Illuminating *Klebsiella pneumoniae* Infections:

A Bioluminescent Platform for Longitudinal Assessment of Antibacterial Activity

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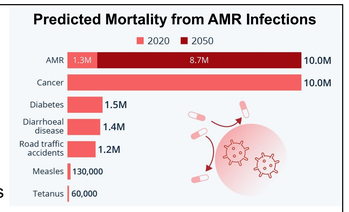
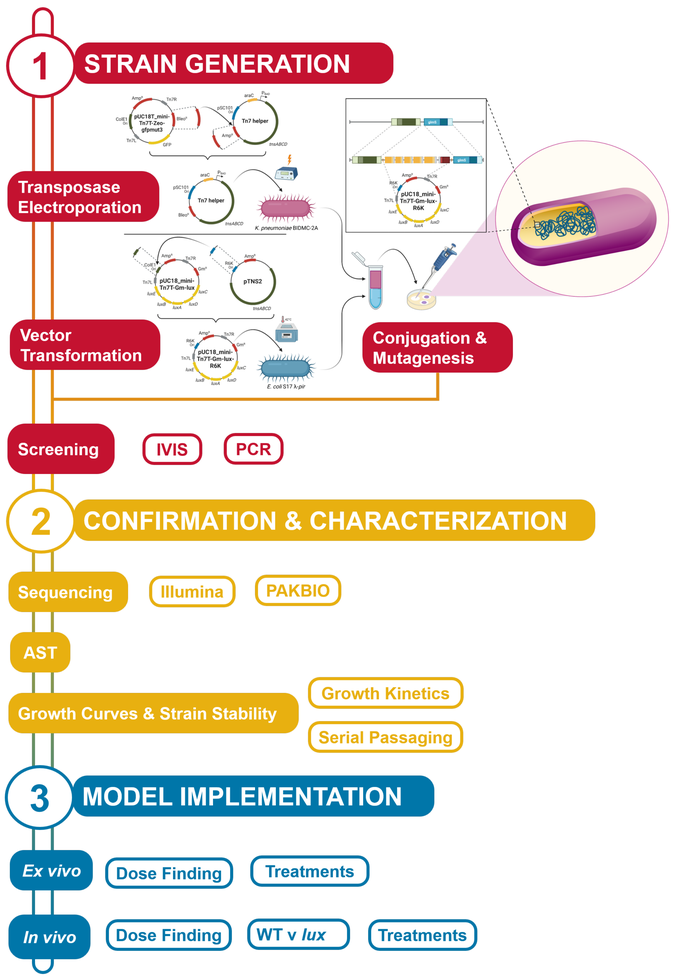
BACKGROUND

- Antimicrobial resistant infections were associated with 4.71 million deaths in 2021 (Naghavi, et al., 2024)
  - *Novel antibacterial solutions to replace or supplement antibiotics are needed.*
- *Klebsiella pneumoniae* is one of the most prevalent gram-negative pathogens isolated from combat wounds
  - ***99.7%*** *of isolates from blast wounds* ***are multidrug-resistant*** (Beley, et al., 2024).
- Current models of *K. pneumoniae* SSTI infections are limited:
  - Utilize laboratory strains which are antibiotic-sensitive and non-clinically relevant.
  - Bacterial enumeration requires serial sacking of animal models.

GOAL: Develop clinically-relevant, luminescent strains of *K. pneumoniae* for *in vitro, ex vivo, and in vivo* assessment of novel antimicrobials.



MATERIALS & METHODS



RESULTS & DISCUSSION

Figure 1: Validation of a bioluminescent *K. pneumoniae* BIDMC-2A strain. WT and *lux* strains were plated on selective agar and imaged under (left) brightfield and (right) an IVIS luminescence imaging system. The *lux* strain produces a strong luminescent signal, while the WT strain shows no detectable signal.

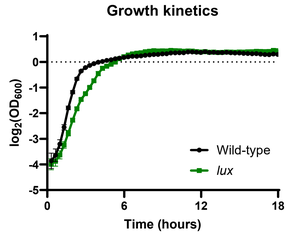


Figure 2: *In vitro* growth kinetics of *K. pneumoniae* BIDMC-2A and BIDMC-2A::Tn7-*lux*. Optical density at 600 nm OD600 was measured over time for the parental BIDMC-2A strain and the bioluminescent derivative grown under identical conditions. Growth kinetics were comparable between strains, with similar exponential growth and stationary phase densities.

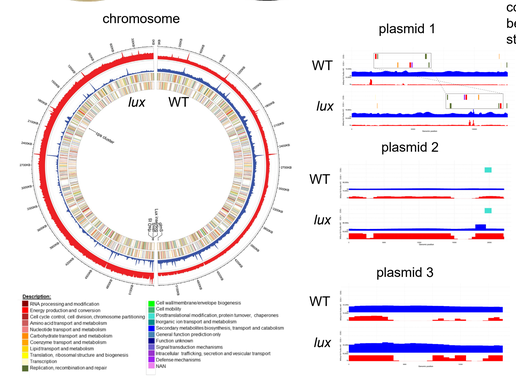


Figure 3: Whole genome alignment of *K. pneumoniae* BIDMC-2A WT and Tn7-*lux* strain. Whole genomes of the WT and *lux* variant were assembled from Illumina and PacBio reads. Integration at the Tn7 site 26 bp downstream of *glmS* was confirmed in the mutant of BIDMC2A::*lux*, and neither the Tn7 helper nor donor plasmids were present.

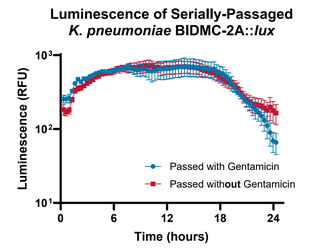


Figure 4: *In vitro* bioluminescence kinetics of *K. pneumoniae* BIDMC2A::Tn7-*lux* after passaging. Bioluminescence (relative fluorescent units, RFU) was measured over time following five serial passages with or without gentamicin in the media. BIDMC2A::Tn7-*lux* exhibited robust and sustained luminescent signal despite the absence of gentamicin.

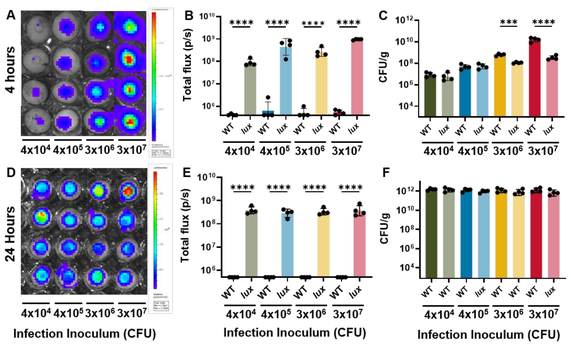


Figure 5: Dose finding for *ex vivo* porcine skin explant infections with *K. pneumoniae* BIDMC2A::Tn7-*lux*. Explants were imaged for **(A,B,D,E)** luminescence and serially processed for **(C,F)** CFU at 4 and 24 hours to identify minimum inoculum concentration and investigate correlation between luminescence and bioburden. Luminescence correlated well (R^2=0.90) with CFU.

Data is presented as the mean ± SD (*n* = 4) with the x-axis set to the limit of detection (LOD). Statistical analysis was performed with a Tukey multiple comparisons test on a one-way ANOVA: *** p < 0.001, **** *p* < 0.0001

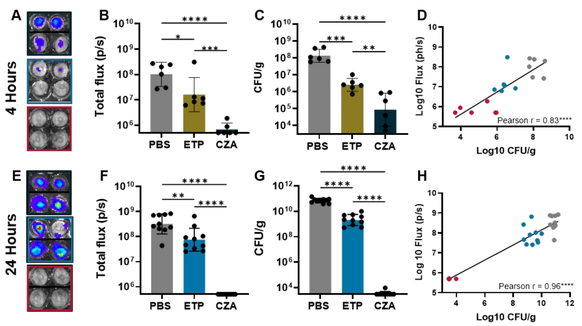
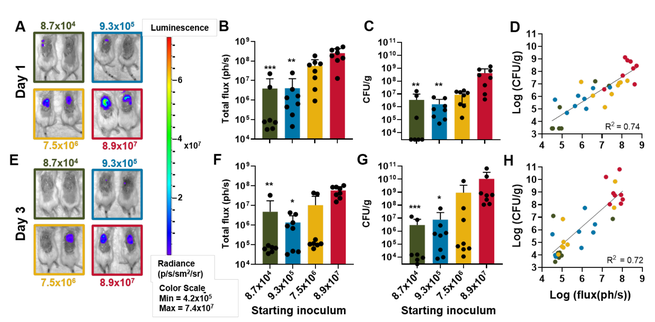


Figure 6: Treatment of *ex vivo* porcine skin explant infected with *K. pneumoniae* BIDMC2A::Tn7-*lux*. Explants were infected with 4x10⁴ CFU and provided treatment according to SoC dosing for each therapeutic. Samples were imaged for **(A,B,E,F)** luminescence, homogenized, and plated to enumerate differences in **(C,G)** CFU bioburden at 4 and 24 hours across treatments. **(D,H)** Correlation was measured between flux and CFU by simple linear regressions.

Data is presented as the mean ± SD (*n* = 10) with the x-axis set to the LOD. Statistical analysis was performed with a Tukey multiple comparisons test on a one-way ANOVA: * *p* < 0.05, ** p < 0.01, *** p < 0.001, **** *p* < 0.0001.

Figure 7: Inoculum determination for *K. pneumoniae* BIDMC-2A::Tn7-*lux* in our *in vivo* murine full thickness dorsal wound model. 6 mm wounds were generated and infected with the bacteria. **(A,B,E,F)** Luminescence and **(C,G)** local bacterial bioburden were measured at the terminal endpoints of 1 and 3 days. 1x10⁸ CFU was selected as the minimum dose to create robust, consistent infections with BIDMC2A::Tn7-*lux*. **(D,H)** Luminescence correlated well with CFU.

Data is presented as the mean ± SD (*n* = 8) with the x-axis set to the LOD. Statistical analysis was performed with a Tukey multiple comparisons test on a one-way ANOVA: * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001.



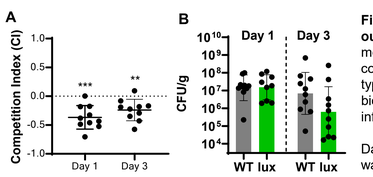


Figure 8: Virulence determination of 5x10⁷ CFU *K. pneumoniae* BIDMC-2A WT and *lux* in our *in vivo* murine full thickness dorsal wound model. (A) Local bacterial bioburden were measured at the terminal endpoints of 1 and 3 days with luminescence used to differentiate colonies. There was a significant reduction in the luciferase colonies as compared to the wild-type. **(B)** However, monoculture infections with WT and lux strains did not show differences in bioburden, indicating that the luciferase mutant is as virulent as the WT in non-competitive infections.

Data is presented as the mean ± SD (*n* = 10) with the x-axis set to the LOD. Statistical analysis was performed with a Tukey *post-hoc* test on a one-way ANOVA: ** *p* < 0.01, *** *p* < 0.001.

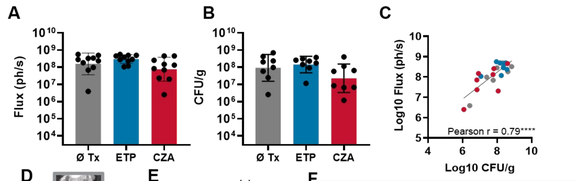


Figure 9: Treatment of *in vivo* full thickness dorsal wound model infected with *K. pneumoniae* BIDMC2A::Tn7-*lux* at 24 hours. 6 mm wounds were infected with 1x10⁸ CFU of bacteria, and mice were provided with treatment according to SoC dosing for each therapeutic. **(A,D)** Luminescence and **(B)** local bacterial bioburden were measured at day 1's terminal endpoint **(C)** Luminescence correlated well with CFU. **(E)** M1 macrophage downregulation by *K. pneumoniae* across all groups is evidenced by Griess results from local wound bed samples, but **(F)** early clearance by ceftazidime-avibactam (CZA) is distinguished by stronger pro-inflammatory cytokines and reduced IL-10.

Data is presented as the mean ± SD (*n* = 8) with the x-axis set to the LOD. Statistical analysis was performed with a Tukey multiple comparisons test on a one-way ANOVA: * *p* < 0.05, ** *p* < 0.01. Cytokines with significant differences between groups (* *p* < 0.05) are shown in panel **(F)**.

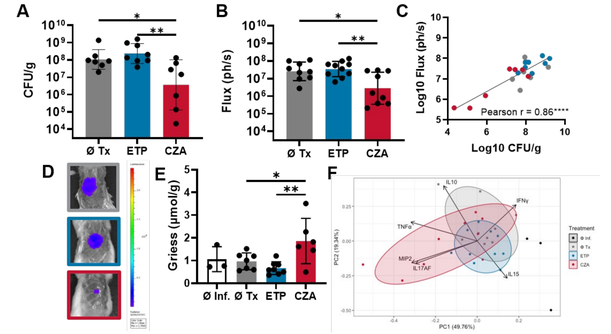


Figure 10: Treatment of *in vivo* full thickness dorsal wound model infected with *K. pneumoniae* BIDMC2A::Tn7-*lux* at 72 hours. (A,D) Luminescence and **(B)** local bacterial bioburden were measured at the terminal endpoint of 3 days. Significant reductions were seen at day 3 with ceftazidime-avibactam (CZA) treatment whereas ertapenem (ETP) was ineffective as the parent strain, BIDMC-2A, is carbapenem resistant. **(C)** Luminescence correlated well with CFU. **(E)** M1 macrophage downregulation, as measured by Griess, is trending downward with CZA treatment given reductions in bioburden and consequential reversal in macrophage polarization. **(F)** IL-10 continues to drive this polarization by *K. pneumoniae* in untreated and ertapenem groups.

Data is presented as the mean ± SD (*n* = 8) with the x-axis set to the LOD. Statistical analysis was performed with a Tukey multiple comparisons test on a one-way ANOVA: * *p* < 0.05, ** *p* < 0.01. Cytokines with significant differences between groups (* *p* < 0.05) are shown in panel **(F)**.

CONCLUSIONS & NEXT STEPS

- **BIDMC-2A::Tn7-*lux*** is a **clinically-relevant** strain of *K. pneumoniae* which can be used to **longitudinally track infections** with a high rate of correlation between luminescence and CFU.
- Other strains of *K. pneumoniae*—including **hypervirulent isolates**—have been successfully **mutagenized** as well, indicating **our methodology is widely deployable against clinically-relevant strains of *Klebsiella*.**

Partners/Collaborators

Thank you to our partners at NMRC-BDRD for their work on assembling the *K. pneumoniae* genomes. We also thank our collaborators at WRAIR for running and analyzing the cytokine and chemokine samples from our *in vivo* studies.

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