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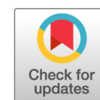

Impact of High-Level Daptomycin Resistance in the *Streptococcus mitis* Group on Virulence and Survivability during Daptomycin Treatment in Experimental Infective Endocarditis

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ABSTRACT Among the viridans group streptococci, the *Streptococcus mitis* group is the most common cause of infective endocarditis. These bacteria have a propensity to be β-lactam resistant, as well as to rapidly develop high-level and durable resistance to daptomycin (DAP). We compared a parental, daptomycin-susceptible (DAP^s) *S. mitis/S. oralis* strain and its daptomycin-resistant (DAP^r) variant in a model of experimental endocarditis in terms of (i) their relative fitness in multiple target organs in this model (vegetations, kidneys, spleen) when animals were challenged individually and in a coinfection strategy and (ii) their survivability during therapy with daptomycin-gentamicin (an *in vitro* combination synergistic against the parental strain). The DAP^r variant was initially isolated from the cardiac vegetations of animals with experimental endocarditis caused by the parental DAP^s strain following treatment with daptomycin. The parental strain and the DAP^r variant were comparably virulent when animals were individually challenged. In contrast, in the coinfection model without daptomycin therapy, at both the 10^6- and 10^7-CFU/ml challenge inocula, the parental strain outcompeted the DAP^r variant in all target organs, especially the kidneys and spleen. When the animals in the coinfection model of endocarditis were treated with DAP-gentamicin, the DAP^s strain was completely eliminated, while the DAP^r variant persisted in all target tissues. These data underscore that the acquisition of DAP^r in *S. mitis/S. oralis* does come at an intrinsic fitness cost, although this resistance phenotype is completely protective against therapy with a potentially synergistic DAP regimen.

KEYWORDS *Streptococcus mitis* group, experimental endocarditis, daptomycin, gentamicin, high-level daptomycin resistance, virulence, fitness

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Among the viridans group streptococci, the members of the *Streptococcus mitis* group are the most frequent cause of human infective endocarditis (IE) and the most common cause of the toxic streptococcal bacteremia syndrome seen in immunocompromised hosts (1–8). This organism is often resistant *in vitro* to β-lactam antibiotics, including penicillin and ceftriaxone (9–16). Moreover, despite uniform *in vitro* susceptibility to vancomycin, patients treated with this agent have had suboptimal outcomes, likely due to vancomycin tolerance (11). This has raised the notion of using daptomycin (DAP) for the treatment of invasive *S. mitis* group infections. Recent studies have somewhat dampened the enthusiasm for the latter approach, as many *S. mitis* group strains have a unique propensity to evolve rapid, durable, and high-level

daptomycin resistance (DAP^r) *in vitro*, *ex vivo*, and *in vivo* (17–19). This study investigated the impact of the acquisition of DAP^r upon both the intrinsic fitness and survivability during treatment with DAP of such strains in a model of IE featuring coinfection with a DAP-susceptible (DAP^s) parental *S. mitis/S. oralis* strain and its *in vivo*-derived DAP^r variant.

(This research was presented in part at the American Society for Microbiology Microbe meeting, Boston, MA, 19 June 2016 [20].)

RESULTS

***In vitro* susceptibility testing.** The DAP, penicillin, and gentamicin (GEN) MICs for the two test strains were as follows: the DAP^s strain had a DAP MIC of 0.5 μg/ml and was not high-level GEN resistant (GEN^r; MIC = 8 μg/ml) but was resistant to penicillin and ceftriaxone (MICs = 8 μg/ml and 4 μg/ml, respectively). The DAP^r strain exhibited high-level DAP^r (MIC > 256 μg/ml), was not high-level GEN^r (MIC = 8 μg/ml), and showed intermediate resistance to penicillin and susceptibility to ceftriaxone (MICs = 0.5 μg/ml and 1 μg/ml, respectively). Of interest, a β-lactam–DAP MIC seesaw effect was observed, paralleling the findings of other studies of DAP^r Gram-positive pathogens (21). For example, in the DAP^s parental strain, the penicillin MIC was 8 μg/ml, but this decreased to 0.5 μg/ml in the DAP^r strain; similarly, the ceftriaxone MIC decreased from 4 in the DAP^s parental strain to 1 μg/ml in the DAP^r strain.

In time-kill synergy studies, only the combination of DAP at 1× MIC plus GEN at either 1/2× MIC or 1× MIC synergistically killed the DAP^s parental strain (Fig. 1A). For the DAP^r strain, there was no synergistic killing observed with any of the antibiotic combinations (Fig. 1B).

IE coinfection model. The results of the IE coinfection model with a challenge with an inoculum of 2 × 10^6 CFU/ml are shown in Table 1. In the absence of antibiotic therapy, both strains induced IE, although the DAP^s parental strain was significantly more competitively fit. For example, in terms of vegetation counts, there was a mean difference of ~4 $\log_{10}$ CFU/g favoring the DAP^s parental strain. This difference was even more magnified in terms of kidney and spleen counts, where the DAP^r strain was apparently unable to hematogenously seed and/or proliferate within these organs.

This reduced competitive fitness was also mirrored when animals were individually challenged with the DAP^r strain at the same 2 × 10^6-CFU/ml inoculum (Table 2). In this scenario, vegetation seeding occurred in all animals, although the median achievable counts were still ~1.5 $\log_{10}$ CFU/g below the count for the parental strain (Table 1). Similarly, seeding to and proliferation within kidneys and spleen occurred with the individual challenge with the DAP^r strain, although this seeding was not uniformly detected in all challenged animals (40% and 60%, respectively).

To examine the impact of the challenge inoculum on competitive fitness, catheterized animals were cochallenged in parallel with an intravenous (i.v.) inoculum of 2 × 10^7 CFU/ml of the DAP^s and DAP^r strains. As seen in Table 3, we saw an outcome very similar to that achieved with the 10^6-CFU/ml coinfection model described above. Thus, the DAP^r strain did infect cardiac vegetations, although it did so at a significantly reduced level compared to that for the DAP^s strain. Moreover, even though both the kidneys and the spleen were seeded by the DAP^r strain in most rabbits, tissue counts were significantly below those of the DAP^s strain.

Table 1 also details the outcome of combined DAP-GEN therapy in animals coinfected with the DAP^s and DAP^r strains at a 2 × 10^6-CFU/ml inoculum. After 48 h of combined treatment, DAP^s parental colonies were completely cleared from all target tissues, leaving only DAP^r colonies surviving in the three target tissues. All DAP^r variants isolated from these target tissues maintained stable, high-level DAP^r at the time of sacrifice, as determined by Etest.

DISCUSSION

Garcia-de-la-Maria et al. have previously shown that *S. mitis* group strains have a unique capacity to evolve stable, high-level DAP^r both *in vitro* and *in vivo* (17). For

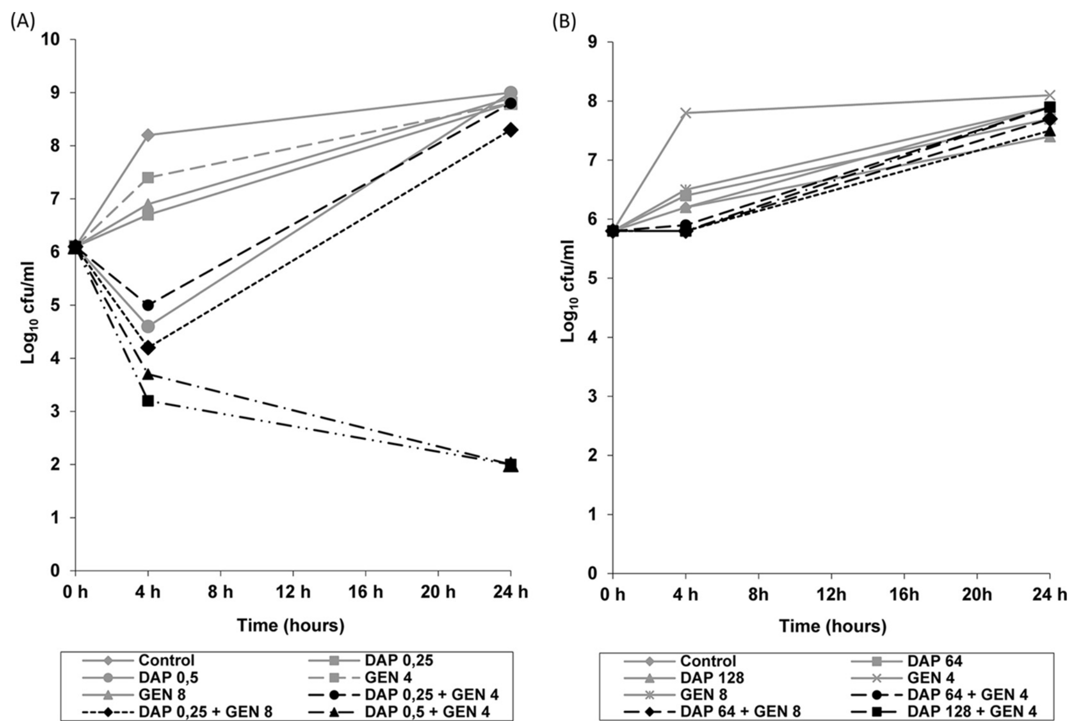

FIG 1 (A) Results of time-kill experiments for DAP[s] strain S.MIT/ORALIS-351 incubated with DAP plus GEN at concentrations of 0.5× MIC and 1× MIC for both antibiotics. (B) Results of time-kill experiments for the DAP[r] variant incubated with DAP plus GEN at concentrations of 64 μg/ml and 128 μg/ml for DAP and 4 μg/ml and 8 μg/ml for GEN. The numbers in the keys are concentrations (in micrograms per milliliter).

example, in a study of 92 *S. mitis* group clinical isolates, this phenotype was identified in ~27% of isolates upon DAP passage *in vitro* (17). We have recently demonstrated that the genetic mechanisms for the development of DAP[r] in *S. mitis/S. oralis* involve the acquisition of loss-of-function single nucleotide polymorphisms (SNPs) within the *cdsA* and *pgsA* loci of the organism (22, 23). These genes encode enzymes which are critical in the biosynthetic pathway for cardiolipin (CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyl-synthetase and CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyl-transferase, respectively). These mutations are associated with a complete loss of cardiolipin and phosphatidylglycerol production (22, 23). Given the critical roles of both cardiolipin and phosphatidylglycerol in the mechanism of action of DAP (24, 25), it is plausible that the mutations in the loci mentioned above are the principal drivers of DAP[r] in our strain after *in vivo* passage. Of interest, this mechanism of DAP[r] differs substantially from the

TABLE 1 *S. mitis/S. oralis* competition *in vivo* in an experimental coinfection model of endocarditis[a]

Study group, strain	Vegetations IR[b]	Vegetations Median (IQR) $\log_{10}$ no. of CFU/g tissue	Kidney IR	Kidney Median (IQR) $\log_{10}$ no. of CFU/g tissue	Spleen IR	Spleen Median (IQR) $\log_{10}$ no. of CFU/g tissue
Rabbits not treated with antibiotics and sacrificed at 24 h						
DAP[s] strain	5/5 (100)	10.1 (9.4–10.2)	5/5 (100)	3.2 (2.7–4)	5/5 (100)	5.3 (4.5–5.6)
DAP[r] strain	4/5 (80)	6.6 (5.7–6.9)	0/5 (0)	0 (0–0)	0/5 (0)	0 (0–0)[f]
P value	1.000	.008	.008	.008	.008	0.008
Rabbits receiving DAP-GEN and sacrificed after 48 h of treatment						
DAP[s] strain	0/6 (0)	0 (0–0)	0/6 (0)	0 (0–0)	0/6 (0)	0 (0–0)
DAP[r] strain	6/6 (100)	8.5 (6.3–9)	5/6 (83)	2.4 (2–2.5)	3/6 (50)	1 (0–3.4)
P value	0.002	0.002	0.015	0.015	0.182	0.180

[a]Competition was between DAP[s] and DAP[r] strains given at an inoculum of 2 × 10^6 CFU/ml.
[b]IR, infection rate, given as the number of animals with infected valve vegetations, kidney, and spleen/total number of animals (percent).

TABLE 2 DAP^r *S. mitis/S. oralis* fitness *in vivo* during challenge in an experimental model of endocarditis[a]

Vegetations		Kidney		Spleen	
IR[b]	Median (IQR) $\log_{10}$ no. of CFU/g tissue	IR	Median (IQR) $\log_{10}$ no. of CFU/g tissue	IR	Median (IQR) $\log_{10}$ of CFU/g tissue
5/5 (100)	8.6 (7.6–8.9)	2/5 (40)	1.4 (0–2.6)	3/5 (60)	2.4 (0–3.2)

[a]The DAP^r *S. mitis/S. oralis* strain was given at an individual inoculum of 2 × 10^6 CFU/ml. Rabbits were not treated with antibiotics and were sacrificed at 24 h postinfection.
[b]IR, infection rate, given as the number of animals with infected valve vegetations, kidney, and spleen/total number of animals (percent).

mechanisms involved in DAP^r in *S. aureus* (charge repulsion) (26–28) and enterococci (antibiotic diversion for *Enterococcus faecalis* and charge repulsion for *E. faecium*) (29, 30). However, little is known about the impacts of DAP^r on innate pathogenicity and the antimicrobial response profiles in the *S. mitis* group.

Many studies have suggested that the acquisition of antibiotic resistance comes with a metabolic fitness cost for the organism (31, 32). This is usually reflected by lower growth rates and/or lower growth yields *in vitro* for such resistant strains compared with those for their respective antibiotic-susceptible parental strains. However, documentation of the fitness costs of antibiotic resistance using *in vivo* virulence experiments in terms of its impact on the organism's (i) transmissibility, (ii) persistence and proliferation within target host tissues, or (iii) ability to evade and survive innate or adaptive immune host defenses is relatively infrequent in the literature (31–33). The current study was designed to quantify the effects of the acquisition of DAP^r in *S. mitis/S. oralis* on both intrinsic virulence and survivability during DAP exposures, using a discriminative model of endovascular infection, IE.

A number of interesting observations emerged from this investigation. First, it seems clear that acquisition of genetic perturbations related to DAP^r does impact the *in vitro* and intrinsic *in vivo* virulence of the DAP^r strain in our model of endovascular infection. Of note, the reduction in the *in vivo* fitness of the DAP^r strain was manifest in all target organs in the IE model, although it was particularly evident in kidneys and spleen. This may reflect an enhanced susceptibility of the DAP^r strains to neutrophil-based host defenses that are replete in the latter organs and accompany abscess formation. Alternatively, this reduced virulence may imply a defect in the seeding of distant target organs by the DAP^r strain, i.e., a perturbation in hematogenous spread from vegetations to these distant organs by non-neutrophil-based mechanisms, such as the elaboration of platelet antimicrobial peptides within cardiac vegetations (34, 35). Second, the apparent *in vivo* fitness defect of the DAP^r strain could not be overcome by merely increasing the challenge inoculum from 10^6 to 10^7 CFU/ml. This suggests that the impact of the DAP^r strain on intrinsic fitness represents a homogeneous and not a heterogeneous population effect. Third, although the DAP^r strain was intrinsically less fit than its parental strain *in vivo*, DAP^r provided the strain with uniform protection against treatment with a combination of DAP-GEN, which synergistically killed the parental isolate.

Garcia-de-la-Maria et al. (17) have previously demonstrated that, in the model of experimental endocarditis caused by strain S.MIT/ORALIS-351, addition of GEN to DAP

TABLE 3 Competitive fitness of DAP^s and DAP^r strains *in vivo* during coinfection challenge in an experimental model of endocarditis[a]

	Vegetations		Kidney		Spleen	
Strain	IR[b]	Median (IQR) $\log_{10}$ no. of CFU/g tissue	IR	Median (IQR) $\log_{10}$ no. of CFU/g tissue	IR	Median (IQR) $\log_{10}$ no. of CFU/g tissue
DAP^s strain	5/5 (100)	8.5 (8.4–8.6)	5/5 (100)	5.0 (4.4–5.4)	5/5 (100)	4.8 (4.8–4.9)
DAP^r strain	5/5 (100)	6.9 (6.7–7.1)	3/5 (60)	1.7 (0.6–1.8)	4/5 (80)	1.6 (1.5–2.0)
P value		0.008	0.492	0.008	1.0	0.008

[a]The challenge inoculum was 2 × 10^7 CFU/ml. Rabbits were not treated with antibiotics and were sacrificed at 24 h postinfection.
[b]IR, infection rate, given as the number of animals with infected valve vegetations, kidney, and spleen/total number of animals (percent).

not only significantly increased the number of vegetations sterilized after 48 h of treatment compared to the number sterilized by DAP alone but also prevented the development of DAP^r in 21 of 23 treated rabbits (91%). Although the mechanisms of DAP^r in the *S. mitis* group seem to differ substantially from those involved in DAP^r in *Staphylococcus aureus* and enterococci, as explained above, there is an interest for future study to look into whether combinations of DAP plus β-lactams, such as ampicillin or ceftriaxone, are synergistic against *S. mitis* and could prevent the development of DAP^r. To this point, Yim et al. (19) recently showed that the combination of DAP plus ceftaroline was synergistic and bactericidal against two prototypic *S. mitis/S. oralis* strains (S.MIT/ORALIS-351 and SF100) in an *ex vivo* model of simulated endocardial vegetations (SEVs) and also prevented the development of DAP^r in both strains.

In conclusion, the acquisition of the DAP^r phenotype affects the virulence of *S. mitis/S. oralis* in experimental IE in terms of a reduction in its *in vivo* fitness in all target organs, especially kidneys and spleen. However, DAP^r variants were able to induce IE, with their survival being amplified in the presence of DAP-GEN combination therapy. Further studies are needed to identify other possibly effective DAP combination therapies that can either prevent the emergence of or enhance the treatment of DAP^r *S. mitis* group variants.

MATERIALS AND METHODS

Microorganisms. We studied a clinically derived parental DAP^s *S. mitis/S. oralis* bloodstream isolate (SMIT-351) from a patient with IE. This strain is virulent in the experimental IE model (17), and it was identified to be an *S. mitis* strain on the basis of standard biotyping and 16S RNA sequencing. Recently, we have had the results of genome sequencing for this strain, and we discovered that this strain is more likely a member of the closely related species *S. oralis*, on the basis of average nucleotide identity (ANI) analysis of the whole-genome sequence. The strain has therefore been renamed S.MIT/ORALIS-351 and is so listed in GenBank. We also studied a stably high-level DAP^r variant strain (strain D_6-6; DAP MIC $>$ 256 μg/ml) isolated from the vegetations of a rabbit with experimental IE after 48 h treatment with DAP alone once daily at 6 mg/kg of body weight/day i.v. (17). According to both determination of the optical density at 600 nm by spectrophotometry and formal counts of the number of CFU per milliliter, the mutant strain (DAP^r) was less fit than the parent strain (DAP^s) over a 24-h time frame *in vitro* in terms of growth kinetics and yield (data not shown).

Antibiotics. DAP powder for *in vitro* testing and animal treatment was supplied by Cubist Pharmaceuticals (Lexington, MA). USP-grade penicillin and gentamicin (GEN) were purchased from Sigma (St. Louis, MO).

***In vitro* susceptibility assays.** DAP, penicillin, and GEN MICs were determined using the broth microdilution method, according to standard recommendations (36). Susceptibility to DAP was tested in Mueller-Hinton broth supplemented with 50 μg/ml of calcium chloride (CAMHB). *Streptococcus pneumoniae* ATCC 49619 served as the quality control strain. DAP MICs were also determined in selected studies by using the Etest method following the manufacturer's recommendations (bioMérieux S.A., Marcy l'Etoile, France).

Time-kill studies. The time-kill methodology was used to test the activity of DAP plus GEN against S.MIT/ORALIS-351 and its DAP^r variant, D_6-6, according to previously described criteria (37). A final inoculum of between 5×10^5 and 7×10^5 CFU/ml was used. Prior to inoculation, each tube of fresh CAMHB plus lysed horse blood at a final concentration of 5% was supplemented with DAP alone or in combination with GEN. For the DAP^s parental strain, the antibiotic concentrations tested were 1/2$\times$ MIC and 1$\times$ MIC for both DAP (0.25 and 0.5 μg/ml, respectively) and GEN (4 and 8 μg/ml, respectively). For the DAP^r strain (DAP MIC $>$ 256 μg/ml), DAP concentrations were adjusted to 64 μg/ml and 128 μg/ml. A tube without antibiotics was used as a growth control. Viability counts were performed at 0, 4, and 24 h as described by Isenberg (38). Drug carryover was addressed by serial dilution plate counting. Bactericidal synergy was defined as a $\geq$2-$\log_{10}$ decrease in the number of CFU per milliliter between the combination antibiotic and the most active agent alone after 24 h; moreover, the number of surviving organisms in the presence of the combination had to be $\geq$2 $\log_{10}$ CFU/ml below the starting inoculum. At least one of the drugs had to be present at a concentration that did not significantly affect the growth curve of the test organism when used alone. Bactericidal activity was defined as at least a 3-$\log_{10}$ reduction in the number of CFU per milliliter at 24 h in comparison with the initial inoculum.

***In vivo* studies. (i) Animal models.** New Zealand White rabbits (body weight, $\sim$2.5 kg) obtained from local breeding sources were housed in the animal facilities located at the Faculty of Medicine from the University of Barcelona and at LA Biomedical Research Institute. They were provided food and water *ad libitum*. This research project fulfills the requirements stipulated in Spanish Royal Decree 223/1988 on the protection of animals used in experiments, and it was approved by the Ethical Committee on Animal Research of the University of Barcelona. In addition, parallel studies performed at the LA Biomedical Research Institute were approved by its Animal Use Committee (IACUC).

(ii) Human pharmacokinetic simulation studies. The antibiotics were administered to animals with IE using a computer-controlled infusion pump system designed to simulate human-equivalent serum

levels following the administration of DAP at the FDA-approved dose for *S. aureus* bacteremia (6 mg/kg) (39) and GEN at the recommended synergistic dose for enterococcal IE (1 mg/kg i.v. every 8 h) (40).

The computer-assisted program procedure has three steps: (i) estimation of antibiotic parameters in the rabbit, (ii) application of a mathematical model to determine the infusion rate required for reproducing human-like pharmacokinetics in animals, and (iii) collection of serum samples to check that the antibiotic levels actually achieved in the animals mimic the desired human pharmacokinetic profiles. These studies have been done previously and reported on elsewhere (37, 39).

(iii) *In vivo* experimental IE model. Experimental aortic valve IE was induced as described previously (41). In brief, an indwelling polyethylene catheter was inserted through the right carotid artery into the left ventricle in anesthetized animals to induce aortic valve trauma; in addition, two catheters for administration of antibiotics were placed into the inferior vena cava through the jugular vein and tunneled subcutaneously to the interscapular region. The external portion of each jugular catheter was connected to a swivel and then to a computer-controlled infusion pump as previously described (41).

At 24 h after placement of the intracarotid catheter, animals were infected via the marginal ear vein with (i) an inoculum of either DAP^s or DAP^r strain at 2×10^6 CFU/ml for assessment of fitness, (ii) a mixed inoculum (ratio, ~1:1) of both strains at 2×10^7 CFU/ml for assessment of fitness at a higher inoculum, or (iii) a mixed inoculum (ratio, ~1:1) of both strains at 2×10^6 CFU/ml for assessment of antibiotic treatment. One milliliter of blood was obtained at 24 h after infection from animals in all groups plus immediately before the initiation of antimicrobial therapy from animals in the treatment groups to confirm the presence of persistent bacteremia (to indicate the successful induction of IE). A group of nontreated infected animals was sacrificed concurrently, and the bacterial densities in vegetations, kidney, and spleen were calculated (see below). The remainder of the animals underwent antibiotic therapy with DAP-GEN, administered for 48 h via the computer-controlled infusion pump system through the indwelling jugular catheter.

After the completion of treatment, six half-lives ($t_{1/2}$s) of both antibiotics (DAP and GEN) were allowed to lapse before the animals were sacrificed in order to avoid antibiotic carryover effects from blood to tissue. This translates to 48 h for DAP ($t_{1/2} = 8$ h) and 9 h for GEN ($t_{1/2} = 1.5$ h). Given the longer half-life of DAP, GEN infusions were continued during the first 15 h. Rabbits were then humanely sacrificed; the heart, spleen, and kidneys were surgically removed; and target tissue samples were obtained: aortic valve vegetations from the heart and tissue samples from the spleen and kidney (41).

Analysis of infected tissues. Target tissue samples were serially diluted and processed for quantitative culture as described before (17). Tissue homogenates were seeded in parallel on plain brain heart infusion agar (BHIA; Oxoid Ltd., Hampshire, England) plates, as well as on BHIA plates containing DAP (8 μg/ml) to individually quantify surviving DAP^s versus DAP^r colonies. Colonies recovered from DAP-containing BHIA plates were also retested in parallel using the DAP Etest to ensure retention of the DAP^r phenotype. Target tissue bacterial counts were expressed as the median and interquartile range (IQR) of the $\log_{10}$ number of CFU per gram of each target tissue. If there was no growth on the quantitative culture plates with tissue homogenates but there was growth in the qualitative culture (for which the rest of the tissue homogenate was cultured in tryptic soy broth for 7 days), that target tissue sample was assigned a value of 2 $\log_{10}$ CFU/g. If there was no growth either in the initial quantitative plate cultures or from the homogenates qualitatively cultured for 7 days, that target tissue sample was assigned a value of 0 and the tissue was considered sterile.

Statistical analysis. The Fisher exact test was used to compare the rates of sterile target tissues between tissues from animals infected with the DAP^r and DAP^s strains. The Mann-Whitney rank sum test was used to compare the values of the $\log_{10}$ number of CFU per gram of target tissues between the different treatment groups. P values of <0.05 were considered significant.

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REFERENCES

1. Fowler VG, Scheld WM, Bayer AS. 2015. Endocarditis and intravascular infections, p 990–1028. *In* Principles and practices of infectious diseases. Elsevier Inc, Amsterdam, The Netherlands.
2. Shelburne SA, Sahasrabhojane P, Saldana M, Yao H, Su X, Horstmann N, Thompson E, Flores AR. 2014. Streptococcus mitis strains causing severe clinical disease in cancer patients. Emerg Infect Dis 20:762–771. https://doi.org/10.3201/eid2005.130953.
3. Freifeld AG, Razonable RR. 2014. Viridans group streptococci in febrile neutropenic cancer patients: what should we fear? Clin Infect Dis 59: 231–233. https://doi.org/10.1093/cid/ciu264.
4. Shelburne SA, Lasky RE, Sahasrabhojane P, Tarrand JT, Rolston KV. 2014. Development and validation of a clinical model to predict the presence of β-lactam resistance in viridans group streptococci causing bacteremia in neutropenic cancer patients. Clin Infect Dis 59:223–230. https://doi.org/10.1093/cid/ciu260.
5. Ahmed R, Hassall T, Morland B, Gray J. 2003. Viridans streptococcus bacteremia in children on chemotherapy for cancer: an underestimated problem. Pediatr Hematol Oncol 20:439–444.
6. Husain E, Whitehead S, Castell A, Thomas EE, Speert DP. 2005. Viridans streptococci bacteremia in children with malignancy: relevance of species identification and penicillin susceptibility. Pediatr Infect Dis J 24: 563–566. https://doi.org/10.1097/01.inf.0000164708.21464.03.
7. Marron A, Carratala J, Gonzalez-Barca E, Fernández-Sevilla A, Alcaide F, Gudiol F. 2000. Serious complications of bacteremia caused by viridans streptococci in neutropenic patients with cancer. Clin Infect Dis 31: 1126–1130. https://doi.org/10.1086/317460.
8. Huang WT, Chang LY, Hsueh PR, Lu CY, Shao PL, Huang FY, Lee PI, Chen CM, Lee CY, Huang LM. 2007. Clinical features and complications of viridans streptococci bloodstream infection in pediatric hemato-oncology patients. J Microbiol Immunol Infect 40:349–354.
9. Prabhu RM, Piper KE, Baddour LM, Steckelberg JM, Wilson WR, Patel R. 2004. Antimicrobial susceptibility patterns among viridans group streptococci isolates from infective endocarditis patients from 1971-1986 and 1994-2002. Antimicrob Agents Chemother 48:4463–4465. https://doi.org/10.1128/AAC.48.11.4463-4465.2004.
10. Doern GV, Ferraro MJ, Brueggermann AB, Ruoff KL. 1996. Emergence of high rates of antimicrobial resistance among viridans group streptococci in the United States. Antimicrob Agents Chemother 40:891–894.
11. Safdar A, Rolston KV. 2006. Vancomycin tolerance, a potential mechanism for refractory gram-positive bacteria: observational study in patients with cancer. Cancer 106:1815–1820. https://doi.org/10.1002/cncr.21801.
12. Hsu R-B, Lin F-Y. 2006. Effect of penicillin resistance on presentation and outcome of nonenterococcal streptococcal infective endocarditis. Cardiology 105:234–239. https://doi.org/10.1159/000091821.
13. Sabella C, Murphy D, Drummond-Webb J. 2001. Endocarditis due to *Streptococcus mitis* with high-level resistance to penicillin and ceftriaxone. JAMA 285:2195.
14. Lonks JR, Dickinson BP, Runarsdottir V. 1999. Endocarditis due to *Streptococcus mitis* with high-level resistance to penicillin and cefotaxime. N Engl J Med 341:1239.
15. Han XY, Kamana M, Rolston KV. 2006. Viridans streptococci isolated by culture from blood of cancer patients: clinical and microbiologic analysis of 50 cases. J Clin Microbiol 44:160–165. https://doi.org/10.1128/JCM.44.1.160-165.2006.
16. Sanchez M, Vicente MF, Cercenado E, de Pedro MA, Gómez P, Moreno R, Morón R, Berenguer J. 2001. Diversity among clinical isolates of penicillin-resistant *Streptococcus mitis*: indication for a PBP1-dependent way to reach high levels of penicillin resistance. Int Microbiol 4:217–222. https://doi.org/10.1007/s10123-001-0040-1.
17. Garcia-de-la-Maria C, Pericas Del Río JM, Castañeda A, Vila-Farrés X, Armero X, Espinal Y, Cervera PA, Soy C, Falces D, Ninot C, Almela S, Mestres M, Gatell CA, Vila JM, Moreno J, Marco A, Miró JM, Hospital Clinic Experimental Endocarditis Study Group. 2013. Early *in vitro* and *in vivo* development of high-level daptomycin resistance is common in mitis group streptococci after exposure to daptomycin. Antimicrob Agents Chemother 57:2319–2325. https://doi.org/10.1128/AAC.01921-12.
18. Akins RL, Katz BD, Monahan C, Alexander D. 2015. Characterization of high-level daptomycin resistance in viridans group streptococci developed upon *in vitro* exposure to daptomycin. Antimicrob Agents Chemother 59:2102–2112. https://doi.org/10.1128/AAC.04219-14.
19. Yim J, Smith JR, Singh N, Hallesy J, Garcia de la Maria C, Bayer AS, Mishra NN, Miro JM, Arias CA, Tran TT, Sullam P, Rybak MJ. 2016. Combinations of daptomycin and ceftaroline or gentamicin against *Streptococcus mitis* in an *in vitro* model of simulated endocardial vegetations, abstr 2016-5703. Abstr ASM Microbe Meet, Boston, MA. American Society for Microbiology, Washington, DC.
20. García de la Mária C, Pericás JM, Armero Y, Moreno A, Mishra NN, Rybak MJ, Tran TT, Arias CA, Sullam PM, Xiong YQ, Bayer A, Miró JM. 2016. High-level daptomycin-resistant (DAP-R) *Streptococcus mitis* is virulent in experimental endocarditis (EE) and enhances survivability during DAP treatment vs. its DAP-susceptible (DAP-S) parental strain, poster P-335. Abstr ASM Microbe Meet, Boston, MA. American Society for Microbiology, Washington, DC.
21. Yang SJ, Xiong YQ, Boyle-Vavra S, Daum R, Jones T, Bayer AS. 2010. Daptomycin-oxacillin combinations in treatment of experimental endocarditis caused by daptomycin-nonsusceptible strains of methicillin-resistant *Staphylococcus aureus* with evolving oxacillin susceptibility (the "seesaw effect"). Antimicrob Agents Chemother 54:3161–3169. https://doi.org/10.1128/AAC.00487-10.
22. Mishra NN, Alvarez DN, Seepersaud R, Tran TT, Garcia-de-la-Maria C, Miro JM, Rybak MJ, Arias CA, Sullam PM, Bayer AS. 2015. Phenotypic and genotypic mechanisms of daptomycin resistance in *Streptococcus mitis*, abstr C1-1364. Abstr 55th Interscі Conf Antimicrob Agents Chemother, San Diego, CA. American Society for Microbiology, Washington, DC.
23. Mishra NN, Tran TT, Seepersaud R, Garcia-de-la-Maria C, Faull K, Yoon A, Proctor R, Miro JM, Rybak MJ, Bayer AS, Arias CA, Sullam PM. 2017. Perturbations of phosphatidate cytidylyltransferase (CdsA) mediate daptomycin resistance in Streptococcus mitis/oralis by a novel mechanism. Antimicrob Agents Chemother 61:e02435-16. https://doi.org/10.1128/AAC.02435-16.
24. Miller WR, Bayer AS, Arias CA. 2016. Mechanism of action and resistance to daptomycin in *Staphylococcus aureus* and enterococci. Cold Spring Harb Perspect Med 6:a026997. https://doi.org/10.1101/cshperspect.a026997.
25. Taylor SD, Palmer M. 2016. The action mechanism of daptomycin. Bioorg Med Chem 24:6253–6258. https://doi.org/10.1016/j.bmc.2016.05.052.
26. Mishra NN, Bayer AS. 2013. Correlation of cell membrane lipid profiles with daptomycin resistance in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 57:1082–1085. https://doi.org/10.1128/AAC.02182-12.
27. Bertsche U, Yang SJ, Kuehner D, Wanner S, Mishra NN, Roth T, Nega M, Schneider A, Mayer C, Grau T, Bayer AS, Weidenmaier C. 2013. Increased cell wall teichoic acid production and D-alanylation are common phenotypes among daptomycin-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates. PLoS One 13:e67398. https://doi.org/10.1371/journal.pone.0067398.
28. Yang SJ, Mishra NN, Rubio A, Bayer AS. 2013. Causal role of single nucleotide polymorphisms within the *mprF* gene of *Staphylococcus aureus* in daptomycin resistance. Antimicrob Agents Chemother 57: 5658–5664. https://doi.org/10.1128/AAC.01184-13.
29. Mishra NN, Bayer AS, Tran TT, Shamoo Y, Mileykovskaya E, Dowhan W, Guan Z, Arias CA. 2012. Daptomycin resistance in enterococci is associated with distinct alterations of cell membrane phospholipid content. PLoS One 7:e43958. https://doi.org/10.1371/journal.pone.0043958.
30. Tran TT, Munita JM, Arias C. 2015. Mechanism of drug resistance: daptomycin resistance. Ann N Y Acad Sci 1354:32–53. https://doi.org/10.1111/nyas.12948.
31. Andersson DI, Levin BR. 1999. The biological cost of antibiotic resistance. Curr Opin Microbiol 2:489–493. https://doi.org/10.1016/S1369-5274(99)00005-3.

32. Andersson DI, Hughes D. 2010. Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol 8:260–271. https://doi.org/10.1038/nrmicro2319.
33. van Hal SJ, Jones M, Gosbell IB, Paterson DL. 2011. Vancomycin heteroresistance is associated with reduced mortality in ST239 methicillin-resistant *Staphylococcus aureus* blood stream infections. PLoS One 6:e21217. https://doi.org/10.1371/journal.pone.0021217.
34. Yeaman MR, Bayer AS. 1999. Antimicrobial peptides from platelets. Drug Resist Updat 2:116–126. https://doi.org/10.1054/drup.1999.0069.
35. Yeaman MR, Bayer AS. 2006. Antimicrobial peptides versus invasive infections. Curr Top Microbiol Immunol 306:111–152.
36. Clinical and Laboratory Standards Institute. 2011. Performance standards for antimicrobial susceptibility testing: 21st informational supplement. M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.
37. Miró JM, García-de la-Mària C, Armero Y, Soy D, Moreno A, del Río A, Almela M, Sarasa M, Mestres CA, Gatell JM, Jiménez de Anta MT, Marco F, Hospital Clinic Experimental Endocarditis Study Group. 2009. Addition of gentamicin or rifampin does not enhance the effectiveness of daptomycin in the treatment of experimental endocarditis due to methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 53:4172–4177. https://doi.org/10.1128/AAC.00051-09.
38. Isenberg HD (ed). 2004. Clinical microbiology procedures handbook, 2nd ed. ASM Press, Washington, DC.
39. Marco F, García-de la-Mària C, Armero Y, Amat E, Soy D, Moreno A, del Río A, Almela M, Mestres CA, Gatell JM, Jiménez de Anta MT, Miró JM, Hospital Clinic Experimental Endocarditis Study Group. 2008. Daptomycin is effective in treatment of experimental endocarditis due to methicillin-resistant and glycopeptide-intermediate *Staphylococcus aureus*. Antimicrob Agents Chemother 52:2538–2543. https://doi.org/10.1128/AAC.00510-07.
40. Baddour LM, Wilson WR, Bayer AS, Fowler VG, Jr, Tleyjeh IM, Rybak MJ, Barsic B, Lockhart PB, Gewitz MH, Levison ME, Bolger AF, Steckelberg JM, Baltimore RS, Fink AM, O'Gara P, Taubert KA, American Heart Association Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young, Council on Clinical Cardiology, Council on Cardiovascular Surgery, Anesthesia and Stroke Council. 2015. Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association. Circulation 132:1435–1486. https://doi.org/10.1161/CIR.0000000000000296.
41. Miro JM, García-de la-Mària C, Armero Y, de-Lazzari E, Soy D, Moreno A, del Rio A, Almela M, Mestres CA, Gatell JM, Jiménez-de-Anta MT, Marco F, Hospital Clinic Experimental Endocarditis Study Group. 2007. Efficacy of televancin in the treatment of experimental endocarditis due to glycopeptide-intermediate *Staphylococcus aureus*. Antimicrob Agents Chemother 51:2373–2377. https://doi.org/10.1128/AAC.01266-06.