the density of microorganisms in the vegetations ($P=.005$) in a larger extent than cloxacillin plus gentamicin. The combination also sterilized more vegetations than daptomycin plus cloxacillin (8/11 [73%] vs 10/11 [91%], respectively), but the difference between them did not reach statistical significance. No recovered isolates showed increased daptomycin MIC.
Discussion

Cloxacillin/nafcillin has been the gold standard treatment for MSSA endocarditis in recent decades. However, mortality rates in patients treated with this antibiotic remain high (2, 3), and no other antibiotic therapy has been shown more effective in reducing the associated undesirable outcomes of MSSA bacteremia and endocarditis (21). This *in vivo* study is the first showing better results with different combinations based on daptomycin than cloxacillin/nafcillin alone or even in combination with gentamicin in an experimental endocarditis caused by MSSA.

It is worth noting that the addition of gentamicin to cloxacillin in native valve endocarditis caused by MSSA is not recommended by current AHA and ESC guidelines (8, 9), as the impact of the aminoglycoside-associated nephrotoxic effect is greater than the clinical impact of the attributed synergistic effect of the combination (22). In our experimental endocarditis model, the addition of gentamicin to cloxacillin significantly reduced the bacterial density in vegetations compared with cloxacillin monotherapy. However, the combination did not show an increased capacity to sterilize endocardial vegetations compared with cloxacillin alone. In addition, when a high inoculum was used, there was a loss of the synergistic and bactericidal effect observed *in vitro*.

Daptomycin combined with fosfomycin or cloxacillin had previously shown *in vitro* synergism and clinical efficacy in case reports of MRSA endocarditis and also *in vivo* in our experimental endocarditis model (10, 13, 16). In a recent clinical trial, the combination of daptomycin plus fosfomycin also showed better clinical efficacy compared to daptomycin alone in the treatment of MRSA bacteremia/endocarditis (23). The current study provides *in vitro* and *in vivo* evidence that both antibiotic regimens
induce increased bactericidal and rapid activity compared with daptomycin alone and
that these combinations are more active than cloxacillin alone or even combined with
gentamicin against MSSA EE. Daptomycin plus either cloxacillin or fosfomycin
showed an increased capacity to sterilize endocardial vegetations compared with
cloxacillin plus gentamicin.

As previously described (16), different mechanisms have been proposed to explain
synergistic activity of cloxacillin with daptomycin: cell-wall charge reduction mediated
by β-lactams, driving an increase in daptomycin binding (10), and the ability to
attenuate staphylococcal virulence and boost innate immunity (24-26). Meanwhile,
synergistic activity with fosfomycin may be explained by fosfomycin PBP-1 inhibition
(15,27,28) and by its ability to modify cell-wall protein composition (12).

This study has some limitations. First, combined therapies using high doses of
daptomycin (10 mg/kg) were not evaluated, although the synergistic effect would
probably be maintained. Second, we did not test neither in vitro nor in vivo efficacy of
daptomycin plus either cloxacillin or fosfomycin against daptomycin non-susceptible
MSSA strains. However, current evidence suggests that daptomycin non-susceptible
strains are extremely unusual in clinical practice (21). And third, although the in vivo
study was performed in only one strain in the animal model, the synergistic and
bactericidal activity of daptomycin combinations were confirmed in vitro in five strains
at both the standard and high inocula, simulating the concentrations reached in valve
vegetations.
In conclusion, the addition of cloxacillin or fosfomycin to daptomycin is synergistic and bactericidal, showing more rapid and potent activity than cloxacillin plus gentamicin for the treatment of MSSA experimental endocarditis, thus supporting the performance of clinical trials to evaluate the use of these combinations in clinical settings.

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**Transparency declarations**

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Merck, Novartis, Pfizer, and ViiV Healthcare, outside the submitted work. All other authors: none to declare.
References


Table 1. Study *S. aureus* strains tested and MICs/MBCs.

<table>
<thead>
<tr>
<th>STRAINS</th>
<th>DAP</th>
<th>CLO</th>
<th>FOM</th>
<th>GEN</th>
<th>VAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSA-143</td>
<td>0.5/0.5</td>
<td>0.12/2</td>
<td>4/8</td>
<td>&gt;64/&gt;64</td>
<td>2/4</td>
</tr>
<tr>
<td>MSSA-175</td>
<td>0.5/0.5</td>
<td>0.5/0.5</td>
<td>4/4</td>
<td>1/2</td>
<td>1/1</td>
</tr>
<tr>
<td>MSSA-678*</td>
<td>1/8</td>
<td>0.25/0.5</td>
<td>8/64</td>
<td>0.25/0.5</td>
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</tr>
<tr>
<td>MSSA-679</td>
<td>0.5/0.5</td>
<td>0.25/0.5</td>
<td>4/32</td>
<td>0.25/0.5</td>
<td>1/2</td>
</tr>
<tr>
<td>MSSA-706</td>
<td>0.5/0.5</td>
<td>0.5/0.5</td>
<td>8/16</td>
<td>0.25/1</td>
<td>0.5/1</td>
</tr>
</tbody>
</table>

*In vivo* study strain
Table 2. *In vitro* time-kill synergy study.

2A. Methicillin-susceptible *S. aureus* (MSSA) Cloxacillin (CLO) + Gentamicin (GEN) Time-Kill Curves (antibiotic concentrations tested at 0.5 x MIC with two different inocula).

<table>
<thead>
<tr>
<th>Strains tested</th>
<th>Baseline (0 hours)</th>
<th>4h</th>
<th>24h</th>
<th>4h</th>
<th>24h</th>
<th>4h</th>
<th>24h</th>
<th>4h</th>
<th>24h</th>
<th>4h</th>
<th>24h</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
<td><strong>Δ Change (x hours)</strong></td>
<td>in log10 cfu/ml</td>
<td>CLO</td>
<td><strong>Δ Change (x hours)</strong></td>
<td>in log10 cfu/ml</td>
<td>GEN</td>
<td><strong>Δ Change (x hours)</strong></td>
<td>in log10 cfu/ml</td>
<td>CLO + GEN</td>
<td><strong>Δ Change (x hours)</strong></td>
</tr>
<tr>
<td>MSSA-143</td>
<td>6.1</td>
<td>+1</td>
<td>+2.9</td>
<td>-1</td>
<td>+1.6</td>
<td>+1</td>
<td>+2.9</td>
<td>-0.9</td>
<td>+1.6</td>
<td>-1.5</td>
<td>+1.2</td>
</tr>
<tr>
<td>MSSA-175</td>
<td>6</td>
<td>+2.2</td>
<td>+3</td>
<td>+0.1</td>
<td>+0.6</td>
<td>-3</td>
<td>+2.3</td>
<td>-3</td>
<td>-4</td>
<td>-1.5</td>
<td>+1.2</td>
</tr>
<tr>
<td>MSSA-678*</td>
<td>6</td>
<td>+2</td>
<td>+3.1</td>
<td>-0.9</td>
<td>+0.6</td>
<td>-1.4</td>
<td>+2.9</td>
<td>-2.2</td>
<td>-3.8</td>
<td>-0.9</td>
<td>+0.6</td>
</tr>
<tr>
<td>MSSA-679</td>
<td>5.9</td>
<td>+2.1</td>
<td>+3.1</td>
<td>+1.6</td>
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<td>+2.8</td>
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<tr>
<td>MSSA-706</td>
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<td>+3.3</td>
<td>-0.2</td>
<td>+2.1</td>
<td>-1</td>
<td>+3.3</td>
<td>-1</td>
<td>-3.4</td>
<td>+1.8</td>
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</tr>
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</table>

**Standard inoculum (10^6 cfu/ml)**

<table>
<thead>
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<th>Strains tested</th>
<th>Baseline (0 hours)</th>
<th>4h</th>
<th>24h</th>
<th>4h</th>
<th>24h</th>
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<th>24h</th>
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<th>24h</th>
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<tr>
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<td>8.3</td>
<td>+0.5</td>
<td>+1.9</td>
<td>-1.5</td>
<td>+1.2</td>
<td>+1.3</td>
<td>+2.8</td>
<td>-1</td>
<td>+1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA-175</td>
<td>8.2</td>
<td>+0.9</td>
<td>+1.5</td>
<td>-0.3</td>
<td>-0.3</td>
<td>+0.3</td>
<td>+1.5</td>
<td>-0.4</td>
<td>-2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA-678*</td>
<td>8.1</td>
<td>+0.8</td>
<td>+1.3</td>
<td>-0.3</td>
<td>+0.9</td>
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<tr>
<td>MSSA-679</td>
<td>8.5</td>
<td>+0.7</td>
<td>+0.8</td>
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<td>+0.5</td>
<td>-0.4</td>
<td>+0.4</td>
<td>-0.6</td>
<td>-1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA-706</td>
<td>8.3</td>
<td>+0.9</td>
<td>+0.8</td>
<td>-0.3</td>
<td>+0.1</td>
<td>0</td>
<td>+0.5</td>
<td>-0.4</td>
<td>-0.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**High inoculum (10^8 cfu/ml)**

* In *vivo* study strain
Table 2. *In vitro* time-kill synergy study.

2B. Methicillin-resistant *S. aureus* (MRSA) Cloxacillin (CLO) + Daptomycin (DAP) Time-Kill Curves (antibiotic concentrations tested at 0.5 x MIC with two different inocula).

<table>
<thead>
<tr>
<th>Strains tested</th>
<th>CONTROL</th>
<th>CLO</th>
<th>DAP</th>
<th>CLO+DAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ Change (x hours) in log_{10} cfu/ml</td>
<td>Δ Change (x hours) in log_{10} cfu/ml</td>
<td>Δ Change (x hours) in log_{10} cfu/ml</td>
<td>Δ Change (x hours) in log_{10} cfu/ml</td>
</tr>
<tr>
<td>Baseline (0 hours)</td>
<td>4h</td>
<td>24h</td>
<td>4h</td>
<td>24h</td>
</tr>
<tr>
<td>Log_{10} CFU/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard inoculum (10^6 cfu/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA-143</td>
<td>5.8</td>
<td>+1</td>
<td>-1.6</td>
<td>0</td>
</tr>
<tr>
<td>MSSA-175</td>
<td>6.1</td>
<td>+2.2</td>
<td>+0.6</td>
<td>-2.3</td>
</tr>
<tr>
<td>MSSA-678*</td>
<td>6.1</td>
<td>+2.1</td>
<td>-1</td>
<td>+0.6</td>
</tr>
<tr>
<td>MSSA-679</td>
<td>6</td>
<td>+2.1</td>
<td>+0.3</td>
<td>-1.8</td>
</tr>
<tr>
<td>MSSA-706</td>
<td>6</td>
<td>+1.4</td>
<td>-0.4</td>
<td>-1.6</td>
</tr>
<tr>
<td>High inoculum (10^8 cfu/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA-143</td>
<td>8</td>
<td>+0.7</td>
<td>-0.2</td>
<td>-1.6</td>
</tr>
<tr>
<td>MSSA-175</td>
<td>8.3</td>
<td>+0.7</td>
<td>-0.3</td>
<td>-1.7</td>
</tr>
<tr>
<td>MSSA-678*</td>
<td>8.2</td>
<td>+0.7</td>
<td>-0.3</td>
<td>-2</td>
</tr>
<tr>
<td>MSSA-679</td>
<td>8.3</td>
<td>+0.8</td>
<td>-0.4</td>
<td>-3.6</td>
</tr>
<tr>
<td>MSSA-706</td>
<td>8.1</td>
<td>+0.9</td>
<td>-1</td>
<td>-1.3</td>
</tr>
</tbody>
</table>

* In vivo study strain
Table 2. *In vitro* time-kill synergy study.

2C. Methicillin-susceptible *S. aureus* (MSSA) Daptomycin (DAP) + Fosfomycin (FOM) Time-Kill Curves (antibiotic concentrations tested at 1 x MIC with two different inocula).

<table>
<thead>
<tr>
<th>Strains tested</th>
<th>CONTROL ( \Delta \text{ Change (x hours)} ) in ( \log_{10} \text{CFU/ml} )</th>
<th>FOM ( \Delta \text{ Change (x hours)} ) in ( \log_{10} \text{CFU/ml} )</th>
<th>DAP ( \Delta \text{ Change (x hours)} ) in ( \log_{10} \text{CFU/ml} )</th>
<th>FOM+DAP ( \Delta \text{ Change (x hours)} ) in ( \log_{10} \text{CFU/ml} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (0 hours)</td>
<td>( \log_{10} \text{CFU/mL} )</td>
<td>4h</td>
<td>24h</td>
<td>4h</td>
</tr>
<tr>
<td>Standard inoculum (10^6 cfu/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA-143</td>
<td>6.1</td>
<td>+1.6</td>
<td>+2.6</td>
<td>-0.2</td>
</tr>
<tr>
<td>MSSA-175</td>
<td>6.1</td>
<td>+2.2</td>
<td>+3</td>
<td>0</td>
</tr>
<tr>
<td>MSSA-678*</td>
<td>6</td>
<td>+2</td>
<td>+3.2</td>
<td>-1.4</td>
</tr>
<tr>
<td>MSSA-679</td>
<td>6.2</td>
<td>+2.2</td>
<td>+2.9</td>
<td>-1</td>
</tr>
<tr>
<td>MSSA-706</td>
<td>6</td>
<td>+1.1</td>
<td>+3</td>
<td>+0.1</td>
</tr>
<tr>
<td>High inoculum (10^8 cfu/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA-143</td>
<td>8</td>
<td>+0.3</td>
<td>+1.1</td>
<td>-0.2</td>
</tr>
<tr>
<td>MSSA-175</td>
<td>8</td>
<td>+1</td>
<td>+1</td>
<td>0</td>
</tr>
<tr>
<td>MSSA-678*</td>
<td>8</td>
<td>+0.7</td>
<td>+1.6</td>
<td>-0.2</td>
</tr>
<tr>
<td>MSSA-679</td>
<td>8.2</td>
<td>+1</td>
<td>+1.1</td>
<td>-1.2</td>
</tr>
<tr>
<td>MSSA-706</td>
<td>8</td>
<td>+0.7</td>
<td>+1.1</td>
<td>-0.6</td>
</tr>
</tbody>
</table>

*In vivo* study strain
Table 3. Treatment of experimental endocarditis caused by MSSA-678

<table>
<thead>
<tr>
<th>Treatment group</th>
<th># rabbits with sterile veg. / # total rabbits (%)</th>
<th>Log$_{10}$ cfu/g vegetation [median (IQR)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (non-treated)*</td>
<td>0/10 (0)</td>
<td>9 (8.1 - 9.3)</td>
</tr>
<tr>
<td>Daptomycin (simulating 6 mg/kg/qd)</td>
<td>0/11 (0)$^a$,$^b$</td>
<td>2 (2 - 3.3)$^c$,$^d$</td>
</tr>
<tr>
<td>Cloxacillin (simulating 2g/4h)</td>
<td>0/10 (0)$^e$,$^f$</td>
<td>3 (2 - 4.5)$^g$,$^h$</td>
</tr>
<tr>
<td>Cloxacillin + Gentamicin (simulating 2g/4h + 1 mg/kg/8h)</td>
<td>3/10 (30)$^e$,$^j$</td>
<td>2 (0.5 - 2.0)$^g$,$^k$,$^l$</td>
</tr>
<tr>
<td>Cloxacillin + Daptomycin (simulating 2g/4h + 6 mg/kg/qd)</td>
<td>8/11 (73)$^a$,$^f$,$^i$</td>
<td>0 (0 - 1)$^c$,$^h$,$^k$</td>
</tr>
<tr>
<td>Fosfomycin + Daptomycin (simulating 2g/6h + 6 mg/kg/qd)</td>
<td>10/11 (91)$^b$,$^j$</td>
<td>0 (0 - 0)$^d$,$^l$</td>
</tr>
</tbody>
</table>

*The control animals were sacrificed 24h after the infection was started; $^a$$P$=.001; $^b$$P$<.001; $^c$$P$=.001; $^d$$P$=.211; $^e$$P$=.001; $^f$$P$=.026; $^g$$P$=.001; $^h$$P$=.086; $^i$$P$=.008; $^j$$P$=.080; $^k$$P$=.005. cfu, colony-forming unit; IQR, interquartile range.