Antagonizing *Klebsiella pneumoniae* using prebiotics and postbiotics under simulated gut conditions

Vijay Baksh*, Dr. Ravinder Nagpal**

* Department of Biological Sciences; Florida State University, Tallahassee, FL

** Department of Health, Nutrition, and Food Sciences; Florida State University, Tallahassee, FL

Abstract

In 2019 alone, approximately 5 million deaths were associated with antibiotic-resistant bacteria, excluding an estimated 1.27 million direct deaths, with projections rising to nearly 9 million annual deaths by 2050. As rates of prolonged hospitalization increase, patient exposure to pathogens such as *Klebsiella pneumoniae* (KP) rises through contact with medical devices of ventilators and catheters. Individuals with metabolic disorders or underlying gastrointestinal dysfunction are especially susceptible to KP colonization and subsequent intestinal translocation due to impaired barrier integrity and reduced microbial diversity, however emerging evidence indicates that KP can translocate across healthy gastrointestinal epithelium to facilitate secondary infections. Additionally, WHO's 2024 bacterial priority pathogen review ranked two different antibiotic-resistant strains of *Klebsiella pneumoniae* within the top 5 highest concerns to public health. This study aims to evaluate microbiome-targeted strategies to suppress KP colonization while preserving beneficial gut flora. Using an in-vitro simulated gut fermentation model, stool inoculate from healthy adults will be antagonized with 3% w/v KP and treated with a combination of probiotics, prebiotics, and postbiotics. Microbial composition will be assessed via 16S rRNA sequencing alongside metabolic shifts using NMR spectroscopy. We hypothesize that KP introduction will induce dysbiosis, while postbiotic and combination treatments will most effectively suppress KP proliferation and restore metabolic homeostasis. These findings may guide development of scalable synbiotic or postbiotic therapeutics to mitigate multidrug-resistant gut colonization in high-risk populations.

Background

Current treatments to bacterial infections rely predominantly on antibiotic administration, while effective in reducing pathogens, these therapies can produce unintended consequences on the body. Broad-spectrum antibiotics used to treat bacterial infections such as those attenuated by *Klebsiella pneumoniae*, can induce microbial dysbiosis within the gut through depleting commensal bacterium (Lin et al., 2017). Additional impacts can include the increase in the permeability of epithelial barrier causing pathogens to translocate to other organs and incite secondary infections or other comorbidities (Wang et al., 2025). Consequently, there is increasing interest in alternative therapeutic approaches such as using Lactic Acid Bacteria to antagonize pathogenic species through the production of organic acids and other antimicrobial metabolites, inhibition of mucosal adherence and biofilm formation, and support of intestinal barrier recovery (Do et al., 2025). More specifically, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* have been shown to inhibit the growth of multi-drug resistant *Klebsiella pneumoniae* under fermentative conditions (Nagpal et al., 2018*). L. rhamnosus* has also been shown to inhibit genes of *K. pneumoniae* that promote biofilm production (Zhang et al, 2024).

Methods

Determine inoculum concentration of *K. pneumoniae*

↓

Collect Stool samples from participants

↓

Stool Inoculation with *K. pneumoniae* and Treatments

↓

Microbiome Sequencing and *K. pneumoniae* quantification using qPCR

↓

Bioinformatics Analysis

Experimental Groups:
Inulin (INU),
Fructooligosaccharide (FOS),
Lactobacillus rhamnosus GG (LGG),
Lactobacillus plantarum (LP),
Heat-Inactivated LP,
Heat-Inactivated LGG,
INU + LP,
FOS + LP,
INU + LGG,
FOS + LGG

Within the generalized process above, we are currently on the third step being anaerobic fecal fermentation where this study will include 10 participants aged 18-35 years old. This study is projected to be concluded by June 2026. The following figures all represent the results from determining the concentration and inoculum amount of *K. pneumoniae*.

Results (optimization pertaining to Step 1)

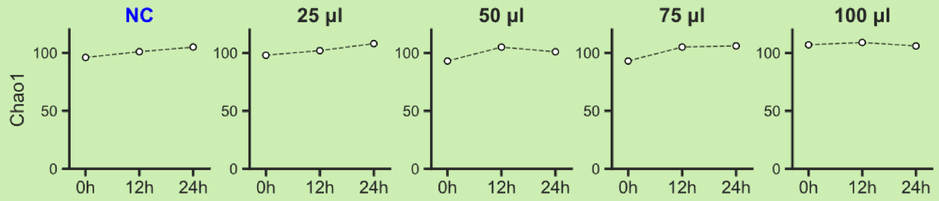


Figure 1. Changes in microbial diversity during fermentation at different volumes of *Klebsiella at* 4.75×10^7 CFU / mL

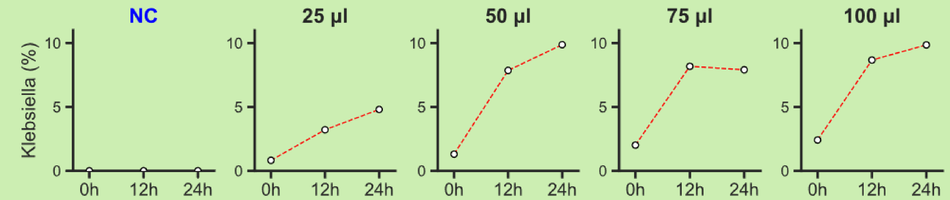


Figure 2. Changes in relative abundance of *Klebsiella* during fermentation at initial volumes of 4.75×10^7 CFU / mL

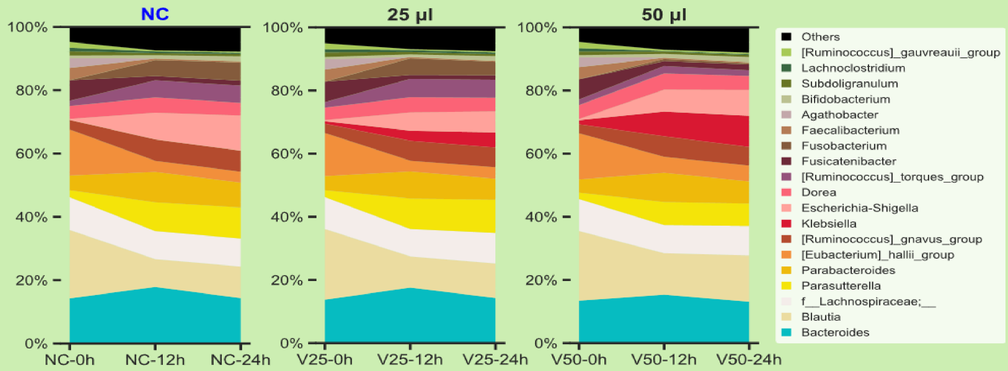
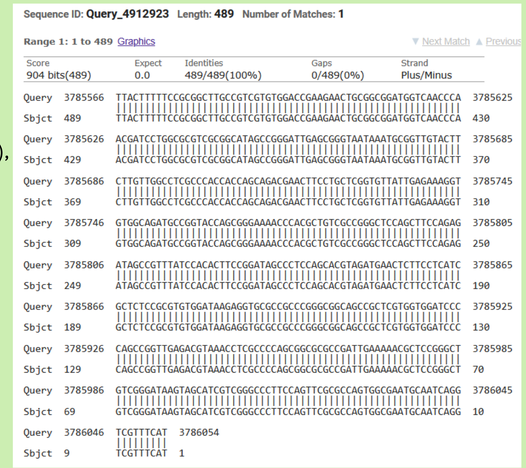


Figure 3. Overall shifts in microbial composition at different volumes of *Klebsiella at* 4.75×10^7 CFU / mL



Sequence ID: **Query_4912923** Length: **489** Number of Matches: **1**

Range 1: 1 to 489 Graphics ▼ Next Match ▲ Previous

Score	Expect	Identities	Gaps	Strand
904 bits(489)	0.0	489/489(100%)	0/489(0%)	Plus/Minus

```
Query  3785566  TTACTTTTTCCGCGGCTTGCCGTCGTGTGGACCGAAGAACTGCGGCGGATGGTCAACCCA  3785625
Sbjct  489      TTACTTTTTCCGCGGCTTGCCGTCGTGTGGACCGAAGAACTGCGGCGGATGGTCAACCCA  430
Query  3785626  ACGATCCTGGCGCGTCGCGGCATAGCCGGGATTGAGCGGGTAATAAATGCGGTTGTACTT  3785685
Sbjct  429      ACGATCCTGGCGCGTCGCGGCATAGCCGGGATTGAGCGGGTAATAAATGCGGTTGTACTT  370
Query  3785686  CTTGTTGGCCTCGCCCACCACCAGCAGACGAACTTCCTGCTCGGTGTTATTGAGAAAGGT  3785745
Sbjct  369      CTTGTTGGCCTCGCCCACCACCAGCAGACGAACTTCCTGCTCGGTGTTATTGAGAAAGGT  310
Query  3785746  GTGGCAGATGCCGGTACCAGCGGGAAAACCCACGCTGTCGCCGGGCTCCAGCTTCCAGAG  3785805
Sbjct  309      GTGGCAGATGCCGGTACCAGCGGGAAAACCCACGCTGTCGCCGGGCTCCAGCTTCCAGAG  250
Query  3785806  ATAGCCGTTTATCCACACTTCCGGATAGCCCTCCAGCACGTAGATGAACTCTTCCTCATC  3785865
Sbjct  249      ATAGCCGTTTATCCACACTTCCGGATAGCCCTCCAGCACGTAGATGAACTCTTCCTCATC  190
Query  3785866  GCTCTCCGCGTGTGGATAAGAGGTGCGCCGCCCGGGCGGCAGCCGCTCGTGGTGGATCCC  3785925
Sbjct  189      GCTCTCCGCGTGTGGATAAGAGGTGCGCCGCCCGGGCGGCAGCCGCTCGTGGTGGATCCC  130
Query  3785926  CAGCCGGTTGAGACGTAAACCTCGCCCCAGCGGCGCGCCGATTGAAAAACGCTCCGGGCT  3785985
Sbjct  129      CAGCCGGTTGAGACGTAAACCTCGCCCCAGCGGCGCGCCGATTGAAAAACGCTCCGGGCT  70
Query  3785986  GTCGGGATAAGTAGCATCGTCGGGCCCTTCCAGTTCGCGCCAGTGGCGAATGCAATCAGG  3786045
Sbjct  69       GTCGGGATAAGTAGCATCGTCGGGCCCTTCCAGTTCGCGCCAGTGGCGAATGCAATCAGG  10
Query  3786046  TCGTTTCAT  3786054
Sbjct  9        TCGTTTCAT  1
```

Figure 4. BLAST analysis of *K. pneumoniae* subsp. Pneumoniae (ATCC 43816 [NCBI accession: CP064352.1]) using the following gene and primers during qPCR:
The *K. pneumoniae* khe gene (NC_016845.1: 309,578–310,066; locus tag KPHS_02720; 489 bp) was used as the target.

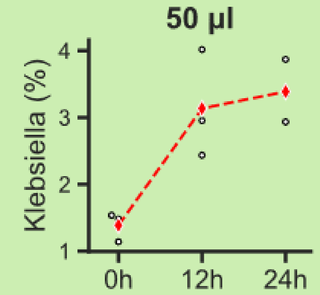


Figure 5. $\boldsymbol{Relative\ abundance\ Calculation\ of\ Kpn} = 2^{-(Ct_{estimated\ Kpn\ 16S} - Ct_{community\ 16S})}$

Conclusions

During the fermentation of *Klebsiella pneumoniae* with healthy stool samples, there exhibited a stable bacterial diversity independent *of K. pneumoniae* concentrations. However, *K. pneumoniae* did proliferate overtime but larger doses did not show to lead to a more exponential growth pattern. From this we conclude the concentration of *K. pneumoniae* may not directly create a dysbiotic microbiome under these circumstances. For the continuation of the experiment, we will be using an inoculation of 50 uL *K. pneumoniae at* 4.75×10^7 CFU / mL .
The experimental group combining *Lactobacillus rhamnosus GG (LGG)* and Inulin is expected to exhibit the greatest proliferation of beneficial bacterial species alongside inhibiting *K. pneumoniae* growth. Amongst the monocultures alone, Heat-Inactivated LGG is expected to have the largest beneficial impact. However, the projection of the order of ranking the experimental groups based on the measurements of microflora diversity, is difficult to predict due to a lack of research surrounding any connection between Inulin and FOS with inhibiting growth of KP.

Acknowledgements

We thank the members of The Gut Biome Lab specifically PhD candidates JooHyeong Kim and Gwoncheol Park for their technical help. This work was supported by the Bess H. Ward Honors Thesis Award.

References

Do. A.D., Quang, H.P., Phan, Q.K. (2025). Probiotic cell-free supernatant as effective antimicrobials against Klebsiella pneumoniae and reduce antibiotic resistance development. International Microbiology (28), 623-632. DOI: 10.1007/s10123-024-00575-x

Nagpal, R., Wang, S., Ahmadi, S., Hayes, J., Gagliano, J., Subashchandrabose, S., Kitzman, D.W., Becton, T., Read, R., Yadev, H. (2018). Human-origin probiotic cocktail increases short chain fatty acid production via modulation of mice and human gut microbiome. Scientific Reports 8 (12649). DOI: 10.1038/s41598-018-30114-4

Zhang, C., Wang, C., Dai, J., Xiu, Z. (2024). The inhibition mechanism of co-cultured probiotics on biofilm formation of Klebsiella pneumoniae. Journal of Applied Microbiology 135 (6). DOI: 10.1093/jambio/lxae138

Wang, X., Fang, Y., Liang, W., Cai, Y., Wong, C.C., et al. (2025). Gut-liver translocation of pathogen Klebsiella pneumoniae promotes hepatocellular carcinoma in mice. Nature microbiology 10, 169 – 184. DOI: 10.1038/s41564-024-01890-9

Lin, S., Chung, P., Wu, Y., Fung, C., Hsu, C., Chen, L. (2017). Inhibition of nitric oxide production reverses diabetes-induced Kupffer cell activation and Klebsiella pneumonia liver translocation. PLoS ONE 12(5). DOI: 10.1371/journal.pone.0177269