MAJOR ARTICLE

Dissecting the Mechanisms of Linezolid Resistance in a *Drosophila melanogaster* Infection Model of *Staphylococcus aureus*

Lorena Diaz,[1,2] Dimitrios P. Kontoyiannis,[3] Diana Panesso,[1,2] Nathaniel D. Albert,[3] Kavindra V. Singh,[2] Truc T. Tran,[2,4] Jose M. Munita,[2,5] Barbara E. Murray,[2] and Cesar A. Arias[1,2]

[1]Molecular Genetics and Antimicrobial Resistance Unit, Universidad El Bosque, Bogota, Colombia; [2]Department of Internal Medicine, Division of Infectious Diseases, University of Texas Medical School at Houston, [3]Department of Infectious Diseases, Infection Control and Employee Health, The University of Texas MD Anderson Cancer Center, and [4]University of Houston College of Pharmacy, Houston, Texas; and [5]Department of Medicine, Clínica Alemana de Santiago, Universidad del Desarrollo, Santiago, Chile

Background. Mini-host models are simple experimental systems to study host-pathogen interactions. We adapted a *Drosophila melanogaster* infection model to evaluate the in vivo effect of different mechanisms of linezolid (LNZ) resistance in *Staphylococcus aureus*.

Methods. Fly survival was evaluated after infection with LNZ-resistant *S. aureus* strains NRS119 (which has mutations in 23S ribosomal RNA [rRNA]), CM-05 and 004-737X (which carry *cfr*), LNZ-susceptible derivatives of CM-05 and 004-737X (which lack *cfr*), and ATCC 29213 (an LNZ-susceptible control). Flies were then fed food mixed with LNZ (concentration, 15–500 μg/mL). Results were compared to those in mouse peritonitis, using LNZ via oral gavage at 80 and 120 mg/kg every 12 hours.

Results. LNZ at 500 μg/mL in fly food protected against all strains, while concentrations of 15–250 μg/mL failed to protect against NRS119 (survival, 1.6%–20%). An in vivo effect of *cfr* was only detected at concentrations of 30 and 15 μg/mL. In the mouse peritonitis model, LNZ (at doses that mimic human pharmacokinetics) protected mice from challenge with the *cfr*+ 004-737X strain but was ineffective against the NRS119 strain, which carried 23S rRNA mutations.

Conclusions. The fly model offers promising advantages to dissect the in vivo effect of LNZ resistance in *S. aureus*, and findings from this model appear to be concordant with those from the mouse peritonitis model.

Keywords. *Staphylococcus aureus*; linezolid; resistance; *Drosophila melanogaster*; *cfr*.

Staphylococcus aureus is an important human pathogen [1], causing infections that are often difficult to treat [2, 3]. Linezolid (LNZ) is a bacteriostatic oxazolidinone used for the treatment of gram-positive bacterial infections [4]. The mechanism of action of LNZ involves inhibition of protein synthesis by interactions with the 50S ribosomal subunit, where it interferes with the positioning of the aminoacyl transfer RNA [5]. Common mechanisms of LNZ resistance in clinical staphylococcal isolates include nucleotide substitutions in genes encoding domain V of the 23S ribosomal RNA (rRNA) (depending on the number of copies of mutated rRNA genes) [6, 7], mutations in genes encoding the ribosomal proteins L3 and L4 (*rplC* and *rplD*, respectively) [8, 9], and methylation of A2503 in the 23S rRNA, which is mediated by Cfr [10, 11], a member of the radical S-adenosylmethionine (SAM) family [12], and also confers resistance to phenicols, lincosamides, pleuromutilins, and streptogramin A antibiotics [13], as well as to 16-membered ring macrolides [14]. The transferable *cfr* gene was initially described in animal staphylococcal isolates [15] and has now been documented in human clinical isolates

Received 27 August 2012; accepted 11 January 2013; electronically published 1 April 2013.

Presented in part: 48th Annual Meeting of the Infectious Diseases Society of America (IDSA), Vancouver, British Columbia, Canada, 21–24 October 2010, Poster 258 and 51st Inter-Science Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Chicago, US, 17–20 September 2011, Poster C1-1781/70.

Correspondence: Cesar A. Arias, MD, PhD, University of Texas Medical School at Houston, 6431 Fannin St, Rm MSB2.112, Houston, TX 77030. (cesar.arias@uth.tmc.edu).

The Journal of Infectious Diseases 2013;208:83–91

DOI: 10.1093/infdis/jit138

Table 1. *Staphylococcus aureus* Bacterial Strains Used in This Study

S. aureus Strain	LNZ MIC, μg/mL[a]	Mechanism of LNZ Resistance	Comment(s)	Reference(s)
NRS119	64	G2576T mutations in all the 5 copies of rRNA genes encoding central loop of domain V of 23S rRNA	Recovered from peritoneal fluid from an 85-year-old man undergoing peritoneal dialysis who developed peritonitis	[7, 31]
004-737X	8	Presence of the *cfr* gene on a plasmid (approximately 55 kb)	Recovered from bronchoalveolar lavage specimen from a 45-year-old women	[16]
004-737X *cfr* cured	2	. . .	In vitro susceptible derivative of 004-737X	This study
CM05	16	Presence of the *cfr* gene on the chromosome in the *mlr* operon located within a 15.5-kb plasmid-like insertion sequence	Recovered from a patient with fatal pneumonia in Colombia	[10, 11, 34]
CM05 *cfr* negative	2	. . .	In vitro susceptible derivative of CM-05	This study
ATCC 29213	2	. . .	LNZ-susceptible control strain	. . .

Abbreviations: LNZ, linezolid; MIC, minimum inhibitory concentration.

[a] Determined by the Etest on Mueller-Hinton agar.

[9–11, 14–18], including those from a hospital outbreak of linezolid resistant *S. aureus* [17].

The common approach to detect antibiotic resistance in clinical practice is based on the determination of minimal inhibitory concentrations (MICs), using conventional in vitro susceptibility tests. Although MIC determination is a very useful tool, there are limitations in drawing in vivo inferences from MICs, because outcomes in patients treated with a particular antibiotic may depend on other factors, such as differences in in vivo expression of resistance genes, the influence of the immune response on bacterial killing, and pharmacokinetic/pharmacodynamic (PK/PD) parameters of a particular drug at the sites of infection [19]. The most tantalizing example of these limitations involves the ongoing controversy of vancomycin breakpoints and patient outcomes of MRSA infection [20].

Based on the above rationale, the possibility of testing antimicrobial resistance using animal models emerges as an attractive alternative to evaluate the host-pathogen interaction. However, important limitations of using mammalian models for this purpose include costs, logistics, turnaround time, and ethical issues. Thus, nonmammalian models have been developed as surrogate hosts for evaluation of bacterial virulence and antimicrobial effectiveness [21–23]. The fruit fly *Drosophila melanogaster* is genetically well defined, has a short generation time, and possesses an innate immune system that is remarkably similar to that of humans [24]. Moreover, the *D. melanogaster* model has been used for the analysis of *S. aureus* pathogenesis, where inoculation results in systemic infection followed by death [25–27]. Previous studies have also shown that this mini-host model is useful for testing the therapeutic response to antifungals [28–30]. In this study, we assessed the in vivo effect of different mechanisms of LNZ resistance in *S. aureus*, using the mini-host model of *D. melanogaster*. We show that important differences in the in vivo therapeutic effect of LNZ are dependent on the specific mechanism of resistance, suggesting that *D. melanogaster* offers promise as a simple and cost-effective model for the in vivo evaluation of antimicrobial resistance.

METHODS

Bacterial Strains and Growth Conditions

Three LNZ-resistant clinical strains of *S. aureus* were evaluated (Table 1): NRS119, which harbors the G2576T substitution in the 23S rRNA genes (all 5 copies) [7, 31]; 004-737X, which carries the *cfr* gene on a plasmid (55 kb) [16]; and CM05, which harbors *cfr* on the chromosome [10, 32]. *S. aureus* ATCC 29213 was also included as a LNZ-susceptible control. LNZ MICs were determined by the Etest. To assess the growth kinetics, bacterial strains were grown in trypticase soy broth (TSB). Overnight cultures were inoculated in TSB, and A_{600} was assessed from 0 to 12 hours and at 24 hours.

Selection of LNZ-Susceptible Derivatives of *S. aureus* 004-737X and CM05 Lacking *cfr*

To obtain a susceptible derivative of 004-737X, which carries the *cfr* gene on a plasmid, we cured the *cfr* plasmid as described previously [33]. Individual colonies were screened by replica plating in Mueller-Hinton (MH) agar containing chloramphenicol (30 μg/mL) and LNZ (4 μg/mL). Additionally, we

identified a derivative of CM05 that spontaneously lost the *cfr* gene, using replica plating of individual CM05 colonies in the presence and absence of LNZ and chloramphenicol, as described before [34]. LNZ-susceptible derivatives from both 004-737X and CM05 were characterized by polymerase chain reaction for detection of the *cfr* gene [15], MIC determination by the Etest, pulsed-field gel electrophoresis (PFGE) [35], and S1 nuclease digestion coupled with PFGE and hybridization with a *cfr* probe [36].

D. melanogaster Infection Model

Wild-type Oregon R *D. melanogaster* flies were maintained using standard procedures for manipulation, feeding, and housing [37]. Flies were infected via thoracic injection with *S. aureus* strains. Bacterial cells from overnight cultures (5 mL of brain heart infusion [BHI] broth) were recovered by centrifugation at 4000 ×*g* for 5 minutes at room temperature. Cells were resuspended in saline (bacterial inoculum of approximately 1×10^8 colony-forming units [CFU]/mL, verified by colony counts on BHI agar). Groups of 25 female flies (2–4 days old) were placed in containers without food for 6 hours and subsequently anesthetized with CO_2. Flies were then infected with the *S. aureus* strains by pricking their dorsal thorax with a needle that had been dipped into the bacterial solution. The infected flies were transferred to tubes containing food mixed with LNZ (human oral suspension of Zyvox, Pfizer, NY) at concentrations of 15, 30, 60, 125, 250, and 500 μg/mL. Flies fed food without LNZ were included as a control in all experiments. Deaths resulting in the first 3 hours after infection were excluded from the survival analysis because they were likely to be caused by the inoculation procedure. Flies were incubated at 30°C, transferred to receive fresh food every 3 days, and monitored for 8 days. Three independent experiments were performed at each antibiotic concentration on different days and at the same time of the day, to eliminate circadian rhythm–associated variability.

Determination of LNZ Toxicity and Concentration in Flies

To determine whether LNZ is toxic to *D. melanogaster*, groups of 30 uninfected flies were fed with food supplemented with LNZ at concentrations of 500, 1000, and 2000 μg/mL and observed for 8 days. To estimate the concentration of LNZ in flies, groups of 300 female wild-type flies were fed with LNZ at concentrations of 0, 30, 60, 125, 250, and 500 μg/mL after a 6-hour starvation period. Subsequently (24 hours after feeding), flies were transferred to empty tubes, weighted, rinsed twice with sterile saline, and macerated in hybridization bags with 400 μL of sterile water. Fly homogenates were centrifuged for 3 minutes at $2348 \times g$. The supernatants were recovered and stored at −4°C while protected from light. A bioassay was carried out using *S. aureus* ATCC 25923, which was grown in MH broth to obtain a cell suspension of 1×10^8 CFU/mL and then diluted to 1×10^7 CFU/mL in MH broth. The bacterial suspension was then spread on MH agar plates and allowed to dry at ambient temperature. Full-thickness holes (6 mm in diameter) were punched in the agar. A total of 60 μL of supernatant from fly homogenates was placed in each well, and plates were incubated aerobically at 37°C for 24 hours. Diameters of circular inhibition zones that developed around the LNZ-containing wells were used to determine the LNZ concentration, using a linear regression model. Each determination was repeated 4 times.

Mouse Peritonitis Model

Female (age, 4–6 weeks), outbred ICR mice (Harlan Sprague Dawley, Houston, TX) were used as previously described [38]. *S. aureus* 004-737X, its susceptible derivative (004-737X *cfr*-cured), and NRS119 were included in these experiments. Inoculation was performed by intraperitoneal injection of 1 mL of bacterial inoculum ($2–4 \times 10^9$ CFU/mL) of the corresponding staphylococcal strains in a suspension containing 50% sterile rat fecal extract, as described previously [38]. Groups of 6 mice in each treatment arm and a group of 3 mice as an infection control were included. Survival was recorded 96 hours after infection. One hour after bacterial inoculations, mice were given LNZ at 80 mg/kg or 120 mg/kg every 12 hours via oral gavage, with treatment continuing for 7 doses on the basis of previously published mouse PK data that correlate with human dosage [39–41]. Surviving animals were euthanized 96 hours after inoculation. Kidneys and spleens were harvested and homogenized in saline. Colony counts on BHI agar from the homogenates were performed to determine bacterial densities in tissues. Experiments were approved by the Animal Welfare Committee, University of Texas Health Science Center at Houston.

Statistical Analysis

Bacterial growth curves were compared for the resistant strains and their susceptible derivatives, using an unpaired 2-tailed *t* test at each time point. Survival curves were plotted using Kaplan-Meier analysis and compared using the log-rank test. Survival rates were compared using the unpaired 2-tailed *t* test. The geometric mean $\log_{10}$ CFU of bacteria recovered from kidneys and spleens were analyzed for statistical significance by analysis of variance with the Tukey post hoc test. The analyses were performed using GraphPad Prism, version 4.0 (GraphPad Software, San Diego, CA). Data were considered statistically significant if P values were $< .05$.

RESULTS

LNZ Protects *D. melanogaster* Against *S. aureus* Infection

Figure 1*A* shows that intrathoracic infection with *S. aureus* ($8 \times 10^7–3 \times 10^8$ CFU/mL) caused approximately 100% mortality by 3 days in the mini-host model regardless of the infecting

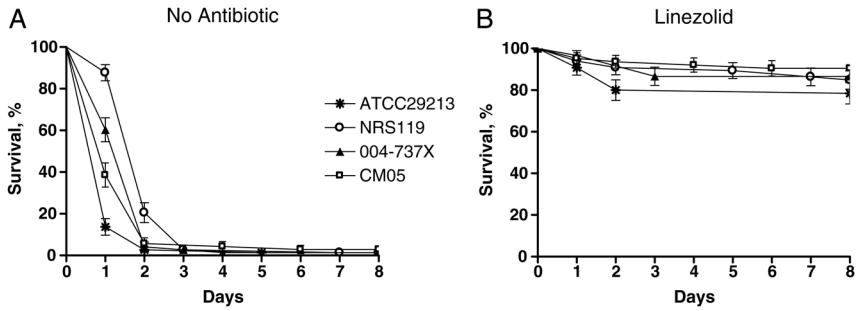

Figure 1. Survival curves of *S. aureus* strains in the mini-host model of *D. melanogaster*. A, survival curves in the absence of antibiotic. B, survival curves in the presence of linezolid (500 μg/mL) administered in the fly food after 6 hours of starvation.

strain. Of interest, *S. aureus* ATCC 29213 resulted in the shortest time to death, with 86% of flies dying by 24 hours ($P \leq .0006$). LNZ was not toxic to *D. melanogaster*, as no lethality was observed after 8 days of administration of food supplemented with LNZ concentrations of 500, 1000, and 2000 μg/mL in the absence of bacterial challenge (data not shown).

To assess the protective effect of LNZ, flies were initially fed food supplemented with LNZ at a final concentration of 500 μg/mL immediately after challenge with different strains of *S. aureus*, continued for 8 days. LNZ at the above concentration fully protected the flies against challenge by all strains regardless of susceptibility to LNZ (survival, 80%–95% at 8 days; $P < .0001$ for all strains, compared with survival in the absence of the antibiotic; Figure 1*B*). These findings indicate that high concentrations of LNZ in flies were sufficient to overcome the in vivo effect of resistance, irrespective of the type of LNZ resistance.

The In Vivo Effect of LNZ Resistance in *D. melanogaster* Is Dependent on Antibiotic Concentration and Mechanism of Resistance

Since we did not observe an in vivo effect of LNZ resistance with antibiotic concentrations of 500 μg/mL added as a supplement to the fly food, we subsequently tested descending amounts of the antimicrobial (250, 125, 60, 30, and 15 μg/mL). Concentrations as little as 15 μg/mL of LNZ in the food protected *D. melanogaster* against challenge by *S. aureus* ATCC 29213 (LNZ susceptible), with survival rates of >78% at 8 days ($P < .0001$, compared with infection in the absence of antibiotic; Figure 2*A*). In contrast, LNZ at concentrations of 15–250 μg/mL failed to protect flies when challenged with NRS119 (MIC, 64 μg/mL), with survival rates ranging from 1.6% to 20% after 8 days of treatment (Figure 2*B*).

Consistent with differences in MICs, the in vivo effect of *cfr* against LNZ was strikingly different from that observed with NRS119 (which harbors the mutational mechanism). Indeed, LNZ remained active against 004-737X (which harbors *cfr* on a plasmid; MIC, 8 μg/mL) at concentrations in food of ≥60 μg/mL (survival rates, ≥80%; Figure 2*C*). The in vivo effect of *cfr*-mediated resistance was only observed at 30 and 15 μg/mL, with survival of 41% and 36%, respectively ($P < .0001$ for both values, compared with a concentration of 60 μg/mL [90% survival]). With CM05, which has only 1 chromosomal copy of *cfr* [32, 33], resistance in vivo could only be identified at the lowest concentration tested (15 μg/mL in the fly food), with a survival rate of only 29% ($P < .0001$, compared with 30 μg/mL; Figure 2*E*). Our findings indicate that *cfr* is less efficient at conferring resistance in vivo, compared with mutations in 23S rRNA.

To establish a direct correlation between the in vivo effect of LNZ against LNZ-resistant *S. aureus* carrying the *cfr* gene, we created LNZ-susceptible derivatives of *S. aureus* 004-737X and CM05 by curing the *cfr* plasmid and selecting cells lacking *cfr*, respectively. The genetic identities of the *cfr*-negative derivatives were confirmed by PFGE in both instances and by S1 digestion for 004-737X (Supplementary Figure 1*A* and 1*B*). The loss of *cfr* did not affect the in vitro growth kinetics of the strains (Supplementary Figure 1*C*), and, as shown in Table 1, the loss of *cfr* reduced the LNZ MIC to levels below the susceptibility breakpoint, confirming the direct correlation between *cfr* and LNZ resistance. Moreover, LNZ at all concentrations protected all *cfr*-negative derivatives (Figure 2*D* and 2*F*), confirming that *cfr* only modestly affected the activity of LNZ in vivo.

Since the in vivo therapeutic effect of LNZ was dependent on the concentration used in the fly food, we developed a bioassay to estimate the amount of the drug in the insect bodies. Using a simple regression linear model constructed with LNZ standard solutions (Supplementary Figure 2), we determined LNZ concentrations in the supernatant of fly homogenates. Table 2 shows the estimated concentrations of LNZ per insect (μg) and per fly homogenate (μg/mL). Our data suggest that the in vivo effect observed is due to the presence of LNZ in tissues and that LNZ is absorbed in *D. melanogaster* in a dose-dependent manner.

Comparison of the Mini-Host Model With a Murine Model of Peritonitis

To compare our mini-host model results with those from a mammalian model of infection, a mouse peritonitis model

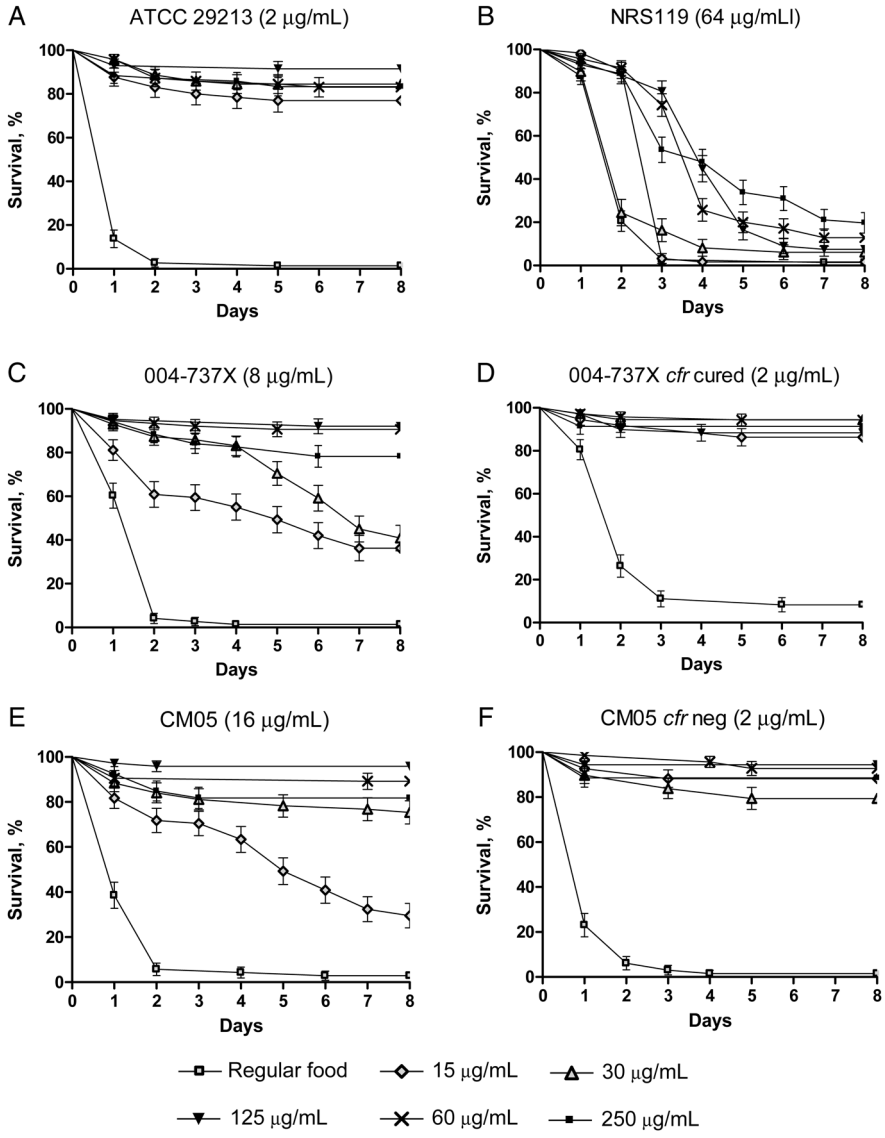

Figure 2. Survival curves of *S. aureus* strains using different concentrations of linezolid. Flies were fed with food supplemented with linezolid at concentrations from 15 to 250 μg/ml; symbols for each concentration, are indicated at the bottom. Each panel shows a single strain using all linezolid concentrations and the control without antibiotic. The name of each strain and its linezolid MIC are indicated at the top of each panel.

Table 2. Determination of Linezolid Concentrations in Flies

LNZ Concentration in Fly Food, μg/mL	Flies Weight, g	Concentration in Supernatant From Fly Homogenates, μg/mL	Amount in Total Volume of Fly Homogenates, μg	Estimated Amount per Fly, μg	Estimated Concentration per mL of Fly Homogenate, μg/mL
30	0.3660	<6	ND	ND	<12.5
60	0.3692	<6	ND	ND	<12.5
125	0.3137	15.42	11.66	0.0388	35.11
250	0.2961	29.58	20.68	0.0689	69.54
500	0.3067	45.59	32.23	0.1074	105.06

Abbreviations: ND, unable to determine.

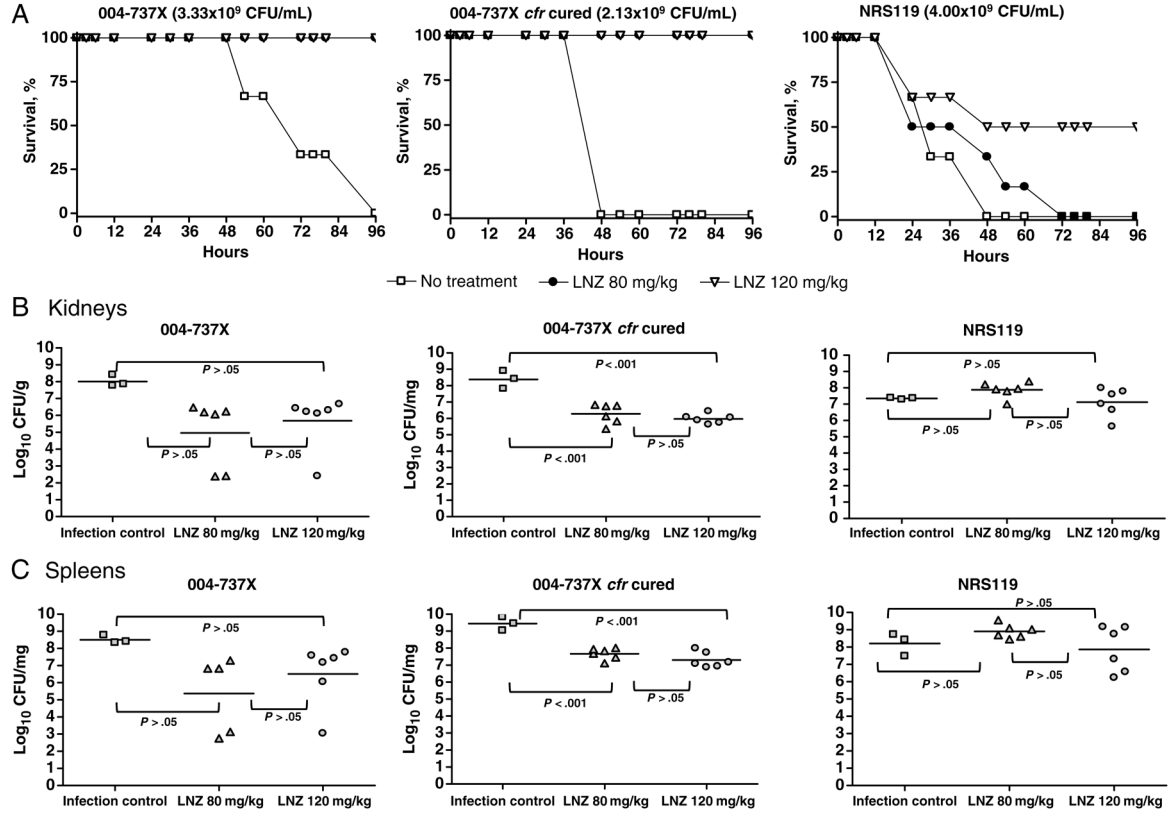

Figure 3. In vivo effect of linezolid against *S. aureus* challenge in a mouse peritonitis model. A, survival curves using the three strains, 004-737X, its susceptible derivative (004-737X cfr cured) and NRS119. Inocula are shown in parenthesis. B, bacterial densities in kidney homogenates and C, bacterial densities in spleen homogenates. For B and C, data are expressed as $\log_{10}$ CFU/mg, horizontal bars represent the geometric mean titers, which were compared by ANOVA with Tukey's post-hoc test. A P value of <.05 was considered significant. Abbreviations: CFU, colony-forming units; LNZ, linezolid.

was used to assess the in vivo efficacy of LNZ against *S. aureus* 004-737X (MIC, 8 μg/mL), a *cfr*-cured derivative of 004-737X (MIC, 2 μg/mL), and NRS119 (MIC, 64 μg/mL). This model has been previously used to evaluate the response to LNZ therapy [40, 41]. The drug was administrated orally via gavage every 12 hours at doses of 80 mg/kg and 120 mg/kg, which, according to previous PK/PD studies, mimic human parameters (peak serum concentrations of LNZ in humans after an oral dose of 600 mg are 15–27 μg/mL) [39–42]. Figure 3 shows that all strains killed the host (100% mortality at 96 hours), using similar inocula in the absence of antibiotic. Mortality at 48 hours in mice infected with NRS119 was 100% as compared to 0% with 004-737X at similar inocula ($P < .0001$), suggesting a difference in virulence between the 2 strains in this model. Moreover, the loss of *cfr* increased the rate of killing of 004-737X, with mortality approaching 100% at 48 hours, suggesting that the *cfr*-containing plasmid (or other plasmids) may affect the in vivo fitness of the strain. Similar to the results reported above for *Drosophila*, *cfr* had no effect on survival at LNZ doses used in the mouse model. Indeed, both dose schemes protected all animals at 96 hours (100% survival). In contrast, the mutational mechanism of resistance present in NRS119 reduced the protection achieved by LNZ therapy, although the effect, as observed in the mini-host model, was concentration dependent (Figure 3*A*). The difference in the in vivo effect of the mechanisms of LNZ resistance was also observed in tissues (Figures 3*B* and 3*C*). LNZ did not produce a significant decrease in colony counts in either spleen or kidney specimens when mice were infected with *S. aureus* NRS119. Interestingly, *cfr* carried by strain 004-737X produced only a weak in vivo effect against LNZ, since the antibiotic reduced bacterial densities in both organs at the tested doses, although the values did not reach statistical significance ($P > .05$). Notably, a significant decrease in bacterial counts in both organs at both dose schemes was observed after loss of the *cfr*-carrying plasmid from strain 004-737X (Figure 3). Our findings suggest that the in vivo effect of the mechanisms of LNZ resistance is comparable between the *D. melanogaster* and mouse models.

DISCUSSION

Animal models to study host-pathogen interactions are important tools in the understanding of virulence and response to antimicrobial therapy. The use of small-mammal infection

models has important limitations, and thus invertebrate models have become popular tools for studying host-pathogen interactions [43]. *D. melanogaster* is an inexpensive model for the study of pathogenesis and antibiotic interactions of gram-positive organisms, including *Listeria monocytogenes* [44], *Bacillus subtilus*, staphylococci, *Enterococcus faecalis*, *Streptococcus pneumoniae,* and *Streptococcus pyogenes* [25, 28]. Moreover, *D. melanogaster* has a fully developed innate immune system comparable to that of mammals [24], and organisms such as *S. aureus* are highly virulent in this model, with low survival rates in the first 48 hours after infection (bacterial inocula, 10^7–10^9 CFU/mL) [25, 45, 46]. Indeed, using the *Drosophila* model, Needham et al were able to study *S. aureus* virulence and the effect of antibiotics such as methicillin and tetracycline, which can rescue flies from *S. aureus* infection [25].

In this work, we adapted a *D. melanogaster* model to assess the efficacy of LNZ therapy and the in vivo effect of mechanisms of LNZ resistance. We aimed to dissect the in vivo role of 2 different mechanisms, namely, mutations in the 23S rRNA genes and Cfr-mediated resistance, which confer different levels of resistance in vitro, and to determine a correlation between our *D. melanogaster* model, MICs, and a mouse model. We chose LNZ since it is an important anti–*S. aureus* antimicrobial agent that can be administered orally with excellent bioavailability in humans. To determine the effect of LNZ against *S. aureus* infection in our mini-host model, we mixed fly food with the antibiotic and assessed the in vivo effectiveness of the drug. Of note, LNZ at 500 μg/mL mixed in the fly food fully protected *D. melanogaster* against *S. aureus* strains, including those that harbor well-characterized mechanisms of resistance, suggesting that high doses of the antibiotic in this model can overcome the mechanism without exhibiting overt toxicity. However, it is important to note that equivalently high linezolid levels in humans may have clinically important toxic effects on bone marrow. Lower concentrations of LNZ in food showed a clear in vivo difference between mutational and *cfr*-mediated mechanisms of LNZ resistance consistent with the MICs. Indeed, LNZ protection against NRS119, which harbors mutations in 23S rRNA genes (MIC, 64 μg/mL) was not seen at concentrations of ≤250 μg/mL in the fly food (survival at 8 days, <20%). Conversely, we only detected lack of LNZ protection in the *cfr*-carrying strains (with lower LNZ MICs) at reduced antibiotic concentrations (30 and/or 15 μg/mL in the food), indicating that the in vivo effect of *cfr*-mediated LNZ resistance is only evident at low concentrations of the antibiotic. Of interest, linezolid protected flies against CM05 at concentrations of 30 and 60 μg/mL, even though the amount of antibiotic detected in fly extracts was below the MIC for the organism. This could indicate that, even at subinhibitory concentrations, LNZ may have a protective effect against this strain. Alternatively, our assay may not correlate with actual concentrations in deep tissues at low doses.

To compare the in vivo effect of resistance in our mini-host model with that of a more established mammal model of infection, we used the mouse peritonitis model, which has been widely used in testing antibiotics [47–49]. We evaluated 2 LNZ regimens that had been previously shown to be effective in mice against intraperitoneal challenge with different *S. aureus* strains [39–41, 48]. Both doses of LNZ protected the animals from death after intraperitoneal challenge of the *cfr*-carrying *S. aureus* strain 004-737X, with 100% survival at 96 hours. Conversely, the effect of the resistance mechanism was evident in strain NRS119, with 50% and 100% mortality at 120 and 80 mg/kg, respectively. On the other hand, *cfr*-mediated resistance had no impact on survival in the mouse model, and a modest (albeit nonstatistically significant) reduction in CFU from tissues was observed. Thus, it is tempting to speculate that *cfr* may impact in vivo bacterial fitness and that LNZ may negatively affect the production of virulence determinants in *S. aureus* [50], preventing progression to an inflammatory cascade that results in clinical symptoms.

The mini-host model is an easy, inexpensive, potentially high-throughput, and reproducible alternative to evaluate the in vivo resistance in *S. aureus*. However, it also has some limitations. First, the amount of LNZ absorbed cannot be precisely determined, and PK/PD determinations would be difficult to establish, although we showed that antibiotic concentrations in fly homogenates correlate with in vivo efficacy. Second, it seems likely that only oral antimicrobials would be evaluable in this model. Third, virulence determinants may target different cells in each model, and therefore the effect of the antibiotic may not be totally comparable. And fourth, despite the considerable similarities in innate immune mechanisms, invertebrate models are not directly comparable with mammalian models. Thus, it is reasonable to expect that some of the virulence attributes of *S. aureus* that affect mammals might not be important in the fly model. Nonetheless, we have shown striking similarities in the in vivo behavior of LNZ resistance mechanisms between our mini-host and murine models, which may possibility lead to use of *D. melanogaster* as a model for dissecting the in vivo effect of different mechanisms of antibiotic resistance in gram-positive pathogens.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgment. We thank David S. Lopez for assistance with statistical analyses and Isabel Reyes for technical assistance.

Financial support. This work was supported in part by Pfizer (an Investigator Initiated Research grant); the National Institute of Allergy and Infectious Diseases, National Institutes of Health (grant R01-AI093749 to C. A. A.); the Instituto Colombiano para el Desarrollo de la Ciencia y Tecnología, Francisco José de Caldas, COLCIENCIAS (graduate scholarship to L. D.); the American Society of Microbiology (Latin American Fellowship for Epidemiology to L. D.); and the Universidad El Bosque (graduate fellowship to L. D.).

Potential conflicts of interest. Dr Arias has received lecture fees, research support, and consulting fees from Pfizer, consulting fees from Cubist, and research support from Astellas, Theravance, and Forest Pharmaceuticals, and he has served as a speaker for Pfizer Novartis, Forest Pharmaceuticals and Cubist. Dr Murray receives grant support from Astellas, Theravance, Forest Pharmaceuticals, and Cubist, and he has served as consultant for Astellas, Theravance, Cubist, Targanta Therapeutics Corporation, Pfizer, Rib-X, AstraZeneca, and Durata Therapeutics. Dr Singh has received research support from Cubist, Astellas, Theravance, and Forest Pharmaceuticals. Dr Kontoyiannis received research support from Merck, Pfizer, and Astellas, and he has served as a speaker for Gilead and as a consultant for Merck. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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