

# Efficacy of oritavancin and comparator agents against vancomycin-resistant *Enterococcus faecium* in an *in vivo* *Galleria mellonella* survival model

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## Abstract

### Objective

The optimal antimicrobial therapy for serious vancomycin-resistant enterococcal (VRE) infections remains unclear. Oritavancin has demonstrated potent bactericidal activity against VanA type VRE *in vitro*. *Galleria mellonella* is a well-established model of both microbial virulence and therapeutic response.

### Methods

5 isolates were used: *E. faecalis* ATCC 29212, *E. faecium* VanA ATCC 700221 (VRE), and 3 *E. faecium* VanA VRE clinical bloodstream isolates. Bacterial inocula were adjusted in an initial pilot study until approximately 90% of larvae died within 72 hours of inoculation. Antibiotic regimens tested were oritavancin (O) 15 mg/kg, daptomycin (D) 10 mg/kg, linezolid (L) 7.5 mg/kg, gentamicin (G) 1.3 mg/kg, ceftriaxone (C) 25 mg/kg, and rifampin (R) 3.75 mg/kg, representing humanized weight-based doses. Groups of 10 larvae were injected with each isolate followed by each antimicrobial agent after approximately 1 hour, with one group of 10 receiving no antimicrobial treatment (untreated group). Uninfected PBS-injected and untouched control groups were included with each experiment. After injection, larvae were incubated at 37°C and survival was measured daily for 7 days. Survival was plotted via Kaplan-Meier curves and groups were compared using the log rank test with Bonferroni correction for multiple comparisons (SPSS version 22, Inc., Chicago, IL).

### Results

Aggregate log-rank pairwise comparisons of each antibiotic compared to the untreated group are shown in Table 1, adjusted for the pathogen being tested. Each antimicrobial agent significantly improved survival over the untreated group. O provided the largest magnitude of survival across all isolates ( $\chi^2=76.9$ ) although strain-to-strain variability in efficacy was observed. D and C afforded the least survival. Compared to the other agents, O increased survival over C, G, and D but not compared to L or R. Mean survival time ranged from 3.2 days for C to 3.9 days for O vs. 2.4 days for the untreated group.

**Table 1. Log Rank Pairwise Comparisons**

Antibiotic	Untreated group	
	$\chi^2$	p-value
C	26.3	0.001
L	58.3	<0.001
G	36.8	<0.001
D	18.3	0.007
R	60.3	<0.001
O	76.9	<0.001

### Conclusion

Compared to the untreated controls, O was the most efficacious agent in this *in vivo* *G. mellonella* survival model, although efficacy was strain-dependent. O provided significantly improved survival over C, G, and D but not over L or R.

## Background

- The optimal antimicrobial regimen for serious VRE infections remains unclear, although combination therapy is often recommended to achieve cidal and improve clinical outcomes
- Resistance rates and reports of therapeutic failures with currently available anti-VRE agents, such as daptomycin, are increasing
- Oritavancin is a novel lipoglycopeptide antimicrobial that has demonstrated bactericidal activity *in vitro* against VanA-type VRE
- Invertebrate models can provide preliminary *in vivo* data quickly and inexpensively
- The innate immune system and immune response to microbial virulence of *Galleria mellonella* is similar to that of mammals
- G. mellonella* have advantages over other invertebrate models in that they can be incubated at human body temperature and both the inoculum and antibiotic dose can be delivered directly to the host body
- The objective of this study was to evaluate the efficacy of ceftriaxone, linezolid, daptomycin, gentamicin, rifampin, and oritavancin against VRE in a *G. mellonella* survival model

## Methods

- A total of 5 organisms were utilized for all experiments:
  - Vancomycin-susceptible *E. faecalis* ATCC 29212
  - VanA-type *Enterococcus faecium* ATCC 700221
  - 3 vancomycin-resistant VanA-type *E. faecium* clinical bloodstream isolates
- MICs were performed in triplicate according to CLSI guidelines
  - 0.002% polysorbate-80 added to all assays containing oritavancin
  - Ca<sup>2+</sup> adjusted to 50 µg/mL for assays containing daptomycin
- G. mellonella* larvae at final instar stage were acquired from wholesaler and used within 7 days
- Groups of 10 healthy larvae weighing at least 250 mg and free of any grey markings were used for each experiment
- Each experiment included a control group of larvae injected once or twice with PBS to assess needle trauma and one untouched control group for attrition
- After injection, larvae were incubated at 37°C and survival was measured daily for 7 days via response to manual stimulation
- Models proceeded in stepwise fashion to ensure lack of toxicity of the antimicrobials and confirm the lethality of the inoculum of each test strain
- For each experiment, larvae were inoculated with the test strain followed by each antimicrobial agent approximately 1 hour after inoculation
- Larvae were injected with humanized weight-based doses of each antibiotic as follows:
  - Ceftriaxone 25 mg/kg
  - Linezolid 7.5 mg/kg
  - Daptomycin 10 mg/kg
  - Gentamicin 1.3 mg/kg
  - Rifampin 3.75 mg/kg
  - Oritavancin 15 mg/kg
- Larvae survival was plotted via Kaplan-Meier method and differences in survival between groups was compared via log-rank test with Bonferroni correction

## Results

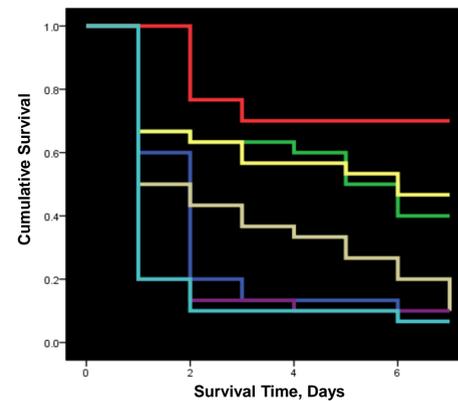
**Table 1. MIC values, in µg/mL, of Enterococcus strains to tested antimicrobials**

Antibiotic	<i>E. faecalis</i> ATCC 29212	<i>E. faecium</i> ATCC 700221	VRE W21579	VRE H19570	VRE S38141
Ceftriaxone	128	>256	>256	>256	>256
Linezolid	2	2	2	2	2
Gentamicin	16	>256	>256	>256	>256
Daptomycin	1	1	1	2	1
Rifampin	2	>256	>256	64	>256
Oritavancin	0.03	0.03	0.06	0.125	0.06

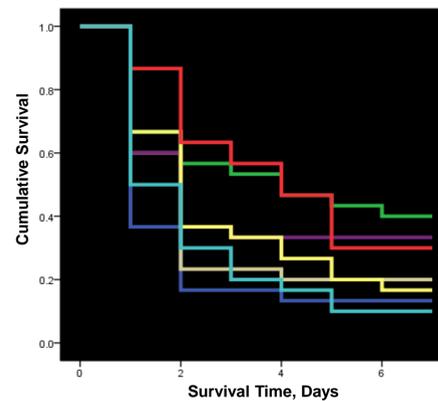
**Table 2. Aggregate log-rank pairwise comparisons of each tested antimicrobial compared to untreated controls**

Antibiotic	$\chi^2$	Untreated Group	P value
Ceftriaxone	26.3		0.001
Linezolid	58.3		<0.001
Gentamicin	36.8		<0.001
Daptomycin	18.3		0.007
Rifampin	60.3		<0.001
Oritavancin	76.9		<0.001

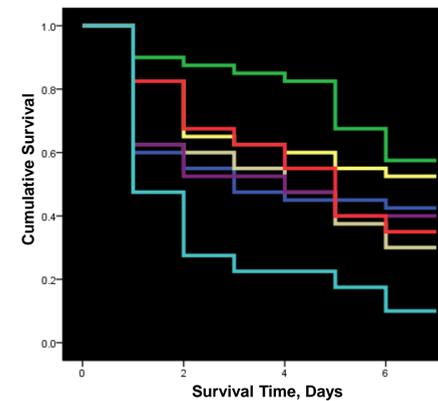
**Figure 1. Kaplan-Meier survival function of *G. mellonella* larvae infected with *E. faecalis* ATCC 29212**



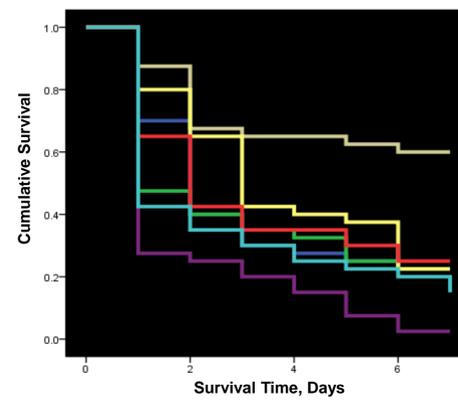
**Figure 2. Kaplan-Meier survival function of *G. mellonella* larvae infected with VRE ATCC 700221**



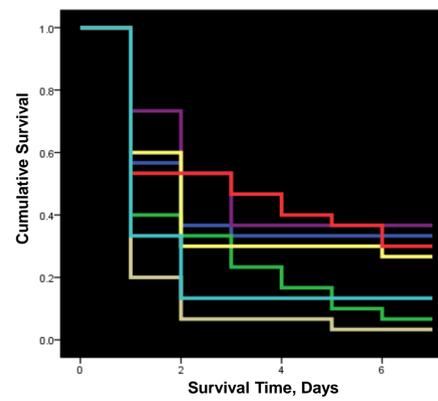
**Figure 3. Kaplan-Meier survival function of *G. mellonella* larvae infected with VRE W21579**



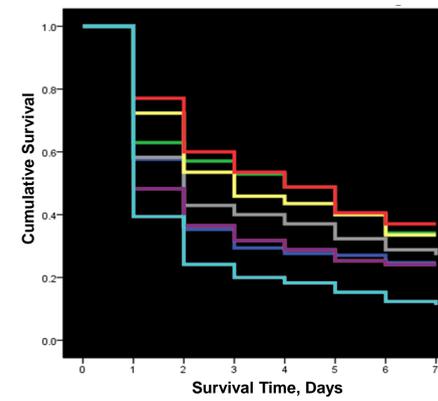
**Figure 4. Kaplan-Meier survival function of *G. mellonella* larvae infected with VRE H19570**



**Figure 5. Kaplan-Meier survival function of *G. mellonella* larvae infected with VRE S38141**



**Figure 6. Aggregate Kaplan-Meier survival function of *G. mellonella* larvae infected with all tested pathogens**



Antibiotic: Ceftriaxone (blue), Linezolid (green), Gentamicin (yellow), Daptomycin (purple), Rifampin (orange), Oritavancin (red), Control (cyan)

**Figure 7. Top: Healthy, uninfected *G. mellonella* larvae. Bottom: *G. mellonella* larvae infected with VRE. The humoral immune response is characterized by melanization and hemolymph coagulation, a process analogous to abscess formation in mammals.**



## Conclusions

- In vitro* susceptibility did not predict efficacy in *G. mellonella* survival model
- Oritavancin provided the highest rate of survival compared to infected untreated control groups
- Oritavancin significantly improved survival over ceftriaxone, gentamicin, and daptomycin
- Efficacy between oritavancin, linezolid, and rifampin was similar
- Significant strain-to-strain variability in efficacy was observed
- G. mellonella* may provide a rapid and cost-effective method to assess antimicrobial activity between *in vitro*, mammalian, and human models
- Further data are needed to validate the results of this study in dose-response experiments and models containing phenotypically diverse strains of VRE

## Disclosures

- This study was supported by The Medicines Company

## References

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